Avis de l'Anses
Saisine n° 2018-SA-0289

Le directeur général

Maisons-Alfort, le 19 avril 2019

AVIS
de l'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail

relatif au caractère perturbateur endocrinien de l'époxiconazole

L'Anses met en œuvre une expertise scientifique indépendante et pluraliste.
L'Anses contribue principalement à assurer la sécurité sanitaire dans les domaines de l'environnement, du travail et de l'alimentation et à évaluer les risques sanitaires qu'ils peuvent comporter.
Elle contribue également à assurer d'une part la protection de la santé et du bien-être des animaux et de la santé des végétaux et d'autre part à l'évaluation des propriétés nutritionnelles des aliments.

Elle fournit aux autorités compétentes toutes les informations sur ces risques ainsi que l'expertise et l'appui scientifique technique nécessaires à l'élaboration des dispositions législatives et réglementaires et à la mise en œuvre des mesures de gestion du risque (article L.1313-1 du code de la santé publique).

Ses avis sont publiés sur son site internet.

La direction de l'évaluation des produits réglementés (DEPR) de l'Anses a été saisie le 08/03/2018 par la directrice générale déléguée en charge du pôle des produits réglementés pour la réalisation de l'expertise suivante : Avis sur le caractère perturbateur endocrinien de l'époxiconazole.

1. CONTEXTE ET OBJET DE LA SAISINE

L'époxiconazole est une substance active antifongique qui fait l'objet de nombreux usages phytopharmaceutiques sur le territoire national.

L'évaluation des risques liés à l'usage des préparations à base d'époxiconazole actuellement le marché a conclu à l'absence d'effet nocif dans les conditions d'emploi prévues par les autorisations de mise sur le marché.

Néanmoins, dans son avis du 7 août 2014 relatif à une demande d'autorisation d'extension majeure du produit OPERA, et après consultation du comité d'experts spécialisé (CES) « Produits phytopharmaceutiques : substances et préparations chimiques », l'Anses notait que « *compte tenu des propriétés de l'époxiconazole, qui présente une activité endocrinienne, l'avis devra être revu après adoption de la règlementation européenne sur les perturbateurs endocriniens* ».

Or, la règlementation européenne sur les perturbateurs endocriniens[1] a été adoptée le 13 décembre 2017 et le document guide[2] permettant son application a été publié le 5 juin 2018.

[1] RÈGLEMENT (UE) 2018/605 DE LA COMMISSION du 19 avril 2018 modifiant l'annexe II du règlement (CE) no 1107/2009 en établissant des critères scientifiques pour la détermination des propriétés perturbant le système endocrinien et RÈGLEMENT DÉLÉGUÉ (UE) 2017/2100 DE LA COMMISSION du 4 septembre 2017 définissant des critères scientifiques pour la détermination des propriétés perturbant le système endocrinien, conformément au règlement (UE) no 528/2012 du Parlement européen et du Conseil

[2] Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009, 5 June 2018

De plus, l'étendue de l'usage de l'époxiconazole entraîne une incertitude sur la réalité du caractère négligeable de l'exposition qui est exigible pour une substance classée R1B approuvée dans le cadre du règlement (CE) N°1107/2009.

Prenant en considération le classement de la substance comme reprotoxique de catégorie 1B et cancérogène de catégorie 2, l'étendue de son usage ainsi que l'adoption du règlement européen sur les perturbateurs endocriniens, il est demandé à la DEPR d'indiquer si l'époxiconazole répond à la définition d'un perturbateur endocrinien (PE) au sens de l'amendement du règlement européen (CE) N°1107/2009 introduisant les critères d'identification pour les perturbateurs endocriniens, adopté par les représentants des Etats Membres. Cette analyse s'appuiera sur le document guide.

2. ORGANISATION DE L'EXPERTISE

L'expertise a été réalisée dans le respect de la norme NF X 50-110 « Qualité en expertise – Prescriptions générales de compétence pour une expertise (Mai 2003) ».

L'analyse a été conduite par la Direction de l'Evaluation des Produits Règlementés selon le document guide pour l'identification des perturbateurs endocriniens dans le contexte des règlements (UE) N°528/2012 et (CE) N°1107/2009 et a été validée par le CES « Produits phytopharmaceutiques : substances et préparations chimiques » lors de sa séance du 23 octobre 2018.

L'Anses analyse les liens d'intérêts déclarés par les experts avant leur nomination et tout au long des travaux, afin d'éviter les risques de conflits d'intérêts au regard des points traités dans le cadre de l'expertise.

Les déclarations d'intérêts des experts sont publiées sur le site internet de l'Anses (www.anses.fr).

3. ANALYSE ET CONCLUSIONS DU CES

Les détails de l'évaluation figurent en annexe 2.

L'époxiconazole est classé reprotoxique de catégorie 1B H360Df (Peut nuire au fœtus. Susceptible de nuire à la fertilité) et cancérogène de catégorie 2 H351 (Susceptible de provoquer le cancer) selon le Règlement (UE) N° 944/2013 amendant le Règlement (CE) N°1272/2008. Cette classification est basée notamment sur des effets adverses qui pourraient être liés à une activité endocrinienne. Le document guide EFSA/ECHA pour l'identification des perturbateurs endocriniens dans le contexte des règlements (UE) N°528/2012 et (CE) N°1107/2009, publié en Juin 2018, a été utilisé pour évaluer le potentiel perturbateur endocrinien de l'époxiconazole.

La majorité des données scientifiques utilisées dans cette évaluation ont fait l'objet d'une revue par les pairs au niveau européen (EFSA et/ou RAC[3] de l'ECHA). De plus, de nouvelles données de la littérature ont été inclues dans l'évaluation générale du poids des preuves. Il est à noter que ces nouvelles données n'ont pas mis en évidence de nouveaux effets adverses ou activité endocrinienne en comparaison des effets/activité déjà discutés au niveau européen mais en ont confirmé certains. En outre, il est important de souligner que ces nouvelles données ne sont pas de nature à remettre en cause les valeurs toxicologiques de référence adoptées au niveau européen.

Dans le cadre de l'application du document guide sur les perturbateurs endocriniens et en considérant les effets adverses et l'activité endocrinienne identifiés, plusieurs modes d'action ont pu être suspectés pour les mammifères, les vertébrés autres que les mammifères ainsi que pour les invertébrés. Il est considéré que, pour ces modes d'action, des données suffisantes sont disponibles et que, selon l'analyse du poids des preuves, il existe suffisamment d'éléments pour

[3] RAC : Risk Assessment Committee

établir la plausibilité biologique du lien entre l'activité endocrinienne et les effets adverses observés.

Ainsi, sur la base du document guide, l'époxiconazole remplit les critères de perturbateur endocrinien pour l'Homme et les organismes non-cibles.

En raison de contraintes de temps, l'application du document a été quelque peu simplifiée en comparaison de ce qui est requis dans ce document et quelques limites peuvent être soulignées :

- La recherche de la littérature ayant permis d'ajouter des publications récentes n'a pas été conduite selon le document guide de l'EFSA sur la revue systématique[4] ;
- Le tableau Excel ayant pour objet de rapporter l'ensemble des paramètres utiles à l'évaluation du caractère perturbateur endocrinien n'a pas été produit ;
- Seuls les résultats positifs (et non les résultats négatifs) ont été généralement rapportés dans les tableaux intégrant les lignes de preuves ;
- L'analyse de la plausibilité biologique a été réalisée uniquement pour certains des modes d'action suspectés.

Ainsi, l'analyse s'est focalisée sur les effets pertinents et les modes d'action pour lesquels les preuves les plus convaincantes étaient disponibles. Néanmoins, l'époxiconazole remplissant les critères de perturbateur endocrinien sur la base de cette analyse, une évaluation supplémentaire n'est pas considérée nécessaire.

4. CONCLUSIONS ET RECOMMANDATIONS DE L'AGENCE

En vertu de la règlementation européenne sur les perturbateurs endocriniens et selon le document guide pour l'identification des perturbateurs endocrinien dans le contexte des règlements (UE) N°528/2012 et (CE) N°1107/2009, l'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail estime que l'époxiconazole remplit les critères de perturbateur endocrinien.

Cette analyse de l'Anses a été transmise à la Commission Européenne dans le cadre de l'article 21 du règlement (CE) N°1107/2009 qui peut permettre de réexaminer, à la lumière des nouvelles connaissances scientifiques et techniques, l'approbation d'une substance active.

Dr Roger GENET

[4] Application of systematic review methodology to food and feed safety assessments to support decision making. EFSA Journal 2010; 8(6):1637

MOTS-CLES

Epoxiconazole, Perturbateur endocrinien, Règlementation européenne, Document guide

Epoxiconazole, Endocrine disruptor, European regulation, Guidance

ANNEXE 1

Saisine de la DEPR par la Directrice Générale en charge du pôle des produits règlementés

anses
agence nationale de sécurité sanitaire alimentation, environnement, travail
Connaître. évaluer. protéger

La Directrice Générale Déléguée
en charges du pôle des produits réglementés

Maisons-Alfort, le 08/03/2018

Note pour Agnès LEFRANC
Directrice de l'évaluation des produits règlementés

Annule et remplace la note du 26/02/2018

Objet : Avis sur le caractère perturbateur endocrinien de l'époxiconazole.

L'époxiconazole est une substance active antifongique qui fait l'objet de nombreux usages phytopharmaceutiques sur le territoire national.

L'évaluation des risques liés à l'usage des préparations à base d'époxiconazole actuellement sur le marché a conclu à l'absence d'effet nocif dans les conditions d'emploi prévues par les autorisations de mise sur le marché.

Néanmoins, dans son avis du 7 août 2014 relatif à une demande d'autorisation d'extension d'usage majeur du produit OPERA, et après consultation du comité d'experts spécialisé « produits phytopharmaceutiques, substances et préparations chimiques », l'ANSES notait que « *compte tenu des propriétés de l'époxiconazole, qui présente une activité endocrinienne, l'avis devra être revu après adoption de la règlementation européenne sur les perturbateurs endocriniens* ».

Or, la règlementation européenne sur les perturbateurs endocriniens a été adoptée le 13 décembre 2017 et le document guide permettant son application est en cours de validation par la commission européenne.

De plus, l'étendue de l'usage de l'époxiconazole entraine une incertitude sur la réalité de l'exposition négligeable des personnes et de l'environnement à ses dangers, exposition négligeable qui est exigible pour une substance classée R1B approuvée dans le cadre du règlement EU 1107/2009

Prenant en considération le classement de la substance comme reprotoxique de catégorie 1b et cancérogène de catégorie 2, l'étendue de son usage, ainsi que l'adoption du règlement européen sur les perturbateurs endocriniens, je vous remercie de bien vouloir indiquer si l'époxiconazole répond à la définition d'un perturbateur endocrinien au sens du projet d'amendement du règlement européen (CE) N°1107/2009[1] introduisant les critères

[1] COMMISSION REGULATION (EU) .../...of XXX amending Annex II to Regulation (EC) No 1107/2009 by setting out scientific criteria for the determination of endocrine disrupting properties

Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail,
14, rue Pierre et Marie Curie, 94701 Maisons-Alfort Cedex
Téléphone : + 33 (0)1 49 77 13 50 - Télécopie : + 33 (0)1 49 77 26 69 - www.anses.fr

d'identification pour les perturbateurs endocriniens, adopté le 13 décembre dernier par les représentants des Etats-membres. Votre analyse s'appuiera sur le projet de document guide[2].

Je vous remercie de me faire part de vos conclusions au plus tard le 30 avril prochain.

Françoise WEBER

Copie : Roger GENET

[2] Guidance for the identification 14 of endocrine disruptors in the context of Regulations (EU) No528/2012 and (EC) No 1107/2009. Draft for public consultation. Drafted by EFSA and ECHA staff, with support from JRC. 7 December 2017.

ANNEXE 2

Epoxiconazole - Assessment of ED properties using the EFSA/ECHA guidance for the identification of endocrine disruptors, October 2018

Epoxiconazole - Assessment of ED properties using the EFSA/ECHA guidance for the identification of endocrine disruptors
October 2018

The EFSA/ECHA Guidance for the identification of endocrine disruptors[5], published in June 2018, was used to assess the endocrine disrupting potential of epoxiconazole. The steps of the analysis presented below follow the ones required by the guidance. It has to be noted that according to the EU Guidance, all the parameters which are useful for the ED assessment, identified in each relevant and reliable study, should be reported in a tabular form. For that purpose, an Excel template is provided with the guidance (Appendix E of the guidance). Nevertheless, due to time constraints, this table was not filled in for the present study.

1. Gather information

Gather all relevant information
All relevant information available for epoxiconazole was gathered:

- Scientific data generated in accordance with internationally agreed study protocols (generally OECD Test Guidelines) and generally performed according to Good Laboratory Practices. These data were extracted from the Draft Assessment Report (DAR) of the substance (Allemagne, 2005) and its Final addendum (Allemagne, 2008) as well as from the CLH report of epoxiconazole (ECHA Committee for risk assessment, 2010).

- Literature data already included in the DAR of epoxiconazole and/or in the CLH report. A more recent search was also performed in order to identify potentially relevant information published after the publication of the DAR, Addendum and CLH report. The search was performed using the Scopus database, considering a period ranging from 2004 to 2018 and the key words epoxiconazole (and its synonyms and CAS numbers) and "endocrine disrupt*".

- Other scientific data, particularly data extracted from ToxCast database.

The EFSA conclusion on the peer review of epoxiconazole (EFSA Scientific Report, 2008) as well as the ECHA RAC Opinions proposing harmonized classification and labelling at EU level for epoxiconazole (RAC (Committee for risk assessment) opinion 2010, 2011 and 2012) were also used as supportive information.
The scientific data already assessed in the DAR/Addendum and/or CLH report were not re-evaluated for the purpose of this case study.

Evaluate relevance and reliability of the data
Data were assessed for their relevance and reliability. All relevant information available in the DAR/Addendum and/or CLH report was also considered relevant by default in this case study. Elements supporting the reliability of scientific data were included in the tables gathering the lines of evidence (see annex).

[5] ECHA (European Chemicals Agency) and EFSA (European Food Safety Authority) with the technical support of the Joint Research Centre (JRC). Andersson N, Arena M, Auteri D, Barmaz S, Grignard E, Kienzler A, Lepper P, Lostia AM, Munn S, Parra Morte JM, Pellizzato F, Tarazona J, Terron A and Van der Linden S, 2018. Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. EFSA Journal 2018;16(6):5311, 135 pp. https://doi.org/10.2903/j.efsa.2018.5311. ECHA-18-G-01-EN.

Extracting and reporting the information
According to the EU Guidance, all the parameters useful for the ED assessment, identified in each relevant and reliable study, should be reported in a tabular form. For that purpose, an Excel template was provided with the guidance (Appendix E of the guidance). Nevertheless, due to time constraints, this table was not filled in for epoxiconazole.

