Supporting Publications
2013:EN-392

EXTERNAL SCIENTIFIC REPORT

CFT/EFSA/PRAS/2012/07-CT 01, 02 and 03

"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"

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Suggested citation: Vv.Aa.; 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. Supporting Publications 2013:EN-392. [88 pp.]. Available online: www.efsa.europa.eu/publications

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SUMMARY

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 the development of such methodologies in 2007. The implementation of the methodologies requires also the establishment of cumulative assessment groups (CAGs) of pesticides on the basis of their toxicological properties. To support this activity EFSA's Pesticides Unit outsourced preparatory work under the Grant Agreement CFP/EFSA/PPR/2009/01. This project was carried out by the Technical University of Denmark, DTU. The project explored the existing data on pesticide active substances in order to identify the toxicological effects and endpoints and where possible data on mechanism or mode of action that can be the basis of a cumulative risk assessment. The final report by DTU contains proposals for cumulative assessment groups of pesticides having specific identified effects and their related endpoints.

EFSA concluded that further consolidation of the outcome of the DTU report needed to be carried out, in particular in the area of neurotoxicity, liver toxicity and toxicity on reproduction and development. Thus, as a follow up of the project carried out by DTU, EFSA launched a call for tender "Toxicological data analysis to support grouping of pesticide active substances for cumulative risk assessment (CRA) of effects on the liver, on the nervous system and on reproduction and development" (CFT/EFSA/PRAS/2012/07).

In order to consolidate the outcome of the report drawn up by DTU, in the present project all the pesticides identified as having effects on reproduction and development, the liver and the nervous system were re-evaluated by the consortium. In addition, all pesticides added to Annex I in the period 31-05-2009 to 31-12-2011, and three pesticides (flurtamone, oxadiargyl and pyridate) not screened by DTU in the absence of DARs, were also evaluated for these effects.

Toxicological analysis of the available regulatory studies provided in support of their approval has been performed for reproductive and developmental toxicity, neurotoxicity and for effects on liver and gallbladder. In total 257 substances were found to have reproductive and developmental toxicity, 67 substances were found to be neurotoxic, and 244 substances to cause effects on the liver and biliary system, including the gallbladder. All the findings (endpoints) that were indicated in the contract as indicative for those effects have been reported for each substance, with their respective NOEL/LOELs. The selection of NOELs and LOELs was performed, as requested by EFSA, without any interpretation on whether an effect is to be considered adverse or not adverse. The identification of key effects appropriate for the establishment of common assessment groups was also not required and therefore not undertaken. It was in fact considered that the establishment of CAGs should be agreed upon by a group of experts rather than be based on the opinion of an individual contractor. However, the data presented in this report provide the basis for addressing both the issue of adversity versus non adversity, and the definition of CAGs.

Critical to these activities is the identification, whenever possible, of the MoA. In the report, established or postulated MoAs have been reported, as well as reference to possible sources of information in this respect, which mostly included the open literature. No in-depth analysis of proposed or postulated MoAs was performed.

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In conclusion, this report provides a comprehensive database that would allow EFSA to access the relevant toxicological information necessary to define the CAGs according to the toxicological criteria that will be adopted.
It is recommended that the data provided in this report be interpreted against information on MoA and the presence of other systemic toxicological effects. Given the present toxicological knowledge, the definition of CAGs will necessarily be the result of an expert weight-of-evidence judgment.

Key words

Cumulative risk assessment, data collection, liver, nervous system, reproduction, development

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BACKGROUND

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. EFSA and the PPR Panel have started the development of such methodologies in 2007. The implementation of the methodologies requires also the establishment of cumulative assessment groups of pesticides on the basis of their toxicological properties. The PPR Panel is currently preparing a new opinion on the identification of pesticides to be included in cumulative assessment groups that will finalize EFSA's work on cumulative risk assessment and facilitate the implementation of cumulative risk assessment in routine MRL-setting. To support this activity EFSA's Pesticides Unit outsourced preparatory work under the Grant Agreement CFP/EFSA/PPR/2009/01. The final report of this project contains proposals for cumulative assessment groups of pesticides. However, after a thorough analysis of the report it became clear that further consolidation of the outcome needs to be carried out on the basis of a robust methodology, in particular in the area of neurotoxicity, liver toxicity and toxicity on reproduction and development.

TERMS OF REFERENCE

This contract was awarded by EFSA to: a consortium of the French Agency for Food, Environmental and Occupational Health and Safety (France), RIVM (The Netherlands), and AOSACCO/ICPS (Italy).

The tasks and responsibilities have been divided amongst the 3 consortium partners:

- Lot 1: RIVM
- Lot 2: AOSACCO/ICPS
- Lot 3: ANSES

ANSES (Antony Fastier) is the lead of the consortium tender.

Contract title: 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

Contract number: CFT/EFSA/PRAS/2012/07

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INTRODUCTION AND OBJECTIVES

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 the development of such methodologies in 2007. The implementation of the methodologies requires also the establishment of cumulative assessment groups (CAGs) of pesticides on the basis of their toxicological properties. To support this activity EFSA's Pesticides Unit outsourced preparatory work under the Grant Agreement CFP/EFSA/PPR/2009/01. This project was carried out by the Technical University of Denmark, DTU. The project explored the existing data on pesticide active substances in order to identify the toxicological effects and endpoints and where possible data on mechanism or mode of action that can be the basis of a cumulative risk assessment. The final report by DTU contains proposals for cumulative assessment groups of pesticides having specific identified effects and their related endpoints.

EFSA concluded that further consolidation of the outcome of the DTU report needed to be carried out, in particular in the area of neurotoxicity, liver toxicity and toxicity on reproduction and development. Thus, as a follow up of the project carried out by DTU, EFSA launched a call for tender "Toxicological data analysis to support grouping of pesticide active substances for cumulative risk assessment (CRA) of effects on the liver, on the nervous system and on reproduction and development" (CFT/EFSA/PRAS/2012/07).

The contract for the project was awarded to a consortium of 3 contractors, i.e. 1) the National Institute for Public Health and the Environment (RIVM), the Netherlands (Lot 1 for neurotoxicity), 2) the International Centre for Pesticides and Health Risk Prevention (AOSACCO/ICPS), Italy (Lot 2 for liver toxicity) and 3) the Agency for Food, Environmental and Occupational Health and Safety (ANSES), France (Lot 3 for reproductive and developmental toxicity).

In order to consolidate the outcome of the report drawn up by DTU, in the present project all the pesticides identified as having effects on reproduction and development, the liver and the nervous system were re-evaluated by the consortium. In addition, all pesticides added to Annex I in the period 31-05-2009 to 31-12-2011, and three pesticides (flurtamone, oxadiargyl and pyridate) not previously screened by DTU, were also evaluated for these effects.

At the kick-off meeting (3-4 September 2012) the objectives and criteria that were presented in the draft technical annex to the call of the tender were discussed. It was noted that the timelines to carry out the work were very short. In view of this, it was agreed between the consortium and EFSA that some of the objectives could be altered.
The most notable alterations were the following:

- It was agreed that the DAR and appertaining documentation (e.g. addenda, technical discussion reports) should be scrutinised in every case. Original studies would only need to be considered when deemed necessary by the contractors.
- The active substances causing specific effects would not have to be listed in a proposed cumulative assessment group.
- No consolidated cumulative assessment groups needed to be provided by the contractors.

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- The contractors weren't required to identify and evaluate information of mode of action. However, any assumed or confirmed mode/mechanism of action reported either in DAR or open literature needed to be reported. It was acknowledged by EFSA that in the case of ANSES they would in most cases not be able to consult also open literature in regard to mode/mechanism of actions based on the sheer amount of substances and the variety of effects they have to deal with.

The outcome of the evaluations for the 3 Lots are presented in 3 subchapters. Each subchapter describes the methodology, individual assessments, results and conclusions for the specific Lot. Each subchapter contains an Appendix with the reporting table, containing the relevant data (e.g. endpoints, NOELs and LOELs) for the specific Lot. The report will therefore contain three reporting tables, one for each Lot

At the end of the report the outcome of the present project is briefly discussed.

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CFT/EFSA/PRAS/2012/07 CT01 (lot1)

LOT 1 - CUMULATIVE RISK ASSESSMENT OF EFFECTS ON THE NERVOUS SYSTEM

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ABSTRACT

In the framework of Regulation (EC) No. 396/2005 on Maximum Residue Levels (MRLs) of pesticides, EFSA and the PPR Panel have started in 2007 the implementation of methodologies required to the establishment of cumulative assessment groups (CAGs) of pesticides on the basis of their toxicological properties. In this context the objective of the contractor RIVM (Lot 1) was to consolidate all available toxicological studies for neurotoxic effects for 67 pesticides identified as having effects on the nervous system by the Technical University of Denmark (DTU) in a previous evaluation of substances included in Annex I of Council Directive 91/414/EEC up to 31st of May 2009. In addition, 60 more recently approved substances (between 31-05-2009 and 31-12-2011) have been evaluated and 3 substances not previously assessed by DTU.

EFSA required the effects to be retrieved according to those previously identified by the DTU; therefore no interpretation on their relevance to establish CAGs was performed. All toxicological studies have been taken into account, including acute and repeated dose studies. For each specific effect a NOEL and a LOEL was identified for the most sensitive species and gender, without interpretation whether an effect is to be considered adverse or not adverse.

All 130 pesticides were evaluated and results are reported in a final database. For 8 pesticides identified as neurotoxicants by DTU no convincing evidence for neurotoxic effects could be found. Argumentations supporting these findings are provided in the report. From the three substances not previously assessed by DTU, one elicited neurotoxic effects. Evaluation of the 60 pesticides added to Annex I during the period 31-5-2009 to 31-12-2011 revealed that nine pesticides had clear neurotoxic properties. Two of these (zinc phosphide and aluminium sulphate) were not included in the final reporting table due to their intended (targeted) use which does not include edible plants. The relevant information on the neurotoxic effects induced by the final selection of 67 pesticides were recorded in the reporting table. For a small number of pesticides MoAs for neurotoxicty were identified. For several pesticides MoAs for their neurotoxicity were suggested in the DARs, but limited evidence to support the suggested MoAs was provided. For the remaining 32 pesticides with potential neurotoxic effects, the specific MoA for neurotoxicity is unknown.

KEY WORDS

Nervous system, acute, repeated dose, NOEL, LOEL, CAG, MoA

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INTRODUCTION AND OBJECTIVES

For neurotoxicity (Lot 1) the objective of RIVM was to analyse all the available toxicological data of the pesticides listed in Annex 9 to call (CFT/EFSA/PRAS/2012/07 to identify relevant neurotoxic effects. These neurotoxic effects were tabulated in an excel file and can be used by EFSA as a basis for establishing refined cumulative assessment groups of pesticides. At the kick-off meeting for this project it was decided that the contractor would not provide proposals for consolidated cumulative assessment groups.

MATERIALS AND METHODS

This project was a follow up of the earlier project performed by the Technical University of Denmark (DTU), in which the pesticides placed on Annex I of the pesticides Directive 91/414/EC at 31-05-2009 were scrutinized for their effects on a extensive range of organs and systems. In their evaluation DTU had identified 67 pesticides with neurotoxic properties. Three pesticides listed on Annex I, i.e. flurtamone, oxadiargyl and pyridate, were not screened by DTU in the absence of DARs at the time. Since the evaluation by DTU another 60 pesticides were added to Annex I during the period 31-5-2009 to 31-12-2011.
In order to consolidate the outcome of the report drawn up by DTU, in the present project RIVM scrutinized all the pesticides identified as being neurotoxic by DTU. In addition, all pesticides added to Annex I in the period 31-05-2009 to 31-12-2011, and flurtamone, oxadiargyl and pyridate were evaluated for neurotoxic effects.

1. Source of information

At the kick-off meeting for this project it was decided that information on toxicological effects would be retrieved from regulatory toxicological studies provided in support of approval and their evaluations under the peer review process of Directive 91/414/EC and Regulation (EC) 1107/2009 (Draft Assessment Reports, additional reports, addenda, evaluation table and discussion table, EFSA Conclusion, Commission reports) as available on the CIRCA website. Original study reports would only be consulted when these sources of information would include insufficient detail to draw conclusions. In addition, JMPR reports were searched for additional information on mode/mechanisms of action (MoA) and for study evaluations not described in the EU Draft Assessment Reports (DAR). When needed, open literature (e.g. PubMed) was searched for additional information, for instance on MoAs.
Information on pesticidal mode of action and chemical class was primarily obtained from either volume 1 of the respective DAR or from the CropLife Australia Management Review Group lists, suggested by EFSA:

- http://www.croplifeaustralia.org.au/files/resistancemanagemen/insecticides/2012%20Insecticide%20Mode%20of%20Action%20Table.pdf
- http://www.croplifeaustralia.org.au/files/resistancemanagemen/herbicides/2012%20-%20Herbicide%20MOA%20Table.pdf
- http://www.croplifeaustralia.org.au/files/resistancemanagemen/fungicides/2012%20Fungicide%20Activity%20Group%20Table.pdf

In case the pesticidal mode of action of the active substance or the chemical class was not clarified in the DAR or listed in either three of the CropLife Australia Management Review Groups lists, several internet sites were consulted (e.g. http://www.plantprotection.org/hrac/MoA.html, http://www.frac.info/frac/publication/anhang/FRAC_Code_List_2007_web.pdf,

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http://glyphosateweedscrops.org/Info/MOA_060807.pdf,
http://www.dropdata.org/RPU/pesticides_MoA.htm)

2. Data collection and reporting

For each of the pesticides indicated above RIVM searched the available information for neurotoxic effects and recorded the data in the reporting table (see Annex I), using the following strategy:

1. All available acute and repeated dose in vivo toxicological studies in mammals were taken into account for identification of neurotoxic effects of the pesticides. In vitro studies were only taken into account when these provided information on the known or presumed neurotoxic MoA.
2. Since the overall objective of the project is to establish common assessment groups for dietary risk assessment, only toxicity studies performed by oral administration (diet, capsule, gavage) were reported. Inhalation studies were considered only for pesticides that are gasses and thus could not be toxicologically tested via the oral route.
3. It was agreed that for neurotoxicity both acute and repeated dose effects would be reported. Therefore 3 additional columns were included in the reporting table, i.e. for acute NOELs, acute LOELs, and remarks concerning the acute effects.
4. As LD_{50} studies are primarily aimed at determining lethality and not describing dose related effects at non-lethal dosages, they were not taken into account, unless, unless dosages were of the same order of magnitude as the dose ranges in the short-term studies.
5. NOELs and LOELs generally were based on the NOELs/NOAELs and LOELs/LOAELs as described in the DAR. In few instances, when the DAR was relatively outdated; more recent evaluations from JMPR were also consulted. Since DARs and JMPR evaluations have been peer reviewed by experts the information provided in these documents was considered scientifically sound and no re-evaluation of the value of the NOEL or LOEL was performed.
6. For each endpoint the lowest NOEL and LOEL were reported, with description and study reference. Higher LOELs and NOELs observed in additional studies were not included in the table.
7. Occasionally, overlapping NOELs/LOELs for a specific endpoint were observed in two or more studies of the same duration in the same species. In such situations, overall NOELs and LOELs based on combined data were determined, provided that the studies were comparable with respect to study design and strain of animal, and provided that there was a margin ≥ 2 between the overall NOEL and LOEL. It is clearly indicated in the table if NOELs/LOELs are based on2 or more studies. In case studies differed in duration and/or animal strain it was considered not appropriate to combine the data from these studies to derive an overall NOEL and LOEL. In these cases both studies were included in the CAG table.
8. For each endpoint only the NOEL and LOEL observed in the most sensitive species were recorded. In case the NOELs in two different species were (almost) identical, the NOELs and LOELS for both species were recorded.
9. In some studies effects that are potentially indicative of neurotoxicity were observed at (near) lethal doses. If in such a case the reviewer considered that these effects could be attributed to general systemic toxicity rather than a neurotoxic property of the pesticide, these effects were not included in the reporting table.
10. RIVM noted that for certain critical endpoints a variety of descriptions are used in the DARs. For such critical endpoints one specific term was included in the table. For example, effects described in the DAR as waddling gait, disturbed balance or loss of coordination were included in the table as the critical endpoint “ataxia”. The specific description of the critical

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endpoint in the study in the DAR was recorded in the remarks column. RIVM considered that such grouping of various descriptions of a critical endpoint will enable directed selections of critical endpoints in Excel and may facilitate the formation of CAGs. An overview of the specific terms used to group effects is presented in Appendix A1.

11. In case in a single study several critical endpoints were observed at the same NOEL/LOEL they were recorded separately in the table (one row for each critical endpoint).
12. In case of dietary studies where NOELs/LOELs can differ for males and females due to differences in dietary intake, the lowest values are recorded in the table.
13. In case a NOEL/LOEL is not the same for males and females the lowest NOEL, belonging to the representative sex, is added to the table and "Based on effects observed in males/females" in the remark column. In case only one sex was studied, this was added to the remarks (only males/females tested).
14. When available, information on possible mode/mechanism of action, reported either in the DAR or from other sources was indicated in the reporting table.
15. For a few pesticides that were identified by DTU as being neurotoxic, RIVM concluded that this was not supported by the available information. These pesticides are not included in the present reporting table. A list with these pesticides, and the reasons for not including them in the reporting table is presented in Appendix B1.
16. Human data were always included in the table, even if the NOELs/LOELs for a certain endpoint were higher than those obtained in animal studies.
17. For a substantial number of pesticides, in particular carbamates and organophosphates, studies on their effect on acetylcholinesterase (AChE) activity were available. For the present evaluation a statistically significant inhibition of ≥ 20% was considered as toxicologically relevant. This is in accordance with guidance that has been developed over the last decade[1] and in is now common practice in many regulatory frameworks. Data for inhibition of erythrocyte and brain AChE were recorded separately.
18. In a number of studies the NOELs were lower than the NOAELs that formed the basis for the ADI or ARfD. In such cases this is indicated in the remarks, together with the value of the NOAEL on which the ADI or ARfD was based.

The selection of NOELs and LOELs was conducted without interpretation whether an effect is to be considered adverse or not adverse, as agreed by the consortium.

RESULTS

The available information on 67 pesticides identified by DTU as neurotoxic, 60 pesticides added to Annex I during the period 31-5-2009 to 31-12-2011, and the pesticides flurtamone, oxadiargyl and pyridate was scrutinized for neurotoxic effects (total number investigated is 130). Evaluation of the latter three pesticides revealed that only pyridate induces potentially neurotoxic effects.

