Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

fosthiazate (ISO); *S*-*sec*-butyl *O*-ethyl (2-oxo-1,3-thiazolidin-3-yl)phosphonothioate

EC Number: -

CAS Number: 98886-44-3

CLH-O-0000007388-63-01/F

Adopted

30 November 2023

P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON FOSTHIAZATE (ISO); S-SEC-BUTYL O-ETHYL (2-OXO-1,3-THIAZOLIDIN-3-YL)PHOSPHONOTHIOATE

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. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

ECHA accepts no responsibility or liability for the content of this table.

Substance name: fosthiazate (ISO); *S*-sec-butyl *O*-ethyl (2-oxo-1,3-thiazolidin-3-yl)phosphonothioate
EC number: -
CAS number: 98886-44-3
Dossier submitter: Germany

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
31.03.2023	France		MemberState	1
Comment received				
No comment.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
29.03.2023	United Kingdom	<confidential>	Company-Manufacturer	2
Comment received				
No comments. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Fosthiazate classification rebuttal (commenting phase) Public.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fosthiazate classification rebuttal (commenting phase)_final.zip				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
29.03.2023	United Kingdom	<confidential>	Company-Manufacturer	3

Comment received

For the proposed classification Repr. 2, H361d (CLH Report page 49-50) the DS makes reference to adverse developmental effects observed in the rabbit developmental toxicity study and in F1 animals from the multi-generation study. A detailed rebuttal with supporting tabulated data is provided in Report No: 0481542-TOX8 A summary of this information is presented below.

In the rabbit developmental toxicity study, groups of 15 or 16 female New Zealand White rabbits were administered by gavage fosthiazate suspended in 0.5% aqueous methylcellulose at dose levels of 0 (control), 0.5, 1.0, 1.5 or 2.0 mg/kg bw/day from Day 6-19 of presumed gestation. The DS notes slightly reduced fetal body weight at highest dose level without any evidence of maternal toxicity. However, statistical analyses (Dunnett's multiple comparison test) of fetal bodyweight data from the rabbit study revealed no significant differences in any treatment group. Furthermore, the difference in mean fetal body weight was very slight (high dose ~5% lower than controls), there was a lack of dose-response relationship, and the high dose values were within the historical control range (mean: 41.0 g, range 38.5-45.0 g, data from 7 studies, 606 rabbit foetuses) for the conducting laboratory.

The DS also refers to the increased number of 'small' fetuses (defined as <32 g by the conducting laboratory) seen in the rabbit developmental toxicity study in their proposal for Repr 2. H361d. However, detailed statistical analysis (Dunnett's multiple comparison test) for the litter incidences of 'small' fetuses revealed no significant difference in any treatment group. It is also noted that when considering the percentage of dams with greater than 10 pups in their litters, this parameter was highest in the high dose group, suggesting that this caused the apparent increase in 'small' pups at the high dose. Notably, when individual data in the main rabbit study are considered, the increase in the number of 'small' fetuses at the high dose level was driven by two litters of 14 pups. The largest litter size in the controls was 12. There was also a lack of similar effects in the range-finding study at a higher dose level (2.5 mg/kg bw/day).

In the main multi-generation study, groups of 25 male and 25 female CD rats were administered fosthiazate in the diet at concentrations of 0 (control), 3, 10, 30 or 100 ppm (equivalent to approximately 0, 0.21, 0.69, 2.09, and 7.21 mg/kg bw/day for males and 0, 0.26, 0.86, 2.62 and 9.34 mg/kg bw/day for females). The dose levels were based on a preceding range-finding study with dose levels of 0, 10, 30, 100 and 300 ppm (equivalent to approximately 0, 0.68, 2.03, 7.09, and 22.64 mg/kg bw/day for males and 0, 0.81, 2.48, 8.93 and 28.19 mg/kg bw/day for females). The DS states that there were adverse effects on litter size, live-birth index, viability index, lactation index, body weight at birth and weaning, onset of eye opening and tooth eruption. Furthermore, the incidence of small pups was affected and cannot be attributed to parental toxicity. However, it is considered that the DS has not fully considered the entirety of the fosthiazate dataset in making these conclusions on the role of maternal toxicity as a factor.

Cholinesterase activity was not measured in the multi-generation study, therefore the levels of cholinesterase inhibition need to be extrapolated from other studies in which cholinesterase activity was measured. In the age-sensitivity study in rats in which pregnant females were treated from Day 6-20 of gestation, the DS notes the toxicologically significant inhibition of erythrocyte cholinesterase activity at 0.7 mg/g bw/day, i.e., maternal cholinesterase inhibition at 0.7 mg/kg bw/day of -73% compared to controls (plasma), -44% compared to controls (erythrocyte (RBC)) and -5% compared

to controls (brain). At 5 mg/kg bw/day, there were marked effects on RBC cholinesterase in dams and also on brain cholinesterase. Values were -95% compared to controls (plasma), -99.5% compared to controls (RBC; all values <LOD (Limit of Detection)) and -90% compared to controls (brain). In the 90-day neurotoxicity study in rats with fosthiazate the high dose was 2.5 mg/kg bw/day. Mean erythrocyte (RBC) cholinesterase values for females at the high dose level were statistically significantly lower than control at Weeks 5, 9 and 14 (76, 68 and 71% of control, respectively). Mean cerebral cortex, cerebellum and brain stem cholinesterase values for high dose females were statistically significantly lower than controls at Weeks 5, 9 and 14 (Week 5 values 56, 74 and 61 % of control, respectively; Week 9 values 45, 58 and 54% of controls, respectively; Week 14 values 33, 52 and 48% of controls, respectively.
The dietary level of 100 ppm (equivalent to 9.34 mg/kg bw/day) in the multi-generation study, is equivalent to a dose level four times higher than that causing toxicologically significant inhibition of cholinesterase activity in the 90-day neurotoxicity study (2.5 mg/kg bw/day) in rats. Hence it can be concluded there would have been marked systemic toxicity (reduced cholinesterase activity) present in the high dose group of the multi-generation study. The effects driving the proposed classification were seen at 30 ppm and above, equivalent to a dose of 2.62 mg/kg bw/day based on pre-pairing data. However, intakes during lactation would be much higher (~2-fold), therefore it can clearly be inferred that significant cholinesterase inhibition is present based on findings in the age sensitivity study and the 90-day neurotoxicity study.
Overall, it is concluded that the proposed classification of Category 2 for reproductive toxicity (Repr. 2, H361d: 'Suspected of damaging the unborn child') is not supported.
For the proposed classification Repr. 2, H361f (CLH Report page 32-33), the DS references the adversity on sexual function and fertility observed in the available multigeneration studies: reduction of number of implantation sites, disturbances in oestrus cycle, prolonged gestation length. A detailed rebuttal with supporting tabulated data is provided in Report No: 0481542-TOX8. A summary of this information is presented below.
The DS notes the prolonged gestation length (and non-significant alterations in oestrus cycles) observed in F0-females at highest dose level of the main multi-generation study which, in their view, cannot be attributed to maternal toxicity. While there was a statistically significant effect on gestation length in the F0 100 ppm group (23.2 days), compared to controls (22.8 days), the change was minimal with no evidence of dystocia. It is noted that no effects on gestation were observed in the range-finding study for reproductive performance, in which groups of 10 male and 10 female CD rats were fed fosthiazate in the diet at concentrations of 0, 10, 30, 100 or 300 ppm fosthiazate i.e., levels similar to and higher than those used in the main multi-generation study. In the range-finding study individual gestation lengths ranged from 22-24 days (mean values for the control and high dose groups were both 23.0 days). It is noted that one high dose female was sacrificed for humane reasons on Day 2 post partum after displaying tremors from day 22 post coitum and a general deterioration in condition and failure of maternal response to the pups.
Published historical control data for gestation length in rats from the conducting laboratories (updated January 2018) reports a mean gestation length of 22.4 days (range 22.1 to 23.0 days). These data combine historical data from studies with (6 studies) and without culling (23 studies), since culling has no impact on gestation length.. Therefore, the range and means are outside these published data for the range-finding study where no treatment related effects on gestation length were noted and also in the main multi-generation study where a statistically significant effect was noted at the high dose.
Overall, taking into account the gestation lengths across both the range-finding study, and the main multi-generation study, there is no clear evidence these changes are treatment-related.