2. Assess the evidence

Assemble, assess, integrate and report the lines of evidence for endocrine activity and adversity
The available information was assembled into lines of evidence depending on whether the parameters correspond to information on EATS (Estrogen, Androgen, Thyroid, Steroidogenic)-related adversity ("EATS-mediated" parameters and parameters "sensitive to, but not diagnostic of, EATS") or endocrine activity ("*in vitro* mechanistic" and "*in vivo* mechanistic").
As required in the ED Guidance, the assessment of the lines of evidence was based on a weight-of-evidence approach, taking into account the available empirical support and expert judgement. The empirical support consists of dose-response, temporal concordance, consistency among studies and species as well as repeatability for the line of evidence. In addition, the expert judgment was also taken into account (e.g. assessment of the concomitant systemic general toxicity, reliability of the studies and of the observed effects).
Although it is highlighted in the ED Guidance that the assembling of lines of evidence should take into consideration all the available evidence (positive and negative) that have been evaluated as relevant and reliable, it is noteworthy that due to time constraints, only the positive effects were generally recorded for epoxiconazole.
For epoxiconazole, three tables assembling the lines of evidence for EAS modalities are proposed: one for mammalian data (Table 1), one for other non-target organisms (vertebrate species only) (Table 2) and one for nematods (Table 3). The tables are presented at the end of the document (Appendix: Tables assembling the lines of evidence). These tables were adapted from those proposed in the ED Guidance, some modifications having been made in order to improve their readability and to facilitate the weight of evidence analysis.

Based on these tables assembling, integrating and assessing the lines of evidence for epoxiconazole, the following listed parameters were considered as supporting evidence in relation to EAS activity/adversity (for more details, please refer to Tables in Appendix):

Evidence of endocrine activity:
- *In vitro mechanistic:*
  - Inhibition of aromatase activity demonstrated *in vitro* in rat, human and porcine cells
  - Inhibition of steroidogenesis in H295R cell line
  - AR antagonism in CHO and MDA-kb2 cells

- *In vivo mechanistic:*
  - Decreased estradiol level in female rat
  - Increased LH level in female rat
  - Increased FSH level in male and female rat
  - Increased testosterone level in female rat and in female guinea pig
  - Androgens: increased level (androstenedione +/- dehydroepiandrosterone) in female rat and in female guinea pig
  - Decreased vitellogenin in plasma in female Zebra fish (*Danio rerio*)

Evidence of endocrine adversity:
- *EAS-mediated parameters:*
  - Ovarian cysts in rat in the two long-term studies
  - Prolonged estrus cycle in rat
  - Reduced number of testicular canaliculi with visible germ cells, reduced spermatid numbers and decreased spermatogenesis in the Japanese quail
  - Reduced number of germ cells and spermatids in the nematode (*Caenorhabditis elegans*), resulting in abnormalities of germ cell development

- Decreased number of mitotic cells, induced abnormalities in the process of germ cell differentiation and reduced number of meiotic cells in the nematode (*Caenorhabditis elegans*)
- Abnormal spermatozoid cells and inhibition of sperm activation in the nematode (*Caenorhabditis elegans*)
- Sex ratio significantly shifted to male in Zebra fish (*Danio rerio*)

- Parameter sensitive to, but not diagnostic of, EAS:

- Ovarian theca granulosa cell tumours and adrenal gland cortex tumours in the female rat
- Decreased incidences of neoplasms in the testes (Leydig cell tumours), adrenal gland medulla (phaechromocytomas, in males) and pituitary gland (adenomas, in females) in rat
- Increased gestation length in rat
- Dystocia observed in rat. Linked to increased gestation length
- Increased time to mating in rat which may indicate irregularities of the oestrous cycle
- Post-implantation losses in rats (late and very late) and rabbits (early)
- Decreased number of live births in rat. Linked to post-implantation loss
- Decreased mean litter size in rat. Linked to post-implantation loss
- Vaginal haemorrhage in rat. Linked to post-implantation loss
- Decreased pup survival in rat
- Increased incidence of a rare malformation, namely cleft palate, in rat. Anasarca (generalised edema) in rat. Thoracic centrum fused with arch in the guinea pig
- Increased placental weight in rat
- Degeneration of the labyrinth and the trophospongium of the placenta in rat
- Decreased number of outcross progeny in the nematode (*Caenorhabditis elegans*)
- Start of spawning delayed in Zebra fish (*Danio rerio*)
- Significant reduced number of eggs and fertilization in Zebra fish (*Danio rerio*)

Regarding thyroid modality, no adverse effect nor activity have been noted in the available dataset, which was otherwise not sufficiently investigated (see below).

3. Initial analysis of the evidence

In accordance with the ED guidance, the first step of the initial analysis of the evidence is to assess whether either EATS-mediated adversity or EATS endocrine activity has been sufficiently investigated. Then, different scenarios, depending on the information available on EATS-mediated adversity and endocrine activity, are proposed.

Have the EATS-mediated parameters been sufficiently investigated? → No

According to the ED Guidance, in order to consider the **EAS-mediated adversity** sufficiently investigated, the following parameters should have been investigated:
- *with regard to humans and mammals (as non-target organisms):* all the 'EAS-mediated' parameters foreseen to be investigated in an extended one-generation reproductive toxicity study (OECD TG 443 (2012); with cohort 1a/1b including the mating of cohort 1b to produce the F2 generation) or a two-generation reproductive toxicity study (OECD TG 416 (2001); test protocol according to the latest version of January 2001).
For epoxiconazole, neither an extended one-generation reproductive toxicity study, nor a 2-generation reproductive toxicity study performed according to the OECD TG dated from 2001, were available. Indeed, the 2-generation study was performed in 1992 according to the OECD TG 416 in force at that time. Although there is a number of published and unpublished studies investigating several parameters relevant for determining the ED potential of epoxiconazole (e.g. anogenital distance), some parameters remained not investigated in the available database (e.g. age at vaginal opening/balano-preputial separation).
- *with regard to other non-target organisms*: the 'EAS-mediated' parameters foreseen to be measured in the Medaka extended one-generation test (MEOGRT, OECD TG 240 (2015)), or alternatively a FLCTT covering all the "EAS-mediated" parameters foreseen to be measured in the MEORGT.

For epoxiconazole due to its endocrine potential various chronic studies in fish were performed. A full life cycle study with sediment under static conditions in zebra fish (*Danio rerio*) in addition to a full life cycle studies under flow-through conditions in fathead minnow (*Pimephales promelas*) are available. Some relevant population effects on 'EAS-mediated' parameters have been observed in these studies (effects on vitellogenin in female and effects on sex ratio in zebra fish).

Furthermore, in order to consider the **thyroid-mediated adversity** sufficiently investigated, the following parameters should have been investigated:
- *with regard to humans and mammals (as non-target organisms)*: the thyroid parameters foreseen to be investigated in the following studies OECD test guidelines 407, 408, 409 (and/or the one-year dog study, if available), 416 (or 443 if available) and 451-3.
With epoxiconazole, although thyroid weight and histopathology seem to have been investigated (protocol and/or results not detailed in the DAR) in the subchronic and chronic toxicity studies in line with requirements of OECD TG in force at the time the studies were performed (early 1990's), thyroid hormone levels (T3, T4 and TSH) were only measured in a developmental rat toxicity study where only one dose level was used.
- *with regard to other non-target organisms*: all the "T-mediated" parameters foreseen to be investigated in the Larval amphibian growth and development assay (LAGDA, OECD TG 241 (2015)), or alternatively, the "T-mediated" parameters foreseen to be investigated in an Amphibian Metamorphosis Assay (AMA, OECD TG 231 (2009)).
For epoxiconazole, only T3 and T4 concentrations were measured in a single non-internationally agreed protocol study in birds. No alterations in serum concentrations of the measured hormones were observed in comparison to control. However, birds are not the most appropriate non-mammalian species to investigate the T modality.

Scenarios
In the ED Guidance, a decision tree is available and different possible scenarios are proposed depending on the outcome to the different questions presented below.

- **EAS parameters:**

Has EAS-mediated adversity been observed? → **Yes**
Based on the tables assessing and reporting the lines of evidence, and as summarized above, it can be seen that EAS-mediated adversity has been observed for both mammalian and non-mammalian species. Therefore, the scenario 2b applies to EAS modalities (adversity based on "EATS-mediated" parameters, not sufficiently investigated).

- **Thyroid parameters:**

Has T-mediated adversity been observed? → **No**
In the case of thyroid modalities, the thyroid-mediated parameters investigated in the available dataset did not indicate adversity.

Has endocrine activity been observed? → **No**
Although endocrine activity was observed for EAS modalities, as evidenced by *in vivo* mechanistic information, no endocrine activity was reported for thyroid modality.

Has endocrine activity been sufficiently investigated? → **No**
As stated in the ED Guidance, to consider the T modality as sufficiently investigated for mammals, the thyroid parameters foreseen to be investigated in the following studies OECD test guidelines 407, 408, 409 (and/or the one-year dog study, if available), 416 (or 443 if available) and 451-3 should have been measured. For non-target organisms other than mammals, T modality was investigated only in birds. Thus, an Amphibian Metamorphosis Assay (AMA, OECD TG 231 (2009)) should, at least, have been conducted. As explained above, all these parameters have not been investigated for epoxiconazole. Therefore, the scenario 2a(iii) applies to thyroid modality (no adversity observed on "T-mediated" parameters, no endocrine activity observed, but not sufficiently investigated). For this scenario, the

next step of the assessment should be to generate further information on thyroid modality. Therefore, the following sections of this document will focus on EAS-modalities.

4. Mode of action analysis

Postulate MoA(s) considering the adversity and/or endocrine activity identified above
As adverse effects were identified for EAS modalities (scenario 2b), a MoA analysis is required. The first step of the MoA analysis is to postulate MoA(s) covering the observed adverse effects and endocrine activity and to consider if available data are sufficient to support the postulated MoA(s). Then, the second step is to establish the biological plausibility for the link between the adverse effects and endocrine activity for the postulated MoA(s), using the Weight of Evidence approach.

4.1. Mode of action analysis for EAS modalities in mammals

- ***Postulated MoA for EAS modalities in mammals:***

First postulated MoA:

	Brief description of key event (KE)	**Supporting evidence**
Molecular Initiating Event (MIE)	Inhibition of aromatase	Inhibition of aromatase activity demonstrated *in vitro* in rat, human and porcine cells, supported by ToxCast data.
KE1	Decreased estradiol level	Decreased estradiol level in rat.
Adverse effects	Post-implantation losses (late resorptions) Placental damages Prolonged estrus cycles Increased gestation length and dystocia	Adverse effects consistently observed in the available published and unpublished *in vivo* rat studies.

In the available *in vitro* and *in vivo* published or unpublished studies, epoxiconazole was demonstrated to be an aromatase inhibitor. Aromatase (CYP19) converts both testosterone and androstenedione to estradiol and estrone respectively during steroidogenesis. An inhibition of aromatase leads to an increased concentration of androgens and a decreased concentration of estrogens, variations that were observed in the rat and/or the guinea pig treated with epoxiconazole.
A depletion in estradiol may be the cause of, at least, the following adverse effects observed in the available *in vivo* toxicity studies conducted on epoxiconazole: post-implantation losses (late resorptions, associated with decreased number of live births, decreased mean litter size, vaginal bleeding), placental damages (increased weight and placental degeneration), prolonged estrus cycle, as well as increased gestation length and dystocia.
In rats, post-implantation losses consisted of late and very late resorptions and were worsened when the duration of exposure was extended to the end of gestation (higher rate and later stages of resorptions). Post-implantation losses cannot be considered as secondary to nonspecific maternal toxicity since they occurred in some studies in the absence of significant maternal toxicity. Post-implantation losses were also not explained by dystocia as, in the prenatal developmental toxicity studies, dams were sacrificed before parturition. Co-administration with ECP (estradiol cyclopentylpropionate) in rats during pregnancy prevents foetal mortality caused by epoxiconazole. There is thus a correlation between the estradiol reduction and the foetal mortality.
Concerning placental damages, increased placental weight was consistently observed in rats. Histopathological examination of the placenta was performed in one study and revealed placental degeneration of the labyrinth and of the trophospongium. The same pattern of alterations was observed in placentas with live fetuses as in placenta with late resorptions, the effects being more pronounced in the latter. Placental damages were linked to depletion in estradiol as supplementation of ECP tended to reduce the severity of degenerative placental damages induced by epoxiconazole.

Second postulated MoA:

	Brief description of key event (KE)	**Supporting evidence**
Molecular Initiating Event (MIE)	Inhibition of aromatase	Inhibition of aromatase activity demonstrated *in vitro* in rat, human and porcine cells, supported by ToxCast data.
KE1	Decreased estradiol and increased testosterone levels	Decreased estradiol level in rat and increased testosterone level in rat and guinea pig.
KE2	Increased LH and FSH levels by feedback response in the hypothalamic pituitary axis	Increased LH and FSH levels observed in rat.
KE3	Continuous stimulations of ovarian cells	Ovarian cysts observed in rat in the 2 available long-term studies.
Adverse effects	Ovarian tumors	Ovarian theca granulosa cell tumours observed in rat in the carcinogenicity study

Depletion of estradiol resulting from aromatase inhibition could trigger a feedback response in the hypothalamic-pituitary axis, resulting in increased LH and FSH levels. Increased level of these hormones were observed in rats treated with epoxiconazole. This finding could lead to a continuous stimulation of ovarian cells, which ultimately results in ovarian tumours.

Other possible MoAs:

The whole database also suggests that other hormones involved in the steroidogenesis are affected by epoxiconazole. Although not consistent across studies, some data suggests a reduction in the activity of the enzymes 11-hydroxylase or 21-hydroxylase, resulting in a decrease of corticosterone and aldosterone. A decrease in these adrenal enzymes may trigger a feedback response in the hypothalamic-pituitary axis resulting in increased ACTH levels. The continuous stimulation of adrenal cortical cells by ACTH could be responsible for the induction of adrenal gland cortex tumours that were observed in female rats in the carcinogenicity study. This mode of action was proposed during the European peer-review of epoxiconazole in view of its approval (DAR 2005, EFSA 2008) based on the results of a mechanistic study performed in rats. Nevertheless, no consistent changes of these adrenal hormones were observed in the whole database.

Also, CYP17 inhibition was suggested in the DAR of epoxiconazole. CYP17 is responsible for the 17α-hydroxylation of pregnenolone and progesterone, leading to production of dehydroepiandrostenedione and androstenedione respectively. Nevertheless, taking into account the whole database, no consistent changes in the levels of progesterone were observed.

Furthermore, epoxiconazole appeared to be an anti-androgenic compound, as androgen-receptor antagonism was observed *in vitro*. However, no clear adverse effects were observed *in vivo* that could be linked to an anti-androgenic activity.

It should be noted that epoxiconazole also induced malformations in rat, in particular cleft palates. This rare malformation is commonly seen with other triazoles. Induction of cleft palates is not considered secondary to other maternal toxic effects. As exposure to epoxiconazole with co-administration of ECP had no impact on the incidence of cleft palates, it was concluded that cleft palate was independent of estradiol regulation. As concluded by the ECHA-RAC in 2012, the mode of action for the formation of cleft palates has not been identified. The likely mode of action suggested by Menegola *et al.* implies an inhibition of the embryonic CYP450 (CYP26) involved in the regulation of retinoic acid, causing craniofacial dysmorphogenesis. An alternative hypothesis involved blockade of IKr potassium channel via hypoxia and/or reactive oxygen species in embryo resulting in embryonic arrhythmia and hypoxia. These modes of action have not been investigated for epoxiconazole and were suggested based on data on other triazoles. As these modes of action are out of the main scope of the ED Guidance

Document and as epoxiconazole was otherwise shown to meet the ED criteria, no further assessment is considered needed on that point.

