Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

dimoxystrobin (ISO); (2*E*)-2-{2-[(2,5-dimethylphenoxy)methyl]phenyl}-2-(methoxyimino)-*N*-methylacetamide; (*E*)-2-(methoxyimino)-*N*-methyl-2-[α-(2,5-xylyloxy)-o-tolyl]acetamide

EC Number: -
CAS Number: 149961-52-4

CLH-O-0000006865-62-01/F

Adopted

8 October 2020

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON DIMOXYSTROBIN (ISO); (2E)-2-{2-[(2,5-DIMETHYLPHENOXY)METHYL]PHENYL}-2-(METHOXYIMINO)-N-METHYLACETAMIDE; (E)-2-(METHOXYIMINO)-N-METHYL-2-[A-(2,5-XYLYLOXY)-O-TOLYL]ACETAMIDE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: dimoxystrobin (ISO); (2E)-2-{2-[(2,5-dimethylphenoxy)methyl]phenyl}-2-(methoxyimino)-N-methylacetamide; (E)-2-(methoxyimino)-N-methyl-2-[a-(2,5-xylyloxy)-o-tolyl]acetamide
EC number: -
CAS number: 149961-52-4
Dossier submitter: Hungary

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
27.08.2019	France		MemberState	1
Comment received				
FR: In the table 1.2, please indicate that the active substance has no relevant impurity.				
Dossier Submitter's Response				
Dimoxystrobin does not contain toxicologically relevant impurities at the specified limit.				
RAC's response				
Thank you very much. Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
27.08.2019	Germany		MemberState	2
Comment received				
The classification with Acute Tox. 4, H332 and H362 (May cause harm to breast-fed children) is supported. The data base is insufficient to conclude that the harmonised classification with Repr. 2, H361d is not justified. With regard to STOT RE2 the comparison with guidance value is currently focused on doses that did not clearly evoke significant/severe effects as requested by the CLP Regulation.				
Dossier Submitter's Response				
Thank you for your comment and support. The responses are given at the corresponding detailed comments.				

RAC's response
Thank you very much for your comment. Please see RAC´s responses under the corresponding hazard classes.

Date	Country	Organisation	Type of Organisation	Comment number
19.08.2019	Germany	<confidential>	Company-Manufacturer	3

Comment received

Page 27; Sperm parameters investigated in the 2-generation toxicity study:
In top dose F0 parental males, percentage of normal sperm and sperm motility were decreased below the HCD range. When evaluating the individual values, one F0 high dose animal #118 with reduced testis and epididymides sizes and oligospermia in epididymides was mainly responsible for the decreased mean sperm motilities and increased number of abnormal sperm. Similar pathology was also seen for control animal #1, which showed epididymidal aspermia and no sperm were detectable (for %normal and abnormal sperm and motility), thus only 24 animals were evaluated. Also, one F1 animal from the 50 ppm dose group (#444) showed aspermia in the pathological investigation and no sperm could be measured (for %normal and abnormal sperm and motility). When the high dose animal #118 was excluded from the calculation all values were within the normal range, and the standard deviation was smaller. The absence of effects in the F1 males further confirms, that spermatogenesis is not affected by treatment with dimoxystrobin.
However, these parameters were not affected and within the historical range, when assessed in the F1 males. An updated table on the sperm parameters ("Sperm parameters of males administered dimoxystrobin") - with added information on standard deviations, historical control data, and number of animals investigated - is provided in the documents sent to EFSA in the context of the AIR III Peer-Review Process. These additional documentation and data is also provided to ECHA via e-mail at the 19th of August.

Page 28, Tables 16 and 17 (mean number of implantations in the 2-generation toxicity study):
The mean number of implantations was statistically significantly reduced in the F1 females and not in the F0 females. The F1 females but not the F0 females had considerably reduced body weights during the premating and the gestation phase. From public literature, there is evidence, that lower body weights (induced by feed restriction) in can induced lower number of implants in rats.
In Terry et al. study (2005) (reference is added), dams with restricted food access that showed a body weight decrease of about 15% during pre-mating and of about 26% during the first gestation week also revealed statistically significantly decreased numbers of implantations.
In this publication access to food was restricted during the pre-mating phase (PMD 8 and 15) and the first week of gestation (GD 7) leading to body weight reductions of -15 to -30%. Body weight was recorded at premating days 8 and 15 (PMD 8 and 15) and gestation day 7 (GD 7) (see Table 14 below). The animals receiving 15 and 10 g food/day, showed reduced body weights between -12 to -26% in the 15 g/day group and -18 to -42% in the 10 g food/day group compared to controls. These two treatment groups had statistically significant lower numbers of implants and numbers of corpora lutea (see Table below):

Table 14: Comparison of selected fertility parameters in animals with restricted access to food (taken from Terry et al., 2005)
Parameter Feed administration

Ad libitum 20g/day 15g/day 10g/day Mating phase no. 20 20 20 13 Body weight PMD 1 256 +/- 13 Within 1% of ad libitum group Body weight PMD 8 Control -6%* -12%* -18%* Body weight PMD 15 Control -6%* -18%* -29%* Body weight GD 7 Control -15%* -26%* -42%* No. copulation positive 20 20 20 8 Pregnancy rate [%]1) 85 85 95 50 No. corpora lutea [mean (SD)] 15.9 (1.4) 16.3 (2.4) 13.2 (1.9)* 12.0 (0.0)* No. implants [mean (SD)] 15.7 (1.6) 15.8 (2.4) 12.2 (3.4)* 9.5 (3.7)* No. viable [mean (SD)] 14.2 (2.4) 15.2 (2.4) 11.5 (3.3)* 6.0 (4.9)* No. dead 0 0 0 0 No. early resorptions [no (SD)] 1.5 (1.8) 0.6 (0.9) 0.7 (0.9) 3.5 (5.7) Pre-implantation loss [mean % (SD)]2) 1.5 (3.7) 3.2 (5.6) 8.1 (22.0) 20.8 (30.8) Post-implantation loss [mean % (SD)]3) 9.4 (11.5) 3.7 (5.9) 5.2 (6.6) 29.6 (47.2) * $p<0.05$ 1) pregnancy rate = (no. gravid/no. copulation positive) x 100 2) pre-implantation loss (per litter, %) = (corpora lutea – implantation sites/corpora lutea) x 100 3) post-implantation loss (per litter, %) = [(implantation sites – viable fetuses)/implantation sites] x 100 The body weight data of the 15 g food/day group are comparable to the females F1 animals dosed with 1200 ppm dimoxystrobin. The adult female F1 body weights during the pre-mating phase of the 2-generation toxicity study were -16 to -34% during the premating phase and -16 - -19% during gestation compared to controls, which gives evidence, that the observed numbers of implants are secondarily related to impaired body weight development, and not a specific indication for a specific effect on reproduction. This is further supported by the fact, that F0 females showed only marginally lower body weights (-3 to -5% in the premating phase and the first week of gestation) and no difference in numbers of implantation compared to concurrent controls were seen in F0 females. A more detailed description of the body weight effects induced by dimoxystrobin treatment is provided in the documents sent to EFSA in the context of the AIR III Peer-Review Process. These additional documentation and data is also provided to ECHA via e-mail at the 19th of August. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Terry et al 2005_feed restriction and fertility.pdf
Dossier Submitter's Response
Thank you for your contribution.
RAC's response
Thank you very much for your contributions. Noted.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
27.08.2019	Germany		MemberState	4