Although as detailed above there was no clear evidence for a treatment related effect on gestation lengths, it is also worth noting the likely marked effects on cholinesterase activity at 100 ppm in the main multi-generation study. A dietary level of 100 ppm or 9.34 mg/kg bw/day in this study, is a dose level four times higher than that the highest dose used in the 90-day neurotoxicity study in rats in which marked cholinesterase inhibition (brain and RBC) was observed. Hence, it can be concluded that marked systemic toxicity would have been present at the high dose level in the multi-generation study. This brings into question the biological relevance of any potential effects on gestation length at this dose level.

The DS also notes the non-significant alterations in estrous cyclicity in F0-females at highest dose level of the multi-generation study. A statistically significant reduction in the number of animals with normal estrous cycle was observed at 10 ppm (below the historical control range). However, a similar effect was not observed at the higher dose levels and an assessment of incidences of acyclic or pseudopregnant females, irregular and extended estrous cycles clearly indicates that the reduction in the number of animals with normal estrous cycle at 10 ppm was not treatment related. The DS notes that the estrous cycle was altered in F0 females and that the proportion of females showing normal estrous cyclicity was lower (albeit with no dose-response relationship) at 10, 30 and 100 ppm. 52-60% of rats showed a normal cycle in these treatment groups, compared with 80% of the control females. Historical control data (HCD) from the conducting laboratory showed a range of 63-100 % for this parameter, with a mean of 86%. At 30 and 100 ppm the percentage of acyclic/pseudo-pregnant females was increased (12 and 24% respectively). These values were within the range of the HCD. Given the likely effects on cholinesterase activities at 100 ppm the biological relevance of any alterations on estrous cyclicity in F0 females at the highest dose level of the multi-generation study is highly questionable.

The DS also cites a reduction in the number of implantation sites (47% of controls) in the high dose group (300 ppm) of the range finding study; and similar observation (~8 %; 12.9 vs 14 in controls) at the high dose of 100 ppm in the main multi-generation study. However, there were no statistically significant differences observed in the P-generation at any dose level. In addition, the number of implants in 100 ppm group in the range-finding study was comparable to the controls. Therefore, it can be concluded that the number of implants was not affected by fosthiazate treatment up to 100 ppm. The reduction of number of implants observed at 300 ppm in the range-finding study was likely a result of the secondary effect from systemic effects (marked inhibition of cholinesterase activities), also noting the likely marked effects on cholinesterase activities at doses of ≥100 ppm. As previously noted, cholinesterase activity was not measured in either the range-finding or the main multi-generation studies, therefore the levels of cholinesterase inhibition need to be extrapolated from other studies. Findings suggest that the reduction in implants is associated with toxicity caused by the anti-cholinesterase activity of fosthiazate.

Overall, it is concluded the proposed classification Category 2 for reproductive toxicity (Repr. 2, H361f: 'Suspected of damaging fertility') is not supported.

For the proposed classification H362: May cause harm to breast-fed children (CLH Report page 55-56) the DS makes reference to postnatal effects on offspring viability and development which they considered to be due to effects on or via lactation. A detailed rebuttal with supporting tabulated data is provide in Report No: 0481542-TOX8 . A summary of this information is presented below.

Based on the range-finding study, there was evidence during necropsy that there are treatment-related effects on mammary milk production, with a high proportion of pups lacking milk in their stomachs. In addition, in females at 100 and 300 ppm from the range-finding study there was an increased incidence of inactive mammary tissue (9/10 and 3/10 females at 100 and 300 ppm, respectively, with no similar findings in any other

group). In the main multi-generation study an absence of milk in stomach at necroscopy was noted at the highest dose level (100 ppm) in offspring culled on Day 4. For pups dying before terminal kill the absence of milk was reported across all groups and the incidence of the finding was comparable across all groups. It should be noted no macroscopic findings in mammary gland were noted at in any group.
The DS refers to effects on 'lactation/milk production' to support the proposal for classification. However, the CLP classification criteria are (1) clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or (2) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk. These criteria do not include behavioural effects (e.g., changes in nursing behaviour) or effects on milk production.
No cross-fostering was performed in the study to provide evidence of substance transfer through milk, therefore the possibility of the active substance or its metabolites reaching the milk during lactation must be considered.
The most relevant study to consider is from the goat metabolism study, also referenced by the DS. In this study lactating goats were dosed orally with 14C-radiolabeled fosthiazate at approximately 10 ppm feed equivalents for four consecutive days to determine the nature and levels of 14C residues in milk and selected tissues. It was shown that fosthiazate was metabolized extensively into natural products and that the 14C-fosthiazate level in milk was very low i.e., less than 0.02 ppm. Therefore, exposure of offspring during lactation as a result of exposure to the mother should be minimal and that exposures for neonatal animals are 200-fold lower than the dams.
Although it cannot be ruled out that neurotoxicity is a cause of pup mortality following in utero exposure, the age-sensitivity study shows no increase in sensitivity of offspring compared to the dams, so this explanation is considered unlikely.
Again, it is important to consider the impact of maternal systemic toxicity. The dietary level of 100 ppm from the multi-generation study, is equivalent to 9.34 mg/kg bw/day in females based on based on pre pairing data. Comparison to the extent of cholinesterase inhibition in both the age sensitivity and 90-day neurotoxicity studies indicates the presence of marked systemic toxicity in the high dose group of the multi-generation study.
Clearly the 300 ppm dose in the reproductive performance range-finding study, equivalent to 28.19 mg/kg bw/day for females, exceeded the MTD based on this analysis, further confirmed by clinical signs in both sexes at the high dose and the premature sacrifice of a female for humane reasons. This female displayed tremors daily from Day 22 post coitum and was killed on Day 2 post partum following continued deterioration in condition and lack of maternal response to the litter. It is noted that the group size in this study was 10/sex, so the level of maternal mortality was 10%, based on the death of the single female. The ECHA CLP guidance states that 'maternal mortality greater than 10 % is considered excessive and the data for that dose level shall not normally be considered for further evaluation'.
In conclusion, it is considered that the inactive mammary tissue observed in the reproductive performance range-finding study was secondary to systemic toxicity as a result of cholinesterase inhibition. Therefore, the effects on the pups are a consequence of marked maternal toxicity (likely RBC and brain cholinesterase inhibition in excess of 40%) and classification is not supported. Overall, the proposed classification: H362: 'May cause harm to breast-fed children is not supported'.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Fosthiazate classification rebuttal (commenting phase) Public.zip

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fosthiazate classification rebuttal (commenting phase)_final.zip

Dossier Submitter's Response

Thank you for this extensive comment and summary of adverse effects observed in studies on reproductive toxicity. However, the DS is still convinced that classification of fosthiazate for reproductive toxicity (sexual function and fertility as well as developmental effects) and for effects on or via lactation is needed.