As a conclusion, it is very likely that epoxiconazole acts on the endocrine system via multiple mode of actions. In the present analysis, the focus was made on the mode of action for which the most convincing evidence is available, i.e. aromatase inhibition.

- ***Sufficient information to support the postulated MoA(s)? → Yes***

Overall, it is considered that the available information is sufficient to support the postulated modes of action implying an inhibition of aromatase activity.

- ***Establish the biological plausibility for the link between the adverse(s) effect(s) and endocrine activity for the postulated MoA(s)***

According to the ED Guidance Document, "*the assessment should consider whether the key event relationship is consistent with what is known in general (biological plausibility) and also what is known for the substance specifically*".
In this document, the biological plausibility is only assessed for the postulated mode of action linking aromatase inhibition to several adverse effects noted in the female rat (post-implantation losses, placental damages, prolonged estrus cycles, increased gestation length and dystocia).
It is noteworthy that, in the OECD AOP Knowledge Base (http://aopkb.org/), no validated AOP are available to support the postulated MoA in rat.

As stated in the ED Guidance Document, the following endpoints should be assessed:
- Biological plausibility for the key event relationships
- Empirical support for dose-response/incidence concordance and temporal concordance for the key event relationship
- Essentiality, consistency, analogy and specificity of the evidence for the association of the KEs with the adverse effect

The following tables summarize the assessment of the biological plausibility for the first postulated MoA in rat described above (MIE: Aromatase inhibition, KE1: Depletion in estradiol, AEs: post-implantation losses, placental damages, prolonged estrus cycles, increased gestation length and dystocia).

Analysis of dose-response and temporal concordance between the KEs in rats:

Dose (mg/kg bw/d)	KE1	Adverse effects			
	Decreased estradiol level	Post-implantation losses (late resorptions)	Placental damages	Prolonged estrus cycles	Increased gestation length and dystocia
0.9	n.i.	n.i.	n.i.	n.i.	- (2-generation study*)
2.3	n.i.	n.i.	n.i.	n.i.	- (2-generation study*)
3.75	n.i.	n.i.	n.i.	n.i.	- (GD7-PND16)
5	n.i.	n.i.	- (GD6-15)	n.i.	n.i.
15	n.i.	- (GD6-15) - (GD7-21 and GD7-PND16)	++ (GD6-15)	n.i.	+ (GD7-PND16) - (GD7-PND16)
20	n.i.	- (GD6-15)	++ (GD6-15)	n.i.	n.i.
23	-68% (GD7-18) -98% (GD7-21)	- (GD7-18) - (GD7-21)	++ (GD7-18) ++ (GD7-21)	+ (F0 and F1, 2-generation study*) (suggested due to increased precoital	+ (F0 and F1, 2-generation study*)

				interval >4days)	
45	n.i.	+ (GD6-15)	++ (GD6-15)	n.i.	n.i.
50	-42% (GD7-21) -93% (GD7-18) -98% (GD7-21)	+ (GD7-21 and GD7-PND16) + (GD7-21) + (GD7-18) + (GD7-21)	++ (GD7-18) ++ (GD7-21)	n.i.	+ (GD7-PND16)
60	n.i.	++ (GD6-15)	++ (GD6-15)	n.i.	n.i.
65	-63% during dioestrus, -86% during prooestrus (4-day)	n.i.	n.i.	+ (4-day)	n.i.
94	-79% during dioestrus, -51% during prooestrus (4-wk)	n.i.	n.i.	++ (4-wk)	n.i.
160	-86% during dioestrus, -79% during prooestrus (4-wk)	n.i.	n.i.	++ (4-wk)	n.i.
180	-80% (GD6-19)	++ (GD6-15) + (GD6-19)	+ (GD6-19) ++ (GD6-15)	n.i.	n.i.

+ indicates effects only observed at the highest tested dose
++ indicates effects observed in a dose related manner
- indicates no effect
n.i. not investigated
GD: Gestation Day
PND: Post-Natal Day
In brackets: duration of exposure. * For the 2-generation study: F0 exposed at least 10 weeks before mating and throughout gestation and lactation periods; F1 exposed *in utero*, and directly from weaning at least 10 weeks before mating and throughout gestation and lactation periods.

Conclusion on the biological plausibility of the link between the adverse effects and the endocrine activity for the postulated MoA:

KERs		
	MIE to KE1	KE1 to AEs
Biological plausibility for the KERs	STRONG – The link between aromatase inhibition and decreased estradiol level is supported by the available knowledge.	STRONG – A decrease in estradiol level is known to alter female fertility and to induce adverse effects on pregnancy in the female rat.
Empirical support for the KERs	STRONG – Epoxiconazole clearly decreases estradiol levels *in vitro* and *in vivo*. *In vitro*, specific studies demonstrated the inhibition of aromatase activity.	MODERATE – Post-implantation losses and prolonged estrus cycles were observed at the same concentrations or above than estradiol levels decreases. Placental damages and increased gestation length/dystocia were observed at lower concentrations than those decreasing estradiol levels. However, this could be explained by the fact that estradiol levels were not measured at these concentrations. Co-administration with ECP (estradiol cyclopentylpropionate) in rats during pregnancy dose-dependently prevents foetal mortality (post-implantation losses/resorptions) and placental damages caused by epoxiconazole.
	MIE	KE1
Essentiality of KEs	No data	Co-administration with ECP (estradiol cyclopentylpropionate) in rats during pregnancy dose-dependently prevents foetal mortality (post-implantation losses/resorptions) and placental damages caused by epoxiconazole.

Consistency	The KE has been observed consistently in different *in vitro* and *in vivo* studies. The pattern of effects is consistent between studies. Consistency across species has also been noted as it was demonstrated that guinea pigs are also sensitive to aromatase inhibition induced by epoxiconazole, i.e. increased level of estradiol precursors (although no related adverse effects have been observed).	
Analogy	Aromatase inhibition is well established for compounds belonging to the same chemical class, i.e. triazole compounds.	
Specificity	In some studies, decreased estradiol levels and related-adverse effects were observed in dams at dose levels that produced no or slight maternal toxicity.	
Identified uncertainties		
Uncertainty 1: Estradiol level (KE1) - in the available dataset, measurements of estradiol level were only investigated from the dose level of 23 mg/kg bw/d onwards, whereas adverse effects were observed from the dose level of 15 mg/kg bw/d onwards. Linked to uncertainty 2.		
Uncertainty 2: Empirical support for KER – a clear dose-concordance cannot be established between KE1 and placental damages or increased gestation length/dystocia due to the lack of estradiol measurement at lower concentrations (see uncertainty 1).		

Overall conclusion on the postulated MoA
The overall biological plausibility is considered strong and is substantiated by a strong/moderate empirical support.

- Human relevance

As reported by ECHA-RAC (2012), rat, guinea pig and non-human primate and human are sensitive to the general mode of action of aromatase inhibition. After epoxiconazole treatment, hormonal changes related to aromatase inhibition were observed in both rat and guinea pig. Furthermore, another anti-aromatase azole compound, inducing early and late resorptions in rats, has also been demonstrated to be embryotoxic in non-human primate (letrozole when administered to baboons during pregnancy induced miscarriages in 62 % of the females during the first half of pregnancy and in 25 % in the second half; a co-treatment with estrogen fully abolishing the adverse effect). Moreover, aromatase inhibitors are widely used for therapeutic uses for its ability to block estrogen production. In accordance with ECHA-RAC opinion, despite differences between species (e.g. hormonal imbalance, placentation), there are similarities in adverse effects on pregnancy between rodents and primates which, with the common mode of action (aromatase inhibition) seen in all test species, reinforce the relevance to humans.

Furthermore, epidemiological studies with azole antifungal drugs showed increased risk of spontaneous abortion following exposure to fluconazole during pregnancy in the Danish Registry-based cohort[6]. A link between *in utero* exposure to itraconazole and higher spontaneous abortions was also suggested in a cohort study in Canada[7] and in another one in Italy[8].

Therefore, the mode of action of aromatase inhibition should be considered relevant for human.

[6] Ditte Mølgaard-Nielsen D, Henrik Svanström H, Melbye M, et al., 2016. Association Between Use of Oral Fluconazole During Pregnancy and Risk of Spontaneous Abortion and Stillbirth. JAMA. 2016;315(1):58-67.

[7] Bar-Oz, B., Moretti, M.E., Bishai, R., et al., 2000. Pregnancy outcome after in utero exposure to itraconazole: a prospective cohort study. Am. J. Obstet. Gynecol. 183, 617–620.

[8] De Santis, M., Di Giannantonio, E., Cesari, E., et al., 2009. First-trimester Itraconazole exposure and pregnancy outcome. A prospective cohort study of women contacting teratology information services in Italy. Drug Saf. 32, 239–244.

- ***Does the available evidence support the biological plausibility for an endocrine MoA?***
  → Yes

The overall biological plausibility is considered strong and is substantiated by a strong/moderate empirical support. Furthermore, the mode of action of aromatase inhibition should be considered relevant for human. It is therefore concluded that the available evidence supports the biological plausibility for an endocrine MoA.

4.2. Mode of action analysis for EAS modalities in non-mammalian organisms (birds and fish)

Fish

AOP 25: Aromatase inhibition leading to reproductive dysfunction for zebra fish (*Danio rerio)*

	Brief description of key event (KE)	**Supporting evidence**
Molecular Initiating Event (MIE)	Inhibition of aromatase	Some effects of epoxiconazole on aromatase activity were observed*
KE1	Reduction, Plasma 17beta-estradiol concentrations	None
KE2	Reduction, Vitellogenin synthesis in liver	None
KE3	Reduction, Vitellogenin accumulation into oocytes and oocyte growth/development	None
KE4	Reduction, 17beta-estradiol synthesis by ovarian granulosa cells	None
KE5	Reduction, Cumulative fecundity and spawning	Significant reduced number of eggs and fertilization rate.
KE6	Reduction, Plasma vitellogenin concentrations	Reduction of plasma vitellogenin concentration in males and females
Adverse effects	Decrease, Population trajectory	The effect at the highest treatment level (0.768 mg/L) was caused by the retardation of spawning.

* effects are observed in rat, human and porcine cells (see above for further details).

This AOP is included in OECD Work Plan.

Supporting evidence available for the key events and the adverse effect was observed in a study (Schaefers C., 2003). This study was GLP and compliant with an internationally agreed study protocol. Epoxiconazole showed effects on sexual development of sensitive fish species, as indicated by the observed alteration of the sex ratio and vitellogenin concentrations. It is reported in the study report of this full life cycle study with zebra fish (*Danio rerio*) that reduced vitellogenin levels in males are of no relevance, however, in females this can lead to impoverished egg nutrient support, which appeared to be the case in the F2-generation at the highest test concentration (0.768 mg/L). This conclusion was agreed by the EU evaluation. In addition to this biochemical marker, the sex ratio appeared to react sensitively if exposure occurred during sensitive life stages. Indeed fish exposed as pre-adults (juvenile development) showed effects on sex ratio at the highest test concentration (768 µg/L) whereas significant effects on this parameter were observed at 192 µg/L if fish were exposed at younger stages. The exposure of the parental generation appeared to be of less importance for the sex ratio of the filial generation.
Similarly in another study performed with fathead minnow (*Pimephales promelas*), the sex ratio appeared to be a sensitive parameter next to growth inhibition of juvenile fish. However, it has to be noticed that there are some uncertainties on the effects on sex ratio observed for fathead minnow. Indeed, there were unusual sex ratios in the control and it is considered that a reliable evaluation of effects on sex ratio is regarded as doubtful for this species.
Because of its specific development of the male gonads from protogyn gonads, zebra fish (*Danio rerio*) can be regarded as particularly sensitive to aromatase inhibition: surpassing a threshold ratio of

testosterone/17b-estradiol seems to be the pathway for transforming protogyn to male gonads. The fathead minnow, although sensitively reacting with respect to growth inhibition, appeared to be less susceptible for endocrine effects caused here by the specific activity of epoxiconazole on aromatase.
In conclusion, epoxicgonazole affects the sexual development of sensitive fish species in the full life cycle study with sediment under static conditions. Indeed, population-relevant effects were observed for concentrations higher than 48 µg/L on sex ratio and higher than 12 µg/L on female vitellogenin. Moreover, at the highest tested concentration (768 µg/L) significant reduction of the number of eggs and fertilization rate, which could be due the retardation of spawning (day of first spawn significantly delayed) were observed and these effects can be considered at potentially adverse for the population of fish.

Birds

AOP 29: Estrogen receptor agonism leading to reproductive dysfunction for Japanese quail (*Coturnix coturnix japonica*)

AOP 29	**Brief description of key event (KE)**	**Supporting evidence**
Molecular Initiating Event (MIE)	Agonism, Estrogen receptor	None
KE1	Reduction, Cumulative fecundity and spawning	None
KE2	Increase, Plasma vitellogenin concentrations	None
KE3	Increase, Vitellogenin synthesis in liver	None
KE4	Increase, Renal pathology due to VTG deposition	None
Adverse effects	Impaired development of, Reproductive organs	Reduced number of testicular canaliculi with visible germ cells

This AOP is still under development but some elements of the overall assessment of the AOP are proposed by the authors of this AOP.

In Japanese quail (*Coturnix coturnix japonica*), supporting evidence available for the adverse effect was observed in 2 tests, one (Chahoud *et al.*, 2004) was not published but was referenced in the original Draft Assessment report of epoxiconazole and the other is a literature data (Konstanze Grote *et al.*, 2008). Both tests are linked since the unpublished test was the dose range finding test and the published study was the definitive test. None of these studies were performed according to Good Laboratory Practices but both were compliant with an internationally agreed study protocol (OECD Guideline 206). Decreased activity of spermatogenesis and/or a reduction of the number of germ cells and spermatids present in testicular tubules were observed in both studies at the same tested dose (50 ppm). In addition reduced number of testicular canaliculi with visible germ cells was observed in the definitive test. These effects, which could be linked to an endocrine disruption of aromatase do not impact the reproductive performance in the study and it is not possible to determine if this could be adverse at the population level. It has also to be noticed that a dose dependent transfer of epoxiconazole into eggs was observed at 50 and 500 ppm without detrimental effects on chicks at the observation termination (14 d after hatchling), which is the normal time to stop observation to be compliant with the OECD guideline 206. However, it would have been interesting to extend the observation period up to the sexual maturation of birds to determine if this exposure at egg stages would have affected the normal development of birds.