Comment received

Adverse effects on development
Based on current knowledge, there are insufficient mechanistic data to clearly demonstrate that the dose dependent incidence of cardiomegaly in the 2-generation study in F1- and F2- offspring is due to the iron deficiency of the lactating dams.

According to the DS Hungary cardiomegaly in pups has been observed in the 2-generation reproductive toxicity study only transiently, because this effect occurred only on day 21 p.n. but neither on day 7 p.n. nor in adults. However, it remains unclear how many pups have been examined on day 4 p.n. The same applies to adult animals. Therefore, the dossier should provide sufficient information on the number of examined pups/adults to come to a clear conclusion on this point.
Whether the cardiomegaly was actually induced by the iron-deficient milk remains uncertain, since there is no information on the iron content in the milk of dimoxystrobin exposed rats. According to Zhang, P. et al, (The effect of serum iron concentration on iron secretion into mouse milk, J Physiol. 2000 Feb 1; 522(Pt 3): 479-491), at least in the mouse milk the iron content is threefold higher than in the serum.
Another possible explanation of the transiently observed cardiomegaly would be that the pups themselves ingest the active substance when start eating other than milk at the age of 12-16 days.
In the cited publication by Roth & Smith (1988), uptake of sodium nitrite during pregnancy and lactation in drinking water induced iron deficiency in lactating dams and in the second week p.n. in offspring. Depending on the dose level of sodium nitrite, following effects were observed in pups: hypochromic microcytic anaemia, reduced growth and mortality. Strong anaemic offspring showed e.g. fatty livers, chylous serum lipaemia and decreased haematopoiesis in the spleen. Except anaemia, these effects were not observed in pups exposed to dimoxystrobin. According to Roth & Smith no defined cardiomegaly was observed, but increased cardiac weights in offspring and dams. In contrast, an increase in heart weights was apparently not observed in adult animals after administration of dimoxystrobin in repeated dose studies.
In two developmental toxicity studies in rabbits, ventricular septal defects were described. In the first study this finding was not clearly dose-dependent (0/0, 3/3, 2/2 and 0/0) (Schilling et al., 2001). However, it should be noted that at maternal toxic dose level of 100 mg/kg bw/d) the post-implantation loss was nearly twice that of the control and therefore ventricular septal defects could have been undetected. In the second study (Schneider et al., 2001) at maternal toxic dose level of 75 mg/kg bw/d both pre- and post-implantation losses were increased and septal defects were observed in 3 animals out of 3 litters (control 1/1). Although these values are within the HCD, the current control groups should not be disregarded, especially since the heart was also identified as a target organ in the 2-generation study.

Conclusion:
In both developmental toxicity studies (rabbit) and the 2-generation reproductive toxicity study (rat) the target organ was the heart. The conclusion of DS Hungary that observed cardiomegaly has been induced during lactation in the 2-generation study is not excluded on our part, but additional data are considered necessary for a clear assessment, e.g. cross-fostering studies as well as the determination of iron and dimoxystrobin in rat milk.

Remark:
In the summary table 24 (p. 31/32) the description of the two-generation reproductive toxicity study appears not correct. The effects at 500 ppm and 150 ppm which occurred in F1 dams and F2 offspring are missing. Furthermore it can be taken from the summary table 24 that at 150 ppm 'impaired body weight and body weight gain' was observed in F1 pups. However, in table 35 (p. 44) there is no statistically significant effect on bw in F1 pups, but there is an effect on bw of F2 pups at 150 ppm (table 16, p. 46).

Adverse effects on or via lactation
In table 16 (p. 46) a statistically significant reduced in bw of F2 pups (150 ppm) was observed on PND 7 which cannot be explained by self-feeding which is argued to start at PND 12. Such an effect was not observed at PND 1 and 4 and also not in F1 dams. Thus, an effect mediated by lactation should be considered.
With reference to the CLP Regulation (criterion b) the transfer in the milk or adverse effects on the quality of the milk have to be taken into account.

Transfer in the milk:
The milk of lactating goats (up to 288 ppm or 10.3 mg/kg bw/d, 5 days) and cows (up to 25 ppm or 0.64 mg/kg bw/d, 30 days) did not contain dimoxystrobin (or metabolites) or very low amounts only. It is argued in the CLH report that the applied doses were very low, however the highest dose applied to goats was in the dose range inducing effects on e.g. body weight in rats. The transfer of dimoxystrobin (or metabolites) into the milk has not been measured in rats. Therefore, and due to known species differences in milk composition, no reliable statement is possible.

Quality of milk:
Decreased iron concentration in milk was observed as consequence of an induced iron deficiency in lactating rats (Roth & Smith, 1988; Anaokar, 1981). Dimoxystrobin decreased iron levels in serum and offspring. Thus, it is considered plausible, that the quality of milk is affected via a decreased iron level. This could explain the above mentioned effect on bw of F2 pups at PND 7. However, determination of iron in rat milk has not been provided.

Conclusion:
Based on the statistically significant reduced body weight observed in pups on PND 7 the additional classification with H362 (may cause harm to breast-fed children) is considered justified.

Dossier Submitter's Response

Thank you for your comment.

