Template for providing comments on CLH report (Proposal for Harmonised Classification and Labelling)

1. Physical/Chemical Properties; Data on application and efficacy; Further Information; Methods of Analysis

Physical and chemical properties of the active substance			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
1(3)	Vol.1, 2.2.1.1.15 – Corrosive to metals	Applicant : The RAR states that for solids, test C.1 (37.4.1.1 of the UN-RTDG) is only necessary when the substance becomes liquid during transport, and that even if this is unlikely for sulcotrione, an appropriate argumentation is missing. According to the applicant, melting temperature of sulcotrione is 139°C whereas according to ECHA's Guidance on the Application of the CLP Criteria, solids having a melting point lower than 55 °C (temperature in the test) must be taken into consideration. This could serve as an appropriate argumentation	

2. Effects on human and animal health

Short-term toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
2 (1)	Vol 3 B.6.3.2.1 Oral 90-day study rat	Applicant: The applicant proposes to complete the study summary add the non-reported parameters, even if there is no statistically significant changes like for cholinesterase.	
2 (2)	Vol 3 B.6.3.2.1 Oral 90-day study rat	Applicant: The applicant does not agree with the RMS that no NOAEL could be derived for male rats due to keratitis in one male observed even at lowest dose level of 50 ppm (3.3 mg/kg bw/d). A relevant NOAEL considering the liver and kidney effects observed at 300 ppm, can be set at 50 ppm, considering the ocular effect observed in rats are not relevant to humans	
2 (3)	Vol 1 Table 2.6-37 (28-day dermal, rats, 2003 and 2009)	Applicant: The applicant disagrees with the RMS in considering liver and kidneys as target organs in these studies.	In the first study conducted in 2003, a statistically significant increase in liver weights was observed in both sexes at the highest dose administered. However, this effect was of small magnitude (approximately 10%) and was not accompanied by any histopathological or biochemical findings. Therefore, it should be considered a non-specific and adaptive response, suggesting that the liver is not a target organ in this study. In the subsequent study conducted in 2009, similar effects were observed in the liver, with increases in relative liver weight observed in male rats at doses of 250 and 1000 mg/kg bw/day. Similarly, this effect was of small magnitude and was not associated with any histopathological findings or changes in biochemical parameters. Hence, it should also be considered an adaptive response to the treatment.

Short-term toxicity

No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
			Increases in kidney weight (abs) were observed in male rats only at 250 and 1000 mg/kg bw/day. However, this significant increase was not associated with treatment-related histopathological findings at the highest dose. Consequently, this effect was not considered adverse and should not be regarded as a target effect for further consideration in the STOT-RE classification. No similar effects in the liver and kidneys were observed in the females. **Organ Weights: Liver and Kidneys**

Dosa levels (mg/kg bw/day)	0	50	100	250	1000
Males					
Liver weight Abs Rel	18.84 4.90	20.50 4.877	19.43 4.930*	21.13 5.034* - (+2.7%)	22.28 5.179** (+5.7%)
Kidney weight Abs Rel	2.84 0.679	3.01 0.719	2.85 0.725	3.14* (+10.6%) 0.748	3.22** (+13.4%) 0.753
Females					
Liver weight Abs Rel	12.06 4.483	12.96 4.571	10.79 4.222	11.47 4.514	11.72 4.533
Kidney weight Abs Rel	2.06 0.767	1.99 0.704	1.91 0.747	1.86 0.733	1.93 0.742

Significantly different from Group 1: *p<0.05,**p<0.01

2 (4)	Vol 1 Table 2.6-37 raw 10 (90-day oral, dogs)	Applicant: According to the RMS the following organs are considered as target organs: eyes, CNS, blood, thymus, digestive tract, lymph nodes, lung.	