We would like to respond to some of the arguments that have been raised by the applicant. Regarding the proposal for developmental effects:

1) Effects seen in rat studies (multi-generation and developmental): The CLP regulation stipulates: "*Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity.*" We do not agree with the applicant that the observed effects such as reductions in post-implantation survival, live birth index, viability index day 4, lactation index, weight at birth and weaning as well as delays in tooth eruption and eye opening can be attributed to maternal toxicity in terms of cholinesterase inhibition. As the applicant already stated, no measurement of cholinesterase was performed in the studies on reproductive toxicity. We agree that from the results from other repeated-dose toxicity studies it is likely that fosthiazate caused inhibition of cholinesterase also in the reproductive toxicity studies. However, this plausible cholinesterase inhibition did not result in neurotoxic symptoms and/or in changes in behaviour in the studies on reproductive toxicity, as far as analysed, and is therefore not relevant as an indication of maternal toxicity. In case the observed adverse effects on reproduction are related to cholinesterase inhibition because cholinergic inhibition is a key event in a biological process involved in reproduction, this is not considered as maternal toxicity or as "*a result of the secondary effect from systemic effects (marked inhibition of cholinesterase activities)",* as postulated by the applicant, but as a mechanism of action which leads to an adverse effect that has to be taken into account.

2) Effects seen in the rabbit developmental toxicity study (Anonymus 6, 1989b): we agree that no dose-dependency for the lowered birth weight in rabbits was observed. Nevertheless, a decrease (-5.4%, within, but close to the minimum range, of HCD) in birth weight was observed at the top dose of 2 mg/kg bw/d. Together with the finding of an increased number of 'small pups' (defined as < 32 g by the conducting laboratory) in the same study, a treatment-related adverse effect on development becomes obvious. The percentage of 'small pups' was increased about 19% at the top dose of 2 mg/kg bw/d (27.7%) in comparison to the control group (8.7%). The value of 27.7% is far outside the range of the HCD (mean: 11.39, range: 2.7 – 21.9). The applicant states, that the finding of 'small fetuses' is due to litter size and that "*the increase in the number of 'small' fetuses at the high dose level was driven by two litters of 14 pups".* It is true that a relationship between litter size and fetal body weight can be expected and that in these mentioned litters the number of small fetuses is the highest observed in the study. However, there are several other litters with the same size (or higher) in the study, where a lower number of small fetuses was observed and where the mean fetal body weight was higher. The mean fetal body weight in three of the seven affected litters from the top-dose group was already below 32 g (i.e. 30.9 g, 31.8 g, 31 g), while litter sizes were 12, 14, 14 and about 57-58% of the fetuses in this litters were affected. In a further litter of the top-dose group, 3 out of 11 fetuses were 'small' and the mean fetal body weight in this litter was only 32.6 g. This leads to the assumption that the reduction in body weight of the 'small fetuses' was drastic or/and also the body weight of the

remaining fetuses was reduced. Unfortunately, no individual data on fetal bodyweight are available, which could be used to further characterise the occurrence of 'small fetuses'. However, the endpoint 'number of small fetuses' was statistically re-analysed with the statistical software R (R Core Team 2022) by the DS. Possible litter effects were taken into account by using generalised estimating equations (GEE) with the fetuses as the statistical unit. Significance of treatment effects was analysed with a post-hoc Dunnett test (one-sided) with respect to the results of GEE analysis. This showed a statistically significant increase of the number of 'small fetuses' (alpha level of 0.05, p-value 0.038) for the top dose (2 mg/kg bw/d) when compared to the control group.
In the range-finding study (limited number of animals used), also an increase of small pups was seen in the 2 mg/kg bw/d treatment group (24.3% vs 17.7% in control), however, without dose-response. Overall, we consider the increase of 'small fetuses' a treatment-related effect.

The main argument of the applicant against a classification for effects on sexual function and fertility is again that the observed effects are "*likely a result of the secondary effect from systemic effects (marked inhibition of cholinesterase activities)"*. As already stated above we do not regard this argumentation as valid.

In addition we would like to respond to some of the arguments that have been raised by the applicant for clarification:

- Regarding the applicant's summary of changes in oestrus cycle (multi-generation study, Anonymus 34, 1990) it is worth to mention that the percentage of females with normal oestrus cycle, 52–60%, was below the range of the HCD (range: 63-100%, mean 86%) in all three dose groups from 10 ppm.

- The parameter implantation sites for the range-finder multi-generation study (Anonymus 25, 1990) was analysed with analysis of variance (ANOVA) with the statistical software R (R Core Team 2022) by the DS. This showed a statistically significant trend (alpha level of 0.05, p-value 0.000383). A post-hoc one-sided Dunnett test (with the statistical software R) for a decrease of the number of implantation sites (alpha level of 0.05, p-value 0.0014) showed a statistically significant decrease of the number of implantations for the top dose when compared to the control group.

- With regard to the reduction in litter size (range-finder multi-generation study), the DS re-evaluated the individual data and notes that the litter size on day 1 reported by the applicant (and reported in the CLH dossier) according to the original study represents the number of born/total pups and not the number of live pups at PND1 and that two total litter losses were not included at the highest dose. The values for the different groups (0, 10, 30, 100, 300 ppm) therefore are:

  - Number of live-born litters (number of pregnant animals): 9 (9)/10 (10)/6 (6)/10 (10)/5 (7),
  - Total on Day 1 (mean ± SD): 12.8 ± 3.6/14.3 ± 3.5/15.3 ± 2.3/12.2 ± 2.6/4.4 ± 3.6,
  - Litter size PND 1 (mean ± SD): 12.2 ± 3.9/14.2 ± 3.4/15.3 ± 2.3/11.3 ± 3.5/3.7 ± 3.8.

The parameter litter size on PND 1 was analysed with the Kruskal-Wallis-test rank sum test for a trend with the statistical software R (R Core Team, 2022) by the DS. This showed a statistically significant trend (α = 0.05, p = 0.0006626). A post-hoc one-sided Dunnett test (with the statistical software R) for a decrease of the litter size on PND 1 (α

= 0.05, p < 1E-04) showed a significantly decreased number of the litter size on PND 1 for the top dose when compared to the control group.
No statistical significant trend was identified for the reduction in implantation sites and litter size in the main multi-generation study (Anonymus 34, 1990).