Another published data is available. This study (Yunhui Li *et al.*, 2016) was not performed according to Good Laboratory Practices, was not compliant with an internationally agreed study protocol and was not performed on a vertebrate species but on a nematode (*Caenorhabditis elegans*). However, effects similar to the ones observed on spermatogenesis in birds were observed in nematodes. Moreover, further parameters were investigated in this publication. Based on these additional results it is possible to indicate that epoxiconazole exposure can result in abnormalities of germ cell development and reduced the number of meiotic cells. Such effects suggest that epoxiconazole may affect the activity of

CYP450 and may inhibit steroidogenic processes such as meiosis-activating sterols. However, it is reported in the publication that the specific sites of action still need further study. It was also reported that epoxiconazole exposure induced noticeable alterations in the size of sperm, suggesting that epoxiconazole may have effects on the process of spermatid budding. Sperm size is associated with sperm competition as larger sperm outcompete smaller sperm prior to fertilization in the nematode *C. elegans* (smaller sperm swimming slower than larger sperm), suggesting that epoxiconazole may cause a decline in sperm competitiveness and consequently in male fertility. Moreover, epoxiconazole exposure can inhibit sperm activation and induced effects on the morphogenesis of sperm pseudopodia (increase in abnormal sperm pseudopodia). All these effects can be linked to an endocrine disruption mode of action and could be considered as population relevant as a significant reduced number of progeny was observed in combination of the above related effects.
It is acknowledged that these effects were observed in an invertebrate study while the analysis should focus on vertebrate but in this case since the effects were similar to those observed in birds, they were considered as supportive evidence to conclude on the endocrine disruptive properties of epoxiconazole.

AOP 25: Aromatase inhibition leading to reproductive dysfunction for Bobwhite quail (*Colinus virginianus*)

	Brief description of key event (KE)	**Supporting evidence**
Molecular Initiating Event (MIE)	Inhibition of aromatase	Some effects of epoxiconazole on aromatase activity were observed*
KE1	Reduction, Plasma 17beta-estradiol concentrations	None
KE2	Reduction, Vitellogenin synthesis in liver	None
KE3	Reduction, Vitellogenin accumulation into oocytes and oocyte growth/development	None
KE4	Reduction, 17beta-estradiol synthesis by ovarian granulosa cells	None
KE5	Reduction, Cumulative fecundity and spawning	
KE6	Reduction, Plasma vitellogenin concentrations	None
Adverse effects	Decrease, Population trajectory	Reduction of the fertility rate (reduced number of hatched chicks per female and 14-day old surviving chicks per female).

* effects are observed in rat, human and porcine cells (see above for further details).

This AOP is included in OECD Work Plan.

In Bobwhite quail (*Colinus virginianus*), supporting evidence available for the adverse effect was observed. This study was compliant with an internationally agreed study protocols.
A reduced but not statistically significant reduction of the fertility rate could be observed, resulting in a reduced number of hatched chicks per female quail and a reduced number of 14-day-old surviving chicks per female quail at the two highest tested doses (50 and 500 ppm). Additionally, at the highest tested dose, compound led to a reduced hatched chick's body weight at day 14. In the lowest tested dose (10 ppm corresponding to 1.0 mg a.s./kg bw/d) no compound related effects were detected. The observed parameters in this study are not sufficient to investigate if the reduction of the chick number are due to an endocrine disruption mode of action. However, it should be considered in parallel with the effects on spermatogenesis observed on the Japanese quail.

5. Overall conclusion on the ED criteria

Epoxiconazole was classified Repr 1B H360Df (May damage the unborn child. Suspected of damaging fertility) and Carc 2 H351 (Suspected of causing cancer) according to Regulation (EU) No 944/2013 amending Regulation (EC) No 1272/2008. This classification was based to some extent on adverse effects which could be related to an endocrine activity. The EFSA/ECHA Guidance for the identification of endocrine disruptors, published in June 2018, was used to assess the endocrine disrupting potential of epoxiconazole.
The majority of the scientific data used to conduct the assessment were peer-reviewed at European level (EFSA and/or ECHA RAC). Furthermore, new literature data have been included in the overall weight of evidence. It is noteworthy that these new data did not identify new adverse effects/endocrine activity compared to those discussed at European level, but confirmed some of them. Moreover, it is important to note that the agreed toxicological reference values are not challenged by these new data.

Following application of the ED Guidance, several modes of action for either mammals, non-mammalian organisms as well as for invertebrates, were postulated for epoxiconazole. It is considered that, for the studied modes of action, sufficient data are available and that, following the weight of evidence analysis, there is sufficient evidence to establish the biological plausibility of the link between the endocrine activity and the observed adverse effects.
Therefore, based on the ED Guidance, epoxiconazole meets the ED criteria for humans and for non-target organisms.

Due to time constraints, the application of the ED Guidance was somewhat simplified compared to what is requested in this document, and some limitations can be highlighted, e.g.:

- the literature search allowing to add recently published data was not performed according to EFSA Guidance on systematic review;
- the Excel table aiming at reporting all parameters useful for the ED assessment was not produced;
- only the positive effects (and not the negative results) were generally recorded in the tables assembling the lines of evidence;
- the biological plausibility analysis was only done for some of the postulated MoA.

Therefore, the focus was made on relevant effects and modes of action for which the most convincing evidence was available. Nevertheless, as epoxiconazole was shown to meet the ED criteria based on this analysis, no further assessment was considered needed.

References

Allemagne, **2005** Epoxiconazole, Draft Assessment Report and Proposed Decision of Germany prepared in the context of the possible inclusion of epoxiconazole in Annex I of Council Directive 91/414/EEC, 18 April 2005.

Allemagne, **2008** Epoxiconazole, Final Addendum to the Draft Assessment Report, Initial risk assessment provided by the Rapporteur Member State Germany for the existing active susbtsance epoxiconazole of the third stage Part A of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC, February 2008.

Christen V, Crettaz P, Fent K. **2014** Additive and synergistic antiandrogenic activities of mixtures of azol fungicides and vinclozolin. *Toxicology and Applied Pharmacology* 279:455-466.

ECHA Committee for risk assessment, **2010** Background document to the opinion of the committee for risk assessment on a proposal for harmonized classification and labelling of epoxiconazole, Annex XV dossier, 17 March 2010.

EFSA Scientific Report, **2008** Conclusion regarding the peer review of the pesticide risk assessment epoxiconazole. *EFSA Journal*, 138, 1-80. http://www.efsa.europa.eu/fr/efsajournal/doc/138r.pdf

Hass U *et al.* **2012** Adverse effects on sexual development in rat offspring after low dose exposure to a mixture of endocrine disrupting pesticides. *Reproductive Toxicology* 34:261-274.

Jacobsen P.R *et al.* **2012** Persistent developmental toxicity in rat offspring after low dose exposure to a mixture of endocrine disrupting pesticides. *Reproductive Toxicology* 34:237-250.

Kjærstad M. B. *et al.* **2010** Endocrine disrupting effects *in vitro* of conazole antifungals used as pesticides and pharmaceuticals. *Reproductive Toxicology* 30:573-582.

Konstanze Grote, Lars Niemann, Britta Selzsam, Wolfgang Haider, Christine Gericke, Matthias Herzler and Ibrahim Chahoud, **2008**, Epoxiconazole causes changes in testicular histology and sperm production in the Japanese quail (*Coturnix Coturnix Japonica*), Environmental Toxicology and Chemistry, Vol. 27, No. 11, pp. 2368–2374, 2008.

RAC (Committee for risk assessment) opinion, **2010** Opinion of the committee for risk assessment on a dossier proposing harmonised classification and labelling at community level, epoxiconazole, CLH-O-0000000630-85-05/F, 17 March 2010

RAC (Committee for risk assessment) opinion, **2011** Opinion on certain scientific study plans in relation to epoxiconazole, ECHA/RAC/A77-O-0000001412-86-02/F, 11 March 2011

RAC (Committee for risk assessment) opinion, **2012** Opinion proposing harmonised classification and labelling at EU level on toxicity on toxicity to reproduction of epoxiconazole, ECHA/RAC/A77-O-0000001412-86-08/F, 28 November 2012.

Rey Moreno M.C *et al.* **2013** Epoxiconazole-induced degeneration in rat placenta and the effects of estradiol supplementation. *Birth Defects Research (Part B)* 98:208-221.

Rieke S *et al.* **2014** Combination effects of (tri)azole fungicides on hormone production and xenobiotic metabolism in a human placental cell line. *Int. J. Environ. Res. Public Health* 11:9660-9679

Rieke S *et al.* **2017** Mixture effects of azole fungicides on the adrenal gland in a broad dose range. *Toxicology* 385:28-37.

Schneider S. *et al.* **2013** Species differences in developmental toxicity of epoxiconazole and its relevance to humans. *Birth Defects Research (Part B)* 98:230-246.

Stinchcombe S. *et al.* **2013** Effects of estrogen coadministration on epoxiconazole toxicity in rats. *Birth Defects Research (Part B)* 98:247-259.

Yunhui Li, Minhui Zhang, Shaojun Li, Rongrong Lv, Pan Chen, Ran Liu, Geyu Liang and Lihong Yin, **2016**, The Use of the Nematode *Caenorhabditis elegans* to Evaluate the Adverse Effects of Epoxiconazole Exposure on Spermatogenesis, Int. J. Environ. Res. Public Health, 13, 993 (doi:10.3390/ijerph13100993).

Appendix: Tables assembling, integrated and assessing the lines of evidence for epoxiconazole

Table 1: Lines of evidence for EAS disruption (mammals)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
Evidence of endocrine activity	In vitro mechanistic	Aromatase	Rat, human and porcine cells		Uptake from the medium		0.01 µmol/L (porcine), 0.1 µmol/L (rat), 1 µmol/L (human)	Inhibition of aromatase activity (decreased oestradiol production using androstenedione as a substrate) Relative sensitivity: human granulosa cells (30% inhibition at 1 µM) < rat granulosa cells (70% inhibition at 0.1 µM) < porcine luteal cells (50% inhibition at 0.01 µM)		Not GLP, not guideline Unpublished study 93.2% purity Results presented by bar graphs (no figures) Not known whether cytotoxicity was measured or not	**Inhibition of aromatase activity demonstrated *in vitro* in rat, human and porcine cells**	S	DAR (Wuttke 1995 TOX2003-1861)
			Rat and human cells		Uptake from the medium		10^{-7} M (rat), 10^{-4} M (human)	Inhibition of the aromatase activity (release of estradiol, in the presence of aromatase substrate 4-androstenedione, determined directly from the culture medium using immunological methods) Relative sensitivity: human granulosa cells < rat granulosa cells		Not GLP, not guideline Unpublished study Purity/batch not specified Cytotoxicity measured		S	DAR (Wuttke 2001 TOX2003-1863)
			Human breast cancer MCF-7 cells		Uptake from the medium		IC50 = 20 µM	Inhibition of testosterone-induced MCF-7 cell proliferation response (aromatase inhibition)		Not GLP, not guideline Published study 99.0% purity Cytotoxicity measured		S	CLH (Kjaerstad 2007) and Kjaerstad 2010
			ToxCast					Active in TOX21_Aromatase_Inhibition (AC50 = 3.8 µM) and NVS_ADME_hCYP19A1 (AC50 = 1.67 µM) Inactive in NVS_ADME_hCYP19A1_Activ	Cytotoxicity limit = 161.93 µM			S	ToxCast data

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
		Steroidogenesis	Rat and porcine cells		Uptake from the medium	1 µmol/L	10 µmol/L	Inhibition of progesterone production in porcine luteal cells at 10 µmol/L, no effect in rat adrenal cells up to 1 µmol/L		Not GLP, not guideline Unpublished study 93.2% purity Results presented by bar graphs (no figures) Not known whether cytotoxicity was measured or not	**Inhibition of steroidogenesis in H295R cell line** Decreased progesterone secretion in human placental cell line and porcine luteal cells but not supported by *in vivo* mechanistic data	S	DAR (Wuttke 1995 TOX2003-1861)
			Rat and porcine cells		Uptake from the medium	0.1 µmol/L	1 µmol/L	Inhibition of cortisol production in porcine adrenal cells at 1 µmol/L, no effect on corticosterone in rat adrenal cells or on prolactin in rat pituitary cells up to 1 µmoL/L		Not GLP, not guideline Unpublished study 93.2% purity Results presented by bar graphs (no figures) Not known whether cytotoxicity was measured or not		S	DAR (Wuttke 1995 TOX2003-1861)
			Human adrenocortical carcinoma cell line H295R		Uptake from the medium		E2: IC50 = 0.2 µM T: IC50 = 5.3 µM	Inhibition of estradiol and testosterone production		Not GLP, not guideline Published study 99.0% purity Cytotoxicity measured		S	CLH (Kjaerstad 2007) and Kjaerstad 2010
			Human placental cell line Jeg-3				10 µM	Decreased progesterone secretion (36%, SS) No change in the estradiol concentration up to 40 µM EPX No steroid biosynthesis dependent gene expression changes up to 40 µM Moderate induction of CYP1A1 with 30 µM (approx 10-fold, SS), no AhR activation but AhR dependent (increased CYP1A1 inhibited by an AhR specific inhibitor)		Not GLP, not guideline Published study Purity: technical grade, purchased from BASF, Lot 5154X Cytotoxicity measured		S	Rieke 2014
			Human adrenocortical carcinoma cell line H295R		Uptake from the medium		?	Reduced testosterone and estradiol levels in the cells Increased progesterone levels		Not GLP, not guideline Published study 99.0% purity Cytotoxicity measured Steroid synthesis assay: no numerical value, only graphs		S	Hass 2012

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
			ToxCast - Human adrenocortical carcinoma cell line H295R				AC50 =1.85 µM =3.59 µM =7.66 µM =4.12 µM =1.38µM =1.89µM =1.28 µM =1.86 µM	Decreased concentrations of: 17α-hydroxyprogesterone Cortisol 11-deoxycortisol Androstenedione Testosterone Oestrone Oestradiol Increased concentrations of: 11-deoxycorticosterone No changes in concentration of: Progesterone				S	ToxCast data
		Estrogen receptor binding/transactivation	Human breast cancer MCF-7 cells		Uptake from the medium		IC50 = 52 µM	Inhibition of 17β-estradiol induced MCF-7 cell proliferation response (anti-estrogenic effect) + Induction of cell proliferation without E2 (6.25-75µM) (weak estrogenic activity)		Not GLP, not guideline Published study 99.0% purity Cytotoxicity measured	Anti-estrogenic and weak estrogenic activities in MCF-7 cells but inactive in ER ToxCast model	E	CLH (Kjaerstad 2007) and Kjaerstad 2010
			ToxCast					ER model: inactive (agonist: 0; antagonist: 0.0372)				E	ToxCast data
		Androgen receptor binding/transactivation	AR-transfected CHO cells		Uptake from the medium		IC50 = 10 µM	AR[9] antagonism in the AR reporter gene assay (Inhibition of the effect of the synthetic androgen R1881)		Not GLP, not guideline Published study 99.0% purity Cytotoxicity measured	**AR antagonism in CHO and MDA-kb2 cells, as well as in a ToxCast bioassay**	A	CLH (Kjaerstad 2007) and Kjaerstad 2010
			AR-transfected CHO cells		Uptake from the medium		LOEC = 1.9 µM	AR antagonism in the AR reporter gene assay		Not GLP, not guideline Published study 99.0% purity Cytotoxicity measured		A	Hass 2012
			MDA-kb2 cells transformed with murine mammalian tumor virus		Uptake from the medium		EC10 = 2.57 µM; EC25 = 2.88 µM; EC50 = 5.15 µM	AR antagonism in the AR reporter gene assay (89% inhibition of the DHT response)		Not GLP, not guideline Published study Unknown purity Cytotoxicity measured		A	Christen 2014