Short-term toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		Although it's evident that the eyes and blood are target organs for specific toxicities of sulcotrione more objective consideration needs to be given to other findings to determine whether they could be considered as indicative of a "target" organ. For instance, assessing the consistency of effects on organs such as the lungs, digestive tract, lymph nodes, thymus, and CNS across studies is essential. It's crucial to ascertain whether these effects might have been influenced by other factors or their significance at doses beyond the maximum tolerated dose (MTD) or doses approaching the limit dose. Given the variability in effects and other factors, the utility of designating certain organs as "target organs" becomes questionable	
2 (5)	Vol 1 Table 2.6-37 raw 12 (1-year oral, dogs)	Applicant: The applicant disagrees with the proposed NOAEL of 50 mg/kg bw/d (see point 3). In addition, the applicant disagrees to consider liver and kidney as target organs.	Considering effect observed in the eyes, there were not considered treatment related by the study director of the study. The increase in hepatic enzymes is temporary and thus cannot be considered as adverse. In addition, the increase of liver weight is not dose related, and there are no histopathological findings, therefore liver cannot be considered as a target organ. Regarding effect observed in kidneys, considering the absence of histopathological findings to corelate the increased kidney weight and the absence of dose response, the adversity of the effect is considered questionable.

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
2 (6	Vol 3 B.6.4.1.4- Study 4 – Bacterial reverse mutation test	Applicant: The applicant disagrees with the RMS to consider this study as fully reliable, considering that the purity (92.4%) was below 95% (lower than minimum required purity) and data on impurities are not available. Other studies conducted in 2007 and 2016 were provided, without deviations and can be considered as more reliable to this old study. This study was with a batch of unknown or different technical specification, and not pertinent to the TS proposed. On this basis the study can be considered as no longer relevant. A new study representative of the ADAMA proposed TS is now available. For these reasons it may be justified to consider the study as supplemental only. An additional new study conducted in 2021 is available and can be provided upon request.	The tested batch used in the study and the result cannot be considered as representative and relevant for the substance of the applicant, considering the low purity and different TS/manufacturing source. In addition, one strain is lacking. A new OECD 471 study was conducted in 2021, with a batch representative of the applicant's proposed TS. Based on the results of the representative study it is concluded that Sulcotrione technical is not mutagenic in the *Salmonella typhimurium* reverse mutation assay and in the *Escherichia coli* reverse mutation assay.
2 (7)	Vol 3 B.6.4.1.10 – Study 10 – In vitro mammalian cell gene mutation	Applicant: The HCD will be provided.	
2 (8)	Vol 3 B.6.4.1.11 – Study 11 – In vitro mammalian cell micronucleus test	Applicant: The HCD will be provided	
2 (9)	Vol 3 B.6.4.1.12 – Study 12 – In vitro chromosome aberration test	Applicant: The applicant agrees with the RMS that the study should be considered as supplemental and performed an *in vitro* micronucleus study in 2021, available upon request.	An OECD 487 study was conducted in 2021, with a representative batch of the applicant. The test item did not induce a statistically significant or biologically relevant increase in the number of binucleated cells with micronuclei in the absence and presence of S9-mix, in either of the two experiments.

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		The original study was with a batch of unknown or different technical specification, and not pertinent to the TS now being proposed. On this basis the study can be considered as no longer relevant to current material. A new study representative of the ADAMA proposed TS is now available.	In conclusion, Sulcotrione technical is not clastogenic or aneugenic in human lymphocytes under the experimental conditions.
2 (10)	Vol 3 B.6.4.1.15- Study 15- Statement on photomutagenicity and QSAR analysis.	Applicant: The applicant disagrees with the RMS that photogenotoxicity cannot be excluded completely. According to the Outcome of the pesticides peer review meeting on general recurring issues in mammalian toxicology EFSA 2016, and based on the existing information, the photogenotoxicity may be excluded.	The photogenotoxicity concern, may be excluded according to the Outcome of the pesticides peer review meeting on general recurring issues in mammalian toxicology EFSA 2016. According to this document, if there is a negative response from the phototoxicity test, no photomutagenicity test is required; Besides, the experts proposed that the phototoxicity test should not be performed if it has been demonstrated that the test material only absorbs at wavelengths lower than 313 nm (instead of 290 nm) and there is insufficient absorption at longer wavelengths to trigger testing in the absence of further guidance. In addition, the applicant already provided QSAR regarding the photogenotoxicity of the active substance. Considering that, sulcotrione was not phototoxic in a study performed according to OECD 432 TG, and there is no relevant absorption above 313 nm. (cf. KCA 2.4_03). Photogenotoxicity potential can be excluded.
2 (11)	Vol 3 B.6.4.2.1- study 1 – micronucleus assay	Applicant: The applicant disagrees with the conclusion of the RMS that sulcotrione is genotoxic/ clastogenic.	In 2022, the applicant performed a combined micronucleus and alkaline comet test with sulcotrione (purity 95.5%). The study was conducted according to OECD guidelines 474 and 489.