Re-evaluation of the 67 pesticides considered to be neurotoxic by DTU led RIVM to the conclusion that 8 of these should not be considered neurotoxic. A list of these pesticides is presented in Appendix B1. In this Appendix for each of these pesticides a justification is given why RIVM did not consider them neurotoxic.

Evaluation of the 60 pesticides substances added to Annex I during the period 31-5-2009 to 31-12-2011 revealed that 51 pesticides either did not induce effects indicative of neurotoxicity, or induced effects (clinical signs), that might be indicative of neurotoxicity, only at lethal or near-lethal

[1] Solecki et al. Guidance on setting of acute reference dose (ARfD) for pesticides. Food and Chemical Toxicology 43 (2005) 1569–1593

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doses. In these cases the doses were much higher than the NOAELs/LOAELs that formed the basis for the ADIs and ARfDs for these substances.
The remaining nine pesticides included in Annex I after 31-5-2009 had neurotoxic properties. One of these, zinc phosphide, is a used as a rodenticide. In the EFSA conclusion on zinc phosphide it is stated that the products are applied in a targeted manner and no significant residues in plants and animal matrices are expected. In addition, aluminium sulphate is only used for flowers that will not be consumed. No residue definitions and MRLs have been set for plant or animal products for these compounds and no consumer risk assessments are required. Therefore data on neurotoxic effects of zinc phosphide and aluminium sulphate are not included in the reporting table.

The relevant information on the neurotoxic effects induced by the 67 pesticides (selection described above) were recorded in the reporting table (excel file) provided by EFSA (see Annex 2).

1. Identified MoAs

Only for a small number of pesticides MoAs for neurotoxicity were identified.

- For organophosphate and carbamate insecticides, nematocides and acaricides the MoA for neurotoxicity is generally accepted to be inhibition of acetyl cholinesterase activity. The binding of organophosphates to AChE is virtually irreversible, whereas the interaction between carbamates and AChE is reversible. As a consequence, the neurotoxic effects observed after exposure to carbamates are generally transient and the NOAELs for acute and repeated dosing are similar. In contrast to this, the neurotoxicity induced by organophasphates are generally of longer duration, and the NOAELs for repeated dosing are lower than those observed after a single exposure.

- For pyrethroid insecticides it is generally accepted that the MoA for neurotoxicity is based on their binding to voltage-sensitive sodium channels in the nerve membrane. The interaction prolongs the open state of the sodium channels resulting in a long lasting prolongation of the transient increase in sodium permeability of the membrane during excitation.

- Neonicotinoid pesticides are nicotinic acetylcholine receptor agonists. Low to moderate activation of these receptors causes nervous stimulation, high levels over-stimulate and block the receptors. Because most neonicotinoids bind much more strongly to insect neuron receptors than to mammal neuron receptors, these insecticides are selectively more toxic to insects than mammals.

- Fipronil disrupts the insect central nervous system by blocking the passage of chloride ions through the GABA receptor and glutamate-gated chloride (GluCl) channels. It is presumed that fipronil may act in mammals through the inhibition of the passage of chloride ions through the GABA receptors. It is noted that GluCl channels do not exist in mammals.

For a number of other pesticides MoAs for their neurotoxic properties were suggested in the DARs. However, generally limited evidence for these suggested MoAs was provided. For 32 pesticides with potentially neurotoxic effects the specific MoA for neurotoxicity is unknown

CONCLUSION AND DISCUSSION

In the present project 67 pesticides that were placed on Annex I at 31-12-2011 were identified as having neurotoxic properties. It appears that many neurotoxic pesticides share common clinical signs. This is not surprising since an effect on the nervous system will often be expressed as behavioural

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changes such as for instance effects on motor activity or motor coordination. However, the underlying causes for a particular behavioural change may be manifold.
In addition, it is noted that clinical signs, that can be indicative of neurotoxicity, can also be a non-specific expression of general systemic toxicity, in particular when the systemic toxicity severely compromises the normal functioning of the organism. As a consequence, it is quite feasible that a number of pesticides included in the reporting table for neurotoxicity on the basis of observed clinical signs in fact may not be neurotoxic, but the induced clinical signs of neurotoxicity are secondary to toxicity in other organs or systems. It can be concluded that great care is needed when considering to base CAGs on the presence of clinical signs by pesticides.

During the kick-off meeting for the project it was decided that the contractors would only provide the NOELs and LOELs for various critical endpoints (in the case of RIVM neurotoxic endpoints) and would not provide consolidated cumulative assessment groups. It was considered that the formation of CAGs for various effects would have to be discussed in and agreed upon by a group of experts rather than be based on the opinion of an individual contractor.
For the same reason, in case in a single study several critical endpoints were observed at the same NOEL/LOEL they were recorded separately (one row for each effect). It is quite possible that the underlying MoA for the different critical endpoints is the same (e.g. salivation and tremor), which might justify recording them together in one row in the reporting table. However, RIVM is of the opinion that the decision whether or not certain critical endpoints could be grouped should also be discussed in and agreed upon by a group of experts rather than be based on the opinion of an individual contractor. Furthermore, the separate recordings of critical endpoints in the excel reporting table allows easy selection of all pesticides and studies in which a particular critical endpoint is observed.

Only for four groups of pesticides clear MoAs for the neurotoxic effects have been identified. Thus, it is generally accepted that certain organophosphates and carbamates act by the inhibition of cholinesterase activity in the nervous system, while pyrethroids are known to cause neurotoxicity by prolonging the opening of sodium channels in the nerve cell membrane. Neonicotinoid pesticides are nicotinic acetylcholine receptor agonists, although the sensitivity of mammals to this neurotoxic action of this group of pesticide appears to be relatively low.
The number of pesticides acting through inhibition of cholinesterase activity or prolonged opening of sodium channels is substantial. Thus, substances acting through one of these MoAs might be considered for placing in a CAG.

For the other suggested MoAs evidence is often limited and often based on effects observed in a single pesticide, so that formation of CAGs is not possible.

It is noted by RIVM that defining a common MoA is not the final possible refinement for grouping several pesticides in a CAG. For instance, pyrethroids are known to exert neurotoxicity by causing long lasting prolongation of the transient increase in sodium permeability of the membrane during excitation. However, on the basis of electrophysiological studies it is possible to distinguish between 2 classes of pyrethroid insecticides: Type I and Type II. Alternatively, based on the binding assay on the alpha-aminobutyric acid (GABA) receptor-ionophore complex, synthetic pyrethroids can also be classified into two types: the alpha-cyano-3-phenoxybenzyl pyrethroids and the non-cyano pyrethroids. Apparently, on a molecular level the MoAs for Type I and Type II pyrethroids or cyano- and non-cyano pyrethroids are different. Functionally, this leads to different behavioural syndromes. This is further complicated by the observation that *in vivo* and *in vitro* studies indicate that simultaneous exposure to more than one pyrethroid often does not show additivity of toxicity, and

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even antagonism has been observed. This was observed in combinations of pyrethroids from the same class (i.e. Type I or Type II) or different classes (i.e. a Type I and a Type II pyrethroid).

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APPENDICES

A1. GROUPING OF EFFECTS/COMMON TERMS

Ataxia
- *altered/abnormal gait,*
- *waddling gait,*
- *gait disturbance*
- *loss of coordination*
- *stiff movements*
- *abasia*
- *tiptoe gait*
- *splayed gait*
- *hypermetria*
- *postural reaction deficits*
- *unsteadiness*

Choreoathetosis
- *convulsions*
- *repetitive pawing motions*
- *writhing*
- *shaking*
- *jerking leg movements*
- *rolling movements*
- *rocking movements*
- *lurching movements.*

Convulsion
- tonic
- clonic
- tonic/clonic

Cognition
- *learning*
- *memory*
- *maze test performance*
- *performance in (active/passive) avoidance test*

Emesis
- *vomiting*
- *retching*

Laboured breathing
- *increased respiratory rate*
- *increased inhalation*
- *laboured respiration*
- *tachypnea*
- *dyspnea*
- *accelerated respiration*

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Motor activity
- *mobility*
- *open field activity,*
- *ambulatory activity*
- *hypo-/hyperactivity*
- *rearing activity*
- *increased latency to first step*
- *inactivity*
- *immobility*
- *recumbency*
- *prostration*

Neuromuscular changes
- *hindlimb extensor strength*
- *fore-limb/hind-limb grip strength*
- *splayed foot*
- *righting ability*
- *splayed legs*
- *landing foot splay*
- *muscle tone*
- *paralysis*
- *paresis*
- *muscle weakness*

Reflex/sensory response
- *hypersensitivity*
- *pupil response*
- *response to touch*
- *startle response*
- *negative air drop*
- *splay reflex*
- *analgesic reflex*
- *response to tail pinch*
- *reactivity to handling*
- *paresthesia (abnormal sensation, as burning, prickling, formication)*
- *arousal*
- *hyperaesthesia*
- *proprioception (unconscious perception of movement and spatial orientation arising from stimuli within the body itself)*

Salivation
- *ptyalism*
- *oral discharge*

Structural changes in nerves or brain
- *nerve/neuronal degeneration*
- *fibre degeneration*
- *axonal degeneration*
- *brain lesion*

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- *necrosis of … cells*
- *increased/decrease size/volume/thickness brain (region)*
- *myelin damage/demyelination*
- *brain vacuolation*
- *swelling of the axons*

Tremor
- *fasciculation*
- *twitching*

Urination
- *urine incontinence*
- *stained/soiled perineum*
- *wet perineum*

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B1. LIST ON NON-NEUROTOXIC SUBSTANCES AFTER RE-EVALUATION

The present EFSA project was partly aimed at consolidating the outcome of the report drawn up by the Technical University of Denmark, DTU. Sixty-seven pesticides were identified as being neurotoxic by DTU. Re-evaluation of these 67 pesticides lead RIVM to the conclusion that 8 of these should not be considered neurotoxic. These pesticides were not included by RIVM in the CAG table for neurotoxicity. Below, for each of these pesticides a justification is given why RIVM did not consider them not neurotoxic.

Dimoxystrobin

Dimoxystrobin is a fungicide. In the DTU report it is concluded that dimoxystrobin belongs to CAG level 4a1a for neurotoxicity on the basis of inhibition of acetylcholinesterase. Indeed, a reduction in serum cholinesterase activity was reported in high dose females. However, there was no effect on erythrocyte cholinesterase activity. The study authors note that the reduced cholinesterase activities in rats are correlated with reduced dietary protein intake.

In a 2 year toxicity study in the rat there was a slightly increased incidence of focal degeneration in the sciatic nerve at the top dose in males (9/20, controls 3/20) and females (6/20, controls 4/20). However the RMS considered this not substance-related because no clear response was seen in the parallel carcinogenicity study with a larger number of rats.

In the EFSA scientific report (46, 1-82, 2005) it is concluded that there were no indications for dimoxystrobin being neurotoxic in acute and repeat dose neurotoxicity studies.

The present reviewers endorse the conclusion that dimoxystrobin is not neurotoxic.

Dinocap

Dinocap is a fungicide and acaricide. In the DTU report it is concluded that dinocap and its metabolites belong to CAG level 2a for functional changes related to the motor division and to CAG level 3f1 for neuronal degeneration.

In the DAR it appears that clinical signs, potentially indicative of neurotoxicity, in LD50 studies and developmental toxicity studies were observed at high, (near) lethal doses, and probably reflect general toxicity. The doses at which these clinical signs were seen were considerably higher than the overall NOAELs on which the ADI and ARfD were based.

Increased incidences of sciatic nerve degeneration and atrophy, observed at the high dose group in a 30 month dietary study in rats were attributed to the markedly increased survival rate and longevity of the high dose animals as compared to control animals (Maita,et al., 1980; described in DAR 2000).

With respect to neurotoxicity the following is concluded in the DAR: No evidence of specific neurotoxicity was observed in acute, subchronic or chronic studies in rodents and dogs. Therefore, studies on neurotoxicity/delayed neurotoxicity are not considered necessary.

The present reviewers conclude that dinocap should not be considered a neurotoxicant.

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Florasulam In an acute toxicity study in the rat salivation, urine and fecal soiling in the perineal area were observed at 1000 mg/kg bw onwards (Gilbert & Yano, 1995; DAR 1999).

In an acute neurotoxicity study in the rat, the results suggested male rats at 2000 mg/kg bw had slightly depressed activity and decreased responsiveness to sharp noise on the day of treatment, although the number of findings were few and within that expected by chance. At 2000 mg/kg bw a decreased motor activity in males on the day of treatment was considered a minimal treatment-related effect. At 1000 mg/kg bw only and increase in perineal staining was observed (Mattsson, Guirk and Yano, 1997; DAR1999). The RMS concluded that there is no need for establishing an ARfD. In the review report for florasulam (SANCO/1406/2001-rev. 6, 18 April 2002) it is stated in the list of endpoints that there is no evidence of neurotoxicity from acute and long-term neurotoxicity studies. The present reviewers endorse this conclusion.

The present reviewer notes that the doses at which minimal clinical signs of toxicity were observed were very high, and much higher than the overall NOAEL of 5 mg/kg bw/day that was the basis or the ADI. The present reviewers conclude that florasulam should not be considered neurotoxic.

Fosetyl aluminium

Fosetyl-aluminium is a fungicide. DTU considered Fosetyl-Al to have nervous system effects at 1250 mg/kg bw/day (NOEL 250 mg/kg bw/day) in one oral 90 day rat study (Coquet, 1973). In this study at 2500 mg/kg bw/day females showed an increase in AChE in ery's (NOEL for this effect 481 mg/kg bw/day). The present reviewers noted that this effect was not statistically, nor toxicologically relevant (increase of 7.7%). No nervous system effects were seen in other acute, subacute, semi-chronic and chronic toxicity studies up to very high dose levels. The present reviewers therefore conclude that fosetyl-aluminium is not a neurotoxic substance.

Phenmedipham

Phenmedipham is a herbicide. According to the DTU report, phenmedipham was identified to increase choline esterase activity in rat brain, red blood cells and plasma.

Upon re-evaluation of the data the present reviewers noted that phenmedipham in a 13-week study in rats induced marginal increases in brain and erythrocyte AChE activity. In a second 13 week study in rats the marginal increase in brain AChE activity was not dose-dependent, while no effect on erythrocyte AChE activity was found. In other short-term, subchronic and chronic studies in rats and dogs no effect on brain and erythrocyte activity was observed. In acute studies with phenmedipham only a few clinical signs were seen following dosing with high doses of phenmedipham. These signs (decreased activity, hunched posture and lethargy) were generally of a non-specific nature and did not include any of the signs associated with carbamate insecticide poisoning.

The present reviewers concluded that phenmedipham should not be considered neurotoxic.

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Prosulfocarb

Prosulfocarb is a herbicide. It was listed in the DTU report in CAG level 4a1a for neurotoxicity (Acetylcholinesterase inhibition).

After acute administration to rats no effect on brain and erythrocyte AChE activity was observed at doses up to 850 mg/kg bw. In an in vitro study only a slight effect on AChE was observed at saturated sulfamocarb concentrations. In a 90-day neurotoxicity study at week 5, increased salivation was observed at week 5 at a dose of 200 mg/kg bw/day, in the absence of an inhibition of AChE activity. Salivation was not affected at week 14, when (only) a 15% inhibition in brain AChE was observed.

Although the RMS considers the increased salivation to be indicative of AChE inhibition, the data do not support this. The carbamates that typically exert their effects through inhibition of AChE activity are N-methyl carbamates. Prosulfocarb is a dipropylthiocarbamate and lacks the typical neurotoxicity of N-methyl carbamates. It is further noted that the overall NOAEL of 0.5 mg/kg bw/day, which is the basis for the ADI is much lower than the doses at which salivation and minor effects on AChE activity are observed.

The present reviewers concluded that prosulfocarb should not be considered neurotoxic

Sulcotrione

Sulcotrione is a herbicide. In three subchronic dog studies (3-month, 4-month, 1-year) clinical signs indicative of neurotoxicity were observed, among others ataxia, tremors and increased reflexes. However, these effects occurred together with other signs such as emaciation and dehydration at high, near lethal doses (300-800 mg/kg bw/day). These doses were considerably higher than the LOAEL of 0.04 mg/kg bw/day from a 2 year study in rats on which the ADI was based. RIVM considered the clinical signs at these doses to be a feature of general systemic toxicity. Similar effects were not observed in studies with rats and mice.

Therefore, the present reviewers concluded that sulcotrione is not a neurotoxic substance.

Triflusulfuron-methyl

Triflusulfuron-methyl is a herbicide. In the DAR (2007) the following is stated: Acute (single dose gavage) and subchronic (90-day feeding) neurotoxicity studies were conducted in rats with triflusulfuron-methyl. In both studies, no clinical or morphological evidence of neurotoxicity was present in male or female rats at any dose tested (up to 2000 mg/kg/day in the acute gavage study and up to 3000 ppm in the subchronic feeding study). In a 2-year feeding study in rats an increased incidence and/or severity of axonal degeneration of the sciatic nerve was observed in male and female rats fed 1500 ppm, the highest concentration tested. These findings were explained by an exacerbation, by some unknown indirect mechanism, of the spontaneous lesion seen commonly in the aging rat. The results of the acute and short-term neurotoxicity studies confirmed that interpretation and strongly suggested that triflusulfuron-methyl is not a neurotoxicant.

Based on the information from the DAR the present reviewers conclude that triflusulfuron-methyl should not be considered neurotoxic.

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CFT/EFSA/PRAS/2012/07 CT02 (LOT 2)

LOT 2 - CUMULATIVE RISK ASSESSMENT OF EFFECTS ON LIVER INCLUDING BILIARY SYSTEM

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ABSTRACT

In the framework of Regulation (EC) No. 396/2005 on Maximum Residue Levels (MRLs) of pesticides, EFSA and the PPR Panel have started in 2007 the implementation of methodologies required to the establishment of cumulative assessment groups (CAGs) of pesticides on the basis of their toxicological properties. In this context the objective of the contractor ICPS (Lot 2) was to consolidate all available toxicological studies for liver, biliary and gallbladder effects for 181 pesticides among those included in Annex I of Council Directive 91/414/EEC up to 31st of May 2009 following a previous evaluation by the Technical University of Denmark (DTU). In addition, 60 more recently approved substances (between 31 05 2009 and 31 12 2011) have been evaluated as well as 3 substances for which no DAR was available at the time of evaluation of the DTU. EFSA required the effects to be retrieved according to those previously identified by the DTU. Therefore no interpretation on their relevance to establish CAGs was performed. Toxicological studies that have been taken into account were both main and preliminary studies (short and long term). For each specific effect a NOEL and a LOEL was identified for the most sensitive species and gender, without interpretation whether an effect is to be considered adverse or not adverse. All 244 pesticides were evaluated and results are reported in a final database. For 15 pesticides no convincing evidence for liver effects could be found. The NOELs for certain effects for 33 pesticides were lower than the ones used to set their peer-reviewed ADI. In addition, for 62 pesticides availability of information on MoA has been reported.

