**Comments on the "CLH report - Proposal for Harmonised Classification and Labelling of 4,4'-sulphonyldiphenol (CAS 80-09-1)" provided by the Belgium FPS Public Health, Food Chain Safety and Environment**

## Introduction:

The below mentioned comments are in response to the submitted harmonized classification and labelling dossier for 4,4'-sulphonyldiphenol (CAS 80-09-1; figure 1) (version number: 2; October 2019).

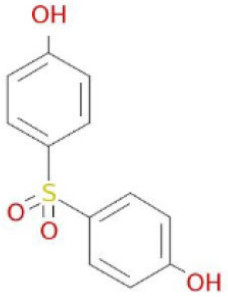


Figure 1: Structural formula of 4,4'-sulphonyldiphenol

The CLH dossier focuses on classification and labeling for reproductive toxicity. Currently, 4,4'-sulphonyldiphenol is self-classified and labelled as Repr. Cat. 2 H361F in two out of the three public REACh registration dossiers. However, the CLH dossier submitter concluded based on the available data that classification and labelling as Repr.Cat 1B H360FD is warranted.

## Comments:

The conclusion of the dossier submitter regarding the harmonized classification and labelling of 4,4'-sulphonyldiphenol was based on effects observed in available animal studies. However, there are several inconsistencies in the reporting of the available data on reproductive toxicity in the CLH dossier, which might lead to misleading conclusions.

1. In the reproductive toxicity study according to OECD TG 421 (Anonymous 12, 2000; page 11) the dossier submitter reported that the relative weights of pituitary and liver was significantly increased and the relative seminal vesicle weight was significantly decreased in male animals.

   Regarding the seminal vesicle weights the original data, however, show, that only the absolute weight but not the relative weight was decreased (dose group 0-3, seminal vesicle abs. weight [g]: 2.825 / 2.718 / 2.860 / 2.428**). A decrease in weight was observed in the highest dose only and, thus, dose-dependency is questionable. This is furthermore supported, because no effects on relative seminal vesicle weights and no histopathological correlates were observed. Historical control data were not provided in the report, but comparable historical control data taken from Charles River Ashland on Crl:CD(SD) rats (time period: 12/2000 – 08/2018; number of studies/control groups: 89/91) show a mean seminal vesicle weight of 2.12 g and a range of 1.82 g to 2.44 g. In conclusion, the effect on absolute seminal vesicle weights was considered as non-adverse and should either be removed from the tabular overview of the screening study results or be described appropriately and accordingly.

2. For the reproductive toxicity study according to OECD TG 443 (Anonymous 13, 2019; page 12) the dossier submitter reported that a significant relative reduction of sperm motility was observed in male animals in all tested dose groups (88, 84*, 85* and 86* %, respectively at 0, 20, 60 and 180 mg/kg bw/d). Furthermore, on page 19 the dossier submitter stated that "*Male reproduction parameters were examined and revealed a significant lower percentage of sperm motility (88, 84*, 85* and 86* % respectively at 0, 20, 60 and 180 mg/kg bw/d).*

*Other parameters were not affected (total spermatids/gram testis, total sperms/gram cauda epididymis, % of abnormal sperms, male mating index and male fertility index).*"

The motility of the sperm was significantly lower in males of test groups 01, 02 and 03 (20, 60 and 180 mg/kg bw/d) compared to controls. However, the lowest value (84% motile sperm) exactly matched the control value in the F1 generation and the overall span of sperm motility in this study reflects a normal range of biological variation in rat (multi)generation studies. No dose-dependency was observed which should have been expected if the effect was treatment-related. Therefore, these statistically significant differences were regarded as incidental and not treatment-related. For the cohort F1A no treatment-related effects were observed as well concerning motility of the sperm and the incidence of abnormal sperm in the cauda epididymides as well as sperm head counts in the testis and in the cauda epididymides. Historical control data of Charles River Ashland, (Crl:CD(SD), OECD 412/422/443) showed a mean of 83.3% and a range of 75.0% to 88.0% supporting the argument that the variation observed was within the normal biological range observed in rat (multi)generation studies.