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		The study is an old study, with deviation and conducted with a batch with a lower purity (92.4%) than the minimum required one. It was with a batch of unknown or different technical specification, and not pertinent to the TS being proposed. On this basis the study can be considered as no longer relevant to current material. A new study representative of the ADAMA proposed TS is now available. In addition to the 2 other existing studies a new *in vivo* micronucleus and comet test is available upon request.	Wistar rats were exposed to concentrations up to 2000 mg/kg bw/d. There was no biological relevant increase in the mean frequency of micronucleated polychromatic erythrocytes in the bone marrow. Moreover, Sulcotrione technical was not genotoxic in the Comet assay in Liver and Duodenum cells.
2 (12)	Vol 3 B.6.4.2.3- Study 3- micronucleus assay	Applicant: The applicant agrees that there is no demonstration of bone marrow exposure in the study and thus performed an additional study: pharmacokinetic study of sulcotrione following oral gavage administration to CD-1 mice (study No. 20300238), to demonstrate that in CD-1 mice there is exposure of bone marrow at 1500 and 3000 mg/kg bw.	A proof of exposure study in rats is available upon request, in conjunction with a new *in vivo* micronucleus/ comet assay that has been performed in rats and is available upon request. Further reasoning is also provided in the Appendix to this commenting section.
2 (13)	Vol 3 B.6.4.2.3- Study 3- micronucleus assay	Applicant: The applicant agrees that there is no demonstration of bone marrow exposure in the study and thus performed an additional study: pharmacokinetic study of sulcotrione following oral gavage administration to CD-1 mice (study No. 20300238), to demonstrate that in CD-1 mice	A proof of exposure study in rats is available upon request, in conjunction with a new *in vivo* micronucleus/ comet assay that has been performed in rats and is available upon request. Further reasoning is also provided in the Appendix to this commenting section.

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		there is exposure of bone marrow at 1500 and 3000 mg/kg bw.	
2 (14)	Vol 1 Table 2.6-41 Row 4	Applicant disagrees to considered the study as acceptable regarding the low purity of the test item -below the minimal required) and considering that the study does not include all the strain required in an Ames test. There are several existing studies considered as more robust. See point 6	
2 (15)	Vol 1 Table 2.6-42 – Row 1	Applicant: The applicant disagrees with the conclusion of the RMS. See point 11	2 (16)
2 (16)	Vol 1 Table 2.6-42 – Row 2	Applicant: The study report is TOX2004-2850 in the DAR (instead of TOX9401326). The study was considered as acceptable by the applicant, in addition a proof of exposure was performed in an additional study that is available upon request.	2 (17)
2 (17)	Vol 1 Table 2.6-42– Row 3	Applicant: In order to consider the study as reliable the applicant performed a study to demonstrate the bone marrow exposure. The study is available upon request.	
2 (18)	Vol 1 2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity/germ cell mutagenicity	Applicant: Regarding the available Ames test, positives results were obtained in 2 old studies, for which were conducted with a material with a purity lower that the minimal required and or of unknow or dissimilar TS.	In 2022, the applicant performed a combined micronucleus and alkaline comet test with sulcotrione (purity 95.5%). The study was conducted according to OECD guidelines 474 and 489.

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		A comet assay (OECD 489) has been performed and is available upon request.	Wistar rats were exposed to concentrations up to 2000 mg/kg bw/d. Sulcotrione technical was not genotoxic in the Comet assay in Liver and Duodenum cells.
2 (19)	Vol 1 2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity/germ cell mutagenicity	Applicant: To answer to the RMS comments that no appropriate *in vivo* follow up test to investigate the induction of gene mutation is available. The applicant performed a combined *in vivo* micronucleus test and comet assay, available upon request.	
2 (20)	Vol 1 2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity/germ cell mutagenicity	Applicant: The Applicant disagrees with the RMS to consider the published literature on the endpoint genotoxicity concerning studies conducted on *Allium cepa* root meristem cells. These data were concluded to be not relevant to human heath risk assessment by the RMS. Moreover, the absence of phototoxicity (OECD 432) as well as the fact that the test material only absorbs at wavelengths lower than 313 nm, allow to conclude that photogenotoxicity potential can be excluded.	
2 (21)	Vol 1 2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity/germ cell mutagenicity	Applicant: The applicant provided genotoxicity studies with batch representative of their active substance (i.e batch 20160103 (new Ames and HPRT tests). Specifications have been updated with the renewal dossier (See Vol. 4), and thus relevance of the tested batch may be reconsidered.	Regarding impurity 2: Genotoxicity studies on impurity 2 are available in the Vol 3, as being a metabolite. The impurity 2 is not expected to be genotoxic based on the available data and a new *in vitro* micronucleus test (OECD 487) available upon request. Therefore impurity 2 is considered sufficiently investigated regarding genotoxicity potential. Impurity 5 is considered as non-relevant by the RMS, based on QSAR analysis performed by the RMS. Therefore, the lacking information