KEY WORDS

Liver, Bile, Gallbladder, NOEL LOEL, Study, CAG

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INTRODUCTION AND OBJECTIVES

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 the development of such methodologies in 2007. The implementation of the methodologies requires also the establishment of cumulative assessment groups (CAGs) of pesticides on the basis of their toxicological properties. To support this activity EFSA's Pesticides Unit outsourced preparatory work under the Grant Agreement CFP/EFSA/PPR/2009/01.
In order to consolidate the outcome of the report drawn up by Technical University of Denmark, DTU, EFSA has launched a call for tender "Toxicological data analysis to support grouping of pesticide active substances for cumulative risk assessment (CRA) of effects on the liver, on the nervous system and on reproduction and development" (CFT/EFSA/PRAS/2012/07). The Annex 9 to the call lists 181 pesticides with liver and biliary effects, which have been identified by DTU among those included in Annex I up to 31 May 2009 and 60 included in Annex I between 31 05 2009 and 31 12 2011 and 3 not previously evaluated by DTU.

In this context the objective of the contractor ICPS (Lot 2) is to evaluate all the available toxicological studies provided in support of pesticides approval in order to identify relevant liver, bile and gallbladder effects; these effects will be taken into account for a further refined cumulative assessment groups of pesticide active substances. For this purpose 181 pesticides included in Annex I of Council Directive 91/414/EEC up to 31st of May 2009 (already evaluated by the Technical University of Denmark, DTU) were re-evaluated by ICPS, and 60 more recently approved substances (between 31 05 2009 and 31 12 2011) were evaluated as well as 3 substances for which no DAR was available at the time of evaluation of the DTU.

MATERIALS AND METHODS

Since the overall objective of the project is to establish common assessment groups for dietary risk assessment only toxicity studies performed by oral administration (diet, capsule, gavage) were reported. In few cases also inhalation studies were considered when the PPP is expected to be applied by fumigation.
For a given toxicological target, there should be several possible CAGs, thus a tiered approach was applied based on the DTU report that identified four levels of CAGs, which would be reflecting increased knowledge on the mode/mechanism of action behind the observed effect:

1) CAG level 1: toxicological target (organ/tissue)
2) CAG level 2: common phenomenological effect (on the toxicological target)
3) CAG level 3: common mode of action
4) CAG level 4: common mechanism of action.

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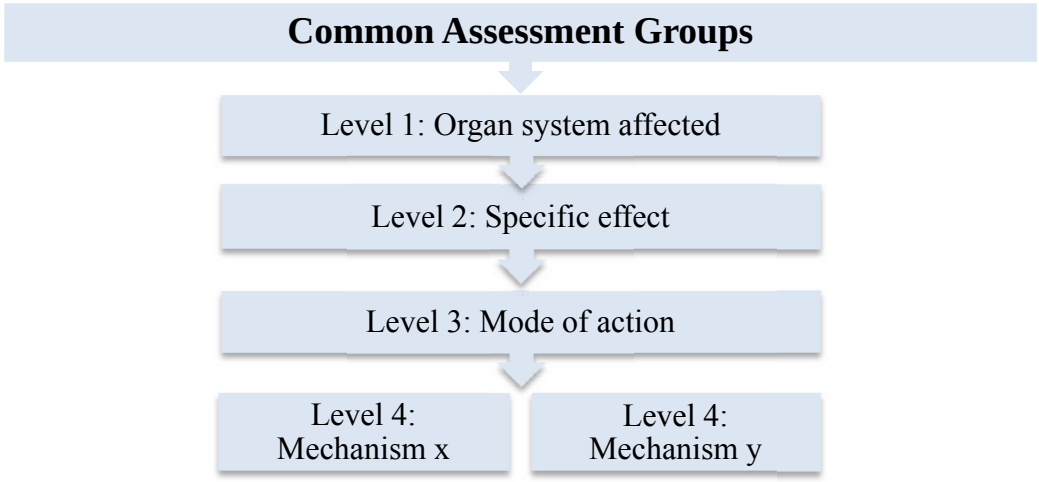

Toxicological effects on liver and bile (CAG level 1) along possible mode/mechanisms of action were identified and reported in accordance to those indicated in the DTU Report as follow:

CAG level 2 liver

- Hypertrophy
- Fatty changes
- Cell degeneration/ cell death
- Inflammation
- Foci of cellular alteration
- Neoplasm
- Lesion of biliary epithelium
- Porphyria
- Cholestasis
- Inclusions
- Karyocytomegaly

CAG level 3 and CAG level 4

Mode and/or mechanisms of action data were as well reported in concordance with the DTU terminology. CAG level 3 and CAG level 4 information were merged together hence, it has been reported whether MoA is known, unknown or presumed. Moreover, when mechanistic studies are available, information on the specific MoA is reported as remark.

The following CAG level 3 and CAG level 4 were considered:

CAG level 3

- Phase I enzyme induction
- Oxidative stress
- Cytotoxicity
- Hormonal changes

CAG level 4

- CYP1A family increase

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- CYP2A family increase
- CYP2B family increase
- CYP2E family increase
- CYP3A family increase
- CYP4A family increase
- Porphyria and cytotoxicity
- Hormonal changes

There are a number of effects that can be considered relevant for humans but nevertheless are not considered relevant for inclusion in a CAG. These non-specific or indirect effects can occur either as a result of an advanced state of a toxic impact/insult i.e. a secondary effect to a specific (direct) effect or as a consequence of high, massive (unrealistic) exposure to a pesticide active substance. The following effects were not taken into account:

- Acute effects
- Clinical observations
- Effects on body weight and related parameters
- Changes in organ weights
- Changes in white blood cell parameters
- Changes in blood and urine clinical biochemistry parameters

In addition, toxicological effects on gallbladder (CAG level 1) were scouted since we have considered it to belong to liver and bile system. Also in this case effects were reported in accordance to those indicated in the DTU Report as follow:

CAG level 2 gallbladder

- Hypertrophy/hyperplasia
- Dysplasia
- Fatty changes
- Fibrosis
- Calculi
- Deposits
- Oedema
- Mucus changes
- Lymphoid changes

These liver, bile and gallbladder specific effects were derived from the DTU and no evaluation on the their relevance towards the identification of CAG has been done.

1. Source of information

Information on toxicological effects were retrieved from available regulatory toxicological studies provided in support of approval and their evaluations under the peer review process of Directive 91/414/EC and Regulation (EC) 1107/2009 (Draft Assessment Reports, additional report, addenda, evaluation table and discussion table, EFSA Conclusion, European Commission Review Report) in the CIRCA website. Where appropriate original studies/data were reviewed.

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When needed open literature via PubMed was scrutinized to address histopathological findings different from those listed in the DTU Report and/or to search for additional information on modes/mechanisms of action. JMPR reports were also consulted for additional information on mode/mechanisms of action.
Information on pesticidal use, mode of action and chemical class was obtained from either volume 1 of the respective DAR or from the Pesticide Properties DataBase (PPDB).

2. Data collection

Toxicological studies that were taken into account for liver, biliary and gallbladder toxicity were both main and preliminary studies, comprising the following time frames and species:

- 28 day (rat, mouse, dog, other);
- 90 day (rat, mouse, dog, other);
- 1 yr. (rat, mouse, dog, other);
- 2 yr./ 18 month (rat, mouse, other);
- Carcinogenicity (rat, mouse);
- Two generation (only when histopathological examination of the liver has been performed);
- Mechanicistic studies (MoA).

Only studies considered to be fully acceptable were taken into consideration. In case of preliminary studies information in the remark column has been provided.

In order to record and retrieve all relevant data that may be used for any further evaluation, a preliminary database (an excel spreadsheet) was developed for each substance (an example is provided as Annex 3 to the report). The excel spreadsheet includes the following information:

- Reference;
- Source of information;
- Year of evaluation of source of information;
- Type of study (duration, species, strain and mode of administration);
- Specific effect;
- Endpoint (name descriptive of the specific effect as reported in DARs);
- Dose levels per gender as reported in DARs;
- NOEL and LOEL from the more sensitive gender and specie;
- MoA (CAGlevel3/CAGlevel4);
- Remarks of MoA;
- Reference of MoA.

In addition, peer-reviewed ADI and ARfD together with the corresponding type of study and the safety factors were included in the excel table. This last piece of information has been added to crosscheck whether the selected NOELs could be lower than the NOAEL used to set ADI and ARfD in the EFSA Conclusions, in such cases it has been pointed out in the remarks, as requested by EFSA.
The following procedure was applied to each substance:

- All studies evidencing liver, bile and gallbladder effects were recorded in an excel spreadsheet (an example is provided as Annex 3 to the report).
- Effects were reported according to DTU liver toxicity list (CAG level 2) and they were scouted in the DARs using the same descriptions listed in the DTU. The descriptions identifying the specific effects were recorded exactly as indicated in DARs.

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- For each specific effect a NOEL and a LOEL has been set based on the lower dose level. Both tabulated data and written conclusions in the report were considered.
- Whenever the observed effect has been observed only in one gender the corresponding gender was reported in the remark column.
- Liver and bile effects considered to be secondary, not relevant and therefore not useful for cumulative risk assessment were not taken into account, as described above.
- NOEL and LOEL of specific effects for which there is no clear evidence of dose relation were not reported.
- Studies on metabolites were considered only when the metabolite itself has been used in the toxicity studies instead of the parent compound due to its high instability.

The selection of NOELs and LOELs was conducted without interpretation whether an effect is to be considered adverse or not adverse, as agreed by the consortium.

3. Data processing

The information registered in the preliminary spreadsheet during data collection has been passed on the final reporting table (Annex 4 of the report), including additional information such as: organ/target system, pesticidal mode, chemical class, chemical name and CAS number.
Liver, biliary and gallbladder specific effects were transferred into the final reporting table. However, since several terms are found in the DARs describing the same specific effect, in the final reporting table only one term has been used to describe the specific effect. A list of such terms (found in the DARs) ascribing to each specific effect can be found in Appendix A2 (for liver and bile) and B2 (for gallbladder).
Data collection performed as described above produced for each pesticide several entries for a specific effect, each of them with the corresponding NOEL and LOEL.

The following criteria were applied to all pesticides to select a unique NOEL and LOEL for each specific effect:

1. Considering that longer-term studies (i.e.1/2 year and 18 month) are generally performed using lower concentrations compared to shorter-term studies (i.e. 28/90 day), priority for setting NOELs and LOELs was given to long-term studies; whenever this assumption was verified.
2. For each specific effect the NOEL and LOEL were set on the most sensitive species and gender.
3. In presence of more than one study with the same time frame exposure (i.e. Two 2yr rat studies), the main criteria to select NOEL and LOEL, was to look for the highest NOEL (in any case lowest of the lowest specific LOEL) and lowest LOEL.
   This strategy has been used with the assumption of the comparability of the studies.
   When this strategy led to select the NOEL and the LOEL from different studies both reference were reported.
4. Occasionally, some liver, biliary and gallbladder effects reported in short-term studies, do not occur in long-term studies. In these cases a note can be found in the remark column of the final table.

RESULTS

All 244 pesticides were evaluated for liver, biliary and gallbladder toxicity according to the methodology previously mentioned. Among these, 60 pesticides were included in Annex I between 31-05-2009 and 31-12-2011 and an additional 3 substances were not previously evaluated by DTU.

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With respect to the 181 (included in Annex I up to 31-05-2009) pesticides that were already analysed by the DTU, a re-evaluation of such pesticides has been performed applying the same methodological criteria followed for the latest Annex I included pesticides (see § 2 and 3). All toxicological studies from the above mentioned source of information were of enough good quality in terms of guide line compliance, acceptability, accuracy and completeness. Therefore, there was no need to look into original studies report/data.
As requested by the EFSA and agreed by the consortium liver, biliary and gallbladder toxicological data and information for all 244 pesticides were reported in the final reporting table (Annex 4). The information included in the final table were the following:

- Active Substance;
- Organ / target system;
- Chemical Class;
- Chemical Name;
- CAS Number;
- Pesticidal Use;
- Pesticidal MoA;
- Study;
- Species;
- Strain;
- Route of administration;
- Type of administration;
- Measured endpoint indicative of a possible common effect;
- Specific NOEL;
- Specific LOEL;
- Remarks;
- Reference;
- Mode/mechanism of action;
- Remarks for mode/mechanism of action;
- Reference MoA;
- Source
- Year of evaluation (publication of conclusion/review report).

Along with all pesticides evaluated, the following 15 (10 from the list evaluated by the DTU and 5 from the list of new substances) resulted not evidencing liver or bile effects, therefore were not included in the final reporting table, as agreed by the consortium:

- *Beta-Cyfluthrin*
- *Bifenox*
- *Chlorpropham*
- *Cyfluthrin*
- *Cyromazine*
- *Lambda-Cyhalothrin*
- *Mecoprop*
- *Methiocarb (aka mercaptodimethur)*
- *Nicosulfuron*
- *Pyridaben*
- *Pyridate*

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- *Sintofen*
- *Tau-Fluvalinate*
- *Z-cypermethrin*
- *Zinc phosphide*

Re-evaluation of all studies of the compounds listed by DTU showed that for about 20% of the studies, NOELs and LOELs identified by the DTU were different from those derived from our analysis. Furthermore, our evaluation identified additional liver and biliary effects for certain pesticides and for few pesticides some effects were not reported (e.g.: Bifenox was reported in the DTU database to elicit only hypertrophic effect, however the 90 day mouse study in which such effect was identified has not been considered acceptable, therefore our evaluation did not assign any effect to this particular pesticide). During the evaluation effects on gallbladder were as well reported.
As mentioned above liver, biliary and gallbladder toxicological effects were scouted according to the groups of effects identified by the DTU (see Material and Methods and Appendix A2 and B2). With regards to the liver neoplasm specific effect, information whether it was in the form of adenomas and/or carcinomas was reported in the remark column, whenever this information was retrievable.
In the DTU database, approximately 70% of the studies reported multiple liver and bile effects with the identification of a unique NOEL and LOEL for all of them. We have noticed that the reported values were selected from the effect having the lowest NOEL and LOEL, therefore for the other effects the reported NOEL and LOEL was not appropriate giving misleading information of the no effect dose. Since our methodology has been based on the concept to assign a unique NOEL and LOEL for each specific effect, these DTU studies were re-evaluated for such purpose.
As requested by EFSA effects with a NOEL lower than the NOAEL used to set peer-reviewed ADI were marked with an asterisk and the corresponding ADI and SF noted in the remark column. The outcome of this evaluation lead to the identification of 33 pesticides.
In addition, for 62 pesticides availability of some information on MoA for liver toxicity has been reported. On the contrary no MoA information for gallbladder effects has been found.
Two pesticides (aluminium sulphate and bromadiolone) showed to have few studies with limited validity or very toxic properties making it extremely difficult to perform a reliable assessment. For these cases more comprehensive information was provided in Appendix C2.
Benzoic acid (already evaluated by DTU) has not been reported since the studies in the DAR were not considered relevant according to our methodology.

CONCLUSIONS

In conclusion, ICPS (Lot 2) has achieved the objectives of the contract. First all available toxicological studies of 244 pesticides on the Annex 9 list to the call for tender for liver and biliary effects have been analyzed. Second, a database with liver, biliary and gallbladder toxicological specific effects has been provided. To reach such objective 244 pesticides were scouted according to the effects previously identified as adequate to set a CAG by the DTU; no interpretation on their relevance towards the identification of CAGs was provided by the contractor. For each effect a NOEL and a LOEL for the most sensitive gender and species has been reported. The selection of NOELs and LOELs was performed, as requested by EFSA, without any interpretation on whether an effect is to be considered adverse or not adverse. The database includes also information on mechanism of action, type of studies, pesticidal use and mode of action, chemical class, making it a valuable source of information for a comprehensive toxicological evaluation.