Therefore, the dossier should provide sufficient information on the number of examined pups/adults to come to a clear conclusion on this point.
Response: According to the study of the 2-generation toxicity study: On day 4 p.p., the individual litters were in general standardized in such a way that, where possible,each litter contained 4 male and 4 female pups (always the first 4 pups/sex and litter were taken for further rearing). If it was not possible in single litters to have 4 pups/sex, it was proceeded in such a way that 8 pups per litter were present for further rearing (e.g. 5 male and 3 female pups). Standardization of litters was not performed in litters with ≤ 8 pups. With the exception of the F1 generation pups, which were chosen as the basis of the F1 generation parental animals, all pups were sacrificed (by means of CO_2) after standardization or weaning. These pups, including stillborn pups and those that died

during their rearing period, were subjected to a macroscopic (external and visceral) examination.
Cardiomegaly is determined at pup necropsy. Cardiomegaly was only seen in F1 or F2 pups sacrificed at PND 21. In none of the F0 or F1 pups, sacrificed or died at PND < 1 (including stillborns) or at PND 4 at any dose group, cardiomegaly was detected. The number of pups undergoing necropsy is indicated in the table below. All PND <1 or PND 4 pups were categorized as "nothing abnormal detected" with the exception of a few pups showing "dilated renal pelvis" (3, 5, 6, 6, 3 in the F1 controls, 50, 150, 500, 1200 ppm dose groups and 3, 1, 2, 3, 1 in the F2 controls, 50, 150, 500, 1200 ppm dose groups) or having shown post-mortem autolysis.

Table: Number of F1 and F2 pups examined for pup necropsy at days < 4 and 4 (for details see individual pup necropsy data pages: 473 – 513 and 744 – 788, study report 2000/1016869)

Death at PND	Controls	50 ppm	150 ppm	500 ppm	1200 ppm
	F1 pups				
< 4	10	16	14	14	32
4	117	113	134	128	112
	F2 pups				
< 4	8	18	16	12	24
4	144	105	136	111	66

All F1 parental animals were necropsied and assessed by gross necropsy: 25 male and 25 female animals of controls and the 50, 150, 500, 1200 ppm dose groups. The only finding to the heart was one isolated heart dilation in one male animal of the 500 ppm dose group. This is considered to be an incidental findings. No further effects in the heart were observed in any of the animals. These observations provide clear evidence, that cardiomegaly is not an effect occurring after in utero exposure, but only after postnatal and/or lactational exposure to dimoxystrobin or to milk containing insufficient iron concentrations. Furthermore, cardiomegaly was shown to be transient.

According to Roth & Smith no defined cardiomegaly was observed, but increased cardiac weights in offspring and dams. In contrast, an increase in heart weights was apparently not observed in adult animals after administration of dimoxystrobin in repeated dose studies.
Heart weights were not determined in the offspring or adult animals of the 2-generation toxicity study. However, it is assumed, that the individual pups – investigated at PND 21, which showed cardiomegaly – also have higher heart weights compared to controls. Heart weights were not determined in the 90-day studies in rats and mice and in the chronic and carcinogenicity rat study. In the chronic mouse study, relative heart weights were slightly increased at the top dose (+15.9%), however in the absence of a histopathological correlate and as the absolute heart weights were slightly decreased compared to controls (- 6%), this was considered to be not treatment-related.

Ventral septum effects in rabbit developmental toxicity studies
The ventral septum effects seen in rabbit development toxicity studies is clearly within historical controls. The heart is a target organ in offspring rats, only when young rats are directly dosed to dimoxystrobin or when offspring animals are dosed to milk with presumably lower iron content, as shown in rat generation toxicity study. In the rat prenatal developmental toxicity study with only in utero exposure, no effects on the heart

were detected. It can thus be concluded, that the heart is not a target organ after in-utero exposure to dimoxystrobin.

Remark on the tables
Thanks for spotting this. Table 24 in the CLH Dossier needs to be updated, also more precisely describing the body weight effects seen in the 150 ppm dose group in the F2 pups only (not in F1 pups), which is due to an overall higher dimoxystrobin intake in F2 pups compared to F1 pups.
The results in Table 24 CLH Dossier are to be corrected into:
1200 ppm (M: 109 mg/kg bw; F, premating: 119; gestation: 103; lactation: 168 mg/kg bw/day) **F0 adults/F1 pups:**
Parental toxicity: reduced food consumption, impaired body weight and body weight gain, increased number of females with stillborn pups (within historical control range), decreased live birth index (within historical controls)
Pup toxicity: increased no. of stillborn (within historical control range), increased number of pups died (within historical control range), impaired body weight and body weight gain starting at PND 4, delays in male and female sexual maturation occurring secondary to decreased offspring body weights, decreased relative weights of thymus and spleen
Pup necropsy observations: hypoplasia of thymus, yellowish liver discoloration, cardiomegaly (in PND 21 pups only; not in PND4 pups), milky fluid in abdomen

1200 ppm (M: 156 mg/kg bw; F premating: 159, gestation: 108; lactation 168 mg/kg bw) **F1 adults/F2 pups**
Parental toxicity: reduced food consumption, impaired body weight and body weight gain, decreased number of implants (secondary to decreased maternal body weight), decreased number of pups delivered (within historical control range)
Pup toxicity: Increased number of pups died (within historical control range), impaired body weight and body weight gain starting PND 4, decreased relative weights of thymus and spleen
Pup necropsy observations: yellowish liver discoloration and cardiomegaly (in PND 21 pups only, not in PND4 pups), hypoplasia of thymus, milky fluid in abdomen

500 ppm (F0 M: 47 mg/kg bw; F premating: 50, gestation: 44; lactation 75 mg/kg bw; F1 M: 62 mg/kg bw; F premating: 64, gestation: 46; lactation 75 mg/kg bw) **F0 and F1:**:
Parental toxicity: reduced food consumption, impaired body weight and body weight gain
Pup toxicity: impaired body weight and body weight gain, delays in male and female sexual maturation occurring secondary to decreased offspring body weight, decreased relative weights of thymus and spleen
Pup necropsy observations: yellowish liver discoloration, cardiomegaly (in PND 21 pups only, not in PND4 pups)

150 ppm (F0 M: 14 mg/kg bw; F premating: 16, gestation: 14; lactation 22 mg/kg bw; F1 M: 18 mg/kg bw; F premating: 19, gestation: 14; lactation 22 mg/kg bw): **F0 and F1:**
Parental toxicity: No treatment-related effect
Pup toxicity: Impaired body weight and body weight gain in F2 pups only (dimoxystrobin intake was higher in F2 compared to F1 pups)
Pup necropsy observations: yellowish liver discoloration in each one F1 and F2 litters, cardiomegaly in one F1 litter (in PND 21 pups only, not in PND 4 pups), not in F2 litter

50 ppm (F0 M: 5 mg/kg bw; F premating: 5, gestation: 5; lactation 8 mg/kg bw; F1 M: 6 mg/kg bw; F premating: 6, gestation: 5; lactation 7
mg/kg bw): **F0 and F1:**

Parental toxicity: No treatment-related effect Pup toxicity: No treatment-related effect NOAEL (Systemic toxicity (parents): 150 ppm (17 mg/kg bw/day) NOAEL (Developmental toxicity): 50 ppm (5 mg/kg bw/day in adults; however, estimated actual doses in pups are 12 mg/kg bw/day due to simultaneous self-feeding) BMD calculations for body weight effects in F1 adult females and F2 pups: BMDL05 (females, PND 21): 25.5-45.3 mg/kg bw, (using measured substance intakes during lactation); BMDL05 (pups, PND 21): 39.8 mg/kg bw (using estimated substance intakes for PND 21)
RAC's response
Thank you very much for your comments. RAC notes that the database still does not allow concluding that cardiomegaly was not an issue of concern. RAC also notes that there is no clear experimental evidence of adverse effect in the offspring due to transfer of dimoxystrobin in the milk or adverse effect on the quality of the milk.