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
			regarding it content in the tested batch 20160103 (new Ames and HPRT tests) is not critical. mpurities 4 and 6 have been removed from the new reference specification proposed by the RMS. It is no longer necessary to include this impurity in the specification based on the data provided by the current applicants and thus that lacking information on the content of these impurities in the Ames and HPRT test performed in 2016 is no critical. In addition, recently performed *in vitro* genotoxicity studies (OECD 471, OECD 487 and OECD 490) with new batch are available upon request. The impurities were analysed in this batch and the analysis is available upon request.
2 (22)	Vol 1 2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity/germ cell mutagenicity	Applicant: The applicant performed a new *in vitro* micronucleus test (OECD 487) to clarify the clastogenic effect of sulcotrione *in vitro.*	A GLP, OECD 487 study was conducted in 2021 with sulcotrione (purity 95.5%), the test item was tested up to 2000 µg/mL for a 3 hours exposure in the absence and presence of S9-fraction, and up to 1400 µg/mL (due to cytotoxicity) for a 24 hours exposure time in the absence of S9-mix. The test item did not induce a statistically significant or biologically relevant increase in the number of binucleated cells with micronuclei in the absence and presence of S9-mix, in either of the two experiments.
2 (23)	Vol 1 2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity/germ cell mutagenicity	Applicant: Regarding existing *in vivo* micronucleus test, proof of exposure is available upon request in addition a new in vivo micronucleus test was performed and available upon request.	In 2022, the applicant performed a combined micronucleus and alkaline comet test with sulcotrione (purity 95.5%). The study was conducted according to OECD guidelines 474 and 489. Wistar rats were exposed to concentrations up to 2000 mg/kg bw/d. There was no biological relevant increase in the mean frequency of micronucleated polychromatic erythrocytes in the bone marrow. Moreover, Sulcotrione technical was not genotoxic in the Comet assay in Liver and Duodenum cells.

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
			A radiolabelled bone marrow proof of exposure study was also conducted prior to the new *in vivo* study. Further rationale on bone marrow exposure is provided in the Appendix to this Section.

Long-term toxicity and carcinogenicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
2 (24)	Vol 3 B.6.5.2 – Study 2 – 2-year study-rat	Applicant: The LOAEL derived by the RMS at 1 ppm based on kidney effects only in males is considered overly conservative. It is seen remarkably frequently in rats and test laboratories are well acquainted with this as a foible when using the rat as a test system and is interpreted accordingly when characterizing the hazard profiles of test items in study reports.	Kidney effects when seen only in male rats are not a specific or sensitive indicator of toxicity. There is considerable literature in the public domain around this topic and the mechanism involved: As the report itself states: "Organ weights generally showed a high variability as may be expected for elderly rats. There was a statistically significant group difference for liver weights in male rats in plastic cages. This was attributable to a single animal (#529) with masses on both medial lobes. The necropsy observations of enlarged kidney, distended renal pelvises and renal cysts showed some increase over control incidences at 10 ppm and above in male rats. These observations were associated with widely variable kidney weights and almost certainly were associated with chronic progressive nephrosis which is commonly seen in older Sprague-Dawley rats, particularly males. Given the high incidence of nephrosis in controls and variability in kidney weights, it is speculative whether the slight increases in incidences of these gross observations were of any biological