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APPENDICES

A2. GROUPING OF LIVER AND BILIARY EFFECTS/COMMON TERMS

1. Hypertrophy
   - *Centrilobular hypertrophy*
   - *Hepatocyte swelling*
   - *Giant cells*
   - *Swelling of liver cells*
   - *Enlargment of hepatocytes*
   - *Proliferation of SER*
   - *Diffuse hypertrophy*
   - *Slight enlargement of centriacinar hepatocytes*
   - *Increased hepatocytes*
   - *Cytomegaly*
   - *Periportal hepatocytes enlargement*

2. Fatty changes
   - *Hepatic steatosis*
   - *Hepatocelullar vacuolization*
   - *Hepatocellular periportal vacuolation*
   - *Hepatocytes vacuolation*
   - *Vacuolisation periportal*
   - *Vacuolization*
   - *Centriacinar vacuolation*
   - *Periacinar vacuolation*
   - *Panacinar vacuolation*
   - *Fat-containing vesicles*
   - *Focal vacuolation*
   - *Fatty vacuolation midzonal*
   - *Fatty vacuolation centriacinar*
   - *Microvesicular vacuolation*
   - *Fine fatty vacuolation*
   - *Large fatty vacuolation*
   - *Lipofuscin*
   - *Cytoplasmatic vacuolation*
   - *Vacuolation punctate periportal*

3. Cell degeneration/ cell death
   - *Centrilobular necrosis*
   - *Necrosis, single cell*
   - *Multifocal cell degeneretion*
   - *Periacinar hepatocytic degeneration*

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- *Focal necrosis*
- *Focal hepatic cystic degeneration*
- *Gallbladder focal necrosis*
- *Spongiosis*
- *Cytoplasmic granulation*

4. Inflammation
   - *Infiltration predominately perivascular*
   - *Pericholangitis*
   - *Cell infiltration*
   - *Focal inflammation*
   - *Perivascular chronic inflammatory foci*
   - *Increased no. of pigmented macrophages*
   - *Granulomatous inflammation*
   - *Histiocytosis, sinusoidal*
   - *Hepatitis acute/multifocal*
   - *Cholangitis*
   - *Leucocytic foci*

5. Foci of cellular alteration
   - *Periacinar hepatocyte eosinophilia*
   - *Clear cell foci*
   - *Basophilic foci*
   - *Eosinophilic foci*

6. Neoplasm
   - *Adenoma*
   - *Carcinoma*
   - *Benign liver cell tumour*
   - *Malignant liver cell tumour*
   - *Hepatoblastoma*
   - *Haemangiosarcoma, malignant, primary: no metastasis*
   - *Haemangiosarcoma, malignant, primary metastasis*

7. Lesion of biliary epithelium
   - *Hyperplasia of the epithelium*
   - *Hyperplasia of the gall-bladder mucosa*
   - *Fibrosis of the gall-bladder and acidophilic epithelial cells of the gall-bladder*
   - *Proliferative changes in bile ducts*
   - *Bile duct dilatation*
   - *Bile duct hyperplasia*
   - *Bile duct proliferation*
   - *Cholangiofibrosis*

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8. Porphyria

9. Cholestasis

10. Karyocytomegaly

    - *Polymorphism in the nucleus*
    - *Multinucleated hepatocytes*

11. Inclusions
    - *Cytoplasmic inclusions*

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B2. GROUPING OF GALLBLADDER EFFECTS/COMMON TERMS

1. Hypertrophy/hyperplasia
   - *Hypertrophy/hyperplasia*
2. Dysplasia
3. Fatty changes
4. Fibrosis
   - *Submucosal fibrosis*
5. Calculi
   - *Choleliths*
   - *Black crystals*
6. Deposits
   - *Concrements*
7. Oedema
   - *Oedema in the walls*
8. Mucus changes
   - *Increased mucus secretion*
   - *Adhesive mucus*
9. Lymphoid changes
   - *Increased lymphoid tissue*
   - *Dilated lymphatic vessel*

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C2. REMARKS ON PESTICIDES

Aluminium sulphate

Short-term studies: the notifier has provided studies deriving from literature. Some of them where summary papers from WHO, ATSDR, EPA and PHG, most of the evaluate aluminium compounds, other than aluminium sulphate.

In short-term toxicity tests with rats, liver, kidney and brain were the target organs of toxicity. The relevant short-term LOAEL of 212 mg/kg bw/day was derived from a 21-day study in rats. These results, however, were not considered relevant for the risk assessment since they were of limited validity.

Bromadiolone

Bromadiolone is very toxic by the oral, dermal and inhalation routes, no classification related to skin or eye irritation, or skin sensitisation is proposed. The active substance belongs to the second generation of long-acting anticoagulant rodenticides. The mode of action is common to the family of anti-vitamin K rodenticides (AVK), i.e. interfering with prothrombin synthesis by blocking the regeneration of vitamin K in the liver, disrupting the clotting mechanisms and increasing the tendency to haemorrhages. This results in decrease of prothrombin time, internal haemorrhages and subsequent death observed in rats, dogs, and other mammalian species. As a consequence bromadiolone is classified as toxic, danger of serious damage to health by prolonged exposure by the oral, dermal and inhalation routes. Bridging the toxicity profile between bromadiolone and the other anticoagulant difethialone was agreed to cover some toxicological endpoints. The overall NOAEL was 0.5 µg/kg bw/day derived from the 90-day toxicity study in rabbit performed with bromadiolone.

As no consumer exposure is envisaged, and as the high toxicity of the substance makes it extremely difficult to perform a reliable assessment, no information on the long-term toxicity and carcinogenicity is required.

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CFT/EFSA/PRAS/2012/07 CT03 (LOT 3)

LOT 3 - CUMULATIVE RISK ASSESSMENT OF EFFECTS ON REPRODUCTION AND DEVELOPMENT

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ABSTRACT

In the framework of Regulation (EC) No. 396/2005 on Maximum Residue Levels (MRLs) of pesticides, EFSA and the PPR Panel have started in 2007 the implementation of methodologies required to the establishment of cumulative assessment groups (CAGs) of pesticides on the basis of their toxicological properties. In this context the objective of the contractor ANSES (Lot 3) was to consolidate all available toxicological studies for reproductive and developmental effects for 197 pesticides among those included in Annex I of Council Directive 91/414/EEC up to 31st of May 2009 following a previous evaluation by the Technical University of Denmark (DTU). In addition, 60 more recently approved substances (between 31 05 2009 and 31 12 2011) have been evaluated.The identification of each reprotoxic endpoint was separated into three categories: developmental toxicity, reproductive toxicity and reproductive organ tumour induction. Toxicological studies taken into account, were short and long term/carcinogenicity studies, one and multi-generation studies, prenatal developmental studies, developmental neurotoxicity studies and mechanistic studies. For each specific reprotoxic endpoint observed in a study, a NOEL and a LOEL was identified, without interpretation of its adversity. All 257 pesticides were evaluated and results are reported in a final database. When available in the DAR or in the open literature, the MoAs of pesticides was reported.

KEY WORDS

CAG, reproductive toxicity, development, NOEL, LOEL, MoA

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INTRODUCTION AND OBJECTIVES

The objective of the contractor ANSES (Lot 3) is to assess all the available toxicological studies provided in support of pesticides approval in order to identify relevant effects on reproduction and development. These effects were tabulated in an Excel file. At the kick-off meeting for this project it was decided that the contractor would not provide proposals for consolidated cumulative assessment groups.

MATERIALS AND METHODS

1. Active substances considered relevant for cumulative risk assessment

All pesticides identified by DTU as being reprotoxic, and the pesticides placed on Annex I between 31-5-2012 and 31-12-2011 had to be scrutinized for developmental of reproductive effects. In total, 254 active substances were assessed and are listed in annex 1of this report. In addition, the three actives substances added by EFSA during the final meeting on November 15, were assessed.

2. Collection of information

2.1. Source of information

Information on toxicological effects for reproductive and developmental toxicity was obtained from Draft Assessment Reports (DAR), DAR addenda and EFSA/ECCO/EPCO peer review reports.
In addition, Competent Authority Report (CAR) for biocide substances and CLH reports (classification and labeling) were consulted for additional relevant toxicological information when available.
In some cases, a search in the open literature, such as PubMed[2], was also performed on potential modes/mechanisms of action (MoA) of the active substances. Finally, available JMPR reports were consulted in order to report additional information on MoA.
The references reported in the Excel file are presented in table 1.

Table 1: Source of information

Reference	
DAR	Draft assessment report, addendum, revised DAR, additional report, EFSA conclusion, evaluation and reporting tables, PRAPeR/EPCO/ECCO reports, Draft Renewal Assessment Report
CAR	Competent authority report (Biocide report) when necessary
JMPR	JMPR report
CLH	CLH report
Open literature	Abstract only or full report
Study report	Full study report

The devTox database[3] was also consulted in order to define the type of developmental anomalies (such as malformations or variations) when no information was available in the DAR.

[2] Pubmed : http://www.ncbi.nlm.nih.gov/pubmed

[3] DevTox: http://www.devtox.org/nomenclature/organ.php

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2.2. Data collection

2.2.1. Chemical structure and pesticidal mode of action

The information that substances contain similar chemical structures or have the same pesticidal mode of action may indicate a possible similar toxicity to experimental animals and humans.

Information on the chemical identity of the active substances was obtained from the DTU report when available. For new active substances chemical name, CAS number and chemical class were extracted from the DAR.

Information on pesticidal use and mode of action was obtained from the DTU report when available. For active substances not reported in this database, the following links, provided by EFSA, were used:

- http://www.croplifeaustralia.org.au/files/resistancemanagemen/insecticides/2012%20Insecticide%20Mode%20of%20Action%20Table.pdf
- http://www.croplifeaustralia.org.au/files/resistancemanagemen/herbicides/2012%20-%20Herbicide%20MOA%20Table.pdf
- http://www.croplifeaustralia.org.au/files/resistancemanagemen/fungicides/2012%20Fungicide%20Activity%20Group%20Table.pdf

However when an active substance was found neither in the DTU database nor in the links above, other sources may have been used (Footprint database[4], pesticidal mode of action proposed in the DAR).

2.2.2. Toxicological studies

The following relevant regulatory toxicological studies provided in support to the active substances approval have been assessed for reproductive and developmental toxicity effects:

- **Short-term, sub-chronic and chronic toxicity studies:** 28-day (rat, mouse, dog, other), 90-day (rat, mouse, dog, other), 1-year (rat, mouse, dog, other). Short-term studies (28-day) provide information on the possible health effect hazards likely to arise from repeated exposure over a relatively limited period of time assessment. Sub-chronic and chronic studies (90-day, 1-year) provide data on the possible health effect hazards from repeated exposure over a prolonged period of time covering post-weaning maturation and growth into adulthood. In these studies, relevant effects on reproductive organs such as relative or absolute weight changes, pathological changes (macroscopic, microscopic) are systematically reported.

- **Long term and carcinogenicity studies**: 2-year, 18-month toxicity studies (rat, mouse). These studies provided information on chronic toxicological effects on reproductive organs and reproductive organ tumor induction.

- **One and multi-generation studies** (rat, mouse). These studies provided information on the effect on the integrity and performance of the male and female reproductive systems, including gonadal function, oestrus cycle, mating behavior, conception, gestation, parturition, lactation, and weaning, and the growth and development of the offspring.

- **Prenatal developmental studies** (rat, rabbits, and guinea-pigs): these studies provided information on the effect on the developing foetuses.

[4] Footprint : http://sitem.herts.ac.uk/aeru/footprint/fr/

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- **Developmental neurotoxicity studies.** These studies provide information on the potential functional and morphological effects on the developing nervous system of the offspring that may arise from exposure *in utero* and during early life. In these studies, relevant effects on physical/developmental landmarks, functional/behavioral endpoints (such as motor activity, motor and sensory function, learning and memory…) were reported.

- **Mechanistic studies**: relevant mechanistic studies evaluating mode/mechanism of action associated to reproductive/developmental or endocrine related effects were reported.

Only the regulatory studies which have been considered as acceptable in the DARs were assessed. Effects observed in studies considered as supportive in the DARs (preliminary studies, open literature) were also reported when considered necessary (effects not covered by the main studies).

Regulatory toxicokinetic studies, acute toxicity studies, genotoxicity studies and studies performed with metabolites have not been assessed.

2.3. Identification of relevant reproductive/developmental endpoints

Reproductive toxicity refers to a wide variety of toxicological effects that may occur in different phases within the reproductive cycle (figure 1). This includes both male and female fertility and developmental toxicity effects.

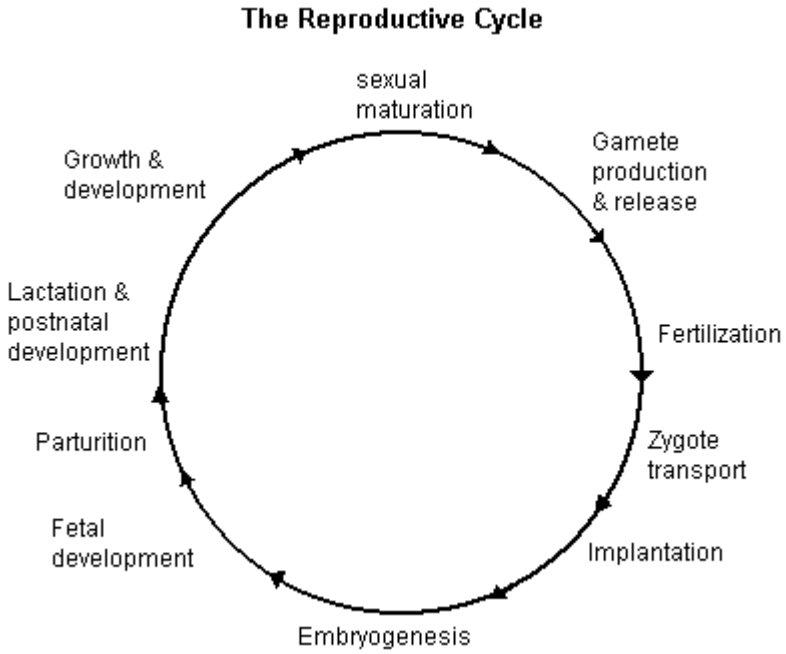

Figure 1: Diagram of the major reproductive life cycle phases, commencing with sexual maturation and moving through fertilization, foetal development, parturition and postnatal development and ending with a sexually mature individual of starting the cycle over again (from Foster et al. 2001).

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Taking into account the Guidance to Regulation (EC) No 1272/2008 on classification, labeling and packaging (CLP) of substances and mixtures, the reprotoxic effects can be structured in the following manners:

- *The developmental effects:* effects on embryonic/foetal development and subsequent growth and development into sexual maturity. This includes the spontaneous abortions (embryo or fetus), resorptions, peri- and postnatal death, low body weight at birth, congenital malformations and alterations of mental and physical development up to and including normal sexual maturity.

- *The reproductive effects:* effects on sexual function and on fertility. This includes effects on sexual behavior, spermatogenesis, oogenesis, fertilization up to and including embryo implantation, parturition, gestation results and any other disturbance in the integrity of the reproductive system. The adverse effects on or via lactation may be included in the reproduction toxicity category.

The frontier of this distinction is not well defined. Indeed, in some case it is not possible to decide whether an observed effect is a sign of impaired fertility or developmental toxicity (or both). For these particular effects, it was decided to report them both in the reproductive and developmental categories. As tumours in reproductive organs may be caused by similar mode of action as other effects in reproductive organs (endocrine related effects), tumours are also analysis.

On this basis, the identification of each toxic endpoint on reproduction/development was separated into three categories:
- Developmental toxicity,
- Reproductive toxicity and
- Reproductive organ tumour induction.

2.3.1. Endpoints for developmental toxicity

The four major manifestations of developmental toxicity are death, structural abnormality, altered growth and functional deficit. Developmental toxicity is investigated in prenatal developmental (teratology) studies and in one- or multi-generation studies. The table 2 presents the relevant endpoint for developmental toxicity identified by the experts.

Table 2: Endpoints for developmental toxicity

Measured endpoints	Details on measured endpoints (examples)
LITTER WITH IMPLANTS	
Prenatal body weight changes	*Foetus/litter*
Delayed prenatal development	*Reduced or non-ossification*
Post-implantation losses	*Early/late resorption/dead foetus/ abortions*
Runts	
Malformations/Anomalies	*When no further details available*
Visceral malformations	*Brain (hydrocephaly, excencephaly…), eye (anophtalmia/microphtalmia…)…*
Skeletal malformations	*Cranio-facial (cleft palate…), Vertebrae, forelimb flexures*
Visceral variations	*Kidney (dilated renal pelvis), urinary tract…*
Skeletal variations	*Vertebrae, supernumerary ribs…*
Mechanistic study endpoints	

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LITTER WITH OFFSPRING	
Postnatal body weight changes	*Body weight gain or body weight loss (At Birth, At PN4, lactation, weaning)*
Delayed postnatal development	*Eye opening, sexual maturation, neurotoxicity…*
Postnatal death	*Birth, PN4, lactation, weaning*
Reduced litter size	
Increased anogenital distance of male offspring	
Decreased anogenital distance of male offspring	
Increased anogenital distance of female offspring	
Decreased anogenital distance of female offspring	
Nipples retention in male offspring	
Nipples retention in female offspring	
Preputial separation of offspring	
Delayed vaginal opening of offspring	
Clinical signs in offspring	
Increased weight of male offspring reproductive organs	*Testes (absolute and/or relative weight), epididymides, seminal vesicles, prostate*
Decreased weight of male offspring reproductive organs	*Testes (absolute and/or relative weight), epididymides, seminal vesicles, prostate*
Increased weight of female offspring reproductive organs	*Ovaries, uterus, vagina, mammary gland*
Decreased weight of female offspring reproductive organs	*Ovaries, uterus, vagina, mammary gland*
Increased weight of offspring endocrine organs	
Decreased weight of offspring endocrine organs	
Increased weight of offspring other organs	
Decreased weight of offspring other organs	
Pathological changes of female offspring reproductive organs	*Ovaries, uterus, vagina, mammary gland*
Pathological changes of offspring endocrine organs	
Pathological changes of male offspring reproductive organs	*Testes (leydig cell hyperplasia…), epididymides, seminal vesicles, prostate*
Pathological changes of offspring other organs	
Altered sperm in male offspring	*Number, mobility, morphology*
Impaired fertility of male offspring	*Sex hormone, fertility index…*
Impaired fertility of female offspring	*Fertility index, oestrus cycle, sex hormone…*
Developmental neurotoxicity	*Behavioural ontogeny, motor activity, learning memory, neuropathology*

Post-implantation loss, determined following delivery of a litter, is the (total number of implantation sites minus number of full-term pups)/number of implantation sites. The endpoint "Post-implantation losses" was included both in the reproductive and developmental categories. The following effects were included in the endpoint *"Post-implantation losses":* abortions, increased early/late resorptions, total resorptions, decreased number of live offspring, stillbirth, foetal death, increased percentage of post-implantation loss.

Litter size is the number of offspring delivered and is measured at or soon after birth. Litter size may include dead as well as live offspring. The endpoint "*Reduced litter size"* was included both in the reproductive and developmental categories.

Runts: fetuses weighting ≤ 70-75% of the mean foetal weight/litter and normally developed. In this endpoint, it was also included stunt fetuses and nanofetuses (foetus weighting less than 60% of the mean fœtal weight in the control group and not associated with achondroplasia).

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Malformations and variations: A malformation is usually defined as a permanent structural change that may adversely affect survival, development, or function. The term *variation* is used to indicate a divergence beyond the usual range of structural constitution that may not adversely affect survival or health. Distinguishing between variations and malformations is difficult since a continuum of responses exists from the normal to the extremely deviant. When information was not clear or not available in the DARs, the DevTox database was used by the experts in order to facilitate the distinction between variations and malformations.

Malformations are separated in two endpoints: "Visceral malformations" and "skeletal malformations". Details on the observed malformations are presented in the excel spreadsheet by organs/tissus target:

- Skeletal malformations: craniofacial (cleft palate); ribs (fused); spinal column (spina bifida); limbs (flexure, polyscelia, ectodactily…).
- Visceral malformations: diaphragm (hernia), brain (hydrocephaly); eye (microphtalmia, anophtalmia); kidney (hydronephrosis); trunk (gastroschisis, omphalocele), general (anasarca)…
- Concerning external malformations, they are included in visceral or skeletal observations.