3. The dossier submitter furthermore reported on page 12 (Anonymous 13, 2019) that the mean number of post-implantation loss was significantly affected (1.5** vs 0.5 in control).

   This should be corrected to "Mean nb of post-implantation loss sign. affected in mid and high dose group (1.3* and 1.5** vs 0.5 in control)" in F0 generation. Historical control data (Charles River Ashland, Crl:CD(SD), female fertility, post-implantation loss/dam) show a mean number of 0.8 (range: 0.4-1.8), however, this effect was dose-dependent, statistically significant and outside the historical control data in Cohort 1B (0.9 / 0.8 / 1.1 / 3.3**).

4. The dossier submitter reported on page 12 (Anonymous 13, 2019) for F1 pups: "Sign. lower tot. nb. of liveborn pups (285* at 180 mg/kg bw/d vs 340 in control) and sign. higher nb of stillborn pups (8* at 180 mg/kg bw/d vs 2 in control)"

   However, the number of stillborn pups in the respective dose groups (0, 20, 60, 180 mg/kg bw/d) was 2, 5, 3, and 8*. Although a significant increase was observed in the high-dose group, no dose-dependency was obvious. The number of eight stillborn pups in the high-dose group seems high, however, this is not an unusual high number in this rat strain, which is supported by six stillborn control pups in the F2 generation. Unfortunately, no historical control data were available from Charles River Ashland in this regard (abs. number of stillborn pups/dam).
   In addition, ratios of liveborn pups/group versus total number of pups delivered/group are not affected (99, 98, 99, 97%). These ratios show clearly that there is no impact of the number of stillborn pups or on the viability of the pups. The significant lower total number of liveborn pups (285* at 180 mg/kg bw/d vs 340 in control) is only the direct consequence of the postimplantation loss. The same finding is observed in the cohort 1B (for F2 pups).

5. The dossier submitter reported on page 13 (Anonymous 13, 2019) and page 37 (table 36) regarding the thymus weights.

   The thymus weights/cohort are summarized in the table 5.1 below and the argumentation should be adopted according to the following arguments: in the F0 generation, contrary to the cohorts 1A and 3, a significant **in**crease (compared to the control group) was observed in both absolute and relative thymus weights, however, only at the mid-dose level (60 mg/kg bw/d). The thymus weight returns to normal value at 180 mg/kg bw/d. Thus, there was no concentration-dependency. In the cohort 1A, there was a **de**crease observed. However, the decrease was observed in the high-dose group, only, and was statistically significant only for the absolute thymus weights (350.9* vs 435.7 in control animals; 81%*), but not for the relative thymus weights (84%). In the cohort 3, however, a similar and statistically significant **de**crease in relative thymus weights (81%*) at 180 mg/kg bw/d was observed in male animals. The absolute thymus weights were not significantly affected (85%). Furthermore, no findings in clinical chemistry were observed in either of the cohorts. By looking at all data related to thymus weight, either in males or females, and whatever the generation (P0, cohort 1A and cohort 3), no clear dose-dependency was observed. Therefore, the decrease

in thymus weight was regarded as an equivocal finding and could be removed from the table which focuses on adverse effects.

Table 5.1: Summary of the thymus weights/cohort:

| | absolute vs control | | | relative vs control | | |
|---|---|---|---|---|---|---|
| | 20 mg/kg bw/d | 60 mg/kg bw/d | 180 mg/kg bw/d | 20 mg/kg bw/d | 60 mg/kg bw/d | 180 mg/kg bw/d |
| F0 | 113% | 113%* | 93% | 116% | 112%* | 96% |
| Cohort 1A | 96% | 100% | 81%* | 98% | 101% | 84% |
| Cohort 3 | 97% | 104% | 85% | 94% | 100% | 81%* |
| Surplus F1 (PND 22) | 124%** | 117%* | 108% | 120%** | 106% | 100% |

With regard to the T-cell dependent antibody response in female animals (dose group 0-3: 13.647 / 8.239 / 9.598 / 14.555), there was neither a dose dependency nor a significant change in T-cell dependent antibody response observed. In consequence, this is considered as non-adverse and not related to treatment with test-substance.