[9] AR: Androgen Receptor

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
			ToxCast					Active in 2 assays: OT_AR_ARSRC1_0960 (AC50=60.26 µM) and Tox21_AR_LUC_MDAKB2_Antagonist (AC50=58.88 µM) Inactive in 7 assays Not tested in 2 assays	Cytotoxicity limit = 161.93 µM			A	ToxCast data
	In vivo mechanistic	Estradiol level	Rat	GD6-19	Oral	<180	180	Decreased estradiol level (-80% vs control) As well as decreased prolactin level (-44/68%)	Dams: At 180 mkd: decreased FC, BW, BWG, corrected net BWG, blood in bedding/vaginal haemorrhages, decreased RBC/platelets, clinical chemistry parameters	GLP, OECD 414 Only 1 dose level; 2 groups with 2 different batches (purity 94.7%, purity 99.8%)	**Decreased estradiol level in female rat**	EAS	DAR (Schneider 2002 TOX2002-2288)
			Rat	GD7-GD21	Oral	<50	50	Decreased estradiol level (-42% vs control)	Dams: At 50 mkd: no effect	Not GLP, no guideline Published study 99% purity Only 1 dose level Individual data not available		EAS	CLH (Taxvig 2008)
			Rat	4-6 days or 4 weeks	Oral	<65	65	Decreased estradiol level in F + decreased prolactin level during prooestrus In M: NSS trend in decreased estradiol level + at the highest dose level (166 mkd) during 4wk: decreased prolactin level	Gavage at 200 mkd for 4-6 days: BW loss (M/F), reduced general state of health (F) Diet at 1500 and 3000 ppm for 4-6 days or 4 weeks (equiv to min. 65 and 70 mkd): reduced FC (M/F)	Not GLP, no guideline Unpublished study Purity/batch not specified Individual data not available for BW and hormone determinations. No data on analytical methods used for hormone determinations. Supplementary study		EAS	DAR (Mellert 1992 TOX2003-1857 Mellert, Hildebrand 1999 TOX2003-1858)
			Rat	GD7-18 and GD7-21	Oral	<23	23	Decreased estradiol level (dose-dependent at GD18, close to 100% at GD21), increased when ECP (synthetic estrogen) is co-administered (dose-dependent)	Dams: At 23 and 50 mkd: decreased FC, decreased carcass weight and corrected net weight gain, decreased platelet count	GLP (except for analysis of hormone levels), followed the general principles of OECD 414 Published studies (BASF) 97% purity Only 2 dose levels		EAS	Rey Moreno 2013 Stinchcombe 2013

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
		Luteinising hormone (LH) level	Rat	GD6-19	Oral	<180	180	Increased LH level (+48% (SS) with batch #1, +34% (NSS) with batch #2)	Dams: At 180 mkd: decreased FC, BW, BWG, corrected net BWG, blood in bedding/vaginal haemorrhages, decreased RBC/platelets, clinical chemistry parameters	GLP, OECD 414 Only 1 dose level; 2 groups with 2 different batches (purity 94.7%, purity 99.8%)	**Increased LH level in female rat**	EAS	DAR (Schneider 2002 TOX2002-2288)
			Rat	4-6 days or 4 weeks	Oral	<65	65	Increased LH level in F; especially during dioestrus	Gavage at 200 mkd for 4-6 days: BW loss (M/F), reduced general state of health (F) Diet at 1500 and 3000 ppm for 4-6 days or 4 weeks (equiv to min. 65 and 70 mkd): reduced FC (M/F)	Not GLP, no guideline Unpublished study Purity/batch not specified Individual data not available for BW and hormone determinations. No data on analytical methods used for hormone determinations. Supplementary study		EAS	DAR (Mellert 1992 TOX2003-1857 Mellert, Hildebrand 1999 TOX2003-1858)
		Follicle stimulating hormone (FSH) level	Rat	4-6 days or 4 weeks	Oral	<65	65	Increased FSH in F; especially during dioestrus Increased FSH in M	Gavage at 200 mkd for 4-6 days: BW loss (M/F), reduced general state of health (F) Diet at 1500 and 3000 ppm for 4-6 days or 4 weeks (equiv to min. 65 and 70 mkd): reduced FC (M/F)	Not GLP, no guideline Unpublished study Purity/batch not specified Individual data not available for BW and hormone determinations. No data on analytical methods used for hormone determinations. Supplementary study	**Increased FSH level in male and female rat**	EAS	DAR (Mellert 1992 TOX2003-1857 Mellert, Hildebrand 1999 TOX2003-1858)
		Testosterone level	Rat	GD7-GD21 and GD7-PND16 (31 days)	Oral	15	50	Increased testosterone level; in dams at GD21 (+105% vs control; at 15: +22%, NSS)	Dams: At 15 and 50 mkd: tendency toward a decreased BWG GD4-PND1 (but not GD7-21) and PND1-13	Not GLP, no guideline Published study 99% purity Only 2 dose levels Low number of litters (9 in the 15 mkd group, 1-2 in the 15 mkd group) Individual data not available	**Increased testosterone level in female rat and guinea pig**	EAS	CLH (Taxvig 2007)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
			Rat	GD7-GD21	Oral	<50	50	Increased testosterone level; in dams at GD21 (+160% vs control)	Dams: At 50 mkd: no effect	Not GLP, no guideline Published study 99% purity Only 1 dose level Individual data not available		EAS	CLH (Taxvig 2008)
			Rat	4-6 days or 4 weeks	Oral	<80	80	Increased testosterone level; in males (4-day exposure: +100% vs control, SS; 4-week exposure: +24% vs control, NSS)	Gavage at 200 mkd for 4-6 days: BW loss (M/F), reduced general state of health (F) Diet at 1500 and 3000 ppm for 4-6 days or 4 weeks (equiv to min. 65 and 70 mkd): reduced FC (M/F)	Not GLP, no guideline Unpublished study Purity/batch not specified Individual data not available for BW and hormone determinations. No data on analytical methods used for hormone determinations. Supplementary study		EAS	DAR (Mellert 1992 TOX2003-1857 Mellert, Hildebrand 1999 TOX2003-1858)
			Guinea pig	GD6-63	Oral	<15	15	Increased testosterone at 15, 50 and 90 mkd	Dams: At 90 mkd: mild anemia	GLP, Modified prenatal study followed OECD 414 Published study 97% purity		EAS	Schneider 2013
			Rat	GD7-18 and GD7-21	Oral	<23	23	Trends towards increased testosterone at GD18 only	Dams: At 23 and 50 mkd: decreased FC, decreased carcass weight and corrected net weight gain, decreased platelet count	GLP (except for analysis of hormone levels), followed the general principles of OECD 414 Published studies (BASF) 97% purity Only 2 dose levels		EAS	Rey Moreno 2013 Stinchcombe 2013
		Steroidogenesis (genes/enzyme changes)	Rat	GD6-19	Oral	<180	180	Decreased progesterone levels (-46/56%)	Dams: At 180 mkd: decreased FC, BW, BWG, corrected net BWG, blood in bedding/vaginal haemorrhages, decreased RBC/platelets, clinical chemistry parameters	GLP, OECD 414 Only 1 dose level; 2 groups with 2 different batches (purity 94.7%, purity 99.8%)	Progesterone: effect not consistent across studies (decreased or increased or no change) **Androgens: increased level (androstenedione**	EAS	DAR (Schneider 2002 TOX2002-2288)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
			Rat	4-6 days or 4 weeks	Oral	<65	65	**In F:** increased androgen (androstenedione, dehydroepiandrosterone) levels, decreased aldosterone level during prooestrus + at the high dose level (160 mkd) during 4wk: decreased corticosterone levels during prooestrus and increased ACTH (oestrus and prooestrus) **In M**: increased androgen levels + at the highest dose level (166 mkd) during 4wk: decreased corticosterone level and increased ACTH *Hypothesis: decrease of the adrenal enzyme activity of either 11- or 21-hydroxylase leading to decreased corticosterone/aldosterone production and then increased ACTH by feedback response in the HP axis*	Gavage at 200 mkd for 4-6 days: BW loss (M/F), reduced general state of health (F) Diet at 1500 and 3000 ppm for 4-6 days or 4 weeks (equiv to min. 65 and 70 mkd): reduced FC (M/F)	Not GLP, no guideline Unpublished study Purity/batch not specified Individual data not available for BW and hormone determinations. No data on analytical methods used for hormone determinations. Supplementary study	**+/- dehydroepiandro-sterone) in female rat and guinea pig** Other steroid hormones: non-consistent changes (corticosterone: increased or decreased, aldosterone: decreased or no change whereas expression of Cyp11b2 was increased, ACTH: increased or no change)	EAS	DAR (Mellert 1992 TOX2003-1857 Mellert, Hildebrand 1999 TOX2003-1858)
			Rat	GD7-GD21 and GD7-PND16 (31 days)	Oral	15	50	Increased progesterone level; in dams at GD21 (+630% vs control; at 15: +250%, NSS)	Dams: At 15 and 50 mkd: tendency toward a decreased BWG GD4-PND1 (but not GD7-21) and PND1-13	Not GLP, no guideline Published study 99% purity Only 2 dose levels Low number of litters (9 in the 15 mkd group, 1-2 in the 15 mkd group) Individual data not available		EAS	CLH (Taxvig 2007)
			Rat	GD7-GD21	Oral	<50	50	No significant effect on progesterone level (+84% vs control; NSS)	Dams: At 50 mkd: no effect	Not GLP, no guideline Published study 99% purity Only 1 dose level Individual data not available		EAS	CLH (Taxvig 2008)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
			Rat	GD7-18 and GD7-21	Oral	<23	23	Trends (without clear dose-dependence) towards decreased progesterone at GD18 and increased progesterone at GD21, and increased androstenedione (ECP (synthetic estrogen)-dependent)	Dams: At 23 and 50 mkd: decreased FC, decreased carcass weight and corrected net weight gain, decreased platelet count	GLP (except for analysis of hormone levels), followed the general principles of OECD 414 Published studies (BASF) 97% purity Only 2 dose levels		EAS	Rey Moreno 2013 Stinchcombe 2013
			Rat	28-day	Oral	5.64	61.79	Increased corticosterone concentration (pronounced (+161%) but not SS) No changes in other steroid hormone concentrations (ACTH, progesterone, aldosterone) Increased expression of Cyp11b2 (aldosterone synthase, converts corticosterone to aldosterone) (2-fold, SS)	Decreased food intake during the first two days Increased circulating cholesterol (+56%) Changes in the expression of genes linked to cholesterol uptake, storage and metabolism (increased expression level of ApoE and Ldlr, reduced expression level of Ces1e, Ehd1, Plin2, Gpc1 and Egr1)	Not GLP, no guideline but study design and parameters analysed stated to be in accordance with OECD 407, except that some organs were excluded (scope = adrenal gland). Published study 97% purity Individual data not available Only in males, 5/grp Correspondence ppm-mkd in Schmidt 2016		EAS	Rieke 2017
			Guinea pig	GD6-63	Oral	<15	15	Increased androstenedione, testosterone and 11-deoxycortisol at 15, 50 and 90 mkd Increased 18-hydroxycorticosterone, corticosterone at the highest dose of 90 mkd only	Dams: At 90 mkd: mild anemia	GLP, Modified prenatal study followed OECD 414 Published study 97% purity		EAS	Schneider 2013
			Guinea pig	GD6-PND21	Oral	15	50	Increased 21-hydroxyprogesterone, as well as progesterone from 50 mkd onwards	Dams: At 90 mkd: slight decreased BWG and FC during gestation	GLP, Pre and post-natal study followed ICH S5A but terminated 3wks after weaning Published study 97% purity		EAS	Schneider 2013

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
Evidence of adversity	EATS-mediated parameter	Ovary histopathology	Rat	104-wk	Oral	9	44	Ovarian cysts; increase at 44 and 89 mkd (incidences of 9, 7, 12, 17**, 18** at 0, 2, 9, 44, 89 mkd)	In females: At 9 mkd: reduced triglycerides (NOAEL = 2 mkd) At 44 mkd: liver (clinical chemistry, hepatocellular hypertrophy), reduced RBC parameters At 89 mkd: reduced FC, BWG, liver (increased W)	GLP, OECD 452	**Ovarian cysts in rat in the 2 long-term studies**	EAS	DAR (Mellert, Hildebrand 1992e TOX2003-1844
			Rat	104-wk	Oral	2	8	Ovarian cysts; increase at 8, 45 and 90 mkd (incidences of 15/49, 18/50, 25*/50, 40**/50, 41**/50 at 0, 2, 8, 45, 90 mkd)	In females: At 8 mkd: ovarian cysts (NOAEL = 2 mkd) At 45 mkd: reduced FC, BWG, liver (increase rel. W) At 90 mkd: liver (increase W, hepatocellular hypertrophy), adrenal gland cortex tumours	GLP, OECD 451		EAS	Mellert, Hildebrand 1992f TOX2003-1845)
		Anogenital distance	Rat	GD7-GD21 and GD7-PND16 (31 days)	Oral	<15	15	Increased anogenital distance (AGD and AGD per cubic root body weight); in female foetuses GD21 at 15 and 50 mkd (same magnitude at the 2 dose levels) and in female pups PND13 at 15 mkd (measurement available for 1 female only at 50 mkd) In males: indications of increased AGD but not consistent across fetuses and pups or across the AGD and AGIndex	Dams: At 15 and 50 mkd: tendency toward a decreased BWG GD4-PND1 (but not GD7-21) and PND1-13	Not GLP, no guideline Published study 99% purity Only 2 dose levels Low number of litters (9 in the 15 mkd groups, 1-2 in the 50 mkd group sacrificed on PND16, 14 in the 50 mkd group sacrificed on GD21) Individual data not available	Increased anogenital distance in male and female fetuses/pups but not reproducible and/or not dose-related + uncertainties related to the low number of animals included in the studies	EAS	CLH (Taxvig 2007)
			Rat	GD7-GD21	Oral	50	>50	No significant effect	Dams: At 50 mkd: no effect	Not GLP, no guideline Published study 99% purity Only 1 dose level Individual data not available		EAS	CLH (Taxvig 2008)
			Rat	GD7-21 and from the day after birth to Pup Day 16	Oral	<3.75	3.75	Increased anogenital distance in male and female pups at birth (AGD and AGD per cubic root body weight); at 3.75 mkd (SS); but no effect at 15 mkd *Hypothesis by the study author: random finding at the low dose or alternatively, the lack of effect at the high dose may be due to a limited group size*	Dams and pups: no effect	Not GLP, no guideline Published study 99% purity Only 2 dose levels Low number of litters (8 in the 3.75 mkd group, 4 in the 15 mkd group) Individual data not available		EAS	Hass 2012