Variations are separated in two endpoints: "Visceral variations" and "skeletal variations". Additional information are presented in the column "Details on measured endpoint" of the excel spreadsheet such as: extra, short or rudimendary ribs, extra lumbar vertebrae (skeletal variations), dilated renal pelvis (visceral variations)… Concerning external variations, they are included in visceral or skeletal observations. Unclassified observations (visceral not classified as variations or malformations (e.g. focal liver necrosis in fetuses) were also included in this endpoint.

When no details on the type of malformations/anomalies were present in the DAR, it was reported in the endpoint "Malformations/anomalies".

Retardations: reduced/incomplete or un-ossification of bones (ribs, sternebrae, skull…) are reported in the endpoint "Delayed prenatal development". It is important to notice that the frontier between "retardation" and "variation" is not well defined.

Postnatal body weight changes: effect observed in pups following birth (litter with live offspring). This end-point includes pup body weight at birth and throughout the lactation period

***Prenatal body weight changes*:** effect observed in fetuses prior to term (litter with implants). This endpoint includes fetal and neonatal weight changes.

Pathological changes in offspring other organs: these findings are reported for offspring only when the observed effects are not seen in parents or are more severe and/or appears at lowest doses.

2.3.2. Endpoints for reproductive toxicity

The reproductive toxicity may be expressed as alterations of the female or male reproductive organs, the related endocrine system, or pregnancy outcomes.
The related endpoints identified by the experts may be separated into three categories: couple-mediated, female-specific and male-specific (Table 3). Couple-mediated endpoints are those in which

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both sexes can have a contributing role if both partners are exposed. Thus, exposure of either sex or both sexes may result in an effect on that endpoint.
Measures of fertility and pregnancy outcome are often obtained from multigeneration reproduction studies.

Table 3: Endpoints for reproductive toxicity

Measured endpoints	Details in measured endpoint *(examples)*
COUPLE-MEDIATED	
Decreased fertility index	
Postnatal death	*At birth (live birth index),* *At PND4 (4-day survival),* *At PND 21 (lactation index)*
Decreased gestation index	
Increased gestation length	*Dystocia*
Decreased mating index	
Pre-implantation losses	*Reduced implantation number*
Post-implantation losses	*Early /late resorptions/dead foetus/ abortions*
Reduced litter size	
Sex ratio	
Other effects on fertility	
Mechanistic study end-points	*Aromatase inhibition, AR/ER receptor binding…*
MALE-SPECIFIC	
Altered male sexual behavior	
Hormonal changes in males	*LH, FSH, testosterone, estrogen*
Decreased weight of male reproductive organs	*Testes, epididymis, seminal vesicles, prostate*
Increased weight of male reproductive organs	*Testes, epididymis, seminal vesicles, prostate*
Pathological changes of male reproductive organs	*Testes (Leydig, Sertoli cells), epididymis, seminal vesicles, prostate*
Altered sperm	*Sperm number (count) and quality (mobility, morphology)*
FEMALE-SPECIFIC	
Hormonal changes in females	*LH, FSH, estrogen, progesterone, prolactin*
Decreased weight of female reproductive organs	*Ovary, uterus, vagina, mammary gland*
Increased weight of female reproductive organs	*Ovary, uterus, vagina, mammary gland*
Pathological changes of female reproductive organs	*Uterus (endometrial hyperplasia, hypoplasia or aplasia…); ovary, vagina, mammary gland*
Altered maternal behaviour	
Oestrus cycle	*Irregular, acyclic, prolonged dioestrous phase, corpora lutea*
Increased placenta weight	*Associated with pathological changes*
Lactation	*Offspring growth or death, milk quality and quantity*

Offspring survival*:* the gestation index and live birth index indicate the viability of young at birth, while the 4-day survival index and lactation index indicate the viability of pups to weaning. Effect observed on live birth index and 4-day survival index was included in the endpoint *"Postnatal death"*. General indices of reproductive function are listed in Appendix C3. The endpoint "Postnatal death" was included both in the reproductive and developmental categories.

Pre-implantation loss: an increase of this parameter could indicate an adverse effect on gamete transport, fertilization process, uterine toxicity, developing blastocyt, or on the process of implantation itself. In the studies (prenatal development) where treatment begins around the time of implantation

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(i.e., day 6 of gestation in the mouse, rat, or rabbit), an increase in pre-implantation loss probably reflects variability that is not treatment related or can reflect very early post implantation losses. In this latter case, the effect was reported in the endpoint "Post-implantation losses".

Changes in weight of male or female reproductive organs: alteration of absolute and/or relative (i.e., adjusted for body weight) organ weights were reported. If these effects occurs with a concomitant reduction in body weight gain, this was specified in the column maternal/parental/general toxicity.
The weights of the prostate and seminal vesicle are androgen dependent and may reflect change in an animal's endocrine status or testicular function. An alteration in the weight of the uterus can also indicate reproductive toxicity: compounds that inhibit steroidogenesis and cyclicity can dramatically reduce the weight of the uterus so that it appears atrophic and small.

***Changes in placenta weight*:** an increased of placenta weight may be associated with aromatase inhibition (Tiboni et al., 2009) and considered as a reproductive effect. On the contrary, decreased placenta weight has been associated with maternal toxicity.

Lactation: reduced growth or decreased survival of pups could be caused by reduced milk availability, palatability or quality, by ingestion of a toxic agent secreted into the milk. Effect observed on lactation index, pup survival, postnatal body weights, milk quantity or quality are included in the endpoint "Lactation".

2.3.3. Endpoints for reproductive organ tumour induction

Table 4: Endpoints for tumour induction

Measured endpoints	Details
Mammary gland tumours	*type*
Ovarian tumours	*type*
Uterus tumours	*type*
Testis tumours	*type*
Prostate tumours	*type*

2.4. Specific NO(A)EL and LO(A)EL

For each endpoint, the NOEL and the LOEL of the effect were reported. The selection of NOELs and LOELs was conducted without interpretation whether an effect is to be considered adverse or not adverse.
For each specific effect, the NOEL and LOEL set were often the same as the NOAEL and the LOAEL established for each endpoint during the European peer-review. However, NOEL and LOEL different from the NOAEL/LOAEL stated during peer-review were reported in the following cases:

- When a statistically significant effects was observed but was not considered as adverse in the DAR and outside the historical control data when available.
- The relation to treatment of the effect was considered equivocal in the DAR and/or has been discussed during expert meeting;
- When strong indications support the relationship between a toxicophore and/or a specific effect, the NOEL/LOEL have been reported (e.g. cleft palate induce by a triazole compound although inside the historical control data and/or effect occurring without dose-relationship);
- Very uncommon effect (e.g. very rare malformations);

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- When the specific effect was observed in several studies/species.

In these cases, relevant comments were presented either in the detail column or in the remark column of the spreadsheet:
- Small effect in comparison to the control group (incidence/severity);
- No statistical significance (but was considered as adverse effect in the DAR)
- No statistical analysis
- Inside historical control data (HCD);
- No dose-relation;
- Only in one study;
- Not considered treatment related in the DAR (e.g. Aged-related);
- No relevance to human;
- Not considered relevant for NOAEL setting.

When the specific NOEL is below the critical NOAEL used to derive the reference values, it has been mentioned in the column "remark" in the excel table.

2.5. Dietary dose conversion (ppm *vs* mg/kg bw/d)

When test substances are expressed as concentration in feed the systemic dose was reported whenever available in the DAR. In the other cases the systemic dose was calculated from the dietary concentration by using the general conversion factors provided in the OECD "guidance noted for analysis and evaluation of chronic toxicity and carcinogenicity studies" (OECD 2002) (Appendix A3). In that case, a comment was reported in the remark column "NOEL/LOEL/converted from ppm".

In the multi-generation studies, mean dose intake could be detailed in function of the exposure periods (pre-mating, gestation and lactation period), sexes (male, female) and generations (F0, F1, F2 …). The NOAEL/LOAEL mentioned in the DAR was taken into account. In other cases, the lowest mean systemic dose was reported in the spreadsheet.

In the case of *in vivo* studies using an inhalation route of administration, concentrations have been reported with the unit (e.g. mg/m^3).

In addition, concentrations have been reported with the unit (e.g. 50µg/L) in the case of *in vitro* studies.

2.6. Maternal/parental/general toxicity

Developmental toxicity endpoints
Because standard study designs require that the top dose exert some minimal indication of maternal toxicity (e.g. 10% reduction in maternal body weight gain), there is sometimes difficulty in distinguish whether a developmental effect seen at such a dose is a direct result of the action of the chemical or an indirect result of general toxicity. The general signs of toxicity are considered non-specific effect and are not appropriate for the establishment of CAGs. Thus, in the case of developmental toxicity endpoints, the maternal toxicity has been reported (clinical signs, mortality, body weight gain, food consumption …).

Reproductive toxicity endpoints
- Effect on offspring

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A direct effect in the offspring via the exposure of the male prior to mating and/or female during pre-mating, gestation and lactation should be distinguish to an indirect result of general toxicity of the parental animals. Thus, the parental toxicity has been reported in the spreadsheet for reproductive endpoints on offspring.

- Effects on adults
The general toxicity observed at the same dose as the reproductive endpoint was reported in the spreadsheet (Body weight alteration, decreased food consumption or water intake).

Tumors induction endpoints
Likewise, the body weight alteration, decreased food consumption or water intake were reported at the dose of the occurring tumors.

2.7. Mode/mechanism of action

Determinations of the mode/mechanism of action by which the substance cause the effect have been reported in the spreadsheet. The (un)certainty of the effect was reported as follow: Known/presumed. When the MoA was unknown, the column in the excel table was not filled.

RESULTS

Excel spreadsheets were established for each individual organ/tissue system (reproductive, developmental or tumour induction toxicity) containing the following information:

- Substance name
- Organ/target system
- Chemical class/chemical name/ CAS number
- Pesticidal use and mode of action
- Study type: duration, species, strain
- Administration: route and type
- Measured endpoint indicative of a possible common effect
- Details on measured endpoint
- Specific NOEL and LOEL
- Maternal/parental/general toxicity
- Remarks
- Mode/mechanism of action
- Reference/source
- Year of peer reviewed

197 pesticides identified by the DTU and 60 pesticides added to Annex I during the period 31-5-2009 to 31-12-2011 were scrutinized for reproductive and developmental effects.
Evaluation of the 60 pesticides added to Annex I during the period 31-5-2009 to 31-12-2011 revealed that 60 pesticides induce effects indicative of a reproductive and/or developmental effects.
In addition, the three actives substances added by EFSA during the final meeting on November 15, were assessed. The spreadsheet is in annex 5.

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1. ELUCIDATION OF MODE/MECHANISMS OF ACTION

Any assumed mode/mechanism of action reported either in DAR or open literature has been duly reported in the Excel spreadsheet. In particular, MoA established within the peer review of pesticide active substance has been taken into account. Details of hypothesis MoAs (Mode/mechanisms of action) for each substance are reported in Appendix B3. This appendix summarizes all the presumed MoAs. However, when the MoAs were based on literature, it should be emphasized that these data were not peer-reviewed (See also 2. limitation). Only those associated with at least one endpoint hypothesized in the DAR and/or in the literature are reported. When a MoA was proposed by the rapporteur member state (RMS) but was not assumed by EFSA peer-review or by JMPR, the MoA has been mentioned in the excel table but not in Appendix B3.

Hypothesis of MoA for developmental and/or reprotoxicity was identified only for a small number of pesticides. Proposed MoA need to be analyzed on a case-by-case basis. They have to be confirmed by other studies and its relevance for human health has to be assessed.

1.1. Presumed MoAs for developmental effects

Some MoAs were presumed for developmental effects:

- Genotoxicity e.g interaction with cell microtubules: effect on the spindle apparatus during tubuline formation in the course of mitosis. This particularly affects proliferative tissues (inhibits mitosis, nuclear division and cytokinesis) inducing effects on embryo/foetal development.

- Anticoagulant: e.g. inhibition of vitamin K epoxide reductase.

- Anaemia: e.g. inhibition of PPO with accumulation of PPIX. This interferes with normal heme synthesis and results in anaemia and hypoxia in the foetus.

- Inhibition/interference with the thyroid hormone homeostasis in pregnant dams:
  In human, hypothyroxinemia early in pregnancy is associated with adverse effects on the developing nervous system. Thyroid-disrupting chemicals are suspected of affecting brain development.

- Inhibition of the embryonic CYP26 degradation of retinoic acid: which then cause dysmorphogenesis (e.g. branchial apparatus).

- Blockade of Ikr potassium (HERG) channel: via hypoxia and/or reactive oxygen species in embryo (based on data for ketoconazole) resulting in embryonic arrhythmia and hypoxia.

- Inhibition of glutamine-synthetase: induce a significant decrease in the level of glutamine in the maternal organism and thus a decreased supply of glutamine, an essential amino acid for the developing embryo, especially during the early stages of development.

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The table 6 presents the list of pesticide sharing the same presumed MoA. Proposed grouping needs to be analyzed on a case-by-case basis as the human relevance and the confirmation of the establishing MoA need to be performed.

Table 5: List of pesticides sharing the same presumed MoAs (developmental effects)

MoA	Name of the pesticides active substances	Developmental effect	Reference
Interruption of thyroid hormone homeostasis	Mancozeb Maneb*	Brain malformations	Axelstad, 2011
Genotoxicity	Carbendazim	Brain and eye malformations	DAR
Anticoagulants	Bromadiolone*	Skeletal anomalies?	DAR
Inhibition of embryonic CYP26 and/or blockade of Ikr potassium (HERG) channel	Flusilazole Epoxiconazole Myclobutanil* Tebuconazole* Metconazole* Cyproconazole* Propiconazole*	Craniofacial or brain malformations	Menegola, 2006
Anaemia	Flumioxazin	Ventricular septeal defects Wavy ribs	DAR
Inhibition of glutamine-synthetase	Glufosinate	Pre- and early post-implantation losses	DAR

* MoA extrapolated from active substances in the same chemical class with a similar pattern of effects. For example, the triazoles Myclobutanil and Tebuconazole, teratogenic effects were not investigated yet but as similar pattern of malformation (cleft palate, hydrocephaly) than other triazoles Flusilazole and Epoxiconale, were observed in rats and rabbits, it was suggested a similar MoA (e.g. inhibition of embryonic CYP26).

1.2. MoA identified for reproductive effects/tumour induction

Some MoAs were presumed for reproductive effects/tumour induction. These data were mainly literature-based and should be interpreted carefully (e.g. *in vitro* studies not always confirmed in *in vivo* studies).

Receptor binding

- Androgen receptor (AR) antagonists: competitive inhibition of the binding of androgens to AR which leads to an inhibition of androgen-dependent gene expression

- Androgen receptor agonists: binding to AR and initiation of transcription of androgen-responsive genes.

- Estrogen receptor (ER) agonists: binding to ER and initiation of transcription of estrogen-responsive genes.

- Estrogen receptor antagonists: competitive inhibition of the binding of estrogen to ER which leads to an inhibition of estrogen-dependent gene expression

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- AhR (Aromatic hydrocarbure receptor) agonists: compounds that act as AhR agonists, such as TCDD and coplanar PCB, have extensive reproductive effects on both males and females, especially when the animals are exposed during early development.

    - Anti-estrogenic effects: the anti-estrogenicity of TCDD are through to be mediated by AhR: anti-estrogenic effects were not retrieved in AhR knockout mice *in vivo* (Buchanan *et al*. 2000) Through AhR is essential for the anti-estrogenicity effect, the precise mechanism by which activation of AhR leads to anti-estrogenic effects is unknown. It has been suggested that the liganded AhR may induced anti-estrogenic effects by binding to dioxin response elements in estrogen responsive genes and physically interfering with the ability of the liganded ER to bind to the DNA and initiate transcription (Safe and Krishnan, 1995).

    - Anti-androgenic effects: TCDD can exerts anti-androgenic effects that were mediated through the AhR: these effects include decreased accessory sex organ weights, delayed preputial separation, decreased testicular sperm production and decreased epididymal sperm storage (reviewed in Roman and Peterson, 1998). However, these effects are not accompanied by decreased androgen production or circulating levels, and they cannot be explained by the antiestrogenic activity of TCDD. The mechanism of this apparent antiandrogenic effect remains to be established.

  Several pesticides (e.g. prochloraz) may induce AhR-mediated transcriptional activity (Kojima *et al*. 2010).

Interruption of steroid hormone homeostasis

- Disruption of enzymes involved in steroid hormone synthesis

    - Inhibition of CYP19 aromatase activity: CYP19 catalyses the conversion of androgens (both testosterone and androstendione) to estrogens, responsible for the homeostatic balance between the male and the female hormones. The inhibition of aromatase leads to an increased concentration of androgens (testosterone) and a decreased concentration of estradiol. The decreased estradiol levels trigger a feedback response in the hypothalamic-pituitary axis resulting in increased LH and FSH levels.

    - Activation of CYP19 aromatase activity (e.g. hepatic induction…).

- Increased catabolism of steroid hormones

    - Renal excretion of 17-β estradiol.

    - Increased oxidation of testosterone by liver microsomal induction: microsomal induction results in increased oxidation of testosterone, leading to disruption of the pituitary-testis hormonal balance.

- Cholesterol stockage disruption

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- Inhibition of esterase activity upon the testes would prevent the release of cholesterol from its storage ester within this cell type and thus decrease the availability of cholesterol for steroidogenesis.

Interruption of amino acid homeostasis

- Inhibition of glutamine synthetase: induce a significant decrease in the level of glutamine in the maternal organism and thus a decreased supply of glutamine, an essential amino acid for the developing embryo, especially during the early stages of development. Glutamine is an essential amino acid for the developing mammalian embryo during the early stages of its development, especially for the successful implantation and maintenance of implantation at those early stages.

Genotoxicity:

- Interaction with cell microtubules: effect on the spindle apparatus during tubuline formation in the course of mitosis. This particularly affects proliferative tissues (inhibits mitosis, nuclear division and cytokinesis) inducing effects on reproductive capacity (sperm production).