Date	Country	Organisation	Type of Organisation	Comment number
29.08.2019	Netherlands		MemberState	5
Comment received				
The NL MSCA agrees with the proposed 'no classification' for adverse effects on sexual function and fertility. It is noted that in the 2-generation study: - a significant decreased number of implantations in the high dose group of F1 dams was observed. As no lab historical control data were available, HC data from literature were used to neutralize this statistically significant observation; - effects on the ovarian follicle were observed (increases F0 and statistical significant decreases in F1) and these effects were regarded to be within the biological variability, but no HC data for this parameter were given. With respect to the removal of the Repr. 2 classification for adverse effects on development and the addition of the Lact. classification, there are some uncertainties. The following is noted: - As described in the CLH-report, current Repr. 2 (H361d) classification is apparently based on effects on body weight, heart (cardiomegaly) and blood (anemia), with an assumed higher susceptibility of offspring compared to adults for these effects. - The results of the prenatal developmental toxicity study in rat do not provide an indication for adverse effects on development. In the rabbit prenatal developmental toxicity, some adverse effects on development are noted. However, these were observed at the high dose only, and at incidences that were within the HCD range, showing no dose-response or not attaining statistical significance. Moreover, the high dose levels were clearly maternally toxic. So, it is agreed that also the prenatal developmental toxicity studies in rabbit do not warrant classification. - When looking at the multigeneration studies, dimoxystrobin treatment resulted in adverse effects on development in both the 2-generation study and the modified one-generation study. No developmental toxicity occurred in the extended one-generation study, though noticing that in this study dimoxystrobin was tested at relatively low dose levels, and that these levels hardly, if at all, induced general toxicity. - Iron-deficient anemia can be considered a general effect of dimoxystrobin, also observed in the repeated dose studies. It is noted that the enhanced one-generation			

study presented similar NOAELs for anemia for both parental as well as offspring. This was however the highest dose tested (4.3 mg/kg bw/d). Therefore, the true NOAEL can be higher and a difference in true NOAELs between parental animals and offspring is possible. - A reduction in pup body weight was noted postnatally with consistency seen over two generations in the 2-generation study and, additionally, also seen in the modified one-generation study. The pup body weight effects were absent at birth. Further, these body weight effects occurred, at least partly, during a time period where milk is the only nutrition source for pups. It is however noted that maternal toxicity was also present during the lactation period in both studies. It may be questioned whether the pup body weight effects should be considered as direct or indirect effects, and thereby affecting the proposed Lact. Classification for this type of effect.
Dossier Submitter's Response
Thank you for your comment and support. *Effects on implantation sites and ovarian follicle count* From public literature, there is evidence that decreased maternal body weight before and during gestation (as observed in feed restriction studies) impacts the numbers of implants and of corpora lutea (see Terry et al., 2005). This further strengthens the case, that a classification for fertility is not justified for dimoxystrobin. *Iron-deficient anemia can be considered a general effect of dimoxystrobin, also observed in the repeated dose studies. It is noted that the enhanced one-generation study presented similar NOAELs for anemia for both parental as well as offspring. This was however the highest dose tested (4.3 mg/kg bw/d). Therefore, the true NOAEL can be higher and a difference in true NOAELs between parental animals and offspring is possible.* The more recently conducted repeated dose and enhanced one-generation toxicity studies were conducted based on requests of a non-European Authority in order to show, that at the dose level of 50 ppm dimoxystrobin does not cause anemia or iron level suppression in young animals and/or offspring. These studied had been conducted in the years 2010/2011. These lower dose level had not been investigated in the originally submitted mechanistic one-generation toxicity study dosed with 150, 500 and 1200 ppm (study conducted in 2000) only. This older mechanistic study had the purpose to further explain the observed cardiomegalies and pale-yellowish livers detected in the PND 21 offspring and contained thus additional hematological investigations in dams and offspring, which are not required parameters in an OECD TG 416. However, indications for anemia had been determined down to the lowest administered dose of dimoxystrobin in dams and offspring and no NOAEL was identified. In the more recent enhanced one-generation toxicity study, no anemia or decreased serum iron levels were detected in dams or offspring at the doses of 50 ppm (the next lower dose of the original 2-generation toxicity study) and thus considered the overall NOAEL for anemia and serum iron level changes) in adults and offspring.
RAC's response
Thank you very much for your comments. RAC notes that the database still does not allow concluding that cardiomegaly was not an issue of concern.

Date	Country	Organisation	Type of Organisation	Comment number
25.08.2019	Germany	<confidential>	Company-Manufacturer	6
Comment received				
Dimoxystrobin is currently under the EFSA pesticide peer review process for reregistration. During the commenting period of the Draft Assessment Report of dimoxystrobin (which is the basis for the Annex I to the CLH Dossier of dimoxystrobin), the notifier/manufacturer was asked to provide additional tabulated summaries/information/data relevant for the assessment of the reproduction toxicity studies (generation toxicity studies in rats and prenatal developmental toxicity studies in rats and rabbits). As these information are also relevant for classification of dimoxystrobin and as EFSA has asked the notifier/manufacturer to provide all information relevant for classification of dimoxystrobin to ECHA, the following additional attachments are provided by the notifier/manufacturer. This is submitted in this way, as the size limitation of the Zipfile of 10 MB was exceeded. - Historical control data rabbit prenatal developmental toxicity studies April 1999 - November 2003 (DocID 2013/1421980) - are submitted in the Zip File 2 - Historical control data rabbit prenatal developmental toxicity studies April 1997 - April 2002 (DocID 2019/2044955 - are submitted in the Zip File 2 - Historical control data rabbit prenatal developmental toxicity studies May 1994 - October 2000 (DocID 2019/2044956 - are submitted in the Zip File 2 ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment ECHA-25 August2019-2.zipx				
Dossier Submitter's Response				
Thank you for your contribution.				
RAC's response				
Thank you very much for your contributions. Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
25.08.2019	Germany	<confidential>	Company-Manufacturer	7
Comment received				
Dimoxystrobin is currently under the EFSA pesticide peer review process for reregistration. During the commenting period of the Draft Assessment Report of dimoxystrobin (which is the basis for the Annex I to the CLH Dossier of dimoxystrobin), the notifier/manufacturer was asked to provide additional tabulated summaries/information/data relevant for the assessment of the reproduction toxicity studies (generation toxicity studies in rats and prenatal developmental toxicity studies in rats and rabbits). As these information are also relevant for classification of dimoxystrobin and as EFSA has asked the notifier/manufacturer to provide all information relevant for classification of dimoxystrobin to ECHA, the following attachments are provided by the notifier/manufacturer: - Compiled document with the additional tabulated summaries/information/data relevant for the assessment of the reproduction toxicity studies (generation toxicity studies in rats and prenatal developmental toxicity studies in rats and rabbits) (containing additional tables on body weight, body weight developments, food consumption, result tables including all relevant historical control data, additional individual data and information to assess sperm parameters, female reproduction and entry into puberty) and acute toxicity studies (DocID 2019/2047163)				