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
		Estrus cyclicity	Rat	4-6 days or 4 weeks	Oral	<65	65	Prolonged estrus cycle (not atrributed to any particular stage of the oestrus cycle as most females showed an irregular pattern); females treated for 4-6 days (65 and 70 mkd via diet; 200 mkd gavage) or 4 weeks (94 and 160 mkd via diet)	Gavage at 200 mkd for 4-6 days: BW loss (M/F), reduced general state of health (F) Diet at 1500 and 3000 ppm for 4-6 days or 4 weeks (equiv to min. 65 and 70 mkd): reduced FC (M/F)	Not GLP, no guideline Unpublished study Purity/batch not specified Individual data not available for BW and hormone determinations. No data on analytical methods used for hormone determinations. Supplementary study	**Prolonged estrus cycle observed in a study considered as supplementary in the DAR, not examined in the 2-generation study. Associated with decreased levels of relevant steroid hormones (DAR).**	EAS	DAR (Mellert 1992 TOX2003-1857 Mellert, Hildebrand 1999 TOX2003-1858)
		Prostate weight	Rat	GD7-21 and from the day after birth to Pup Day 16	Oral	3.75	15	Increased prostate weight in male offspring PND16 at the highest dose level (+41% compared to controls)*(n=6 at 3.75 mkd, n=3 at 15 mkd)*	Dams and pups: no effect	Not GLP, no guideline Published study 99% purity Only 2 dose levels Low number of litters (8 in the 3.75 mkd group, 4 in the 15 mkd group) Individual data not available	Increased prostate and epididymis absolute weight in male offspring in the absence of histopathological findings, uncertainties related to the low number of animals.	EAS	Jacobsen 2012
		Epididymis weight	Rat	GD7-21 and from the day after birth to Pup Day 16	Oral	3.75	15	Increased epididymis weight in male offspring PND16 at the highest dose level (+18% compared to controls) *(n=6 at 3.75 mkd, n=3 at 15 mkd)*	Dams and pups: no effect	Not GLP, no guideline Published study 99% purity Only 2 dose levels Low number of litters (8 in the 3.75 mkd group, 4 in the 15 mkd group) Individual data not available		EAS	Jacobsen 2012
	Parameter sensitive to, but not diagnostic of, EATS	Tumours type	Rat	104-wk	Oral	8	45	Ovarian theca granulosa cell tumours; increase at 45 and 90 mkd (incidences of 2/49, 4/50, 2/50, 10*/50, 13*/50 at 0, 2, 8, 45, 90 mkd)	In females: At 8 mkd: ovarian cysts (NOAEL = 2 mkd) At 45 mkd: reduced FC, BWG, liver (increase rel. W) At 90 mkd: liver (increase W, hepatocellular hypertrophy), adrenal gland cortex tumours	GLP, OECD 451	**Ovarian theca granulosa cell tumours and adrenal gland cortex tumours in the female rat, liver tumours in the male and female mouse.** **Decreased incidences of neoplasms in the testes (Leydig cell tumours), adrenal gland medulla**	N	DAR (Mellert, Hildebrand 1992f TOX2003-1845)
			Rat	104-wk	Oral	45	90	Adrenal gland cortex tumours, increase at the highest dose in F (adenoma 3/50, 1/50, 1/50, 3/49, 10/50 and carcinoma 0, 0, 0, 0, 2/50 at 0, 2, 8, 45, 90 mkd)	In females: At 8 mkd: ovarian cysts (NOAEL = 2 mkd) At 45 mkd: reduced FC, BWG, liver (increase rel. W) At 90 mkd: liver (increase W, hepatocellular hypertrophy), adrenal gland cortex tumours	GLP, OECD 451		N	DAR (Mellert, Hildebrand 1992f TOX2003-1845)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
			Rat	104-wk	Oral	34	69	Decreased incidences of neoplasms in the testes (Leydig cell tumours)	In males: At 34 mkd: reduced FC, BWG, liver (hepatocellular hypertrophy, eosinophilic and mixed cell foci) At 69 mkd: liver (increase W,fatty changes)	GLP, OECD 451	**(phaechromocytomas, in male) and pituitary gland (adenomas, in female) in rat**	N	DAR (Mellert, Hildebrand 1992f TOX2003-1845)
			Rat	104-wk	Oral	1	6	Decreased incidences of neoplasms in the adrenal gland medulla (phaechromocytomas) in males	In males: At 34 mkd: reduced FC, BWG, liver (hepatocellular hypertrophy, eosinophilic and mixed cell foci) At 69 mkd: liver (increase W,fatty changes)	GLP, OECD 451		N	DAR (Mellert, Hildebrand 1992f TOX2003-1845)
			Rat	104-wk	Oral	45	90	Decreased incidences of neoplasms in the pituitary gland (adenomas) in females	In females: At 8 mkd: ovarian cysts (NOAEL = 2 mkd) At 45 mkd: reduced FC, BWG, liver (increase rel. W) At 90 mkd: liver (increase W, hepatocellular hypertrophy), adrenal gland cortex tumours	GLP, OECD 451		N	DAR (Mellert, Hildebrand 1992f TOX2003-1845)
			Mouse	78-wk	Oral	M: 28.1 F: 42.4	M: 72.2 F: 214.4	Liver tumours in M and F at the highest dose (in M: adenoma 0, 0, 0, 0, 0, 3 and carcinoma 1, 0, 0, 0, 3, 33* at 0, 0, 0.12, 0.69, 28.1, 72.2 mkd) (in F: adenoma 0, 0, 0, 0, 0, 5 and carcinoma 0, 1, 1, 1, 1, 33* at 0, 0, 0.22, 0.92, 42.4, 214.4 mkd)	In males: At 28.1 mkd: reduced BWG, liver (increased W, eosinophilic foci), testes (deposition of amyloid) (NOAEL = 0.69 mkd) At 72.2 mkd: liver (focal necrosis, hyperplasia, tumours) In females: At 42.4 mkd: reduced BWG, liver (increased W) (NOAEL = 0.92 mkd) At 214.4 mkd: liver (focal necrosis, hyperplasia, eosinophilic foci, deposition of amyloid, tumours), ovary (deposition of amyloid)	GLP, OECD 451		N	DAR (Mellert, Hildebrand 1992g TOX2003-1846)
		Adrenals weight	Rat	2-generation	Oral	2.3	23	Decreased adrenal weight; F0/F1 males	Parents: At 23 mkd: decreased FC (F0 females, F1 M/F), BW (F1 M), liver (increased W in F1 F, decreased fatty changes in F1 M) (NOAEL = 2.3 mkd)	GLP, OECD 416	Effect on adrenals weight not consistent across studies (decreased or increased).	N	DAR (Hellwig, Hildebrand 1992 TOX2003-1847)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
			Rat	28-day	Oral	5.64	61.79	Decreased absolute and relative adrenal weight, SS (-35%)	Decreased food intake during the first two days No changes in other steroid hormone concentrations Increased circulating cholesterol (+56%) Changes in the expression of genes linked to cholesterol uptake, storage and metabolism (increased expression level of ApoE and Ldlr, reduced expression level of Ces1e, Ehd1, Plin2, Gpc1 and Egr1)	Not GLP, no guideline but study design and parameters analysed stated to be in accordance with OECD 407, except that some organs were excluded (scope = adrenal gland). Published study 97% purity Individual data not available Only in males, 5/grp Correspondence ppm-mkd in Schmidt 2016		N	Rieke 2017
			Rat	GD7-18 and GD7-21	Oral	<23	23	Increased adrenal weight Study author: "Might suggest a stress response to EPX treatment"	Dams: At 23 and 50 mkd: decreased FC, decreased carcass weight and corrected net weight gain, decreased platelet count	GLP (except for analysis of hormone levels), followed the general principles of OECD 414 Published studies (BASF) 97% purity Only 2 dose levels		N	Stinchcom be 2013
			Guinea pig	GD6-63	Oral	50	90	Increased absolute and relative adrenal weight (slight but SS) According to the study authors: "might reflect either an altertion in the steroid hormone production of the adrenals or potential consequences of maternal stress"	Dams: At 90 mkd: mild anemia	GLP, Modified prenatal study followed OECD 414 Published study 97% purity		N	Schneider 2013