Long-term toxicity and carcinogenicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
			significance. In females, organ weights were not significantly different from control." (Reference is made to Anver MR & Cohen BJ (1979) The Laboratory Rat. Volume 1. But more recent descriptions which match the finding with sulcotrione very well can be found in e.g. Gad, S. C. (2016). *Animal models in toxicology.* 3rd Ed CRC press.)
2 (25)	Vol 1 Table 2.6-37 raw 4 (supplementary 2-year oral, rat)	Applicant: We would suggest that the renal findings represent more a flaw of the test system, rather than a "toxicity" of the test item hence 1 ppm could be the NOAEL rather than the LOAEL See point 24	
2 (26)	Vol 1. Table 2.6-46 raw 2 (supplementary 2-year oral, rat)	Same comments as point 25 (Vol 1 Table 2.6-37 raw 4)	
2 (27)	Vol 1. 2.6.5.1 Short summary and overall relevance of the provided information on long-term toxicity and carcinogenicity	Applicant: We would suggest that the renal findings represent more a flaw of the test system, rather than a "toxicity" of the test item hence 1 ppm could be considered a NOAEL rather than a LOAEL, and a BMD analysis would then not be necessary. See point 25	

Reproductive toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
2 (28)	Vol 3 B.6.6.1.1 - Study 1	Applicant: agrees with RMS in establishing the parental LOAEL at 10 ppm (0.6 mg/kg bw/d)	**Liver effects**

Reproductive toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		based on effects observed in males at 10 ppm and above, encompassing ocular lesions, increased kidney weight and histopathological finding in the kidneys. Nonetheless, Liver effects at 10 ppm are more appropriately regarded as adaptive response. Further details regarding the data are given in column 3. Also, Kidney effects are more likely a flaw of the test system than any indicator of a specific toxicity of sulcotrione.	In males, liver weights (absolute and relative) were significantly increased in P0 and P1 generations at 10 ppm and above, without dose response relationship. This changes in liver weight were not correlated to treatment-related histopathological effects. Slight increase incidence of mild vacuolation of hepatocytes was present in P0 treated animals but this was statistically significant only at mid-dose (225 ppm). For female, statistical increase of liver weight (abs. and rel.) is only observed at 225 ppm or above in P1 and at 5000 in P0, in addition there are no histopathological finding in the liver. The increase in absolute liver weight is not clearly dose-related in P1 females. The following sentence should be modified as followed to include the absence of dose-related effects in absolute liver: "In the P1 generation relative liver weight was also statistically significant increased at 225 ppm (+9%) and 5000 ppm (+12%)." Should be replaced by "In the P1 generation **absolute and** relative liver weight was also statistically significant increased at 225 ppm (**+13% and** +9% respectively) and 5000 ppm (**+8% and** +12% respectively), without a clear dose response relationship." This increase in liver weights was no associated to any treatment-related histopathological findings and should be considered as an adaptive response.
2 (29)	Vol 3 B.6.6.1.1 - Study 1	Applicant: The RMS only partly agreed with the conclusion of applicant, setting instead a NOAEL for fertility at 225 ppm (14 mg/kg bw/d) based on decreased fertility for the second mating of both generations (P0: -16 %, P1: -12 %) at 5000 ppm. The applicant disagrees with this conclusion	NOAEL **for fertility** was set up at 225 ppm based on slight decrease in fertility index observed in the second mating of the F1 animals (64% at top dose) but neither in F2 (1st and 2nd mating) or in F1 generation (first mating) (84%) at top dose. This decrease was of small magnitude and not consistent between the two generations, nor between the two matings of the same generation. It was therefore concluded that this was

Reproductive toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		considering that no adverse effects were observed in both males and females in this 2-generation study. Further details regarding the data are given in column 3.	not a treatment-related effect. This conclusion is also reinforced by the second study, conducted in the same laboratory, under similar experimental conditions, one year later, in which a fertility index of 68% was obtained in F1 animals from the control group, indicating that the fertility index of 64% is most likely within the range of the laboratory's historical values. In the study report the authors concluded that "No significant treatment-related effects were observed in mating and fertility indices for males and mean gestation length for females of either generation. The gestation index and mean gestation length for females did not differ from controls."
2 (30)	Vol 3 B.6.6.1.2 – Study 2	Applicant: The RMS only partly agreed with the conclusion of applicant and set up the parental NOAEL at 1 ppm (0.063 mg/kg bw/day) based on corneal opacities, increased kidney weights and urinary tract anomalies, which occurred in males at 10 ppm (0.65 mg/kg bw/d) and in females at 225 ppm (16.5 mg/kg bw/d). The applicant agrees partially with this conclusion considering corneal opacities and excluding the kidney effects related to alpha-2µglobuline nephropathy, a known mode of action occurring only in male rats (not in female rats or in mice), considered to be non-relevant in human. Further details regarding the ocular lesions data are given in column 3. See also comments about use of male rat kidney effects in driving NOAELs above. See point 25.	Ocular lesions were observed in both generations. Sulcotrione-induced ocular opacity was evident at 109 days of age, only at 225 ppm P0 animals with an increased number of affected animals at 161 days of age in both 225 ppm groups. No findings were observed for P0 animals at 1 or 10 ppm at any point. For P1 males, some animals of the 225 groups had corneal opacity at Day 21 (weaning) while no males were affected with similar opacity at 10 ppm. Although a few animals showed opaque eyes with opacity ≥ focal-moderate, at day 56 and day 104 (2/25 and 3/25, respectively), this effect is not statistically significant and none of the P1 males showed similar effect at termination (0/25). Only few P1 males of the 10-ppm group (3/25) had keratitis, all grade 1. Histopathology observations in P0 and P1 males