6. On page 16 the dossier submitter reports with regard to the food consumption: “A statistically significant decrease of food consumption was only observed at day 3 of the premating period in males and females at the high dose group (24.3 mg/kg bw/d vs 30.7 mg/kg bw/d in control group and 14.8 mg/kg bw/d vs 20.2 mg/kg bw/d in control group, respectively in males and females).”

   The units are not reported in a correct way. This sentence should be corrected to: “A statistically significant decrease of food consumption was only observed at day 3 of the premating period in males and females at the high dose group (24.3 g/animal/d vs 30.7 g/animal/d in control group and 14.8 g/animal/d vs 20.2 g/animal/d in control group, respectively in males and females).”

7. On page 16 the dossier submitter reports with regard to the number of implantation sites “Furthermore, a declining tendency in the number of implantation sites [..]”

   This statement is questionable and should be modified. The number of implantation sites in the respective dose-groups (group 0-3: 15.2 / 14.6 / 15.4 / 13.7) was not altered in a dose-dependent manner and there were no statistically significant changes observed. The numbers of implantation sites were well within historical control data (Charles River Ashland, Crl:CD(SD), OECD 412/422/443 implantation sites/dam: mean = 15.2 (range: 12.3 - 17.8). The numbers of implantation sites/dam were also within historical control data from CitoxLab (France; time period 02/2016 to 04/2020; rat strain: Sprague-Dawley, RjHan:SD (Rats CD®) (from Janvier or Charles River); two-generation reproduction toxicity studies (OECD 413)): F0: mean = 15.0 (range 13.8 - 16.0), F1: mean = 14.1 (range 12.1 - 15.3).

8. On page 18 a mistake in table 12 should be corrected:

| | males | | | |
|---|---|---|---|---|
| Dose level (mg/kg bw/d) | 0 | 10 | 60 | 300 |
| Pituitary rel. (*$10^{-3}$) | 2.89 | 2.63 | 2.88 | ~~4.43**~~ 3.43** |

9. On page 18 a mistake in table 13 should be corrected:

| Dose level (mg/kg bw/d) | | 0 | 10 | 60 | 300 |
|---|---|---|---|---|---|
| Cecum | Diffuse hyperplasia, mucosal epith. | 0/12 | / | 0/1 | ~~11/12**~~ 11/12*** |

***P<0.001

10. On page 19 the dossier submitter reported on test-substance related mortality: “Regarding the F0 parental generation, 1 female of the low dose group was sacrificed on study day 63 due to poor general condition.”

    This suggested that mortality was related to treatment. However, test-substance treatment clearly did not induce mortality in any study performed with 4,4’-sulphonyldiphenol. This should be unmistakably stated throughout the complete CLH report on 4,4’-sulphonyldiphenol.

11. On page 20 the dossier submitter reported regarding the mean number of F1 pups: “Regarding offspring examination, the mean number of F1 pups per dam was lower at the mid and high doses. Moreover, the mean pup body weight was significantly higher at the 2 highest dose levels.“

    Although the mean number of F1 pups delivered per dam (average litter size) was lower in the mid- and high-dose groups (14.9 / 14.0 / 13.5 and 12.7 pups/dam), the decrease versus the control group did not reach statistical significance. In addition, (as discussed in the section 4), the decrease of the mean number of pups is the direct consequence of the postimplantation loss (as there is no significant decrease of the number of implantation sites). This should be added in the CLH report. Furthermore, it should be reflected that all changes were within the historical control data (Charles River Ashland, Crl:CD(SD), OECD 412/422/443 mean number of pups born: mean = 14.3 (range: 12.1 - 15.9)).