- Historical control data rabbit prenatal developmental toxicity studies April 1999 - November 2003 (DocID 2013/1421980) - are submitted in the Zip File 2 - Historical control data rabbit prenatal developmental toxicity studies April 1997 - April 2002 (DocID 2019/2044955 - are submitted in the Zip File 2 - Historical control data rabbit prenatal developmental toxicity studies May 1994 - October 2000 (DocID 2019/2044956 - are submitted in the Zip File 2 - Historical control data rat prenatal developmental toxicity studies January 1994 - June 1999 (DocID 2019/2044957 - Historical control data pup necropsy observations from reproduction toxicity studies January 2008 - December 2014 (DocID 2019/2046312) - Benchmark Dose Calculations on body weight effects in dams and offspring of the 2-generation toxicity study (DocID 2015/1172904) - Benchmark Dose Calculations on body weight effects in dams and offspring of the 2-generation toxicity study (EPA BMDS Software 3.1.1) (DocID 2019/2046321) - Graphical analysis of individual male entry into puberty (preputial separation - PPS) data correlated with body weight (1200 ppm dimoxystrobin dose vs control) (DocID 2019/2044949) - Graphical analysis of individual male entry into puberty (preputial separation - PPS) data correlated with body weight (500 ppm dimoxystrobin dose vs control) (DocID 2019/2044950) - Graphical analysis of individual female entry into puberty (Vaginal opening - VO) data correlated with body weight (1200 ppm dimoxystrobin dose vs controls) (DocID 2019/2044951) - Graphical analysis of individual female entry into puberty (Vaginal opening - VO) data correlated with body weight (500 ppm dimoxystrobin dose vs controls) (DocID 2019/2044952) Publications: - Terry et al 2005 (DocID 2019/2045629) - Da Silva Faria et al 2004 (DocID 2019/2045813) - Carney et al 2004 (DocID 2004/1041034 - Chernoff et al 2009 (DocID 2009/1132002) - Melching-Kollmuss et al 2014 (DocID 2014/1326033) ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment ECHA-25 August 2019-1.zipx
Dossier Submitter's Response
Thank you for your contribution.
RAC's response
Thank you very much for your contributions. Noted.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
25.08.2019	Germany	<confidential>	Company-Manufacturer	8
Comment received				
A more detailed summary of the acute toxicity studies has also been provided during the EFSA pesticide peer review process and is submitted with the word document (DocID 2019/2047163) included in the zip file. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment ECHA-25 August 2019-1.zipx				

Dossier Submitter's Response
Thank you for your contribution.
RAC's response
Thank you very much for your contributions. Noted.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
27.08.2019	Germany		MemberState	9
Comment received				
The comparison with the guidance value (p. 72/73) is focussed on haematological effects of the modified one-generation reproduction toxicity study at 500 ppm (57 mg/kg). It should be noted that the small reduction in MCV and MCH in males occurred only at day 29 and not at day 98 (table 18 (p. 47/48)). The small reduction in MCH and MCHC in females was only observed at day 100 (not at day 29). A STOT RE-classification needs significant or severe effects which are not clearly given here. It is furthermore argued in the CLH-report (p. 73) that the anaemia was not completely reversible after a recovery period. This was observed in rats receiving a higher dose of 4500 ppm (3 w treatment, 2 w recovery). The calculated dose was 232 mg/kg (p. 34). In parallel a further group received 4500 ppm for 5 w (calculated dose: 264 mg/kg). The observed haematological were pronounced and are described as severe anaemia in the CLH-report. The guidance value (STOT RE 2) for a 4 w study is 30 < C ≤ 300 mg/kg. Adjusting the dose of the 5 w group (264 mg/kg) to 4 w a dose of 330 mg/kg is calculated slightly above the guidance value. Adjusting the dose of the 3 w group (232 mg/kg) to 4 w a dose of 174 mg/kg results well in the range of the guidance value. Conclusion: With regard to STOT RE 2 the comparison with guidance value is currently focused on doses that did not clearly evoke significant/severe effects as requested by the CLP Regulation. It is recommended to consider not only the doses of the modified one-generation reproduction toxicity study, but also those of the other studies (e.g. short-term toxicity studies in rats, mice and dogs) in case of significant/severe toxicity.				
Dossier Submitter's Response				
Thank you for your comment. 90-day rat study: 103/121 mg/kg bw: thickening of duodenal mucosa in few males and females; mean corpuscular volume (MCV)↓in females (-3%, statistically significant); These doses are slightly above the trigger for STOT RE 2; 21/24 mg/kg bw: thickening of duodenal mucosa in one female. This is not significant/severe toxicity. 90-day mouse study: No substance-related effects at 206/318 mg/kg bw. 90-day dog study: 36.8/37.7 mg/kg bw: unspecific toxicity (body weight, food consumption, diarrhea, vomitus), statistically significant mean corpuscular volume at at least one time point (MCV)↓ (-6%), mean corpuscular haemoglobin (MCH)↓ (-7%), mean corpuscular haemoglobin concentration (MCHC)↓ (-7%) 1-year dog: 22.3/22.7 mg/kg bw: unspecific toxicity, no effects on hematology. Chronic rat and mouse studies: Alle effects were clearly above the triggers for classification with STOT RE 2 of 12.5 mg/kg bw.				

RAC's response
Thank you very much. RAC notes that large reductions in iron concentration in blood are resulting in modest (not higher than 20%) alterations in heametological parameters and moreover the effect does not progress over time. Thus RAC notes that these effects are not of sufficient severity to warrant classification as STOT RE.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
27.08.2019	France		MemberState	10
Comment received				
FR agrees with the proposal of classification for environmental hazards and the proposals of acute and chronic M factors.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
The support is noted.				