Overall, our proposal for classification as toxic for fertility (Repr. 2, H361f) is maintained. Another MS commented that a classification as Repr. 1B, H360f should be considered, primarily based on the effects from the dose range-finding study (see comment no.6). We agree, that, in particular with respect to the reduction in implantation sites, Cat. 1B should be discussed by RAC as these effects cannot be attributed to maternal toxicity, which was obvious before mating only in terms of isolated clinical signs at the top dose level. Also effects from the multi-generation study (i.e. changes in oestrus cycle and reductions in implantation sites and litter size) did not occur in the presence of maternal toxicity.

3) Effects on or via lacatation (May cause harm to breast-fed children (H362)): Again, the applicants's argumentation that inactive mammary tissue was "*secondary to systemic toxicity as a result of cholinesterase inhibition*" is not considered valid. No neurotoxic effects interfering with nursing behaviour were reported.

Regarding the applicant's argumentation that the CLP classification criteria "*do not include…effects on milk production*" we would like to draw the applicant's attention to the fact that the Guidance on the Application of the CLP Criteria states: *"A substance which does not cause overt toxicity in the mother but which interferes with milk production or quality will normally be classified for effects on or via lactation because in this case the effect on lactation is most likely a direct substance-related effect."* Effects on mammary tissue (inactive mammary tissue) were observed and absence of milk in the stomach was reported for offspring. Therefore, we consider a classification for effects on or via lactation (Lact., H362) still justified.

RAC's response

Thank you for the thorough comments on the reproductive toxicity assessment following exposure to fosthiazate. RAC has carefully assessed the information on reproductive toxicity and the outcome on classification and labelling is available in the RAC Opinion.

Date	Country	Organisation	Type of Organisation	Comment number
29.03.2023	United Kingdom	Health and Safety Executive	National Authority	4

Comment received

Reproductive Toxicity – Sexual Function and Fertility
We note that there is no further information on the reduction of implantation sites and it is therefore unclear whether this would be an effect on sexual function and fertility or on development. To help discriminate, it would be useful to include information on corpora lutea counts for the multigeneration reproductive toxicology studies.

Dossier Submitter's Response

Thank you for the comment. However, no information on corpora lutea counts is available from the dose range-finding generation study or multi-generation studies and therefore, no conclusion on pre-implantation loss can be drawn. In general, effects prior to implantation are regarded as attributable to effects on sexual function and fertility. Effects after implantation can be influencend by altered sexual function and fertility as well as

developmental effects. In both generational studies, a reduction in the number of mean implantation sites as well as a reduction of litter size was observed. The magnitude of the reduction of litter size was in both studies (slightly) higher than that of the reduction of the number of implantation sites. The reduction in implantation sites is interpreted as an impairment of sexual function and fertility. The reduction in litter size is mainly interpreted as a consequence of the reduction in the number of implantations and therefore also as an impairment of sexual function and fertility. However, also developmental toxicity can additionally be involved here and a clear discrimination does not seem possible.
RAC's response
Noted. RAC agrees with the DS that in general, effects prior to implantation are regarded as attributable to effects on sexual function and fertility.

Date	Country	Organisation	Type of Organisation	Comment number
31.03.2023	France		MemberState	5
Comment received				
Was not reviewed.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
31.03.2023	Sweden		MemberState	6
Comment received				
The Dossier Submitter (The Federal Institute for Occupational Safety and Health in Germany) proposes a harmonised classification for fosthiazate in Repr. 2, H361fd and Lact., H362. Sexual function and fertility The Swedish CA suggests that classification as Repr. 1B, H360F should be considered. This is based primarily on the approximately 50% reduction of mean implantation sites (7.6 ± 4.3 vs. 14.3 ± 3.8 in the control group) and litter size (6.2 ± 2.5 vs. 12.8 ± 3.6) in the 300 ppm dose group in the dose range finding study, in absence of adverse maternal toxicity (Anonymous 25, 1990). Furthermore, in the 2-generation reproduction toxicity study (Anonymous 34, 1990), the mean number of implantation sites and litter size were reduced at 100 ppm, without adverse maternal toxicity, however no higher dose group was included in the study. Development The Swedish CA considers that the effects on development observed in the dose range finding study (Anonymous 25, 1990) and the 2-generation reproduction toxicity study (Anonymous 34, 1990) warrant classification as Repr. 1B, H360D. This is primarily based on the evidence of effects on prenatal survival. More specifically, the adverse effects on development include: - In the 300 ppm dose group in the dose range finding study in rats (Anonymous 25, 1990) there was a reduction in post-implantation survival (58% vs. 89% in control) and live birth index (84% vs. 96% in control) in the absence of significant maternal toxicity. - Effects on development were observed also in the F1-generation of the 2-generation				

reproduction toxicity study (OECD TG 416) in rats (Anonymous 34, 1990), despite the lower dose used compared to the dose range finding study. At the highest dose group of 100 ppm, there was a statistically significant reduction in live birth index (92% vs. 99% in control) and birth weight (89% of control) in the absence of significant maternal toxicity.
- Findings in the developmental toxicity study (OECD TG 414) in rabbit (Anonymous 6, 1989b), were an increased number of small fetuses (+19%) and lower foetal birth weights (-5.4%) at 2 mg/kg bw/day, in the absence of maternal toxicity.

In addition to the developmental effects described above, there were very clear evidence of effects on postnatal survival and growth in the 2-generation reproduction toxicity study and in the dose range finding study. For example, all pups in the highest dose group of 300 ppm were dead by day 3 in the dose range finding study. The postnatal mortality likely involves the effects on maternal lactation, although potential impact on prenatal development cannot be excluded. However, the observed effects on post-implantation survival, live birth index and birth weight cannot be attributable to effects on lactation.

In four developmental toxicity studies of rats and rabbits (Anonymous 6, 1989b; Anonymous 5, 1989a; Anonymous 24, 1989; Anonymous 41, 1990) the treatment periods were shorter than what is required by the current test guidelines. The Dossier Submitter concludes that: "As there are some uncertainties related to the data base, classification with Category 1B is not applicable". We do not support this argumentation as the shorter treatment periods do not reduce the credibility of the observed effects. On the contrary, observation of effects already after a shorter treatment period indicates that even more severe effects could be elicited in studies using the currently required exposure duration. Furthermore, the 2-generation reproduction toxicity study performed according to OECD TG 416 and the dose range finding study showed clear effects on development.

Lactation
The Swedish CA supports the proposal to classify fosthiazate as Lact., H362, based on the clear evidence of an adverse effect on postnatal growth and survival, in combination with the observed effects on mammary tissue of the dams and effects on the lactation index.