12. On page 23 the dossier submitter reported: “However, a slightly reduced number of females with liveborn pups was observed at 60 and 180 mg/kg bw/d. The oestrous cycle was also modified at the highest dose level. Furthermore, the mean number of implantation sites tended to decrease at 180 mg/kg bw/d.“

    With regard to the number of females with liveborn pups (dose group 0-3: Cohort 1B: 24 / 24 / 21 / 21) the number of females with liveborn pups was slightly reduced at the mid and high dose, but this change was not statistically significant, and no obvious dose dependency was observed. This should be reflected in the report.

    Oestrous cycle data, generated during the last 3 weeks prior to mating to produce the F2 litter, revealed regular cycles in the females of all test groups including the control. The mean oestrous cycle duration in the different test groups was comparable: 3.9 days in control group, 4.0 in the low- and mid-dose group and 4.5 days in the high-dose group. This variation is not statistically significant. Furthermore, all changes were within the historical control data (Charles River Ashland, Crl:CD(SD), mean estrous cycle length (days), OECD 412/422/443: mean = 4.2 (range: 3.9 - 5.2)). The mean oestrous cycle length was also within historical control data from CitoxLab (France; time period 02/2016 to 04/2020; rat strain: Sprague-Dawley, RjHan:SD (Rats CD®) (from Janvier or Charles River); two-generation reproduction toxicity studies (OECD 413)). The historical control data from CitoxLab (France) gave a range 4.0 - 5.8 (F0 generation: mean = 4.9 (range of individual values: 3.0 - 18.0 / range of study means: 4.0 - 5.8)) for F0 generation and 4.4 - 4.9 (F1 generation: mean = 4.5 (range of individual values: 3.0 - 9.5 / range of study means: 4.4 - 4.9) for F1 generation.

13. On page 25 a mistake in table 23 should be corrected:

| Dose level (mg/kg bw/d) | 0 | 30 | 100 | 300 |
|---|---|---|---|---|
| Fertility index (%) | 100 | 90 | 100 | ~~60~~ 80 |

14. With regard to the uterus weight the dossier submitter reported on page 25: “Furthermore, uterus weight was increased in females exposed to 300 mg/kg bw/d. Microscopic examination revealed also changes in these organs as well as in mammary gland in males (see table 25).”. Another statement was included on page 44: “Uterus weight was increased in females exposed to 300 mg/kg bw/d (0.197, 0.224, 0.224 and 0.307, respectively at 0, 30, 100 and 300 mg/kg bw/d). Microscopic examination revealed also changes in these organs as well as in mammary gland in males.”

    The uterus weight was changed only in the high-dose group, but this alteration was without statistical significance (dose groups 0-3: abs. weights [g]: 0.601 / 0.669 / 0.648 / 0.850; rel. weights [%]: 0.157 / 0.224 / 0.224 / 0.307). Therefore, a dose-response relationship is questionable. The above-mentioned microscopic examinations should reflect that in the high-dose group 2/10 animals with eosinophil infiltrates in the uterus were observed, but that such effect was also observed in the control group in 1/10 animals. Unfortunately, no historical control data from Charles River were available to further interpret the relevance of this finding.

15. On page 25 a mistake in the headline of table 24 should be corrected: “Organ weight data (in g)” has to be changed in “Relative organ weight data (in %)”

16. On page 26 the dossier submitter stated “A significantly lower bwg value was noted in males of the highest dose level (no further information available).“

    This should be further specified. The body weight of male animals on day 28 was not significantly changed (dose group 0-3: body weight [g]: 487.4 / 489.1 / 450.7 / 430.1), but the body weight change of male animals between days 0-28 was changed (dose group 0-3: body weight change [g]: 120.3 / 110.3 / 74.9 / 57.5*).