Table 6 presents the list of pesticide and sharing the same presumed MoA for a reproductive effect. Only pesticides with detailed MoA pathways are presented. In the case where no clear MoA was obtained because of an early interruption of the steroid hormone homeostasis, the effect where reported separately as "Steroid hormone homeostasis disruptors" in the table. The association of the presumed MoAs with the endpoints needs to be interpreted carefully and in a case-by-case basis. In particular, the human relevance and the MoA confirmation need to be performed.

Table 6 summarizes all the MoA presumed in the DAR or in the literature. Only those which may be associated with at least one endpoint are reported.

Table 6: List of pesticide sharing the same identified MoAs (Reproductive effects)

MoAs	Pattern of toxicity	Name of the pesticide active substances
Anti-estrogenic/androgenic action	ER antagonist	Prochloraz, myclobutanil
	Aromatase inhibitor	Prochloraz, fluzilazole, epoxiconazole, tebuconazole, triflusulfuron, propiconazole
	AhR agonist	Prochloraz, epoxiconazole
	AR agonist	Myclobutanil
Anti-androgenic/estrogenic action	Aromatase inducer	Thiacloprid, benthiavalicarb, propamocarb, pirimicarb
	ER agonist	Chlorpyrifos, pirimiphos-methyl, tebuconazole, methiocarb, deltamethrin
	AR antagonist	Chlorpyrifos, pirimiphos-methyl, bupirimate, prochloraz, epoxiconazole, linuron, cypermethrin, methiocarb, deltamethrin, diuron, propiconazole

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	AhR agonist	Prochloraz, epoxiconazole
	Inhibition of esterase activity	Molinate
	Progesterone hydroxylation Inhibitor	Prochloraz
Steroid hormone homeostasis disruptors	Adrenal enz. activity (CYP 17?)	Epoxiconazole, tebuconazole, myclobutanil
	Inh. malate deshydrogenase mitochondrial	Spirodiclofen
	Increased oxidation of testosterone	Propyzamide
Genotoxicity	Interaction with cell microtubules	Carbendazim
Interruption of amino acid homeostasis	Inhibition of glutamine synthetase	Glufosinate

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1.3. Conclusion on the elucidation of the MoA

Concerning the developmental toxicity, a same effect could be induced by different MoA. It is not known if these effects could be cumulative as the key events and the pathways may be very different. Furthermore, very few mechanism studies were performed to understand the MoA. When available, grouping pesticide by MoA might be performed.
It was in fact considered that the establishment of CAGs should be agreed upon by a group of experts rather than be based on the opinion of an individual contractor.

For certain chemical classes (e.g triazole) associated with a particular developmental effect (e.g brain and cranio-facial malformations), grouping based on extrapolation from a well studied reference substance to others can be made.

Concerning the reproductive toxicity, several MoA could be presumed for a substance. For example, a pesticide active substance can act directly on one or several receptors (e.g. antagonist AR, agonist AhR) and while also disrupting the hormone synthesis (e.g. aromatase inhibition, progesterone hydroxylation inhibition). In this case, it is difficult to link a specific MoA to a specific observed effect.

This is particularly true for substances acting on the early synthesis of the steroid hormone (e.g. substance affecting the steroidogenesis before CYP 17 enzyme), which could impact testosterone, estradiol and progesterone. In this case, several target organs could be identified. Therefore, effect observed with this MoA could not be grouped with other MoA identified in this report. However, an observed effect (e.g. Leydig cell hyperplasia) could be induced by either a specific anti-androgenic action (e.g. *via* AR antagonism) or a disruption synthesis of the steroid hormones. In this case the grouping by MoA might be less relevant. Indeed, it has recently been shown that substances acting via different MoA (e.g. anti-androgenic substances) could act on the same organ and lead to cumulative effects (Jacobsen *et al*., 2012).
On a case-by-case basis, the recommendation would thus to perform CAGs for reproductive toxicity on specific effects (e.g. Leydig cells tumours) and/or MoA if confirmed.

2. Limitations

The analysis of the toxicological data provided needs to be performed in a weight-of-evidence basis and expert judgment.

In fact, developmental and reproductive endpoints need to be interpreted together with maternal/parental toxicity or general toxicity.

Since some endpoints were not consistent across the studies and between sexes and/or species (e.g. weight changes of reproductive organs without pathological effects), the relevance of the effect needs to be assessed. Considering that the absence of an effect has not been reported, some results should be carefully assessed when it has not been confirmed in other studies (e.g more recent guideline studies). Furthermore, in some older DARs, the study summaries are reported very poorly and the relevance of the effect and the NOAEL/LOAEL should be analyzed with caution.

The identification of a MoA for the active substances was limited. In fact, within the peer review of pesticide active substances little mechanistic data/information was available, in particular for old active substances.

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The majority of the postulated MoAs were literature based. These data need to be taken cautiously, particularly when the protocol of the mechanistic study was not clearly reported. Furthermore, *in vitro-in vivo* extrapolation may sometimes not be relevant. For example, a pesticide proven to be an AR antagonist *in vitro* may not be toxic *in vivo* as no endpoints associated with the supposed MoA were observed in the regulatory toxicity studies. The extrapolation may be sometime challenging because of the lack of *in vivo* metabolism in the *in vitro* studies, a different affinity to the receptor *in vivo* or some deficiencies in the regulatory studies (old protocols, non investigated endpoints (e.g. AGD distance)). Furthermore, interaction with receptors agonism/antagonism was the main data in the literature, identification of other MoA (e.g. effect on progesterone) is lacking and would need further investigations.

CONCLUSIONS AND RECOMMENDATIONS

Toxicological analysis of the available regulatory studies provided in support of their approval has been performed for reproductive and developmental toxicity. All the findings (endpoints) were reported for reproductive, developmental and tumour induction for each substance. The identification of key effects appropriate for the establishment on common assessment groups would need to be performed. This should be performed on a weight of evidence analysis of the provided data.

MoA have been reported for each substance when available. The identification of MoA for active substances from the peer review of pesticide active substances was limited. The majority of the postulated MoA were literature-based. Proposed MoA need to be analyzed carefully in order to analyze the human relevance of the MoA, and the confirmation of the establishing MoA. When literature-based, confirmation of the MoA would be needed (e.g. reproductibility of the effect, protocol in accordance with regulatory requirements)

For developmental toxicity, few mechanistic studies were performed to understand the MoA. When MoA are presumed, grouping pesticide by MoA might be performed. However, grouping by effect would be also relevant when no MoA were presumed.

For reproductive toxicity, grouping by specific effect is recommended as an effect could be induced by several MoA that might be cumulative. Nevertheless, it was in fact considered that the establishment of CAGs should be agreed upon by a group of experts rather than be based on the opinion of an individual contractor.

Finally, the grouping of pesticide active substances based on MoA will be probably improved with time and the better understanding of the MoA.

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APPENDICES

APPENDIX A3	Conversion factors provided by the OECD
APPENDIX B3	Elucidation of mode/mechanism of action of effects on reproduction and development
APPENDIX C3	Calculated indices in reproductive studies

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A3. APPENDIX: CONVERSION FACTORS PROVIDED BY THE OECD

ENV/JM/MONO(2002)19

TABLE 1:

APPROXIMATE RELATION OF PARTS PER MILLION IN DIET TO MG/KG BODY WEIGHT/DAY*

Animal	Weight (kg)	Grams food consumed per day (liquids omitted)	Type of diet	1 ppm in food equivalent to, in mg/kg bw/d	1 mg/kg bw/d equivalent to, in ppm of diet
Mouse	0.02	3	Dry laboratory chow diets	0.150	7
Chick	0.40	50		0.125	8
Rat, young	0.10	10		0.100	10
Rat, older	0.40	20		0.050	20
Guinea pig	0.75	30		0.040	25
Rabbit	2.00	60		0.030	33
Dog	10.00	250		0.025	40
Cat	2	100	Moist semi-solid diets	0.050	20
Monkey	5	250		0.050	20
Dog	10	750		0.075	13
Man	60	1500		0.025	40
Pig or Sheep	60	2400	Relatively dry grain-forage mixtures	0.040	25
Cattle, maintenance	60	7500		0.015	65
Cattle, fattening	500	15000		0.030	33
Horse	500	10000		0.020	50

* From Lehman (1954), as reproduced in IPCS Environmental Health Criteria Monograph No. 70 (WHO, 1987).

As outlined in IPCS Environmental Health Criteria Monograph No. 104 (WHO, 1990), if dietary intake is measured, JMPR evaluations indicate that X ppm in the food is **equal** to Y mg/kg bw, but if there is inadequate food intake data and the tabulated conversion factors are used, it is reported that X ppm in the food is **equivalent** to Y mg/kg bw.

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B3. Appendix: elucidation of presumed MoA of effects on reproduction and development

SA (chemical class)	Hypothesized MoA	Proposed pattern of toxicity effects	Detailed MoA	Reference
FLONICAMID (Pyridine compound)	Anti-estrogenic	Interference with steroid hormone homeostasis Renal findings (renal tubular vacuolation), Higher excretion of17-β-estradiol ↓17-β-estradiol, ↑ LH/FSH positive feedback	Treatment-related findings (decreased weight of ovary in females, decreased weight of uterus in offspring, delayed vaginal opening) likely related to the reduced blood 17β-estradiol level. The absence of male serum gonadotrophin concentrations changes suggests that the primary effect is one of the reduction in the concentration of circulating 17β-oestradiol, rather a direct stimulation of female gonadotrophin secretion. This effect may be related to the renal findings (renal tubular vacuolation, kidneys are the target organ of flonicamid) at this dose level, inducing higher urine volume and higher excretion of 17β-oestradiol. The slight increase in LH/FSH levels may also reflect a positive feedback at this dose-level in the 17β-estradiol response to decrease 17β -estradiol levels. Peer review: The variations observed in some hormone levels in females (reduced 17β-estradiol, increased LH and FSH) were considered not adverse taking into account the fluctuations of hormone levels in untreated animals at different sampling times and the lack of variations after dietary administration of flonicamid for 28 or 90 days.	DAR
PROCHLORAZ (Imidazole)	Anti-androgenic	AR antagonism ↓testosterone	Prochloraz act through several endocrine disrupting mechanisms, and induce various endocrine disrupting effects. Prochloraz is a potential endocrine disrupter exerting antiestrogenic (weak) and antiandrogenic activity but also can inhibit aromatase activity (Andersen et al., 2002 and Vinggaard et al., 2002). Thus, Prochloraz possesses the ability to enhance biological effects via cellular pathways due to its AhR-agonist/antagonist, antiestrogenic, antiandrogenic and aromatase inhibiting effects.	DAR

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	Steroid synthesis disruptor	Steroid synthesis inhibitors →**Progesterone hydroxylation inhibitor** Inh of P450c17 (17α–hydroxylase/17,20-lyase) ↑progesterone, ↓testosterone foetal	Prochloraz displays 2 anti-androgenic mechanisms. Firstly it is reported to be an androgen receptor antagonist in vitro and in vivo. Prochloraz was also found to feminize the male rat offspring in vivo by significantly reducing the AGD and increased nipple retention after gestational exposure to 50 and 150 mg/kg. Secondly prochloraz is reported to reduce foetal testosterone production in vivo and in vitro which may impact male reproductive performance. Prochloraz has also been shown to inhibit aromatase activity. This may contribute to the effect by inhibiting the conversion of testosterone into oestradiol and thereby reducing oestradiol levels. The anti-estrogenicity and AhR agonist activity of Prochloraz may partly be related to its potential to induce AhR-mediated CYP 1A1 and CYP 1B1 expression, which may cause increased liver weight. These activities may be associated to the extended gestation and parturition.	
	Anti-estrogenic	ER antagonist (very weak) Aromatase inhibitor (CYP19 aromatase) Decreased conversion of testosterone to estrogens ↓estrogen AhR agonist		
DINOCAP (dinitrophenol)	ATP-synthesis inhibitor	Pattern of effects observed in mice with thiabendazole (another ATP-synthesis inhibitor):	**MoA hypothesis: Developmental toxicity due to an energy-deficient intrauterine environment caused by uncoupling of cellular oxidative phosphorylation?** A prenatal dose of thiabendazole, an ATP-synthesis inhibitor, induced a deformity involving reduced limb size in mice fetuses (Ogata et al., 1984), and ATP levels in fore- and hindlimb buds of fetuses were related to the incidence of this deformity (Tsuchiya & Tanaka, 1985). Role of mitochondria in mediating apoptotic signals (green & Kromer 2004, little &Mirkes 2002) => programmed cell death (PCD) is an essential component of normal physiological processes such as embryogenesis and normal tissue development (Vaux & Korsmeyer 1999). Some studies showed a positive correlation between mitochondrial uncoupling activity and PCD (Maccarrone et al. 2001;	Open-literature (low confidence)

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			2003). 2,4-dinotrophenol (analogue of dinocap) enhanced the fas apoptotic signal in Jurkat Bcl-2 cells (Linsinger et al.1999), however the link between malformations and mitochondrial uncoupling activity is still poorly understood. (Illivicky and Casida 1969. Matsumoto et al. 2011 (Available from: http://www.intechopen.com/books/herbicides)	
BENTHIAVALICARB (carbamate)	Estrogenic	Aromatase induction: weak ↑ estradiol levels	Benthiavalicarb increases liver microsome aromatase activity (36%). However no effect on the aromatase activity was noted in both ovary and uterus. No effect on oestradiol progesterone LH serum levels including the top dose. No effect on uterus and ovary size. => DAR conclusion: In the rat, the induction of uterine tumours at the top-dose was not sufficiently explained by neither excessive toxicity nor a possible endocrine disrupting effect of the substance.	DAR
PROPAMOCARB (carbamate)	Estrogenic	Aromatase induction: weak	Weak aromatase induction in human placenta microsome (Andersen et al. 2002). Enhanced ER transactivation activity (when added with E2) (Andersen et al. 2002)	Open literature (1 publication)
PIRIMICARB (carbamate)	Estrogenic	Aromatase induction: weak	Weak aromatase induction in human placenta microsome (Andersen et al. 2002) Enhanced ER transactivation activity (when added with E2) (Andersen et al. 2002	Open literature (1 publication)
METHIOCARB (carbamate)	Estrogenic/ anti-androgenic	ER agonist/ antagonist AR: weak	Enhanced ER transactivation activity, reduced androgen induced response in the AR transactivation assay (Andersen at al. 2002)	Open literature
DICLOFOP (carbamate)	Anti-estrogenic (via an inhibition of aromatase?)	PPAR α agonist (strong)	Induction of proliferation of peroxisomes. Mechanistic studies presented in the DAR showed that diclofop can act as a PPAR α agonists. Toxic effects are sometines reported on Leydig and pancreas cell with PPAR α agonists. However, induction of proliferation of peroxisomes has been reported only in rodent. Hypothesis of MoA: Recent studies have reported that MEHP and troglitazone (a drug of TZDs), which are the ligands for PPARα and PPARγ, decreased the mRNA level of aromatase, an estrogen synthesis enzyme, in ovarian cell lines (Lovekamp-Swan et al., 2003 and Mu et al., 2000), and fenofibrate, a ligand for PPARα, inhibited the gene expression of	DAR/open literature

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			aromatase in the mouse ovary (Toda et al., 2003). Therefore, the compounds activating PPARα and PPARγ may disrupt the endocrine systems as well as ER agonists and AR antagonists.	
FLUSILAZOLE (triazole)	Anti-estrogenic	Aromatase inhibition Decreased conversion of androgens to estrogens ↓estrogen	Anti-estrogenic acticity mediated through inhibition of aromatase activity. Testicular adenoma: The interference of flusilazole with hypothalmic-pituitary-gonadal (HPG) axis was suggested as a possible mechanism of testicular tumour induction. Some evidence in support of this theory was provided by a comparative study with ketoconazole. Flusilazole did cause a slight reduction in both serum and testicular testosterone and a dose-dependent decrease in serum estradiol.	DAR
	Anti-androgenic	↓testosterone		
	Teratogenicity	Inh. embryonic CYP26 Inh. Degradation retinoic acid → Cranio-facial and brain malformations	Teratogenicity study Cranio-facial and brain malformations : cleft palate, hydrocephaly The suggested mechanism for the teratogenicity effects involves the inhibition of embryonic CYP26 degradation of retinoic acid (Menegola, 2006)	
EPOXICONAZOLE (triazole)	Anti-estrogenic	Aromatase inhibition Inh convertion androstendione and testosterone to estradiol: ↓oestradiol ↓ maternal placental oestradiol ↑ progesterone, testosterone HPT-feed-bach : ↑FSH/LH AhR agonist	Aromatase converts both testosterone and androstendione to estradiol. The inhibition of aromatase leads to an increased concentration of androgens and a decreased concentration of estradiol. The decreased estradiol levels trigger a feedback response in the hypothalamic-pituitary axis resulting in increased LH and FSH levels. Depletion of estradiol levels results from epoxiconazole aromatase inhibition to placental damage and to late fetal death. Estradiol co-treatment prevents late foetal resorptions and significantly reduced the placental damage.	DAR CLH Open literature
	Steroid synthesis	Steroid synthesis modulator Inh adrenal enz. Activity (11-	Reduction of adrenal enzymes activity would result in a decreased of corticosterone and aldosterone production, without affecting	

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	disruptor	12-hydroxylase), CYP 17? ↓aldosterone, corticosterone HPT feed-back: ↑ ACTH ↑FSH Higher steroid production in adrenal	testosterone synthesis? The decrease in adrenal steroid levels triggers a feedback response in the hypothalamic-pituitary axis resulting in increased ACTH levels	
	Anti-Androgenic	AR-antagonist		
	teratogenicity	HERG channel blocker (Weak) → hypoxia/ROS → dysmorphogenesis OR Inh. embryonic CYP26 →Inh degradataion retinoic acid → dysmorphogenesis	Suggested: a) Inhibition of embryonic CYP26 degradation of retinoic acid which then cause dysmorphogenesis (e.g. branchial apparatus). b) An alternative hypothesis involving blockade of Ikr potassium (HERG) channel via hypoxia and/or reactive oxygen species in embryo (based on data for ketoconazole) resulting in embryonic arrhythmia and hypoxia. Epoxiconazole may be a weak Ikr potassium channel inhibitor (mechanistic study, IC50=45.43µM). Epoxiconazole caused dysmorphogenesis of cultured embryos observed together with abnormal neural crest distribution. Dysmorphogenesis effects were not reproduced in rat embryos during in vivo exposure. The cleft palates were not prevented by estradiol co-treatment Also, marked reduction of estradiol and progesterone and massive placental change is likely to contribute to the formation of cleft palate.	
PROPICONAZOLE	Anti-estrogenic	Aromatase inhibition Induction 17β-estradiol and testosterone	Induction 17β-estradiol and testosterone (Kjaerstad, 2010)	DAR/Open literature
	Anti-Androgenic	AR-antagonist	Anti-androgenic in an androgen receptor reporter gene assay (Kjaerstad 2010).	
	Teratogenicity	Not investigated for propiconazole. May be via a blockade of	Teratogenicity study ↑ Cranio-facial and brain malformation : cleft palate Other malformations: eye	