Dossier Submitter's Response

Thank you for the comment and the summary of effects relevant for classification for reproductive toxicity. We could agree with the reasoning that uncertainities related to the data base were identified (i.e. shorter treatment as foreseen by the current test guidelines) do not affect the credibility of the effects seen in the study. This should be taken into account by RAC when discussing the appropriateness of Cat 1B or Cat 2 (also for effects on sexual function and fertility).

RAC's response

Thank you for the comments on the reproductive toxicity assessment following exposure to fosthiazate. RAC has carefully assessed the information on reproductive toxicity and the outcome on classification and labelling is available in the RAC Opinion.

Date	Country	Organisation	Type of Organisation	Comment number
30.03.2023	Belgium		MemberState	7

Comment received

Sexual function and fertility :
In the range finding generation study (Anonymous 25, 1990), the number of implantation sites and litter size on day 1 was reduced at the highest tested dose (300 ppm). Decrease

was not dose-related, however the modification at the highest dose was severe (7.6 compared to 14.4 in control for implantation sites and 6.2 compared to 12.8 in control for litter size). Regarding these 2 parameters, no information about statistical analysis was available.
In the multigeneration study (Anonymous 34, 1990), even if dose was lower than the range finding study, the number of implantation sites and litter size were slightly reduced. However, parameters did not exhibit significant modification or dose-related modification and no information about HCD was available.
In the multigeneration study (Anonymous 34, 1990), in the F0, the number of normal oestrus cycle was only significantly affected at 10 ppm. Change was also observed at the 2 highest doses, but reduce was not significantly modified (% of female per group with normal oestrus cycle: 80, 80, 52, 56 and 60 %, respectively at 0, 3, 10, 30 and 100 ppm). Furthermore, at the 3 highest doses, the number of female with normal oestrus cycle was lower than the range of HCD (63 to 100 %), while the number of females with normal oestrus cycle in control groups was within the range of HCD. In the F1 generation, oestrous cycle examination did not reveal significant modification (% of female with a normal oestrus cycle of 72, 88, 68 and 88 %, respectively at 0, 3, 10 and 30 ppm).
CLH report mentioned a significant alteration of the gestation length at the highest dose. Based on the available results, a calculated mean gestation length was of 22.82, 21.6, 23.14, 22.925 and 23.175, respectively at 0, 3, 10, 30 and 300 ppm. At 10 ppm, gestation length was higher as 2 females had a gestation of 24.5 days.
Based on the observed effects, a classification as Repr. 2 H361f seems appropriate.

Development :
Pups viability index was severely reduced in 2 studies.
In the range finding generation study (Anonymous 25, 1990): 90, 90, 73, 13 and 0 %, respectively at 0, 10, 30, 100 and 300 ppm. Modification was clearly dose-related and no pups survived until lactation day 4 at the highest dose.
In the multigeneration study (Anonymous 34, 1990): 94, 97, 96, 86** and 44** %, respectively at 0, 3, 10, 30 and 100 ppm. A trend to dose-related decrease was observed and change was significantly at the 2 highest doses.
In the preliminary teratology study (Anonymous 5, 1989a), performed in rabbits, an increased incidence of small pups was observed at the mid dose group (outside the range of HCD) (17.7, 7.2, 24.3 and 13 %, respectively at 0, 1, 2 and 2.5 mg/kg bw/d), however change was not dose-related and no modification was noted at the highest dose. While, in the teratology study (Anonymous 6, 1989b), increased incidence of small pups was noted at the highest dose and the modification was clearly outside the range of HCD (8.7, 13.6, 20.8, 16.2 and 27.7 %, respectively at 0, 0.5, 1, 1.5 and 2 mg/kg bw/d). No information was available regarding the statistical analysis.
The duration of exposure of the prenatal developmental toxicity was shorter than recommended by current guideline and CLH mentioned that "there are some uncertainties related to the data base, classification in Category 1B is not applicable". However, BE CA is of the opinion that viability index was severely decreased in 2 studies and a discussion regarding a possible classification in category 1B is warranted.

Dossier Submitter's Response

Thank you for the comment and summary of effects relevant for classification for reproductive toxicity.

We agree with the MS as well as with the reasoning made by an additional MS (please see comment no. 6) that uncertainities related to the data base were identified (i.e. shorter treatment as foreseen by the current test guidelines) do not affect the credibility of the effects seen in the study. This should be taken into account by RAC when discussing the

appropriateness of Cat 1B or Cat 2. It should also be considered that developmental effects were seen in two different species (rat and rabbit).
RAC's response
Thank you for the comments on the reproductive toxicity assessment following exposure to fosthiazate. RAC has carefully assessed the information on reproductive toxicity and the outcome on classification and labelling is available in the RAC Opinion.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
29.03.2023	United Kingdom	<confidential>	Company-Manufacturer	8
Comment received				
No comments. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Fosthiazate classification rebuttal (commenting phase) Public.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fosthiazate classification rebuttal (commenting phase)_final.zip				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
31.03.2023	France		MemberState	9
Comment received				
Was not reviewed.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
30.03.2023	Belgium		MemberState	10
Comment received				
Acute toxicity – oral route : Based on the results of the 2 available studies, the proposal for classification as Acute Tox. 3 H301 is supported. Furthermore, as the lowest LD50 was of 57 mg/kg bw, the proposed ATE value is supported by BE CA. Although no impact on the proposed classification, the estimated LD50 in male in the second acute oral toxicity study (Anonymous 13 (1989a) is questionable. As no mortality was observed at 81 mg/kg bw and all animals died at the next treated dose of 102 mg/kg bw, a LD50 between 81 and 102 mg/kg bw seem more appropriate than a LD50 of 104 mg/kg bw as proposed in the CLH dossier. Acute toxicity – dermal route : Based on the available study, the LD50 value for dermal toxicity is >200 and <2000 in				

females (LD50 of 861 mg/kg bw in females). Therefore, the proposal for classification as Acute Tox. 3 H311 and the ATE of 861 mg/kg bw are supported. Acute toxicity – inhalation route : Based on the available study, the LC50 value for inhalation toxicity is >0.5 and <1.0 mg/L (LC50 of 0.56 and 0.83 mg/L respectively in females and males). Therefore, the proposal for classification as Acute Tox. 3 H331 and the ATE of 0.56 mg/kg bw are supported.
Dossier Submitter's Response
Thank you for the comment and the support regarding acute toxicity via dermal and inhalation routes. Regarding the oral acute toxicity we agree that an LD50 of 104 mg/kg bw for males is not appropriate.
RAC's response
Noted.