Date	Country	Organisation	Type of Organisation	Comment number
27.08.2019	Germany		MemberState	11
Comment received				
The German CA agrees with the proposal of classification for environmental hazards as Aquatic Acute 1 (H400), Aquatic Chronic 1 (H410) and the acute/chronic M-factor of 100. In the chronic fish study (Anonymous 1999b, 1999/10521) there is at 0.001 mg/L a 30 % decrease in wet weight and a 13 % decrease in body length, both significantly different compared to the control. Additionally there is a 13 % lower survival of young fish in the study at 0.01 mg/L. The LOEC in the study is determined at the tested concentration of 0.001 mg/L and the NOEC at 0.000316 mg/L. This NOEC should be used for the chronic classification.				
Dossier Submitter's Response				
Thank you for your support for the environmental classification and the acute/chronic M-factor of 100. *In the chronic fish study (Anonymous 1999b, 1999/10521) ... The LOEC in the study is determined at the tested concentration of 0.001 mg/L and the NOEC at 0.000316 mg/L. This NOEC should be used for the chronic classification*. As part of a recent Request for Additional Information, the Applicant submitted new information to EFSA regarding the study by Anonymous 1999b, 1999/10521. The test laboratory prepared a GLP-compliant amendment to this report (Anonymous 2019, 2019/2047112) which identified two critical points with the original report. First, the original statistical evaluation used the individual fish as the statistical unit to evaluate this study, contrary to current guideline recommendations which require that the replicate aquarium mean value is used as the statistical unit. Based on a proper statistical evaluation using the replicate aquarium as the statistical unit, the LOEC was 0.00316 mg/L and the NOEC was 0.001 mg/L. Second, the control fertility rate was incorrectly reported as 74% in the original report, but a re-evaluation of the raw data showed that the correct fertility rate was 94%. Considering				

this fertility rate, the calculated hatching success in the control replicates as well as the mean hatching success were **below the control survival validity criterion** stipulated in the OECD TG 210 (1992) (>66%), the US EPA OPPTS 850.1400 (1996) (>66%) and OECD TG 210 (2013) (≥75%). A study which does not meet test guideline validity criteria should not be considered as basis for classification.

Nevertheless, this new information does not change the overall classification.

RAC's response

The support is noted. Agree with the DS response. Based on the data submitted by the applicant as part of a recent Request for Additional Information to EFSA, the study has hatching success of 66% which is below to the criteria set up in OECD TG 210 (2013) (≥75%) and equal to the criteria set up in the OECD TG 210 (1992) (>66%) and the US EPA OPPTS 850.1400 (1996) (>66%) guidelines. The rest of the validity criteria on dissolved oxygen concentration, the water temperature range, the use of analytical measure of the test concentrations and the post hatching success (93.5% for this study) are fulfilled. In respect to the temperature, the temperature variation (2 °C) is within the range prescribed in the guidelines (OECD2010=± 1.5, EPA OPPTS 850.1400=± 2). Also, a 0.5 °C deviation measured on a single day isn't expected have a significant effect on trout hatchery. The study also resulted in a hatching success of 66% which is below to the criteria set up in OECD TG 210 (2013) (≥75%) and equal to the criteria set up in the OECD TG 210 (1992) (≥66%) and the US EPA OPPTS 850.1400 (1996) (≥66%) guidelines. For controls the range of hatching success for the four replicates was 51.1% to 76.6% at day 39 and based on the viable fertilised eggs at day 14 (94%). It's worth noting that in the RAR2017 the hatching success was reported to be 83.8% at 39 days and thus the study was not questioned for validity. Based on the abovementioned discussion of the data the ELS study by Anonymous, 1999(a) the study still could be considered as valid for classification purposes, but with some reservations.

Date	Country	Organisation	Type of Organisation	Comment number
30.08.2019	Belgium		MemberState	12

Comment received

BE CA supports the proposal of environmental classification of Dimoystrobin:
Aquatic Acute 1, H400 and M=100
Aquatic Chronic 1, H410 and M=100

Aquatic acute toxicity
Even though it has no impact on the proposed M-factor because the result is in the same range of the key algae study, we don't agree that the reliable and valid acute guideline study with Crassostrea virginica cannot be used for classification purposes. In the CLP-guidance (I.3.1. Acute toxicity) it is mentioned that data on other species (than fish, crustacea and algae/aquatic plants) shall also be considered if the test methodology is suitable. The results with the highest toxicity should than be chosen.

Aquatic chronic toxicity
Idem for the chronic Chironomus riparius study which should not systematically be disregarded for classification purposes as mentioned in the CLH report.
However, for this specific case we are of the opinion that the Dohmen G.P, 2001study which uses spiked water is not suitable for chronic classification purposes:
- The test is performed under static regime. Only initially measured concentrations are

given in the CLH report and annex I. Because the substance disappeared rapidly from the water phase (only 18-32% detected after 28d) to the sediment, a NOEC based on the end of test concentration (if available) would have been more preferable (CLP guidance, I.4.3).
- furthermore, the substance has the potential to adsorb to sediment particles (Koc between 195.8-935.3 mg/L) and thus exposure via sediment (ingestion) cannot be ruled out.

Furthermore we question the 97dNOAEC of 0.001mg/L in the Anonymous, 2000/5125-study with Oncorhynchus mykiss where it is concluded that there is no statistically or biologically significant difference to the control after taking into account of the higher number of surviving fish and the total biomass and length (sum of weight and length per replicate) at 0.001 mg/L. Nevertheless without this correction a significant effect on body length was seen at 0.001 mg/L. Therefore we are of the opinion that the 97dNOEC to be used for classification and labelling should be 0.000316 mg/L instead of 0.001 mg/L. This will however not impact the proposed chronic M-factor of 100 (NRD, 0.0001<NOEC≤0.001).

Even though not a key study, it would have been more appropriate to base the results of the Lemna gibba study on the geometric mean concentration instead of the initial concentration because concentrations were not maintained throughout the test. In annex I of the CLH report it is mentioned that analysed concentration were 88.8% to 101.5% of nominal at test initiation and from 59.4% to 98.4% of nominal at test termination. It is however not stated which exposure concentration(s) dropped under the 80% of nominal.