17. On page 31 a typo in table 31 should be corrected:

| | males | | | |
|---|---|---|---|---|
| Dose level (mg/kg bw/d) | 0 | 100 | 300 | 1,000/600 |
| D 42 | 351.4 | 343.8 | 326.0 | ~~293.3**~~ 294.3** |

18. On page 34 the dossier submitter stated: “In the EOGRTS (Anonymous 13, 2019), the number of implantation sites was moderately modified only in the cohort 1B (13.7 vs 15.2 at 180 and 0 mg/kg bw/d, respectively). However, the DS wants to highlight that this effect appeared at a much lower dose than in Anonymous 12 (2000) and in Anonymous 14 (2017).”

    The number of implantation sites should be reflected considering all available information. In the P0 generation the number of implantation sites was 15.3, 14.8, 14.9, and 14.3 (dose group 0-3: 0, 20, 60, and 180 mg/kg bw/d), and in the F1 generation 15.2, 14.6, 15.4, and 13.7 (dose group 0-3: 0, 20, 60, and 180 mg/kg bw/d). The historical control data (Charles River Ashland, Crl:CD(SD), OECD 412/422/443) showed a mean number of implantation sites/dam of 15.2 (range: 12.3 - 17.8). The numbers of implantation sites were also within historical control data from CitoxLab (France; time period 02/2016 to 04/2020; rat strain: Sprague-Dawley, RjHan:SD (Rats CD®) (from Janvier or Charles River); data from two-generation reproduction toxicity studies (OECD 413)): F0: mean = 15.0 (range 13.8 - 16.0); F1: mean = 14.1 (range 12.1 - 15.3) The putative decrease in the high-dose group was

neither statistically significant, nor was an obvious dose dependency observed and the data were clearly within the historical control range.

Also the second statement of the dossier submitter does not reflect the available data appropriately, because the number of implantation sites did not decrease at much lower doses compared to other studies. The first study (Anonymous 12, 2000 – Japan) showed numbers of implantation sites of 15.9, 13.3, 14.8, and 10.7 at 0, 10, 60, and 300 mg/mg bw/d, respectively. The second study (Anonymous 14, 2017 – OECD 422) showed numbers of implantation sites of 15.8, 15.0, 15.5, and 10.4** at 0, 30, 100, and 300 mg/kg bw/d, i.e. the statement should be that on a screening level (OECD 422) the number of implantation sites is affected at (much) higher doses than in the higher tier OECD 443 study.

19. On page 35 of the CLH dossier the dossier submitter stated: "All these severe effects cannot be explained by maternal toxicity as general condition of the animals was unaffected by the treatment in all studies. Animals only exhibited excessive salivation just before or immediately after exposure to the test substance in all studies. Moreover, body weight examination showed only slight variations during the premating and mating periods that cannot be accounted for these effects. Severe decreased number of implantation sites and severe higher oestrous duration were observed in three different studies."

    As mentioned above, the only significant decrease of implantation sites outside the historical control data was observed at 300 mg/kg bw/d on a screening level (OECD 422), therefore above statement should be rephrased in a more balanced way. The same holds true for the oestrous cycle length. The mean oestrous cycle length (days) in the OECD 443 study was 3.9, 3.9, and 4.1* for the P0 generation, 4.1, 4.1, 4.1, and 4.1 days for cohort 1A and 3.9, 4.0, 4.0, and 4.5 days for cohort 1B (dose groups 0-3). The oestrus cycle length for cohort 1B will subsequently be amended to 3.9, 4.0, 4.0, and 4.1 (see below[1]). The screening studies showed values of 4.08, 4.01, 4.14, and 5.57** days (OECD 421) and 4.02, 3.97, 4.01, and 5.16* days (OECD 422) (dose groups 0-3), however, the high dose group was much higher at 300 mg/kg bw/d on screening level (OECD 421 and 422) instead of 180 mg/kg bw/d (OECD 443). The historical control data (Charles River Ashland, Crl:CD(SD), OECD 412/422/443) gave a range of 3.9 – 5.2 days (mean = 4.2 days). The historical control data from CitoxLab (France; time period 02/2016 to 04/2020; rat strain: Sprague-Dawley, RjHan:SD (Rats CD®) (from Janvier or Charles River); data from two-generation reproduction toxicity studies (OECD 413)) gave a range of 4.0 - 5.8 (F0 generation: mean = 4.9 (range of individual values: 3.0 - 18.0 / range of study means: 4.0 - 5.8) for F0 generation and 4.4 - 4.9 (F1 generation: mean = 4.5 (range of individual values: 3.0 - 9.5 / range of study means: 4.4 - 4.9) for F1 generation. Overall, there were no significant effects observed on mating, fertility, gestation index, and duration of gestation, which questions whether classification with Category 2 for fertility would be sufficient.