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
29.03.2023	United Kingdom	<confidential>	Company-Manufacturer	11
Comment received				
No comments. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Fosthiazate classification rebuttal (commenting phase) Public.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fosthiazate classification rebuttal (commenting phase)_final.zip				
Dossier Submitter's Response				
Noted				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
29.03.2023	United Kingdom	Health and Safety Executive	National Authority	12
Comment received				
Serious Eye Damage/Eye Irritation We note that there was systemic toxicity observed in the first eye irritation study (Anonymous 17, 1989e), with 1 animal found dead and 2 animals killed in extremis after 5hrs. Systemic toxicity and lethality via the eye are intrinsic hazards of some organophosphates. Therefore, we suggest considering whether the additional hazard statement of "EUH070 – Toxic by eye contact" should apply.				
Dossier Submitter's Response				
Thank you for the comment and suggestion. We agree and have already proposed the additional hazard statement "EUH070 – Toxic by eye contact" (please see section 13 (additional labelling) of the CLH report).				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
31.03.2023	France		MemberState	13
Comment received				
Was not reviewed.				
Dossier Submitter's Response				
Noted				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
30.03.2023	Belgium		MemberState	14
Comment received				
Based on the available results of the Anonymous 17's study, score of conjunctival redness ≥2 observed in the 3 surviving animals and effects fully reversible within 21 days, the proposal to classify as Eye irrit. 2 H319 is supported.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
29.03.2023	United Kingdom	<confidential>	Company-Manufacturer	15
Comment received				
No comments. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Fosthiazate classification rebuttal (commenting phase) Public.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fosthiazate classification rebuttal (commenting phase)_final.zip				
Dossier Submitter's Response				
Noted				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
31.03.2023	France		MemberState	16
Comment received				
Was not reviewed.				
Dossier Submitter's Response				
Noted				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
30.03.2023	Belgium		MemberState	17
Comment received				
Based on the available effects observed in different acute studies, BE CA supports the proposal to classify as STOT SE 1.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
31.03.2023	France		MemberState	18
Comment received				
Was not reviewed.				
Dossier Submitter's Response				
Noted				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
29.03.2023	United Kingdom	<confidential>	Company-Manufacturer	19
Comment received				
For the proposed classification H373 'May cause damage to organs through prolonged or repeated exposure' based on adrenal gland effects (CLH Report page 110) the DS makes reference to the effects on the adrenals seen in rats, mice and dogs in all repeated dose studies, with dogs the most sensitive species. A detailed rebuttal with supporting tabulated data is provide in Report No: 0481542-TOX8. A summary of this information is presented below. The DS proposes Category 2 (STOT RE 2, H373), taking into consideration changes in adrenals observed in dogs (28-/90-day studies) at dose levels which meet Category 1 (STOT RE 1, H372). However, the DS also takes into account that the adrenal effects observed in the 28- and 90-day studies were not significantly increased in the 1-year dog study at comparable dosages, therefore Category 2 (STOT RE 2, H373) was considered more appropriate based on the CLP guidance (Guidance on the Application of the CLP Criteria (2017) which states "If there are differences in effects at the GV (Guidance Value) between studies with different duration then more weight is usually given to studies of longer duration (28 days or more)". The lowest relevant effect level in the rat was ≥53.6 ppm (4.12 mg/kg bw/d) from the 90-day oral study in rats with vacuolation of the adrenal cortex in males. Cholinesterase investigations in this study after 12 weeks showed marked reductions in brain cholinesterase at dose levels of ≥53.6 ppm in males (≥23% reduction compared to controls) and females (≥66% reduction). Erythrocyte cholinesterase was significantly reduced in males (≥57% reduction) and females (≥71% reduction) at dose levels of ≥53.6 ppm in both sexes at the end of 12 weeks. These data are supported by the 90-				

day neurotoxicity study in rats with fosthiazate where there were significant effects on cholinesterase activities at the highest dose level of 2.5 mg/kg bw/day; mean erythrocyte cholinesterase values for females were statistically significantly lower than control at Weeks 5, 9 and 14 (24, 32 and 29% reductions, respectively). In addition, mean cerebral cortex, cerebellum and brain stem cholinesterase values for high dose females were statistically significantly lower than control at Weeks 5, 9 and 14 (Week 5 were 44, 26 and 39% reductions, respectively; Week 9 were 55, 42 and 46% reductions, respectively and for Week 14 were 67, 48 and 52% reductions, respectively).
Based on these data it is clear for all the oral rat studies that the adrenal effects were only seen in the presence of significant systemic toxicity (cholinesterase inhibition).
This is also true for the 28-day dermal study where vacuolation of the zona fasciculata was seen in males (4/5) and females (2/5) with increased organ weight in both sexes at the high dose of 250 mg/kg bw/day. Clinical chemistry investigations in this study revealed at doses ≥25 mg/kg bw/day that erythrocyte cholinesterase was markedly reduced in males (≥55% reductions). At ≥2.5 mg/kg bw/day erythrocyte cholinesterase was markedly reduced in females (≥21% reductions). Brain cholinesterase showed treatment-related reduction in both sexes at dose levels of ≥25 mg/kg bw/day.
The DS concluded that the LOAEL in the 28-day dog study was 0.54 mg/kg bw/day based on histopathological changes in the adrenals of both sexes, however this NOAEL was not confirmed in the 90-day and one-year studies in dogs. In the 28-day study histopathological examination of the adrenal glands revealed cell enlargement and pallor in the zona fasciculata of the adrenal cortices in 2 males at 0.54 mg/kg bw/day, in all males and one female at 5.4 mg/kg bw/day and in all dogs i.e. both sexes at 26.8 mg/kg bw/day. Erythrocyte cholinesterase was markedly reduced in both sexes at dose levels of ≥5.4 mg/kg bw/day (≥77% reduction) compared with controls. Brain cholinesterase was reduced at dose levels of ≥5.4 mg/kg bw/day (≥33% reduction in males and ≥49% reduction in females) in both sexes compared with controls.
In a re-evaluation of adrenal slides from the 90-day and 1-year oral studies in dogs by a panel constituted by the applicant, it was concluded that the severity of cytoplasmic hypertrophy and/or increased pallor of the cells in the zona glomerulosa and fasciculata of the adrenal cortex was only increased in the high dose groups (5.4 mg/kg bw/day for the 90-day study, and 5 mg/kg bw/day for the 12 month study). In the 90-day dog study erythrocyte and brain cholinesterase were markedly reduced in both sexes compared with concurrent controls at the highest dose level of 5.4 mg/kg bw/day (erythrocyte cholinesterase reductions of 67.9% for males and 66.2 % for females, brain cholinesterase reductions of 22.6% for males and 31.6% for females). In the one-year study inhibition of plasma cholinesterase activity was observed at 0.5 mg/kg bw/day and above in both sexes at 3, 6, 9 and 12 months. However, erythrocyte and brain cholinesterase activities were not affected.
The DS also notes an increased incidence of corticomedullary pigmentation of the adrenal cortex and mineralization of pigmented cells of the adrenal cortex observed in the 104-week carcinogenicity study in the CD-1 mouse. In this study there were increased adrenal weights at the high dose 300 ppm in both sexes (30.5 mg/kg bw/day (males) and 39.17 mg/kg bw/day (females)) with histopathological changes in the adrenal cortex (corticomedullary pigmentation of the adrenal cortex) in females at 100 ppm (10.4 mg/kg bw/day) and in males at 300 ppm (30.5 mg/kg bw/day). In a supplementary histopathological assessment of the adrenal glands of selected females from the mouse carcinogenicity study eight adrenals from four high dose females that were reported with the corticomedullary pigment and up to nine adrenals from six control females; where possible, three reported with the corticomedullary pigment and three reported without the pigment were examined. The cortico-medullary pigment reported in the Aughton, 1990 study was PAS (periodic acid/Schiff staining) positive, LZN (Long Zeihl Neelson stain) positive, Schmorl's negative and Perls' negative indicating that the pigment is likely to be