Some editorial or/and minor comments :
- Table 39: summary of relevant information on bioaccumulation
BCF of 48 should read BCF of 84
- Table 40: summary of relevant information on acute aquatic toxicity
Difference in testing regime (static, flow through) between table and description of study
- Anonymous 1998/10601
- Anonymous 2000/5125
- Anonymous 2000/5092
- Annex 1, 4.5.2 Study 2 – Development and emergence toxicity study in Chironomus riparius
Typo : Guideline 204? However this guideline is a fish, Prolonged Toxicity Test: 14-Day Study
Table 41 of the CLH report however mentions that the study is performed according to the BBA draft guideline (1995) which mainly forms the basis for OECD 219 (sediment-water Chironomid Toxicity Testing using spiked water)

Dossier Submitter's Response

Thank you for your support for the environmental classification.

Aquatic Acute 1, H400 and M=100
Aquatic Chronic 1, H410 and M=100
Agreed.

…we don't agree that the reliable and valid acute guideline study with Crassostrea virginica cannot be used for classification purposes. In the CLP-guidance (I.3.1. Acute toxicity) it is mentioned that data on other species (than fish, crustacea and algae/aquatic plants) shall also be considered if the test methodology is suitable.

We agree that the language in the CLP-guidance allows data on other species to be considered if the test methodology is suitable. However, the test method is not completely suitable for an acute aquatic endpoint. Although the test method is identified as an acute test it determines both lethal (mortality) and sub-lethal (growth) endpoints. Only the lethal endpoint should be considered for acute toxicity whereas the sub-lethal endpoint should be considered as a chronic effect.

However, for this specific case we are of the opinion that the Dohmen G.P, 2001 study which uses spiked water is not suitable for chronic classification purposes…
Agreed.

Furthermore we question the 97dNOAEC of 0.001mg/L in the Anonymous, 2000/5125-study with Oncorhynchus mykiss…
As part of a recent Request for Additional Information, the Applicant submitted new information to EFSA regarding the study by Anonymous 1999b, 1999/10521. The test laboratory prepared a GLP-compliant amendment to this report (Anonymous 2019, 2019/2047112) which identified two critical points with the original report.

First, the original statistical evaluation used the individual fish as the statistical unit to evaluate this study, contrary to current guideline recommendations which require that the replicate aquarium mean value is used as the statistical unit. Based on a proper statistical evaluation using the replicate aquarium as the statistical unit, the LOEC was 0.00316 mg/L and the NOEC was 0.001 mg/L.

Second, the control fertility rate was incorrectly reported as 74% in the original report, but a re-evaluation of the raw data showed that the correct fertility rate was 94%. Considering this fertility rate, the calculated hatching success in the control replicates as well as the mean hatching success were **below the control survival validity criterion** stipulated in the OECD TG 210 (1992) (>66%), the US EPA OPPTS 850.1400 (1996) (>66%) and OECD TG 210 (2013) (≥75%). A study which does not meet test guideline validity criteria should not be considered as basis for classification.

Nevertheless, this new information does not change the overall classification.

Even though not a key study, it would have been more appropriate to base the results of the Lemna gibba study on the geometric mean concentration instead of the initial concentration because concentrations were not maintained throughout the test.
Agreed. However, **the study does not meet the validity criteria** in the current test guideline EPA 850.4000 (2012) because the doubling time of number of fronds in the control is higher than 2.5 days (observed 2.9 and 3.2 days for the time period of 0-7 and 0-14 days separately) and should therefore not be considered for classification.

Some editorial or/and minor comments:
- Table 39: summary of relevant information on bioaccumulation
BCF of 48 should read BCF of 84
Agreed.

Table 40: summary of relevant information on acute aquatic toxicity
Difference in testing regime (static, flow through) between table and description of study
- Anonymous 1998/10601
- Anonymous 2000/5125
- Anonymous 2000/5092

The narrative description of the exposure regime in the CLH report is incorrect for these studies. The correct testing regime for the mentioned studies are indicated in Table 40 as follows: - Anonymous 1998/10601 - static - Anonymous 2000/5125 – flow through - Anonymous 2000/5092 – flow through *Annex 1, 4.5.2 Study 2 – Development and emergence toxicity study in Chironomus riparius* *Typo: Guideline 204? However this guideline is a fish, Prolonged Toxicity Test: 14-Day Study. Table 41 of the CLH report however mentions that the study is performed according to the BBA draft guideline (1995) which mainly forms the basis for OECD 219 (sediment-water Chironomid Toxicity Testing using spiked water).* Agreed. OECD TG 204 is incorrect. The correct guideline reference is OECD TG 219.
RAC's response
The support is noted. Agree with the DS response. See also response on comment 11.

Date	Country	Organisation	Type of Organisation	Comment number
24.08.2019	United Kingdom		MemberState	13
Comment received				
Dimoxystrobin (EC: -; CAS: 149961-52-4) Chronic toxicity to fish: The basis of the proposed Aquatic Chronic classification is the 97 d NOAEC mortality of 0.0010 mg/L for Oncorhynchus mykiss, which is expressed as a nominal concentration (Anon., 1999). The study report determined a NOEC for fish mean body length which was 0.000316 mg/L (nominal). The CLH report cites information presented in the DAR (2005) which rejects this NOEC endpoint on the basis that the effect, while statistically significant, is minimal and there is no statistical difference when the sum of fish length is considered. The endpoint based on 'sum of lengths' is unusual and is not in the OECD 201 test guideline. Rather, the mean or median values for the length of the fish in each test group should be statistically compared to the control according to OECD 201. Equally, it is unclear what the sum of fish lengths approach represents because neither the raw data nor a detailed description are presented in the DAR or the CLH report. We note that the 2017 RAR does not consider the sum of fish lengths approach and includes the NOEC for fish mean body length at 0.000316 mg/L. We consider that the NOEC for fish length of 0.000316 mg/L based on mean body length is more appropriate than the present mortality NOEC as mean length at all test concentrations above 0.000316 mg/L with surviving fish were statistically significantly lower than the control and there is a negative concentration-response relationship. In addition, actual concentrations ranged from 51.5-158.2% of the nominal (RAR, 2017) and therefore, geometric mean measured concentrations should be used. Algae toxicity (Wyskiel et al., 2000): The key endpoint for the proposed Aquatic Acute classification is a 72 h ErC50 of 0.0078 mg/L for Navicula pelliculosa based on mean measured concentrations (Wyskiel et al., 2000). Measured concentrations ranged from 85.1-112.8% of nominal concentrations at test initiation and from 101.2-173.3% of the nominal at test termination at 120 h. The CLH				