20. On p. 38 the dossier submitter reported: "However, the maternal care was not affected. "

    This statement is misleading and should be removed, because maternal care cannot be examined in an OECD 414 study as animals were killed on GD 20.

21. On page 42 the dossier submitter reported: "Moreover, the mean pup body weight was significantly higher at the 2 highest dose levels (see table 44)."

    The statement should include that higher body weights were secondary and due to reduced litter size.

22. On page 44 the dossier submitter reported: "At PND 21, a higher body weight value was noted in male pups of the low dose group (+ 6.6 % compared to the control group)."

[1] EFSA checked the raw data of the study and concluded that one animal should not be included in the calculation of the oestrus cycle length of cohort 1B. During the observation period this animal showed only once the beginning of a cycle (a proestrous) but a cycle end (another proestrous or estrous) was never detected. Thus, the analysis software took the whole observation period as one very long cycle, which explains the higher average and standard deviation in table IA-141 of the original report. The value that EFSA calculated is correct and the report will be amended accordingly.

The statement should include that there was no dose dependency and no statistical significance. The male body weights at PND21 were +6.6% / -1.5% / +2.3% compared to control.

23. On page 45 the dossier submitter reported: “In the prenatal developmental toxicity study (Anonymous 19, 2014), the mean percentage of post-implantation loss was increased at the highest dose group (4.7, 3.9, 3.9 and 6.3 %, respectively at 0, 30, 100 and 300 mg/kg bw/d).”

    The historical control data of the test lab (BASF, Wistar, OECD 414) showed a mean percentage of 7.1% and a range of 4.7% to 12.0%. Therefore, the observed differences are considered to reflect the normal range of fluctuations for animals of this strain and age. Furthermore, no dose-dependency was observed. The statement should be adopted.

## Summary and Conclusion:

For assessing the hazard class ‘toxicity to reproduction’, the most relevant and conclusive study for regulatory purposes is the Extended One-Generation Study according to OECD TG 443 (EOGRTS) (Anonymous 13, 2019). This study has been requested for 4,4’-sulphonyldiphenol by the eMSCA in the substance evaluation process “REACH substance evaluation (CoRAP)”. The study was recently available and was already used by the dossier submitter (CA Belgium) in the above discussed CLH dossier for DHDPS.

Overall in the recent version of the CLH report there are several inconsistencies in the reporting of the data on reproductive toxicity. Those inconsistencies should be adopted and reflected in the discussion. Data on historical control data should be added and discussed in order to allow a fair and objective presentation of available data and to avoid a misleading interpretation.

Although some effects observed in the EOGRTS study could be useful for classification and labelling of 4,4’-sulphonyldiphenol, the results did also show that 4,4’-sulphonyldiphenol can be used safely: the OECD443 study clearly demonstrates a robust no observed adverse effect level (NOAEL). This NOAEL is subsequently used as point of departure for risk assessment and derivation of a derived no effect level (DNEL) as a safe exposure level for risk assessment, by using appropriate assessment factors according to EChA guideline. This DNEL will be included in the subsequent REACh registration dossier update of 4,4’-sulphonyldiphenol and will be included in the communicated safety data sheets as well. This way, safe use conditions will be defined and communicated throughout the trade chain.