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		HERG channel blocker or inhibition of embryonic CYP26 (like other triazoles)?		
AZIMSULFURON (Sulfonylurea)	?	↓estradiol (plasma) ↑LH	Similar profile of hormonal, spermatogenic and accessory gland changes as flutamid (according to the study director), antiandrogenic effect, However, several effects commonly associated with aromatase inhibitor exposure were not observed in azimsulfuron studies. For example, absent were impacts on gestational length, dystocia, pre- and post-implantation losses, Leydig cell hyperplasia and adenomas. Therefore, the mechanism of action for azimsulfuron has not been clearly defined by the existing studies. Although, azimsulfuron administration produces effects in common with antiandrogenic compounds and aromatase inhibitors, there may be other plausible mechanisms of action. A direct effect on Sertoli cells may be a plausible mechanism.	DAR
SPIRODICLOFEN (Tetronic acid)	Steroid synthesis disruptor	<u>Steroid synthesis disruptor</u> Inh malate deshydrogenase mitochondriale Disturbance NADPH generation ↑LH ↓estradiol, progesterone ↓testosterone Stimulation of Leydig cells → hypertrophy → hyperplasia → tumors	**Presumed MoA for Leydig cells tumours:** hormone-mediated effects (inh of steroid hormone biosynthesis, increase LH release in male dog, decrease in levels of progesterone and estradiol in female rat) via disturbance of NADPH generation in mitochondria and cytoplasma through inhibition of malate deshydrogenase(all assumption not experimentally verified). This triggers cascade of hormone-mediated events. Do not act via androgen or estrogen-receptor mediated mechanism. Do not interact with enzymes involved in hormone steroid biosynthesis. Increase level of gonadotrophins then results in chronic stimulation of testicular leydig cells resulting in hypertrophy, hyperplasia and tumor formation. Hormone mediated-non genotoxic mechanism.	DAR
LINURON (urea)	Anti-androgenic/other	<u>AR antagonist (weak affinity)</u> Inh of androgen induced gene expression Hypersecretion LH ↑ testosterone <u>Other mechanisms</u>	**Presumed MoA:** Linuron increased the incidence of testicular tumors in rats. Linuron may produce this effect via an endocrine alteration because prolonged stimulation of rat Leydig cells results in hyperplasia and adenoma formations. Linuron had effects on the tested but the MoA and the relation to pituitary function remained unclear.	DAR/ open literature

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			The MoA of hyperplasia/uterus tumors is unknown	
MANCOZEB (Dithiocarbamate)	Teratogenicity	<u>Interruption of thyroid hormone homeostasis</u> Based on the toxic effect of the metabolite ETU	Head malformations	Open literature
PROPYZAMIDE	Interruption of steroid hormones homeostasis	↑ testosterone oxidation capacity in microsomes ↑ LH/FSH positive feedback ↑ estradiol and corticosterone	**Presumed MoA:** Microsomal induction results in increased oxidation of testosterone, leading to disruption of the pituitary-testis hormonal. <u>Mechanistic studies</u> In the 13 week study, propyzamide treatment (329 mg/kg) resulted in increased serum LH and FSH, increased absolute and relative (to body) liver weight, increased microsomal protein content, increased oxidation of testosterone, increased activity of cytochrome-P450 and -B5, and NADPH-cyochrome-c- reductase, increased gross pathology of the liver (enlarged/dark), increased relative (to body) testicular weight, and increased testicular interstitial cell hyperplasia and testicular neoplastic effects. <u>Regulatory studies</u> 2-y rats :↑ testes enlarged, ↑ testes benign interstitial tumours	DAR
CHLORPYRIFOS	Estrogenic	<u>ERα agonist</u> ↓ testosterone, FSH, LH <u>AR antagonist</u>	ERα agonist (Kojima, 2010)	Open literature
	Anti-androgenic		AR antagonism (Wiswanath, 2010)	
CHLORPYRIFOS-METHYL	Anti-androgenic	↓ testosterone	- ↓ testosterone (Jeong, 2006) - ↓ the testosterone propionate-stimulated weight of accessory sex organs in Hershberger assay (no effect with chlorpyrifos-methyl alone) (Kang, 2004)	Open literature
PIRIMIPHOS-METHYL	Estrogenic	<u>ERα agonist</u>	ERα agonist (Kojima, 2010)	Open literature
	Anti-	<u>AR antagonism</u>	AR antagonism (Orton, 2011)	

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	androgenic			
CARBENDAZIM	Interaction with cell microtubules	Effect on the spindle apparatus during tubuline formation in the course of mitosis	**Presumed MoA:** Effect on the spindle apparatus during tubuline formation in the course of mitosis. This particularly affects proliferative tissues (inhibits mitosis, nuclear division and cytokinesis) inducing effects on reproductive capacity (sperm production) and embryo/fetal development. Hormonal changes in males (increased testosterone) is due to shifts in the normal feedback from the testes that are independent on testosterone but linked to alterations in Sertoli cell-pituitary signaling	DAR
FLUMIOXAZIN	Anemia	Inhibition of PPO with accumulation of PPIX interfering with normal heme synthesis and resulting in anaemia.	**Presumed MoA:** Hypoxia produced by anaemic condition in the foetus induces post-implantation losses and growth retardation. Hypoxia in fetal tissues is followed by suppressed liver function and a decrease in protein synthesis inducing wavy ribs. Ventricular septeal defects are explained by a compensation for the anaemia by pumping greater volume of blood leading to mechanical distortion of the heart.	DAR
IPRODIONE	Steroid synthesis disruptor	↓ testosterone, ↑ LH and FSH ↓ progesterone	Mechanistic studies - ↑ LH and FSH, ↓ testosterone DAR: Inhibition of steroid/testosterone secretion (action at the level of testosterone biosynthesis), action at androgen receptor not convincing. AIR (2012): Applicant's proposal: Inhibition of cholesterol transport. This mechanism should be further discussed in an expert meeting. Literature - ↓ testosterone and progesterone (Blyston, 2007) Iprodione affects steroidogenesis within the testis possibly through enzyme inhibition of the steroidogenic pathway before CYP17	DAR/Open literature
THIACLOPRID	Estrogenic	Increased aromatase activity	Regulatory studies - Uterine adenocarcinomas (rats) - Ovarian luteomas (mice)	DAR

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			Mechanistic studies - ↑ aromatase activity in the liver, not in the ovary (mice and rats) - ↑ metabolism of steroid testosterone (induction of enzymes which catalyze the metabolism of testosterone to androstenedione) (rats) - ↓estradiol/progesterone ratio, slight ↑ in progesterone (consequence of feedback mechanism) (mice) **Presumed MoA:** Induction of the microsomal liver enzymes included. This results in increased plasma estradiol levels and continuous stimulation of the uterine endometrium, which may explain the increased incidence of uterine adenocarcinomas in old and acyclic rats. Ovarian tumours observed in mice are regarded as secondary to the hormonal imbalance induced by the liver enzyme induction. It is known that increased estradiol levels in mice but not in rats produces a positive feedback response via prolactin release (Saade et al., 1989) which may explain the increased incidence of ovarian tumours instead of uterine tumours as in rats.	

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GLUFOSINATE	Decreased glutamine	Glutamine synthetase inhibition	**Presumed MoA:** Glutamine synthetase inhibition (enzyme catalyzing glutamate in glutamine): Induce a significant decrease in the level of glutamine in the maternal organism and thus a decreased supply of glutamine, an essential amino acid for the developing embryo, especially during the early stages of development. Glutamine is an essential amino acid for the developing mammalian embryo during the early stages of its development, especially for the successful implantation and maintenance of implantation at those early stages. During the early pre-implantation stages of the development, glutamine plays an important role in the energy metabolism of the developing embryo. Further into the development, after implantation, glutamine is replaced by glucose as the predominant exogenous energy substrate for the developing embryo. The developing embryo is dependent on the external supply of glutamine by the maternal organism as a source for energy substrate for its metabolism	DAR
MOLINATE	Anti-androgenic	Inhibition of esterase activity ↓ testosterone, progesterone, 17alpha-hydroxyprogesterone and androstenedione	Regulatory studies - Ovaries: increased weight, vacuolation, hyperplasia/hypertrophy *Accumulation of lipids due to inhibition of esterase activity* - Altered sperm (decreased motile sperm, abnormal heads) and degeneration of testes *Effect on the mid-stage of spermatogenesis (during stages VII to VIII, sperm are release from the Sertoli cells) is indicative of a disruption of testosterone production and/or release by the Leydig cells.* - ↓ mating index - Pre-implantation losses Mechanistic studies - ↓ testosterone, progesterone, 17alpha-hydroxyprogesterone and androstenedione without effect on plasma cholesterol concentration - ↓ fertility index	DAR

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			Presumed MoA: Block in neutral cholesterol ester hydrolase (nCEH) and decrease in the availability of cholesterol for steroidogenesis. Sulphur oxidation of molinate (to molinate sulfoxide) is required to elicit inhibition of esterase activity upon the testes within Leydig cells. Such an action would prevent the release of cholesterol from its storage ester within this cell type (reaction catalyzed by nCEH). Therefore, inhibition of nCEH would prevent the testis from providing the surge of testosterone needed for sperm release from Sertoli cell, resulting in dysfunction in the normal development of the sperm membrane (head lesion) and thus causing the reduction in fertility in rats. Increased abnormal sperm morphology and decrease in sperm viability, motility and concentration induces a decreased fertility and an increased pre-implantation loss. The major source of cholesterol in rats is from HDLs in plasma hydrolyzed within the cell cytosol by nCEH, contrary to man for which the majority of the cholesterol is obtain from LDLs (inhibition of cytosolic nCEH by molinate sulfoxide if unlikely).	
BROMADIOLONE	Antivitaminic K	Vitamin K deficiency	Possible developmental toxicant (read-across data from warfarin)? Warfarin causes a specific kind of embryopathy when administered to humans in the first trimester. The deformities consist of skeletal anomalies including severe nasal hypoplasia, stippled epiphyses and hypoplasia of the extremities. The mechanism behind these anomalies is considered to be the vitamin K-deficiency. Vitamin K is essential for clotting proteins in the liver. However, extrahepatic tissues/organs i.e. cartilage and bone also contain vitamin K dependent proteins. Studies have been conducted with Warfarin together with high doses of vitamin K. This treatment leads to an extrahepatic vitamin K deficiency, while the vitamin K dependent processes in the liver of the dams would have been preserved. In these studies the teratogenic effects of Warfarin was confirmed. Without vitamin K supplementation and adapted study protocol, results on the teratogenic effects of Warfarin have been equivocal. Based on Warfarin data, human fetuses also seem to be much more vulnerable to vitamin K deficiency than rodent fetuses.	DAR/CAR

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DIMETHOATE	Anti-androgenic	↓ testosterone, ↓ LH	Interference with pituitary (reduction of number of pituitary LH cells) or direct toxicity on testes? (Verma, 2009)	Open literature
FENBUTATIN OXIDE	Steroid synthesis disruptor?	Inhibition of testicular steroidogenic enzyme (3β-HSD and 17β-HSD) activity ↓ testosterone, ↑ FSH and LH	↓ testosterone, ↑ FSH and LH, inhibition of testicular steroidogenic enzyme (3β-HSD and 17β-HSD) activity - *Responsible for the regulation of testicular testosterone synthesis* (Reddy 2006)	Open literature
BUPIRIMATE	Anti-androgenic	AR antagonism	Potential AR antagonist activity (Aït-Aïssa, 2010)	Open literature
TRIFLUSULFURON	Anti-estrogenic	Weak aromatase inhibitor → disruption of the hypothalamic-pituitary-testis axis	MoA: *In vivo* and *in vitro* mechanistic studies have been conducted in order to elucidate the mechanism for the induction of Leydig cell adenomas. Based on these studies, it was concluded that triflusulfuron methyl is a relatively weak aromatase inhibitor (hepatic microsomes and cultures Leydig cells). Consistent with its low potency, triflusulfuron methyl induced Leydig cell adenoma only in rats (a highly sensitive species) and only following long-term administration of relatively high doses. Several key events were identified as part of the continuum associated with the aromatase mode of action. These included the following: binding to Cytochrome P450 and producing the characteristic Type II binding spectra associated with competitive inhibitors of aromatase; reduced serum estradiol and subsequent increased LH (since estradiol serves as a negative feedback loop for LH secretion, the decreased estradiol level is thought to increase LH level); sustained elevation of LH responsible of increased Leydig cell hyperplasia and subsequently Leydig cell adenomas.	DAR
CYPERMETHRINE / Zeta-CYPERMETHRIN (pyrethroid)	Anti-androgenic	AR antagonist (weak)	cypermethrin can act as AR antagonist (Du 2010; Zhang 2008, Xu et a. 2008)). Cyfluthrin induced hER-mediated estrogenic activity(Kojima 2010)	Open literature
	Estrogenic	ER agonist (weak)		
CYFLUTHRIN / Beta-CYFLUTHRIN	Anti-androgenic	AR antagonist (moderate)	AR antagonist: blockage of the 5-dihydrotestosterone (DHT)-induced AR activity (Zhang 2008)	Open literature

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(pyrethroid)	Estrogenic	ER agonist (weak)	Cypermethrin induced hER-mediated estrogenic activity(Kojima 2010)	
MYCLOBUTANIL	Steroid synthesis disruptor	Disruption of testosterone homeostasie → CYP17-hydroxylase/17,20 lyase (CYP17A1) inhibition	**Presumed MoA**: Disruption of the HPG axis: increased testosterone will result in reduced gonadotropin-releasing hormone (GnRH) synthesis and release from the hypothalamus and reduced synthesis and release of LH and FSH from the anterior pituitary.	DAR/open literature
	Anti-estrogenic/ Androgenic	ER antagonist/ AR agonist	Myclobutanil have capacity to bind both ERalpha (antagonist) and AR (agonist). (Okubo et al. 2004).	
	Terato	Not investigated for myclobutanil. May be via a blockage of HERG channel blocker or inhibition of embryonic CYP26 (like other triazoles)?	Teratogenicity study ↑ Cranio-facial and brain malformation : hydrocephaly, craniorachischisis	
TEBUCONAZOLE	Anti-oestrogenic (including aromatase inhibition)	Aromatase inhibition Inh convertion androstendione and testosterone to estradiol: ↓oestradiol ↑ progesterone,	Inhibition of the response induced both by 17 beta estradiol and testosterone. Inhibition of aromatase. (such as other conazoles).(Kjaerstad 2010) Aromatase converts both testosterone and androstendione to estradiol. The inhibition of aromatase leads to an increased concentration of androgens and a decreased concentration of estradiol. The decreased estradiol levels trigger a feedback response in the hypothalamic-pituitary axis resulting in increased LH and FSH levels	DAR/open literature
	Steroid synthesis disruptor	Disruption of testosterone homeostasie ↑ both progesterone and 17α-hydroxyprogesterone → CYP17-hydroxylase/17,20 lyase (CYP17A1) inhibition ? ↓ estradiol and testosterone ↑ progesterone	Presumed MoA: Increased plasma concentration of progesterone n the dams. In utero exposure to natural or synthetic progesterone can induce hypospadia in male mice, and the synthetic progesterone medroxyprogesterone acetate feminize male and virilizes female genitalia (Willingham et al., 2006). Thus, the high maternal progesterone concentration is likely to be involved in the virilizing effect on the female offspring.	

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	Anti-androgenic	AR antagonist	Inhibition of the response induced by androgens (such as other triazoles epoxyconale and propiconazole) (Kjaerstad 2010).	
	Teratogenicity	Not investigated for tebuconazole. May be via a blockade of HERG channel blocker or inhibition of embryonic CYP26 (like other triazoles)?	Teratogenicity study ↑ Cranio-facial and brain malformation : cleft palate, exencephaly, meningocele, hydrocephaly Other malformations: limb (peromelia, malrotation), eye (agnathia, anophtalmia)	

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C3. APPENDIX: CALCULATED INDICES IN REPRODUCTIVE STUDIES

Selected indices that may be calculated from endpoints (Guideline for reproductive risk assessment, EPA/630/R-96/009, October 2009)

MATING INDEX

$$\frac{\text{Number of males or females mating}}{\text{Number of males or females cohabited}} \times 100$$

Note: Mating is used to indicate that evidence of copulation (observation or other evidence of ejaculation such as vaginal plug or sperm in vaginal smear) was obtained.

FERTILITY INDEX

$$\frac{\text{Number of cohabited females becoming pregnant}}{\text{Number of nonpregnant couples cohabited}} \times 100$$

Note: Because both sexes are often exposed to an agent, distinction between sexes often is not possible. If responsibility for an effect can be clearly assigned to one sex (as when treated animals are mated with controls), then a female or male fertility index could be useful.

GESTATION (PREGNANCY) INDEX

$$\frac{\text{Number of females delivering live young}}{\text{Number of females with evidence of pregnancy}} \times 100$$

LIVE BIRTH INDEX

$$\frac{\text{Number of live offspring}}{\text{Number of offspring delivered}} \times 100$$

SEX RATIO

$$\frac{\text{Number of male offspring}}{\text{Number of female offspring}}$$

4-DAY SURVIVAL INDEX (VIABILITY INDEX)

$$\frac{\text{Number of live offspring at lactation day 4}}{\text{Number of live offspring delivered}} \times 100$$

Note: This definition assumes that no standardization of litter size is done until after the day 4 determination is completed.

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LACTATION INDEX (WEANING INDEX)

$$\frac{\text{Number of live offspring at day 21}}{\text{Number of live offspring born}} \times 100$$

Note: If litters were standardized to equalize numbers of offspring per litter, number of offspring after standardization should be used instead of number born alive. When no standardization is done, measure is called weaning index. When standardization is done, measure is called lactation index.

PREWEANING INDEX

$$\frac{\text{Number of live offspring born} - \text{Number of offspring weaned}}{\text{Number of live offspring born}} \times 100$$

Note: If litters were standardized to equalize numbers of offspring per litter, then number of offspring remaining after standardization should be used instead of number born.