Dose levels (ppm)	0	1	10	225	225-P
Number of animals examined	25	25	25	25	25

<table>
<tr><th colspan="4">Reproductive toxicity</th></tr>
<tr><th>No.</th><th>Column 1
Reference to assessment report</th><th>Column 2
Comment (restricted to 500 characters, ca.10 lines)</th><th>Column 3
Further explanations</th></tr>
<tr><td></td><td></td><td></td><td>
<table>
<tr><td>P
0</td><td>Subacute Keratitis
Grade 1&2
Grade 3
Grade 4</td><td></td><td></td><td></td><td>1
1
2</td><td>1
4
3</td></tr>
<tr><td></td><td>Chronic Keratitis
Grade 1&2
Grade 3
Grade 4</td><td></td><td></td><td></td><td>1
2</td><td>2</td></tr>
<tr><td>P
1</td><td>Subacute Keratitis
Grade 1&2
Grade 3
Grade 4</td><td></td><td></td><td></td><td>1
10
10</td><td>6
3</td></tr>
<tr><td></td><td>Chronic Keratitis
Grade 1
Grade 2
Grade 3
Grade 4</td><td></td><td></td><td>3</td><td>2
5
1</td><td>1
1
13
5</td></tr>
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The applicant considers although the incidence of eye lesions in P1 males is low and not statistically significant, it is treatment related, and then 10 ppm may be considered as a conservative LOAEL for P1 males, while 10 ppm is a clear NOAEL for P0 males and P0&P1 females, under the experimental conditions of this 2-generation toxicity study.

An expert statement/ position paper on eye lesions, to justify the opinion that it is not relevant for human including an extensive literature search on this mechanism may be provided.
</td></tr>
<tr><td>2 (31)</td><td>Vol 3 B.6.6.1.2 – Study 2</td><td>Applicant: disagrees with the developmental NOAEL set up at 1 ppm (0.063 mg/kg bw/d). This NOAEL was based on a statistically increase of dilated renal pelvis observed at 10 ppm only in the F1 weanlings. This finding is not consistent between</td><td>In both generations, the major contributing anomalies were renal pelvis and ureter dilatations, and convoluted ureters. Findings at 4 days of age were not consistent between the two generations and most of the findings below 225 ppm did not persist to weaning.</td></tr>
</table>

Reproductive toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		F1 and F2 generations and has not been reported properly (errors in the number of affected pups). Further details regarding the renal pelvis data are given in column 3. See also comments above.	Combined urinary tract findings were statistically significantly increased when compared to control and on a *per litter* base at 225 ppm both in the F1- and in the F2-generation. No statistically significant increase in combined urinary tract findings were observed at 1 and 10 ppm in both generations, as showed in the tables below. In addition, based on the review of F1 individual data, it appears that certain observations were duplicated, leading to a distortion in the count of pups exhibiting an increase in dilated renal pelvis (please see the table below). In the F1 generation, specifically at PND22 (weanling), 11 pups out of 107 (10.3%) demonstrated dilated renal pelvis at 10 ppm, instead of the 13 reported in Table B6.6-26. Similarly, 7 pups, instead of 8, exhibited dilated kidney pelvis at 1 ppm. It is therefore necessary to revise the Table 6.6-26 accordingly, extending the revision to F2 generation and including other histopathological findings. **At PND21 (weanling) – F1**