ceroid, which is a spontaneous age-related finding in CD-I mice. The DS concluded that result suggests that fosthiazate exacerbated this background change. Although there were no measurements of cholinesterase activities in this study it is very likely that there would have been marked changes at least at the higher dose levels The achieved mean daily intakes were 1.02, 3.1, 10.3 and 30.5 mg/kg bw/day in males and 1.11, 3.2, 10.4 and 39.17 mg/kg bw/day at the achieved dose levels of 10.7, 32.2, 107 or 322 ppm). Systemic toxicity was clear evident at the high dose (300 ppm) where overall body weight gain was markedly reduced (43%) compared with the combined control mean. The Guidance on the Application of the CLP Criteria Guidance to Regulation (EC) No 1272/2008 clearly states (Table 3.9.2.2. Classification criteria for substances) 'one shall carefully evaluate the data and, where possible, not include secondary effects'.
It is considered highly plausible that the adrenal effects are secondary to stress induced by cholinesterase inhibition where STOT SE classification is already required. The link between stress and changes in the adrenals has been reviewed and documented in literature and by the National Toxicology Program (https://ntp.niehs.nih.gov/nnl/endocrine/adrenal/cxvacuol/index.htm). Whilst adrenal changes alone are not conclusive evidence of stress, it is considered that the concurrent effects on cholinesterase elicited by fosthiazate provide a weight of evidence that the adrenal effects are attributable to a stress response.
In addition to the above considerations, it should also be noted that the observed changes in adrenal weight were frequently found in CD rats and were within the intrinsic biological variability. To support this point the retrievable additional historical control data (HCD) closest to the relevant time frame have been submitted from the conducting laboratory (Anonymous 2022a, Anonymous 2022b and Anonymous 2022c).
The data from the 90-day rat study were compared to HCD on Sprague-Dawley rats from 11 sub-chronic 90-day studies conducted between 1992-1995, provided by the laboratory (LabCorp 2022a). Again, the data include all retrievable information from the closest possible time frame and from the CRO where the relevant studies were conducted. The overall mean absolute adrenal weight for male and female rats was found to be 0.054 and 0.068 g, but according to these data can be as high as 0.078 and 0.116 g, respectively (maximum value found within the 11 studies). This leads to an overall mean relative adrenal to body weight of 0.011 and 0.023 % for male and female rats, respectively and to the highest individual value of 0.018 and 0.038 % for male and female, respectively. Of note, all statistically increased absolute and relative adrenal weight changes found in the 90-day rat study were within the range of the corresponding HCD, and are therefore within the normal biological variability of this organ.
The data from the main two-generation study were compared to HCD on Sprague-Dawley rats from 11 sub-chronic 90-day studies conducted between 1992-1995 (Anonymous 2022a), and a single OECD TG 416 study, also provided (Anonymous 2022b, Anonymous 2022c).
In the main two-generation study, statistically significant adrenal weight changes were found in female rats only. The overall mean absolute adrenal weight for female rats was found to be 0.068, 0.067 and 0.062 g for the sub-chronic and the OECD 416 study (F0 and F1 generation), respectively, but according to these data sets can be as high as 0.116, 0.092 and 0.092 g, respectively. This leads to an overall mean relative adrenal to body weight of 0.023, 0.021 and 0.018 for the respective studies mentioned above, and to the highest individual value of 0.038, 0.042 and 0.027 %, respectively. Again, all absolute and relative adrenal weight changes found in main two-generation study were well within the range provided by the corresponding historical control datasets. The same was true for the pre-pairing body weights as well as the preparing feed consumption except for the high-dose F0 group which had a feed consumption marginally above the HCD maximum value.
It is also questionable whether the effects seen on the adrenals constitute a 'significant

toxic effect' as stipulated in the CLP Guidance, given the lack of any functional changes i.e. lack of any clear effects on sterol metabolism. Such effects would have impacted fertility and given the potential influence on cholesterol metabolism, which is fulcrum to healthy eyes and would have resulted in eye effects expressed as cataract formation, which dogs are known to be particularly sensitive. There is no evidence that fosthiazate has an effect on sterol metabolism (i.e., findings in the eyes (cataract), serum cholesterol levels, clinical indications of liver damage and liver histopathology such as steatosis.1. Overall, the proposed classification with Category 2 (STOT RE 2, H373) based on changes in the adrenals is not merited, given these effects are; (i) likely to be secondary to neurotoxicity and (ii) the effects on the adrenals have no functional consequence and therefore not considered to be a 'significant toxic effect' as stipulated in the ECHA CLP Guidance. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Fosthiazate classification rebuttal (commenting phase) Public.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fosthiazate classification rebuttal (commenting phase)_final.zip
Dossier Submitter's Response
Thank you for the comment. Again, as in the case of reproductive toxicity, we consider the applicant's argumentation that the effects observed in the adrenals are "*secondary*" to neurotoxicity/systemic toxicity/stress (as a result of cholinesterase inhibition) and should therefore not be considered not sufficiently supported by the data. If the adrenal effects are related to cholinesterase inhibition (because cholinergic inhibition is a key event in these adverse effects), this should be considered as a mechanism of action in the WoE analysis. Adrenal weights were affected in all analysed animal species and also histopathological changes were identified in rats, mice and dogs. The DS considers this a significant toxic effect and therefore we keep our original proposal that fosthiazate should be classified as STOT RE 2 (H373) with the adrenals as target organ. The mechanistic considerations are in fact lending further support to this proposal.
RAC's response
Thank you for the comments on the STOT RE assessment following exposure to fosthiazate. RAC has carefully assessed the information on STOT RE and the outcome on classification and labelling is available in the RAC Opinion.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
29.03.2023	United Kingdom	<confidential>	Company-Manufacturer	20
Comment received				
No comments. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Fosthiazate classification rebuttal (commenting phase) Public.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fosthiazate classification rebuttal (commenting phase)_final.zip				
Dossier Submitter's Response				
The referenced attachment does not contain new information concerning the the classification as hazardous to the aquatic environment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
29.03.2023	United Kingdom	Health and Safety Executive	National Authority	21

Comment received

Fosthiazate (ISO) (EC: 619-377-3; CAS: 98886-44-3):
We note a long-term toxicity to C. riparius study (Anon., 1999) is available indicating no effects for the tested exposure range. Given fosthiazate is a nematicide and insecticide, it would be useful for the DS to present additional information to support the study endpoint including details of tested concentrations, effects and analytical verification.

Given long-term ECx endpoints with confidence intervals are preferred to NOECs for hazard classification, please can the DS confirm if an EC10 is available from the OECD TG 211 study (Anon., 1994).

We also note algal endpoints are based on 120-hour observations while 72- or 96-hour endpoints are preferred for hazard classification. While we recognise, it is unlikely to impact the hazard classification proposal, are 72-/96-hour endpoints available / able to be calculated?