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GENERAL CONCLUSIONS

Toxicological analysis of the available regulatory studies provided in support of their approval has been performed for reproductive and developmental toxicity, neurotoxicity and for effects on liver and gallbladder. In total 257 substances were found to have reproductive and developmental toxicity, 67 substances were found to be neurotoxic, and 244 substances to cause effects on the liver and biliary system, including the gallbladder. All the findings (endpoints) that were indicated in the contract as indicative for those effects have been reported for each substance, with their respective NOEL/LOEL. The selection of NOELs and LOELs was performed, as requested by EFSA, without any interpretation on whether an effect is to be considered adverse or not adverse. The identification of key effects appropriate for the establishment of common assessment groups was also not required and therefore not undertaken. It was in fact considered that the establishment of CAGs should be agreed upon by a group of experts rather than be based on the opinion of an individual contractor. However, the data presented in this report provide the basis for addressing both the issue of adversity versus non adversity, and the definition of CAGs.

Critical to these activities is the identification, whenever possible, of the MoA. In the report, established or postulated MoAs have been reported, as well as reference to possible sources of information in this respect, that mostly included the open literature. No in-depth analysis of proposed or postulated MoAs was performed.

In conclusion, this report provides a comprehensive database that would allow EFSA to access the relevant toxicological information necessary to define the CAGs according to the toxicological criteria that will be adopted.

It is recommended that the data provided in this report be interpreted against information on MoA and the presence of other systemic toxicological effects. Given the present toxicological knowledge, the definition of CAGs will necessarily be the result of an expert weight-of-evidence judgment.

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ABBREVIATIONS

ACCase: Acetyl co-enzyme A carboxylase

AChE: Acetylcholinesterase

ADI: Acceptable Daily Intake

AGD: Ano-Genital Distance

AhR: Aromatic hydrocarbon Receptor

ALS: Acetolactate synthase

ANSES: Agence Nationale de Sécurité Sanitaire de l'alimentation, de l'environnement et du travail (French Agency for Food and Occupational Health & Safety)

AR: Androgen Receptor

ARfD: Acute Reference Dose

BW: Body Weight

BWG: Body weight gain

CAG(s): Cumulative Assessment Group(s)

CAR: Cumulative Risk Assessment

CRA: Cumulative Risk Assessment

CYP: Cytochrome P450

DAR: Draft Assessment Report

DTU: Technical University of Denmark

EFSA: European Food Safety Authority

ER: Estrogen Receptor

FSH: Follicle-stimulating Hormone

GABA: Gamma-aminobutyric acid

GD: Gestational Day

GluCl: Glutamate-gated chloride

HC: Historical Control

HCD: Historical Control Data

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HPPD:	4-Hydroxyphenyl pyruvate dioxygenase
ICPS:	International Centre for Pesticides and Health Risk Prevention (Italy)
LH:	Luteinizing hormone
LO(A)EL:	Lowest Observed (Adverse) Effect
MKD:	Mg/Kg Bw/D
MoA:	Mode/Mechanism of Action
MRL:	Maximum Residue Level
NA:	Not Available
NO(A)EL:	No Observed (Adverse) Effect
NOEL:	No Observed Effect Level
PND:	Postnatal Day
PP:	Postpartum
PPIX:	Protoporphyrinogen IX
PPO:	Protoporphyrinogen oxidase
PPP:	Plant Protection Product
PPR:	Panel on Plant Protection Products and their Residues (EFSA)
PPS:	Preputial separation
RIVM:	Rijksinstituut voor Volksgezondhied en Milieu (Dutch National Institute for Public Health and the Environment)
SF:	Safety factor
TCDD:	2,3,7,8-tetrachlorodibenzo-p-dioxin

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ANNEXES

ANNEX 1: LIST OF PESTICIDE ACTIVE SUBSTANCES TO BE INCLUDED IN CAGS

Substances	Category	Neuro	Liver	Repro
1-Methyl-cyclopropene	PG		Y	Y
1-Naphthylacetamide (1-NAD)	PG	N	Y	Y
1-Naphthylacetic acid (1-NAA)	PG	N	Y	Y
2,4-D	HB, PG	Y	Y	Y
2,4-DB	HB		Y	Y
2-Phenylphenol (incl. sodium salt orthophenyl phenol)	FU		Y	Y
6-Benzyladenine	PG	N	Y	Y
Abamectin (aka avermectin)	AC, IN	Y	Y	Y
Acetamiprid	IN	Y	Y	Y
Acibenzolar-S-methyl (benzothiadiazole)	PA		Y	Y
Aclonifen	HB		Y	Y
Alpha-Cypermethrin (aka alphamethrin)	IN	Y	Y	
Aluminium phosphide	IN, RO			
Aluminium sulphate	BA	Y*	Y	Y
Amidosulfuron	HB			Y
Amitrole (aminotriazole)	HB			Y
Azimsulfuron	HB		Y	Y
Azoxystrobin			Y	Y
Beflubutamid	HB		Y	Y
Benalaxyl	FU		Y	Y
Benfluralin	HB	Y	Y	Y
Bensulfuron	HB		Y	Y
Bentazone	HB		Y	Y
Benthiavalicarb	FU		Y	Y
Benzoic acid	BA, FU, OT	Y	Y	Y
Beta-Cyfluthrin	IN	Y	Y	Y
Bifenazate	AC		Y	
Bifenox	HB		Y	Y
Bispyribac	HB	N	Y	Y
Boscalid	FU		Y	Y
Bromadiolone	RO	N	Y	Y
Bromoxynil	HB		Y	Y
Bromuconazole	FU	N	Y	Y

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Substances	Category	Neuro	Liver	Repro
Bupirimate	FU	N	Y	Y
Buprofezin	IN	N	Y	Y
Calcium phosphide	RO			Y
Captan	FU		Y	Y
Carbendazim	FU		Y	Y
Carbetamide	HB	Y	Y	Y
Carboxin	FU	N	Y	Y
Carfentrazone-ethyl	HB		Y	Y
Carvone	PG			
Chloridazon (aka pyrazone)	HB		Y	Y
Chlormequat (chloride)	PG	Y		Y
Chlorothalonil	FU		Y	Y
Chlorotoluron	HB		Y	Y
Chlorpropham	PG, HB	Y	Y	Y
Chlorpyrifos	IN, AC	Y		Y
Chlorpyrifos-methyl	IN, AC	Y		Y
Chlorsulfuron	HB		Y	Y
Cinidon ethyl	HB		Y	Y
Clethodim	HB	N	Y	Y
Clodinafop	HB		Y	Y
Clofentezine	AC		Y	Y
Clomazone	HB		Y	Y
Clopyralid	HB		Y	Y
Clothianidin	IN	Y	Y	Y
Copper compounds	FU		Y	Y
Cyazofamid	FU			Y
Cyclanilide	PG		Y	Y
Cycloxydim	HB	N	Y	Y
Cyflufenamid	FU		Y	Y
Cyfluthrin	IN, AC	Y	Y	Y
Cyhalofop-butyl	HB		Y	
Cymoxanil	FU	Y	Y	Y
Cypermethrin	IN, AC	Y	Y	Y
Cyproconazole	FU	N	Y	Y
Cyprodinil	FU		Y	Y
Cyromazine	IN		Y	Y
Daminozide	PG			
Dazomet	NE, FU, HB, ST	N	Y	Y
Deltamethrin	IN	Y	Y	Y

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Substances	Category	Neuro	Liver	Repro
Desmedipham	HB	Y	Y	Y
Dicamba	HB	Y	Y	Y
Dichlorprop-P	HB		Y	Y
Diclofop	HB	N	Y	Y
Diethofencarb	FU	N	Y	Y
Difenoconazole	FU		Y	Y
Diflubenzuron	IN		Y	Y
Diflufenican	HB		Y	Y
Dimethachlor	HB		Y	Y
Dimethenamid-P	HB		Y	Y
Dimethoate	IN, AC	Y		Y
Dimethomorph	FU		Y	Y
Dimoxystrobin	FU	N		Y
Dinocap	FU, AC	N	Y	Y
Diquat (dibromide)	HB, DE			
Dithianon	FU	N	Y	Y
Diuron	HB		Y	Y
Dodemorph	FU		Y	Y
Dodine	FU	N	Y	Y
Epoxiconazole	FU		Y	Y
Esfenvalerate	IN	Y		Y
Ethephon	PG	Y	Y	Y
Ethofumesate	HB		Y	Y
Ethoprophos	NE, IN	Y	Y	Y
Ethoxysulfuron	HB		Y	Y
Etofenprox	IN		Y	Y
Etoxazole	IN		Y	Y
Etridiazole	FU	N	Y	Y
Famoxadone	FU		Y	Y
Fenamidone	FU		Y	Y
Fenamiphos (aka phenamiphos)	NE	Y		Y
Fenazaquin	AC	N	Y	Y
Fenbuconazole	FU	N	Y	Y
Fenbutatin oxide	AC	N	Y	Y
Fenhexamid	FU		Y	Y
Fenoxaprop-P	HB		Y	Y
Fenoxycarb	IN	N	Y	Y
Fenpropidin	FU	Y	Y	Y
Fenpropimorph	FU	Y	Y	Y

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Substances	Category	Neuro	Liver	Repro
Fenpyroximate	AC		Y	Y
Fipronil	IN	Y	Y	Y
Flazasulfuron	HB		Y	Y
Flonicamid (IKI-220)	IN	N	Y	Y
Florasulam	HB	N	Y	
Fluazifop-P	HB	N	Y	Y
Fluazinam	FU		Y	Y
Fludioxonil	FU		Y	Y
Flufenacet (formerly fluthiamide)	HB	Y	Y	Y
Flumioxazin	HB		Y	Y
Fluometuron	HB	N	Y	Y
Fluopicolide	FU		Y	Y
Fluoxastrobin	FU		Y	Y
Flupyrsulfuron-methyl (DPX KE 459)	HB		Y	Y
Fluquinconazole	FU	Y	Y	Y
Flurochloridone	HB	N	Y	Y
Fluroxypyr	HB			Y
Flusilazole	FU		Y	Y
Flutolanil	FU		Y	
Flutriafol	FU	N	Y	Y
Folpet	FU			Y
Foramsulfuron	HB			
Forchlorfenuron	PG			Y
Formetanate	IN, AC	Y	Y	Y
Fosetyl-aluminium	FU	N		Y
Fosthiazate	NE	Y		Y
Fuberidazole	FU		Y	Y
Gibberellin	PG		Y	Y
Glufosinate	HB	Y		Y
Glyphosate (incl trimesium aka sulfosate)	HB		Y	Y
Haloxyfop-P (Haloxyfop-R)	HB	N	Y	Y
Hexythiazox	AC, IN	N	Y	Y
Hymexazol	FU	N	Y	Y
Imazalil (aka enilconazole)	FU		Y	Y
Imazamox	HB			
Imazaquin	PG			Y
Imazosulfuron	HB		Y	Y
Imidacloprid	IN	Y	Y	Y
Indoxacarb	IN	Y		Y

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Substances	Category	Neuro	Liver	Repro
Iodosulfuron-methyl-sodium	HB		Y	Y
Ioxynil	HB		Y	Y
Iprodione	FU		Y	Y
Iprovalicarb	FU		Y	Y
Isoproturon	HB		Y	Y
Isoxaben	HB	N	Y	Y
Isoxaflutole	HB	Y	Y	Y
Kresoxim-methyl	FU		Y	
lambda-Cyhalothrin	IN	Y	Y	Y
Lenacil	HB		Y	Y
Linuron	HB		Y	Y
Lufenuron	IN	Y	Y	Y
Magnesium phosphide	IN, RO		Y	
Malathion	IN, AC	Y	Y	Y
Maleic hydrazide	PG		Y	
Mancozeb	FU	Y	Y	Y
Maneb	FU	Y	Y	Y
MCPA	HB		Y	Y
MCPB	HB		Y	Y
Mecoprop	HB		Y	Y
Mecoprop-P	HB		Y	Y
Mepanipyrim	FU		Y	Y
Mepiquat	PG	Y		Y
Mesosulfuron	HB			
Mesotrione	HB		Y	Y
Metalaxyl-M	FU		Y	
Metaldehyde	MO	Y	Y	Y
Metamitron	HB		Y	Y
Metazachlor	HB		Y	
Metconazole	FU, PG		Y	Y
Methiocarb (aka mercaptodimethur)	IN, MO,RE	Y	Y	Y
Methomyl	IN	Y		
Methoxyfenozide	IN		Y	
Metiram	FU	Y	Y	Y
Metosulam	HB	N	Y	Y
Metrafenone	FU		Y	Y
Metribuzin	HB		Y	Y
Metsulfuron-methyl	HB			Y
Milbemectin	IN		Y	Y

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Substances	Category	Neuro	Liver	Repro
Molinate	HB	Y	Y	Y
Myclobutanil	FU	N	Y	Y
Napropamide	HB	N	Y	Y
Nicosulfuron	HB			
Oryzalin	HB	N	Y	Y
Oxadiazon	HB		Y	Y
Oxamyl	IN, NE	Y		Y
Oxasulfuron	HB	Y		Y
Oxyfluorfen	HB	N	Y	Y
Paclobutrazol	PG	N	Y	Y
Penconazole	FU		Y	Y
Pencycuron	FU	N	Y	Y
Pendimethalin	HB		Y	
Penoxsulam	HB	N	Y	Y
Pethoxamid	HB		Y	
Phenmedipham	HB	N		Y
Phosmet	IN	Y	Y	Y
Picloram	HB		Y	
Picolinafen	HB			Y
Picoxystrobin	FU			
Pirimicarb	IN	Y		Y
Pirimiphos-methyl	IN	Y		Y
Prochloraz	FU	N	Y	Y
Profoxydim	HB	N	Y	Y
Prohexadione-calcium	PG			
Propamocarb	FU	Y		Y
Propaquizafop	HB			Y
Propiconazole	FU		Y	Y
Propineb	FU	Y	Y	
Propoxycarbazone	HB		Y	Y
Propyzamide	HB		Y	Y
Proquinazid	FU	N	Y	Y
Prosulfocarb	HB	N	Y	Y
Prosulfuron	HB		Y	Y
Prothioconazole	FU		Y	Y
Pymetrozine	IN		Y	Y
Pyraclostrobin	FU, PG		Y	Y
Pyraflufen-ethyl	HB		Y	
Pyrethrins	IN	Y	Y	

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Substances	Category	Neuro	Liver	Repro
Pyridaben	AC, IN	N	Y	Y
Pyrimethanil	FU		Y	Y
Pyriproxyfen	IN		Y	Y
Quinmerac	HB	N	Y	Y
Quinoclamine	HB, AL	Y	Y	Y
Quinoxyfen	FU		Y	Y
Quizalofop-P (covers also Quisalofop - ethyl and - tefuryl)	HB		Y	Y
Rimsulfuron (aka renriduron)	HB		Y	Y
Silthiofam	FU		Y	Y
Sintofen (aka Cintofen)	PG	N	Y	Y
S-Metolachlor	HB		Y	Y
Sodium 5-nitroguaiacolate	PG		Y	Y
Sodium hypochlorite	BA			Y
Sodium o-nitrophenolate	PG		Y	Y
Sodium p-nitrophenolate	PG		Y	Y
Spinosad	IN	Y	Y	Y
Spirodiclofen	AC, IN	N	Y	Y
Spiroxamine	FU		Y	Y
Sulcotrione	HB	N	Y	Y
Sulfosulfuron	HB			
Sulfuryl fluoride	IN	Y	Y	Y
tau-Fluvalinate	IN	Y	Y	Y
Tebuconazole	FU		Y	Y
Tebufenozide	IN	N	Y	Y
Tebufenpyrad	AC		Y	Y
Teflubenzuron	IN		Y	
Tefluthrin	IN	Y	Y	Y
Tepraloxydim	HB		Y	Y
Terbuthylazine	HB	N	Y	Y
Tetraconazole	FU		Y	Y
Thiabendazole	FU		Y	Y
Thiacloprid	IN	Y	Y	Y
Thiamethoxam	IN		Y	Y
Thifensulfuron-methyl	HB			Y
Thiophanate-methyl	FU		Y	Y
Thiram	FU	Y	Y	Y
Tolclofos-methyl	FU	Y	Y	Y
Tolylfluanid	FU, AC		Y	Y

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Substances	Category	Neuro	Liver	Repro
Tralkoxydim	HB		Y	Y
Triadimenol	FU	Y	Y	Y
Tri-allate	HB	Y	Y	Y
Triasulfuron	HB		Y	Y
Triazoxide	FU	N	Y	Y
Tribenuron (aka metometuron)	HB		Y	Y
Triclopyr	HB		Y	Y
Trifloxystrobin	FU		Y	Y
Triflumizole	FU	N	Y	Y
Triflumuron	IN	N	Y	Y
Triflusulfuron	HB	N	Y	Y
Trinexapac (aka cimetacarb ethyl)	PG		Y	Y
Triticonazole	FU		Y	Y
Tritosulfuron	HB		Y	Y
zeta-Cypermethrin	IN	Y	Y	Y
Zinc phosphide	RO	Y*	Y	Y
Ziram	FU, RE	Y	Y	Y
Zoxamide	FU		Y	Y
Flurtamone	HB	N		
Oxadiargyl	HB	N		
Pyridate	HB	Y		

N Substances evaluated but considered not to be included in CAG (see Appendix B1, Lot 1).
* Substance not included in the reporting table for neurotoxic substances, based on intended uses (see Results of Lot 1).

Colour code	
Substances with white background	List of pesticide active substances to be included in CAGs (included in Annex I up to 31 December 2011)
Substances with yellow background	Substances included in Annex I between 31 05 2009 and 31 12 2011 and not included by DTU
Substances with green background	Substances included in Annex I but not assessed by DTU

Category
AC - Acaricide
AT - Attractant
BA - Bactericide
EL - Elicitor
FU - Fungicide
HB - Herbicide
IN - Insecticide

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MO - Molluscicide
NE - Nematicide
OT - Other treatment
PA - Plant activator
PG - Plant growth regulator

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ANNEX 2: RIVM FINAL REPORTING TABLE

ANNEX 3: ICPS PRELIMINARY REPORTING TABLE

ANNEX 4: ICPS FINAL REPORTING TABLE

ANNEX 5: ANSES FINAL REPORTING TABLE

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