report does not mention analytical measurement during the study. Therefore, we are uncertain of how the 72 hour mean measured concentrations were calculated and whether they are reliable. Please can you describe how 72 hour mean measured endpoints were calculated?
The RAR includes cell data for the 3 solvent controls and procedural controls. The initial cell concentration was less than the OECD TG 201 recommendation but we note that cell numbers did meet the x16-fold increase over 72 hours. We note that the 24 hour cell measurements are all quoted as <10000 – please can you clarify if actual cell counts were taken at this time point or if the methodology was unable to consider <10000 cells. Also, other cell counts are identical for different replicates at the same time. Whilst this may be the case, please can you provide details of the counting method and its suitability? This information is relevant to confirm the validity of the study controls.
A 120 hour NOErC of 0.0012 mg/L based on mean measured concentrations was obtained from the study. We are unsure how suitable this value is for classification purposes because the exposure period is longer than the standard 72 – 96 hour duration and we are unclear if controls met both validity criteria and exponential growth over the whole exposure period up to 120 h.
From Table B.9.2.6.2-1 in the RAR (2017), it is unclear what the 72 hour NOErC was. Please can the DS provide a 72 h NOErC and / or EC10? Depending on information to support the use of 72 hour mean measured concentrations, it may be appropriate to consider the surrogate approach using the 72 hour ErC50 from this study if a 72 hour NOErC/EC10 cannot be determined.

Dossier Submitter's Response

Thank you for your comment.

Chronic toxicity to fish:
Agreed, the endpoint based on 'sum of lengths' is an unusual metric for chronic fish toxicity and is not mentioned in the OECD TG 210. As part of a recent Request for Additional Information, the Applicant submitted new information to EFSA regarding the study by Anonymous 1999b, 1999/10521. The test laboratory prepared a GLP-compliant amendment to this report (Anonymous 2019, 2019/2047112) which identified two critical points with the original report.

First, the original statistical evaluation used the individual fish as the statistical unit to evaluate this study, contrary to current guideline recommendations which require that the replicate aquarium mean value is used as the statistical unit. Based on a proper statistical evaluation using the replicate aquarium as the statistical unit, the LOEC was 0.00316 mg/L and the NOEC was 0.001 mg/L.

Second, the control fertility rate was incorrectly reported as 74% in the original report, but a re-evaluation of the raw data showed that the correct fertility rate was 94%. Considering this fertility rate, the calculated hatching success in the control replicates as well as the mean hatching success were **below the control survival validity criterion** stipulated in the OECD TG 210 (1992) (>66%), the US EPA OPPTS 850.1400 (1996) (>66%) and OECD TG 210 (2013) (≥75%). A study which does not meet test guideline validity criteria should not be considered as basis for classification.

Nevertheless, this new information does not change the overall classification.

Algae toxicity (Wyskiel et al., 2000):
The CLH report does not mention analytical measurement during the study. Therefore,

we are uncertain of how the 72 hour mean measured concentrations were calculated and whether they are reliable. Please can you describe how 72 hour mean measured endpoints were calculated?

Exposure concentrations were determined during the study at 0 and 120-h and are summarised below along with the mean. Since concentrations remained stable over 120-h in all but the lowest treatment, they are considered an accurate representation of the mean exposure concentrations at 72-h.

Table 2. Measured concentrations of BAS 505 F during the toxicity test with the freshwater alga, *Navicula pelliculosa*.

Nominal Concentration of BAS 505 F (µg/L)	Measured Concentration of BAS 505 F (µg/L)			
	0 Hour	120 Hour	Mean	% Recovery
Test Media				
0 (control)	ND[1]	ND[3]	ND	---
0 (solvent control)	ND	ND	ND	---
0.86	0.97	1.19, 1.49[4]	1.22	142
1.7	1.81	1.72	1.77	104
3.4	3.66	3.52	3.59	106
6.5	5.53	6.60	6.07	93
13	13.9	13.7	13.8	106
Blank				
0	ND	---	ND	---
Matrix Spike[2]				
3.4	---	4.00		
	---	4.20	4.10	121
Laboratory Control Spike[2]				
3.4	3.65	4.04	3.85	113

[1] ND = none detected at or above the limit of quantitation of 0.50 µg/L.
[2] Matrix spike samples and laboratory control samples were prepared with the test substance used to formulate the test concentrations (purity = 96.0%).
[3] Analysis resulted in a value of 0.42 µg/L, which is greater than the LOD but less than the LOQ.
[4] Due to the high initial analytical value, the sample was re-analyzed (the cause of the increased analytical value in the re-analysis is not known). All values were used in the calculation of the mean.

BASF Reg. Doc. Number 2000/5128
BASF Study Number 97132
T.R. Wilbury Study Number 1329-BA 18 of 35

We note that the 24 hour cell measurements are all quoted as <10000 – please can you clarify if actual cell counts were taken at this time point or if the methodology was unable to consider <10000 cells.

The 24-h cell counts were made using a haemocytometer. No further details are provided in the study report regarding counts <10000 cells/mL or on detection limitations.

A 120 hour NOErC of 0.0012 mg/L based on mean measured concentrations was obtained from the study. We are unsure how suitable this value is for classification purposes because the exposure period is longer than the standard 72 – 96 hour duration and we are unclear if controls met both validity criteria and exponential growth over the whole exposure period up to 120 h. *From Table B.9.2.6.2-1 in the RAR (2017), it is unclear what the 72 hour NOErC was. Please can the DS provide a 72 h NOErC and / or EC10? Depending on information to support the use of 72 hour mean measured concentrations, it may be appropriate to consider the surrogate approach using the 72 hour ErC50 from this study if a 72 hour NOErC/EC10 cannot be determined.* The OECD TG 201 validity criteria were intended to be evaluated against algal growth over a period of 72-h, so it is not appropriate to use these criteria to evaluate growth parameters over 120-h. The 72-h control growth parameters met the guideline validity criteria. As part of a recent Request for Additional Information, the Applicant submitted new information to EFSA on the study by Wyskiel et al., 2000 (2000/5128) which included a calculation of ECx endpoints based on mean measured concentrations (Kaiser, 2019; 2019/1006413). 72-h ErC10 = estimate is not reliable as it is below the lowest tested concentration 72-h ErC20 = 0.0018 mg BAS 505 F/L (95% CI: 0.0013 to 0.0023) 72-h ErC50 = 0.0078 µg BAS 505 F/L (95% CI: 0.00617 to 0.00986) – from original report. A 72-h NOErC was not determined.
RAC's response
Agree with the DS response and see also response on comment 11 for the comment regarding the Anonymous 1999b, 1999/10521 study.

CONFIDENTIAL ATTACHMENTS
1. ECHA-25 August2019-2.zipx [Please refer to comment No. 6]
2. ECHA-25 August 2019-1.zipx [Please refer to comment No. 7, 8]
3. Terry et al 2005_feed restriction and fertility.pdf [Please refer to comment No. 3]