Dossier Submitter's Response

Thank you for your comment. In the following, we provide additional information:

- **OECD 219 - *C. riparius*:**

Chemical analysis via LSC and HPLC showed recovery of total radioactivity > 80 % in all tested samples. No radioactivity was detected in the control samples. The radioactivity in the water phase decreased over the course of the experiment. The radioactivity in pore water remained ± constant from day 7 on. The radioactivity in sediment increased over the course of the experiment. Further information is given in the following table.

Nominal concentrations [µg a.s./L]	Day 0 water [µg/L]	Day 0 pore water [µg/L]	Day 0 sediment [µg/kg]	Day 0 Total radio-activity [%]	Day 7 water [µg/L]	Day 7 pore water [µg/L]	Day 7 sediment [µg/kg]	Day 7 Total radio-activity [%]	Day 24 water [µg/L]	Day 24 pore water [µg/L]	Day 24 sediment [µg/kg]	Day 24 Total radio-activity [%]
6	6	0.8	n.d.	101.2	5.6	3.9	n.d.	96.4	5.2	3.9	n.d.	86.9
13	13.0	n.d.	n.d.	103.3								
25	25.1	1.9	0.9	97.5	22.1	13.8	4.2	99.1	17.1	15.7	22.4	81.0
50	50.4	n.d.	n.d.	96.0								
100	100.3	11.4	2.9	95.9	88.7	50.1	55.4	98.5	82.3	63.2	86.1	90.8

n.d. not determined

No statistically significant effects on emergence or development were observed up to the highest tested concentration. A slight decrease in emergence rate (approx. 11 %) was visible at the highest tested concentration, but the applied statistical test (Dunnet-test, α = 0.05, one-sided smaller) did not detect a significant difference. EC_x-calculations were not provided with the study report. Additional calculations by the DS did not yield a reliable EC_{10} (likely due to the overall low effects observed in the study, leading to high uncertainty of the calculated EC_x) and the NOEC is the preferable endpoint. Further details on emergence rate and development are given in the following table:

Nominal concentrations [µg a.s./L]	Sum of inserted larvae	Sum of emerged midges	Emergence rate$_{arc*}$ (+ standard deviation)	Development rate (+ standard deviation)
Control	80	80	1.51 (0.113)	0.07 (0.000)
6	80	76	1.38 (0.137)	0.07 (0.001)
13	80	76	1.38 (0.137)	0.07 (0.001)
25	80	76	1.38 (0.137)	0.07 (0.001)
50	80	79	1.51 (0.113)	0.07 (0.001)
100	80	71	1.24 (0.098)	0.07 (0.000)

*Emergence rate was arcsin-transformed

- **OECD 211 – *D. magna*:**

In the study report EC_{50} values are given (89 µg/L), which are however only calculated via the geometric mean of the highest and second-highest concentration. Additional EC_x recalculations by the DS showed low reliability due to very wide and overlapping confidence intervals (likely due to the very steep concentration-response relationship). The estimated EC_{10} (log-logistic model) was 62.89 µg/L (CI: 11.68 – 114.1 µg/L), but the NOEC = 60 µg/L was preferred as endpoint for this study.

- **OECD 201 – *R. subcapitata*:**

No relevant effects on algal cell number could be observed at any of the sampled time points. Therefore, the E_rC_{50} and E_bC_{50} are also > 4.51 mg a.s./L for 72 h and 96 h. The choice of time point does not affect the overall endpoint.

Overall, the provided additional information does not lead to changes of the proposed classification.

RAC's response

RAC supports that in the study with *C. riparius* no significant effects were seen on emergence or development after study duration.

RAC agrees that the NOEC is the preferred endpoint for the study conducted according to OECD TG 211 as effects were only seen in the highest test concentration. The EC10 shows low reliability du to the very wide confidence intervals. RAC notes that both values for NOEC and EC10 are in the same range coming to the same classification.

RAC supports that in the study with *R. subcapitata* no effects were seen at any time point during the study indicating that the E_rC_{50} and E_bC_{50} is also > 4.51 mg a.s./L after 72 and 96 hours.

Date	Country	Organisation	Type of Organisation	Comment number
23.03.2023	United Kingdom	Health and Safety Executive	National Authority	22

Comment received

Fosthiazate (ISO) (EC: 619-377-3; CAS: 98886-44-3):
We note a long-term toxicity to C. riparius study (Anon., 1999) is available indicating no effects for the tested exposure range. Given fosthiazate is a nematicide and insecticide, it would be useful for the DS to present additional information to support the study endpoint including details of tested concentrations, effects and analytical verification.

Given long-term ECx endpoints with confidence intervals are preferred to NOECs for hazard classification, please can the DS confirm if an EC10 is available from the OECD TG 211 study (Anon., 1994). We also note algal endpoints are based on 120-hour observations while 72- or 96-hour endpoints are preferred for hazard classification. While we recognise, it is unlikely to impact the hazard classification proposal, are 72-/96-hour endpoints available / able to be calculated?
Dossier Submitter's Response
Please refer to our response to the previous comment 21.
RAC's response
Please see response above on comment 21.

Date	Country	Organisation	Type of Organisation	Comment number
31.03.2023	France		MemberState	23
Comment received				
No comment.				
Dossier Submitter's Response				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
30.03.2023	Belgium		MemberState	24
Comment received				
Based on the results of the aquatic toxicity test on the most sensitive species (Daphnia magna with 48h EC50 = 0.28 mg/L and 21d NOEC = 0.06 mg/L), the fact that the substance is not rapidly degradable, it is justified to classify, following the classification criteria of regulation 1272/2008, as Aquatic Acute 1, H400 and Aquatic Chronic 1, H410. In view of the proposed classification and toxicity band for acute toxicity between 0.1mg/L and 1 mg/L, an M-factor for acute toxicity of 1 is warranted. A M-factor of 1 for chronic toxicity is justified based on the non-rapidly degradability of the substance and a chronic toxicity band of 0.01 and 0.1 mg/L. In conclusion : we support the proposed environmental classification.				
Dossier Submitter's Response				
Thanky you for your support.				
RAC's response				
Thanky you for your support.				

OTHER HAZARDS AND ENDPOINTS – Physical Hazards

Date	Country	Organisation	Type of Organisation	Comment number
31.03.2023	France		MemberState	25
Comment received				
Was not reviewed.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
29.03.2023	United Kingdom	<confidential>	Company-Manufacturer	26
Comment received				
No comments. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Fosthiazate classification rebuttal (commenting phase) Public.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fosthiazate classification rebuttal (commenting phase)_final.zip				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

PUBLIC ATTACHMENTS
1. Fosthiazate classification rebuttal (commenting phase) Public.zip [Please refer to comment No. 2, 3, 8, 11, 15, 19, 20, 26]

CONFIDENTIAL ATTACHMENTS
1. Fosthiazate classification rebuttal (commenting phase)_final.zip [Please refer to comment No. 2, 3, 8, 11, 15, 19, 20, 26]