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
		Adrenals histopathology	Rat	28-day	Oral	5.64	61.79	Atrophy of the *zona fasciculata* (5/5 animals)	Decreased food intake during the first two days No changes in other steroid hormone concentrations Increased circulating cholesterol (+56%) Changes in the expression of genes linked to cholesterol uptake, storage and metabolism (increased expression level of ApoE and Ldlr, reduced expression level of Ces1e, Ehd1, Plin2, Gpc1 and Egr1)	Not GLP, no guideline but study design and parameters analysed stated to be in accordance with OECD 407, except that some organs were excluded (scope = adrenal gland). Published study 97% purity Individual data not available Only in males, 5/grp Correspondence ppm-mkd in Schmidt 2016	Effect on adrenal histopathology not consistent across studies (no effect in the regulatory studies).	N	Rieke 2017
			Guinea pig	GD6-PND21	Oral	15	50	Vacuolation of the zona fasciculata, increased incidence and severity of multifocal areas of cytoplasmic changes in the deep zona fasciculata According to the study authors: "might reflect either an alteration in the steroid hormone production of the adrenals or potential consequences of maternal stress"	Dams: At 90 mkd: slight decreased BWG and FC during gestation	GLP, Pre and post-natal study followed ICH S5A but terminated 3wks after weaning Published study 97% purity		N	Schneider 2013
		Gestation length	Rat	2-generation	Oral	2.3	23	Increased gestation length (23 or 24 days); in 9 F0 (to F1a) and 6 F1 (to F2) dams	Parents: At 23 mkd: decreased FC (F0 females, F1 M/F), BW (F1 M), liver (increased W in F1 F, decreased fatty changes in F1 M) (NOAEL = 2.3 mkd)	GLP, OECD 416	**Increased gestation length in rat, consistent across studies. Probably due to interference with parturition-inducing signals (DAR)**	N	DAR (Hellwig, Hildebrand 1992 TOX2003-1847)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
			Rat	GD7-GD21 and GD7-PND16 (31 days)	Oral	15	50	Increased gestation length (mean: 23.7 days); highest dose level	Dams: At 15 and 50 mkd: tendency toward a decreased BWG GD4-PND1 (but not GD7-21) and PND1-13	Not GLP, no guideline Published study 99% purity Only 2 dose levels Low number of litters (9 in the 15 mkd groups, 1-2 in the 50 mkd group sacrificed on PND16, 14 in the 50 mkd group sacrificed on GD21) Individual data not available		N	CLH (Taxvig 2007)
			Rat	GD7-21 and from the day after birth to Pup Day 16	Oral	3.75	15	Increased gestation length (mean: 23.8 days); highest dose level, SS	Dams: No maternal toxicity	Not GLP, no guideline Published study 99% purity Only 2 dose levels Low number of litters (8 in the 3.75 mkd group, 4 in the 15 mkd group) Individual data not available		N	Hass 2012
		Dystocia	Rat	2-generation	Oral	2.3	23	Dystocia; 2 F0 dams died with dystocia Vaginal haemorrhages in 6 F0 and 1 F1 dams	Parents: At 23 mkd: decreased FC (F0 females, F1 M/F), BW (F1 M), liver (increased W in F1 F, decreased fatty changes in F1 M) (NOAEL = 2.3 mkd)	GLP, OECD 416	**Dystocia observed in rat, consistent across studies. Linked to increased gestation length.**	N	DAR (Hellwig, Hildebrand 1992 TOX2003-1847)
			Rat	GD7-GD21 and GD7-PND16 (31 days)	Oral	15	50	Dystocia; highest dose level	Dams: At 15 and 50 mkd: tendency toward a decreased BWG GD4-PND1 (but not GD7-21) and PND1-13	Not GLP, no guideline Published study 99% purity Only 2 dose levels Low number of litters (9 in the 15 mkd groups, 1-2 in the 50 mkd group sacrificed on PND16, 14 in the 50 mkd group sacrificed on GD21) Individual data not available		N	CLH (Taxvig 2007)
		Time to mating	Rat	2-generation	Oral	2.3	23	Increased precoital interval >4 days (duration of a normal oestrus cycle); in 3 F0 and 4 F1 mating pairs	Parents: At 23 mkd: decreased FC (F0 females, F1 M/F), BW (F1 M), liver (increased W in F1 F, decreased fatty changes in F1 M) (NOAEL = 2.3 mkd)	GLP, OECD 416	**Increased time to mating which may indicate irregularities of the oestrous cycle**	N	DAR (Hellwig, Hildebrand 1992 TOX2003-1847)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
		Fertility	Rat	2-generation	Oral	2.3	23	Trend towards decreased fertility indices in the high dose group; males (SS only in F2 mating group, NSS in F1a and F1b) and females (NSS), however, according to the DAR "*when F1a, F1b and additional matings with untreated partners were considered in combination, fertility was proven for all F0 parental animals*".	Parents: At 23 mkd: decreased FC (F0 females, F1 M/F), BW (F1 M), liver (increased W in F1 F, decreased fatty changes in F1 M) (NOAEL = 2.3 mkd)	GLP, OECD 416	Decreased fertility indices but according to the DAR, the fertility was proven for all F0 parental animals. Uncertainties in the interpretation of this finding (study report not available).	N	DAR (Hellwig, Hildebrand 1992 TOX2003-1847)
		Post-implantation loss	Rat	GD6-15	Oral	20	60	Post-implantation loss; marginal increase (NSS) at 60 and 180 mkd Increased resorptions (early) at 180 mkd	Dams: At 20 mkd: decreased corrected net BWG At 60 mkd: decreased BW/BWG At 180: BW loss, clinical signs	GLP, OECD 414 Acceptable as a range-finding study	**Post-implantation losses in rats (late and very late) and rabbits (early)** **Worsened when the duration of exposure is extended to the end of gestation (higher rate of resorptions, later stages of resorptions observed)** **Not considered secondary to maternal toxicity (e.g. absence of significant maternal toxicity in Taxvig studies) and not a consequence of dystocia (resorptions observed in dams sacrificed before paturition).**	N	DAR (Hellwig, Hildebrand 1989 TOX2003-1848
			Rat	GD6-15	Oral	15	45	Post-implantation loss; marginal increase at 45 mkd Increased resorptions (especially late) at 45 mkd	Dams: At 45 mkd: decreased FC and BWG (GD6-8) (NOAEL = 15 mkd)	GLP, OECD 414		N	Hellwig, Hildebrand 1990b TOX2003-1849)
			Rat	GD6-19	Oral	<180	180	Post-implantation loss (40-60% vs 10% in controls) Increased resorptions (especially late)	Dams: At 180 mkd: decreased FC, BW, BWG, corrected net BWG, blood in bedding/vaginal haemorrhages, decreased RBC/platelets, clinical chemistry parameters	GLP, OECD 414 Only 1 dose level; 2 groups with 2 different batches (purity 94.7%, purity 99.8%)		N	DAR (Schneider 2002 TOX2002-2288)
			Rat	GD7-GD21 and GD7-PND16 (31 days)	Oral	15	50	Post-implantation loss (mainly very late resorptions), highest dose level (also NSS at 15)	Dams: At 15 and 50 mkd: tendency toward a decreased BWG GD4-PND1 (but not GD7-21) and PND1-13	Not GLP, no guideline Published study 99% purity Only 2 dose levels Low number of litters (9 in the 15 mkd groups, 1-2 in the 50 mkd group sacrificed on PND16, 14 in the 50 mkd group sacrificed on GD21) Individual data not available		N	CLH (Taxvig 2007)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
			Rat	GD7-GD21	Oral	<50	50	Post-implantation loss (late and very late resorptions)	Dams: At 50 mkd: no effect	Not GLP, no guideline Published study 99% purity Only 1 dose level Individual data not available		N	CLH (Taxvig 2008)
			Rabbit	GD7-19	Oral	20	80	Post-implantation loss Increased resorptions (early) (total litter loss in 3 does); highest dose level	Dams: At 20 mkd: decreased FC,BWG (NOAEL=5mkd)	GLP, OECD 414		N	DAR (Hellwig, Hildebrand 1990a TOX2003-1851)
			Rat	GD7-18 and GD7-21	Oral	23	50	Post-implantation loss (late resorptions) Vehicle: corn oil or CMC At 23 mkd: NSS Co-administration of ECP (GD7-21, EPX 50 mkd) (synthetic estrogen) reduced post-implantation losses and late resorptions	Dams: At 23 and 50 mkd: decreased FC, decreased carcass weight and corrected net weight gain, decreased platelet count	GLP (except for analysis of hormone levels), followed the general principles of OECD 414 Published studies (BASF) 97% purity Only 2 dose levels		N	Rey Moreno 2013 Stinchcombe 2013
		Number of live births	Rat	2-generation	Oral	2.3	23	Increased number of stillborn pups; F1 and F2 Decreased gestation index	Parents: At 23 mkd: decreased FC (F0 females, F1 M/F), BW (F1 M), liver (increased W in F1 F, decreased fatty changes in F1 M) (NOAEL = 2.3 mkd)	GLP, OECD 416	**Decreased number of live births, consistent across studies. Linked to post-implantation loss.**	N	DAR (Hellwig, Hildebrand 1992 TOX2003-1847)
			Rat	GD6-19	Oral	<180	180	Decreased number of live fetuses	Dams: At 180 mkd: decreased FC, BW, BWG, corrected net BWG, blood in bedding/vaginal haemorrhages, decreased RBC/platelets, clinical chemistry parameters	GLP, OECD 414 Only 1 dose level; 2 groups with 2 different batches (purity 94.7%, purity 99.8%)		N	DAR (Schneider 2002 TOX2002-2288)
			Rat	GD7-GD21 and GD7-PND16 (31 days)	Oral	15	50	Increased frequency of stillbirths; highest dose level (increased "born dead per litter" and decreased "born alive per litter")	Dams: At 15 and 50 mkd: tendency toward a decreased BWG GD4-PND1 (but not GD7-21) and PND1-13	Not GLP, no guideline Published study 99% purity Only 2 dose levels Low number of litters (9 in the 15 mkd groups, 1-2 in the 50 mkd group sacrificed on PND16, 14 in the 50 mkd group sacrificed on GD21) Individual data not available		N	CLH (Taxvig 2007)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
			Rat	GD7-18 and GD7-21	Oral	23	50	Decreased number/percent of live fetuses Vehicle: corn oil or CMC	Dams: At 23 and 50 mkd: decreased FC, decreased carcass weight and corrected net weight gain, decreased platelet count	GLP (except for analysis of hormone levels), followed the general principles of OECD 414 Published studies (BASF) 97% purity Only 2 dose levels		N	Rey Moreno 2013 Stinchcombe 2013
		Litter size	Rat	2-generation	Oral	2.3	23	Decreased mean litter size (liveborn pups); F1	Parents: At 23 mkd: decreased FC (F0 females, F1 M/F), BW (F1 M), liver (increased W in F1 F, decreased fatty changes in F1 M) (NOAEL = 2.3 mkd)	GLP, OECD 416	**Decreased mean litter size, consistent across studies. Linked to post-implantation loss.**	N	DAR (Hellwig, Hildebrand 1992 TOX2003-1847)
			Rat	GD7-GD21 and GD7-PND16 (31 days)	Oral	15	50	Decreased live litter size; highest dose level	Dams: At 15 and 50 mkd: tendency toward a decreased BWG GD4-PND1 (but not GD7-21) and PND1-13	Not GLP, no guideline Published study 99% purity Only 2 dose levels Low number of litters (9 in the 15 mkd groups, 1-2 in the 50 mkd group sacrificed on PND16, 14 in the 50 mkd group sacrificed on GD21) Individual data not available		N	CLH (Taxvig 2007)
		Pup survival index or litter viability	Rat	2-generation	Oral	2.3	23	Decreased viability index; F1b, F2 Decreased lactation index; F2 Increased mortality during rearing; F2	Parents: At 23 mkd: decreased FC (F0 females, F1 M/F), BW (F1 M), liver (increased W in F1 F, decreased fatty changes in F1 M) (NOAEL = 2.3 mkd)	GLP, OECD 416	**Decreased pup survival, consistent across studies.**	N	DAR (Hellwig, Hildebrand 1992 TOX2003-1847)
			Rat	GD7-GD21 and GD7-PND16 (31 days)	Oral	15	50	Postnatal deaths of pups, highest dose level	Dams: At 15 and 50 mkd: tendency toward a decreased BWG GD4-PND1 (but not GD7-21) and PND1-13	Not GLP, no guideline Published study 99% purity Only 2 dose levels Low number of litters (9 in the 15 mkd groups, 1-2 in the 50 mkd group sacrificed on PND16, 14 in the 50 mkd group sacrificed on GD21) Individual data not available		N	CLH (Taxvig 2007)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
		Litter/pup weight	Rat	2-generation	Oral	2.3	23	Decreased pup BW gain days 4-21	Parents: At 23 mkd: decreased FC (F0 females, F1 M/F), BW (F1 M), liver (increased W in F1 F, decreased fatty changes in F1 M) (NOAEL = 2.3 mkd)	GLP, OECD 416	Effect on litter/pup weight not consistent across studies (decreased or increased).	N	DAR (Hellwig, Hildebrand 1992 TOX2003-1847)
			Rat	GD7-GD21	Oral	<50	50	Increased fetal weight; males and females	Dams: At 50 mkd: no effect	Not GLP, no guideline Published study 99% purity Only 1 dose level Individual data not available		N	CLH (Taxvig 2008)
		Presence of anomalies (external, visceral, skeletal)	Rat	2-generation	Oral	2.3	23	Anasarca; 1 F1b, 3 F2 (from 3 litters) Cleft palate; 1 F1b	Parents: At 23 mkd: decreased FC (F0 females, F1 M/F), BW (F1 M), liver (increased W in F1 F, decreased fatty changes in F1 M) (NOAEL = 2.3 mkd)	GLP, OECD 416	**Increased incidence of a rare malformation, namely cleft palate, in rat. High incidence in the presence of maternal toxicity or repeated observation of isolated cleft palates in rats at doses without maternal toxicity. Not considered secondary to other maternal toxic effects.** **Anasarca (generalised edema) in rat, rare malformation observed in 2 studies (not clear if secondary to difficulty in**	N	DAR (Hellwig, Hildebrand 1992 TOX2003-1847)
			Rat	GD6-15	Oral	60	180	Cleft palate; 50% fetuses and 90% litters at the highest dose	Dams: At 20 mkd: decreased corrected net BWG At 60 mkd: decreased BW/BWG At 180: BW loss, clinical signs	GLP, OECD 414 Acceptable as a range-finding study		N	DAR (Hellwig, Hildebrand 1989 TOX2003-1848)
			Rat	GD6-15	Oral	15	45	Skeletal variations; rudimentary cervical and/or accessory 14th ribs	Dams: At 45 mkd: decreased FC and BWG (GD6-8) (NOAEL = 15 mkd)	GLP, OECD 414		N	DAR (Hellwig, Hildebrand 1990b TOX2003-1849)
			Rat	GD6-19	Oral	<180	180	Cleft palate: 3 fetuses from 2 litters Anasarca: 7 fetuses from 6 litters Absent or small tuberositas deltoidea and other skeletal malformations/variations especially in a batch with higher content of impurities	Dams: At 180 mkd: decreased FC, BW, BWG, corrected net BWG, blood in bedding/vaginal haemorrhages, decreased RBC/platelets, clinical chemistry parameters	GLP, OECD 414 Only 1 dose level; 2 groups with 2 different batches (purity 94.7%, purity 99.8%)		N	DAR (Schneider 2002 TOX2002-2288)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
			Rat	GD6-15	Dermal	400	1000	Cleft palate: 1 fœtus at the highest dose level Skeletal variations: rudimentary cervical and/or accessory 14th ribs (cervical ribs (12 fetuses/9 litters), 14th ribs (10 fetuses/7 litters) At 400: same effects but not considered adverse	Dams: Up to 1000 mkd: no effect	GLP, OECD 414	**parturition or teratogenic effect)** **Thoracic centrum fused with arch in the guinea pig.**	N	DAR (Hellwig, Hildebrand 1993 TOX2003-1850)
			Guinea pig	GD6-63	Oral	15	30	Thoracic centrum fused with arch; dose-related increased fetal and litter incidence in all groups, SS litter incidence at 50 and 90 mkd	Dams: At 90 mkd: mild anemia	GLP, Modified prenatal study followed OECD 414 Published study 97% purity		N	Schneider 2013
		Placental weight	Rat	GD6-19	Oral	<180	180	Increased placental weight	Dams: At 180 mkd: decreased FC, BW, BWG, corrected net BWG, blood in bedding/vaginal haemorrhages, decreased RBC/platelets, clinical chemistry parameters	GLP, OECD 414 Only 1 dose level; 2 groups with 2 different batches (purity 94.7%, purity 99.8%)	**Increased placental weight in rat, consistent across studies.**	N	DAR (Schneider 2002 TOX2002-2288)
			Rat	GD6-15	Oral	5	15	Increased placental weight	Dams: At 45 mkd: decreased FC and BWG (GD6-8) (NOAEL = 15 mkd)	GLP, OECD 414		N	DAR (Hellwig, Hildebrand 1990b TOX2003-1849)
			Rat	GD6-15	Dermal	400	1000	Increased placental weight	Dams: Up to 1000 mkd: no effect	GLP, OECD 414		N	DAR (Hellwig, Hildebrand 1993 TOX2003-1850)
			Rat	GD6-15	Oral	<20	20	Increased placental weight	Dams: At 20 mkd: decreased corrected net BWG At 60 mkd: decreased BW/BWG At 180: BW loss, clinical signs	GLP, OECD 414 Acceptable as a range-finding study		N	DAR (Hellwig, Hildebrand 1989 TOX2003-1848)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
		Placental histopathology	Rat	GD7-18 and GD7-21	Oral	<23	23	Dose-dependent severity of the degeneration of the labyrinth and the trophospongium, more pronounced in placentae with late resorptions than in placentae with live fetuses Co-administration of ECP (GD7-21, EPX 50 mkd) (synthetic estrogen) tends to reduce the severity of degenerative placental changes	Dams: At 23 and 50 mkd: decreased FC, decreased carcass weight and corrected net weight gain, decreased platelet count	GLP (except for analysis of hormone levels), followed the general principles of OECD 414 Published studies (BASF) 97% purity Only 2 dose levels	**Degeneration of the labyrinth and the trophospongium of the placenta in rat**	N	Rey Moreno 2013
		Vaginal haemorrhage	Rat	2-generation	Oral	2.3	23	Vaginal haemorrhages in 6 F0 (2 of these died of severe dystocia) and 1 F1 dams (did not deliver pups after prolonged gestation)	Parents: At 23 mkd: decreased FC (F0 females, F1 M/F), BW (F1 M), liver (increased W in F1 F, decreased fatty changes in F1 M) (NOAEL = 2.3 mkd)	GLP, OECD 416	**Vaginal haemorrhage in rat, consistent across studies. Linked to post-implantation loss.**	N	DAR (Hellwig, Hildebrand 1992 TOX2003-1847)
			Rat	GD6-19	Oral	<180	180	Blood in bedding and/or vaginal haemorrhage in 8 dams	Dams: At 180 mkd: decreased FC, BW, BWG, corrected net BWG, blood in bedding/vaginal haemorrhages, decreased RBC/platelets, clinical chemistry parameters	GLP, OECD 414 Only 1 dose level; 2 groups with 2 different batches (purity 94.7%, purity 99.8%)		N	DAR (Schneider 2002 TOX2002-2288)
			Rat	GD6-19	Oral	45	60	Vaginal haemorrhages in 1 dam at 60 mkd and in 4 dams at 75 mkd shortly before terminal sacrifice	Dams: At 45 mkd: decreased FC, decreased RBC/Hb/Ht, clinical chemistry parameters At 60 mkd: decreased BWG GD19-20, decreased corrected net BWG, vaginal haemorrhages At 75 mkd: increased liver W	Not GLP, OECD 414 with deviations (litter data and fœtal parameters not investigated) Unpublished study Acceptable for characterisation of maternal toxicity		N	DAR (Schneider 2002 TOX2003-1852)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for EATS-related adversity assessment	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indica-tive of	Reference
			Rat	GD7-18 and GD7-21	Oral	23	50	Vaginal haemorrhage in dams treated from GD7 to GD21 only, observed at the end of gestation (GD18 to GD21) (13/23 with corn oil as vehicle, 11/23 with CMC as vehicle) Also observed in ECP (synthetic estrogen) co-administration groups: 8/14 in control, 6/16 with 0.5 µg/d ECP, 4/16 with 1 mg/d ECP. Necrobiotic placenta which could be associated with blood coagulum around placenta at necropsy	Dams: At 23 and 50 mkd: decreased FC, decreased carcass weight and corrected net weight gain, decreased platelet count	GLP (except for analysis of hormone levels), followed the general principles of OECD 414 Published studies (BASF) 97% purity Only 2 dose levels		N	Stinchcom be 2013

Table 2: Lines of evidence for EAS disruption (non-mammalian organisms)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for assessment of adversity	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indicative of	Reference
Evidence of EATS-mediated adversity	EATS-mediated parameter	Histopathology (thyroid gland, gonad)	Japanese quail (*Coturnix coturnix japonica*)	3 weeks	oral (diet)	10 ppm	50 ppm	Effects observed on testes. Reduced number of testicular canaliculi with visible germ cells.	Effects on testes (histopathology).	Study not GLP, compliant with OECD 206 Batch identified (purity 99.2%)	**The effects on testis and on sperm could be linked to effects on aromatase.**	E, A, T	Konstanze Grote *et al.* 2008
			Japanese quail (*Coturnix coturnix japonica*)	3 weeks	oral (diet)	10 ppm	50 ppm	Reduction in spermatid number.	Reduction in spermatid number.	Study not GLP, compliant with OECD 206 Batch identified (purity 99.2%)			Konstanze Grote *et al.* 2008
			Japanese quail (*Coturnix coturnix japonica*)	3 weeks	oral (diet)	10 ppm	50 ppm	Decreased activity of spermatogenesis in 3 out of 6 males in the two upper dose groups.	Spermatogenesis is affected.	Study not GLP, compliant with OECD 206		N	DAR (Chahoud, I, ; Niemann, L., Selzsam, B., Gericke, C, Haider, W. 2004)
	Parameter sensitive to, but not diagnostic of, EATS	Reproductive performance	Japanese quail (*Coturnix coturnix japonica*)	3 weeks	oral (diet)	500 ppm	-	The reproductive success as well as all reproductive parameters to be determined according to the OECD guideline were not statistically significant or dose related affected up to the highest dose of 500 ppm.		Study not GLP, compliant with OECD 206	The adverse effect on testis and sperm did not impact the reproductive success of birds.	N	DAR (Chahoud, I, ; Niemann, L., Selzsam, B., Gericke, C, Haider, W. 2004)
		Time to maturity (time to first spawn)	Zebra fish (*Danio rerio*)	-water sediment study	water	192 µg/L	768 µg/L	Start of spawning delayed at 768 µg/L (fish exposed as fertilized eggs).	Day for start of spawning delayed.	GLP, OECD 219	**Population relevant effects. The later start of spawning and the reduced**	N	DAR (Schaefers C. 2003)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for assessment of adversity	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indicative of	Reference
		Reproduction (fecundity, fertility)	Zebra fish (*Danio rerio*)	-water sediment study	water	192 µg/L	768 µg/L	Significant reduced number of eggs and fertilization rate at 768 µg/L (fish exposed as fertilized eggs).	Reduction of the reproductive performance	GLP, OECD 219	**number of eggs and fertilization rate could be due to delayed maturation of fish.**	N	DAR (Schaefers C. 2003)
Evidence for endocrine activity	in vivo mechanistic	Estradiol, testosterone and thyroid hormone levels measurements (egg yolk, adult, thyroid hormone from thyroid gland)	Japanese quail (*Coturnix coturnix japonica*)	3 weeks	oral (diet)	500 ppm	-	No alterations in serum concentrations of the measured hormones (Estradiol and testosterone as well as T3 and T4) as compared to control.		Study not GLP, compliant with OECD 206 Batch identified (purity 99.2%)	No effects in the concentrations of the investigated hormones.	E, A, T, S	Konstanze Grote *et al.* 2008
		VTG in females	Zebra fish (*Danio rerio*)	water sediment study	water	12 µg/L (initial concentration)	48 µg/L (initial concentration)	Vitellogenin in females decreased significantly and dose-related from 12 µg/L for fish exposed as juvenile 69 d old).	Vitellogenin concentration in plasma is affected.	GLP, OECD 219	**The overall EC10 for vitellogenin in females is 30 µg/L. Effects on vitellogenin in females can lead to impoverished egg nutrient support.**	E, A, S	DAR (Schaefers C. 2003)
			Zebra fish (*Danio rerio*)	water sediment study	water	48 µg/L (initial concentration)	192 µg/L (initial concentration)	Vitellogenin in females decreased significantly and dose-related from 48 µg/L for fish exposed as pre-adults 85-125 d old).	Vitellogenin concentration in plasma is affected.	GLP, OECD 219		E, A, S	DAR (Schaefers C. 2003)
			Zebra fish (*Danio rerio*)	-water sediment study	water	192 µg/L (initial concentration)	768 µg/L (initial concentration)	Vitellogenin in females decreased significantly at 768 µg/L (fish exposed as fertilized eggs).	Vitellogenin concentration in plasma is affected.	GLP, OECD 219		E, A, S	DAR (Schaefers C. 2003)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for assessment of adversity	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indicative of	Reference
		VTG in males	Zebra fish (*Danio rerio*)	-water sediment study	water	12 µg/L (initial concentration)	48 µg/L (initial concentration)	Vitellogenin in males decreased significantly and dose-related from 48 µg/L on, the according EC10 was in the range of 4-6 µg/L (fish exposed as fertilized eggs).	Vitellogenin concentration in plasma is affected	GLP, OECD 219	There were no effects on the body weight, body length and fertility of the adult fish and therefore the parameter "vitellogenin reduction in males" is of no relevance at the population level	E, A, S	DAR (Schaefers C. 2003)
	EATS-mediated parameter	Sex ratio male biased)	Zebra fish (*Danio rerio*)	-water sediment study	water	48 µg/L (initial concentration)	192 µg/L (initial concentration)	Sex ratio was significantly shifted to male (fish exposed as fertilized eggs).		GLP, OECD 219	**Population relevant effects and a EC10 value of 30 µg/L could be determined.**	E, A	DAR (Schaefers C. 2003)
			Zebra fish (*Danio rerio*)	-water sediment study	water	48 µg/L (initial concentration)	192 µg/L (initial concentration)	Sex ratio was significantly shifted to male (fish exposed as juveniles (69 d old)).		GLP, OECD 219		E, A	DAR (Schaefers C. 2003)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for assessment of adversity	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indicative of	Reference
			Zebra fish (*Danio rerio*)	-water sediment study	water	192 µg/L (initial concentration)	768 µg/L (initial concentration)	Sex ratio was significantly shifted to male (fish exposed as pre-adults 85-125 d old)).		GLP, OECD 219		E, A	DAR (Schaefer)s C. 2003
Evidence for adversity	Parameter sensitive to, but not diagnostic of, EATS	Egg production	Mallard duck (*Anas platyrhynchos*)	22 weeks	oral (diet)	150 ppm	500 ppm	Not statistically significant reduced number of eggs laid but considered to be probably of biological importance.	Non-significant reduction of the reproductive performance	GLP, EPA 71-4	Effects probably of biological importance but no sufficient evidence to state on its adversity.	N	DAR (Johnson A.J. et al. 1993)
		Egg viability (% viable embryo of egg set)	Mallard duck (*Anas platyrhynchos*)	22 weeks	oral (diet)	150 ppm	500 ppm	Not statistically significant reduced proportion of viable embryos of eggs set but considered to be probably of biological importance.	Non-significant reduction of the reproductive performance	GLP, EPA 71-4		N	DAR (Johnson A.J. et al. 1993)
		Viable embryos	Mallard duck (*Anas platyrhynchos*)	22 weeks	oral (diet)	150 ppm	500 ppm	Not statistically significant reduced number of viable embryos per female but considered to be probably of biological importance.	Non-significant reduction of the reproductive performance.	GLP, EPA 71-4		N	DAR (Johnson A.J. et al. 1993)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for assessment of adversity	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indicative of	Reference
		Hatchability	Bobwhite quail (*Colinus virginianus*)	29 weeks	oral (diet)	10 ppm (1 mg a.s./kg bw/d)	50 ppm	Not statistically significant reduction of the number of hatched chicks per female quail.	Non-significant reduction of the fertility rate	GLP, EPA 71-4 (high mortality due to injury was observed in the test but the test remains reliable)	-	N	DAR (Munk R. et al. 1994)
		Number of 14-day old survivors	Bobwhite quail (*Colinus virginianus*)	29 weeks	oral (diet)	10 ppm (1 mg a.s./kg bw/d)	50 ppm	Not significant reduction number of 14-day-old surviving chicks per female quail.	Non-significant reduction of the fertility rate	GLP, EPA 71-4 (high mortality due to injury was observed in the test but the test remains reliable)	-	N	DAR (Munk R. et al. 1994)
		Bodyweight	Bobwhite quail (*Colinus virginianus*)	29 weeks	oral (diet)	50 ppm	500 ppm	Reduced hatched chick's body weight at day 14.	Reduced bodyweight.	GLP, EPA 71-4 (high mortality due to injury was observed in the test but the test remains reliable)	-	N	DAR (Munk R. et al. 1994)
			Japanese quail (*Coturnix coturnix japonica*)	3 weeks	oral (diet)	10 ppm	50 ppm	Dose-dependent and statistically significant body weight loss of the male birds.	Bodyweight loss.	Study not GLP, compliant with OECD 206	-	N	RAR (Chahoud, I, ; Niemann, L., Selzsam, B., Gericke, C, Haider, W. 2004)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for assessment of adversity	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indicative of	Reference
			Rainbow trout (*Oncorhynchus mykiss*)	97 days	water	16.7 µg/L	50 µg/L	52 %reduction for the body weight compared to the control.	Reduced bodyweight.	GLP, OECD 210	-	N	RAR (Munk R. 1996)
		Length	Rainbow trout (*Oncorhynchus mykiss*)	97 days	water	16.7 µg/L	50 µg/L	17 % reduction for the body length compared to the controls.	Reduced body length.	GLP, OECD 210	-	N	RAR (Munk R. 1996)
			Fathead minnow (*Pimephales promelas*)	24 weeks (post-hatch) of F1 generation	water	10 µg/L	30 µg/L	Growth reduction during the early phases of the F1-generation (day 23 and 54).	Reduced growth.	GLP, EPA 71-5	NOAEC is estimated to be 10 µg/L.	N	RAR (Zok S. 2003)
			Zebra fish (*Danio rerio*)	water sediment study	water	192 µg/L	768 µg/L	Reduced length at the end of early life stage period (day 28) and at the end of juvenile growth period (fish exposed as fertilized eggs).	Reduced body length.	GLP, OECD 219	-	N	RAR (Schaefers C. 2003)
		Survival	Rainbow trout (*Oncorhynchus mykiss*)	28 days	water	68.1 µg/L	147 µg/L	0% mortality at 68.1 µg/L, 5% mortality at 147 µg/L and 40% at 316 µg/L.		GLP, OECD 204	-	N	RAR (Munk R. 1990)
			Rainbow trout (*Oncorhynchus mykiss*)	28 days	water	50 µg/L	100 µg/L	0% mortality at 50 µg/L and 5% mortality at 100 µg/L.		GLP, OECD 204	-	N	RAR (Munk R. 1992)
		Behaviour	Rainbow trout (*Oncorhynchus mykiss*)	28 days	water	-	68.1 µg/L	Reduced feed consumption observed at all tested concentrations and no feed consumption was observed at 316 µg/L and at all the higher tested concentrations	Reduced feed consumption.	GLP, OECD 204	-	N	RAR (Munk R. 1990)
			Rainbow trout (*Oncorhynchus mykiss*)	28 days	water	50 µg/L	100 µg/L	Reduced or no feed consumption observed at 100 µg/L.	Reduced feed consumption.	GLP, OECD 204	-	N	RAR (Munk R. 1992)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for assessment of adversity	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indicative of	Reference
		Residues in eggs and liver	Rainbow trout (*Oncorhynchus mykiss*)	97 days	water	16.7 µg/L	50 µg/L	Reduced food consumption, swimming near the water surface increasing in strength and quality with the duration of the study could be observed.		GLP, OECD 210	-	N	RAR (Munk R. 1996)
	-		Japanese quail (*Coturnix coturnix japonica*)	3 weeks	oral (diet)	-	-	A dose-dependent transfer of epoxiconazole into the eggs as well as residues in the liver of treated adult animals was proven.	Residues of epoxiconazole in eggs and liver.	Study not GLP, compliant with OECD 206	No detrimental effects on chicks observed at test termination (14 d after hatchling)	N	RAR (Chahoud, I, ; Niemann, L., Selzsam, B., Gericke, C, Haider, W. 2004)

Table 3: Lines of evidence for EAS disruption (invertebrates)

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for assessment of adversity	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indicative of	Reference
Evidence of EATS-mediated adversity	EATS-mediated parameter	Histopathology (gonad)	Nematode (*Caenorhabditis elegans*)	48 h	Test medium	1 µg/L	10 µg/L	Epoxiconazole significantly decreased the number of total germ cells.	Spermatogenesis.	Study not OCDE/GLP	**Epoxiconazole can reduce the number of germ cells and result in abnormalities of germ cell development.**	E,A,S	Yunhui Li et al., 2016
			Nematode (*Caenorhabditis elegans*)	48 h	Test medium	0.1 µg/L	1 µg/L	Epoxiconazole significantly decreased the number of spermatids.	Spermatogenesis.	Study not OCDE/GLP		E,A,S	Yunhui Li et al., 2016
		Mitotic cells	Nematode (*Caenorhabditis elegans*)	48 h	Test medium	1 µg/L	10 µg/L	Epoxiconazole significantly decreased the number of mitotic cells.		Study not OCDE/GLP	**Epoxiconazole significantly decreased the number of mitotic cells.**	E,A,S	Yunhui Li et al., 2016
		Meiotic cells	Nematode (*Caenorhabditis elegans*)	48 h	Test medium	1 µg/L	10 µg/L	Epoxiconazole significantly decreased the number of meiotic cells.		Study not OCDE/GLP	**Epoxiconazole induced abnormalities in the process of germ cell differentiation and reduced the number of meiotic cells. Epoxiconazole can affect the activity of CYP450 and inhibit steroidogenic processes such as meiosis-activating sterols.**	E,A,S	Yunhui Li et al., 2016

	Line of evidence	Parameter	Species	Exposure	Route of exposure	NOAEL for the effect mg/kg bw/d	LOAEL for the effect mg/kg bw/d	Observed effects	General systemic toxicity relevant for assessment of adversity	Reliability	Conclusion (effects supporting evidence which cannot be avoided are reported in bold)	Indicative of	Reference
		Sperm morphology	Nematode (*Caenorhabditis elegans*)	48 h	Test medium	-	0.1 µg/L	Epoxiconazole significantly decreased the diameter and cross-sectional area of spermatids.	Morphology of spermatids.	Study not OCDE/GLP	**Abnormal spermatozoid cells**	E,A,S	Yunhui Li et al., 2016
		Sperm motility	Nematode (*Caenorhabditis elegans*)	48 h	Test medium	1 µg/L	10 µg/L	Percentage of spermatid activation significantly decreased.	Sperm activation	Study not OCDE/GLP	**Sperm activation was inhibited by epoxiconazole exposure.**	E,A,S	Yunhui Li et al., 2016
			Nematode (*Caenorhabditis elegans*)	48 h	Test medium	10 µg/L	-	Percentage of sperm normal transfer was not evidently altered. Sperm migration seems to be not significantly affected.		Study not OCDE/GLP	-	E,A,S	Yunhui Li et al., 2016
	Parameter sensitive to, but not diagnostic of, EATS	Reproduction (fecundity, fertility)	Nematode (*Caenorhabditis elegans*)	48 h	Test medium	0.1 µg/L	1 µg/L	The number of outcross progeny significantly decreased.	Decrease in the reproduction performance (reduced number of progeny).	Study not OCDE/GLP	**The decrease of the reproduction success is observed at exposure level inducing effects on sperm production and quality.**	N	Yunhui Li et al., 2016
Evidence for adversity	General systemic toxicity	Gene expression	Nematode (*Caenorhabditis elegans*)	48 h	Test medium	-	0.1 µg/L	Expression of gene *daf-3*, *daf-4* and *daf-5* was significantly increased.		Study not OCDE/GLP	-	N	Yunhui Li et al., 2016
			Nematode (*Caenorhabditis elegans*)	48 h	Test medium	1.0 µg/L	10 µg/L	Expression of gene *daf-1*and *daf-7* was significantly increased.		Study not OCDE/GLP	-	N	Yunhui Li et al., 2016