Dose Levels (ppm)	0	1	10	225	225a
Litters examined	17	21	20	21	23
Fetuses observed	78	107	107	124	126
Litters Normal	17	21	20	21	22
Fetuses Normal	73	96	91	85	103
Mean litter %	94.41	90.63	84.33	68.18 **	78.84
SD	10.44	12.37	21.95	26.95	30.33
Urinary tract findings combined					
Litters	4	7	7	18	11
Fetuses	5	9	12	38	21

Reproductive toxicity

No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations

Mean Litter %	5.59	7.86	12.05	31.06 **	19.89
SD	10.44	12.50	20.05	26.64	30.25
Kidney pelvis dilated					
Litters	3#	5#	6#	15#	9#
Fetuses	3 (3#)	8 (7#)	13 (11#)	37 (33#)	17 (17#)

#Based on individual data (Appendix Z)
[a]Animals housed in polycarbonate cages (vs hanging wire cages)
** significant different from 0 ppm dose level, p<0.01, Mann-Whitney U test, two sample rank test (from the study report)

At PND21 (weanling) – F2

Dose Levels (ppm)	0	1	10	225	225a
Litters examined	21	16	21	19	20
Fetuses observed	157	128	161	149	158
Litters Normal	21	16	20	19	20
Fetuses Normal	140	113	138	113	125
Mean litter %	88.49	88.28	82.14	75.60 *	79.20 *
SD	12.63	12.47	21.95	27.83	15.76
Urinary tract findings combined					
Litters	12	9	11	16	16
Fetuses	17	15	23	36	32
Mean Litter %	11.51	11.72	17.86	24.40 *	20.18 *
SD	12.63	12.47	27.83	15.99	14.76

[a]Animals housed in polycarbonate cages (*vs* hanging wire cages); * significant different from 0 ppm dose level, p<0.05, Mann-Whitney U test, two sample rank test (from the study report) .

Reproductive toxicity

No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
2 (32)	Vol 3 B.6.6.2.2 – Study 2	Applicant: The study 2 was considered by the RMS as supplementary based on the study design and the top dose used for the main study. The study was performed in 1988 according to the OECD TG guideline of 1981 TG where the recommended exposure time was from GD6 up to GD18 for rabbit. The top dose selected was selected based on a dose range finding study that clearly show adverse effect (mortality and decreased body weight gains) at 500 and 1000 mg/kg bw/day. Consequently, the highest dose selected for the main study was 300 mg/kg bw/day. Further details regarding the renal pelvis data are given in column 3.	Since 2001, the guideline clearly recommends examining the effects from preimplantation, when appropriate, through the entire period of gestation to the day before caesarean section. Therefore, the study 2 could be considered as supplementary. Nevertheless, the top dose selected for the main study was supported by clear adverse effects reported in the DRF study (see table below)

Dose level (mkd)	0	100	200	500	1000
Mated females	6	6	6	6	6
Death	0	0	0	**1**	**5**
Dams with live fetuses on GD29 (%)	6 (100)	6 (100)	5 (83)	4 (80)	1 (17)
Complete litter loss	0	0	1	0	0
Examined Females	6	6	5	5	1
Corpora lutea (mean/litter)	10.3	12.5	12.8	11.3	10
SD	1.8	2.9	2.2	0.5	0
Implantation (mean/litter)	10.0	10.2	10.0	10.3	10.0
SD	3.7	2.8	3.8	0.5	0
Live litter size (mean/litter)	7.8	9.8	9.2	9.8	8.0
SD	3.5	2.4	3.4	0.5	0
Foetal body weight (g)	39.46	39.49	38.60	36.84	43.11
SD	4.68	8.38	8.05	3.60	0

Therefore, given the increase in mortality rates observed at 500 and 1000 mg/kg bw/day, along with significant decreases in body weight gains at 1000 mg/kg bw/day, it was deemed necessary to set the maximum dose level for the main study below 500 mg/kg bw/day. Consequently, the highest dose chosen was 300 mg/kg bw/day. While the limited number

Reproductive toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
			of pregnant females precluded robust statistical analysis or definitive conclusions, no teratogenic effects were reported at any dose level up to 1000 mg/kg bw/day in this dose-range finding study.
2 (33)	Vol 3 B.6.6.2.2 – Study 2	Applicant: Applicant disagrees with the developmental NOAEL set up by the RMS at 30 mg/kg bw/d based on increased incidences of soft tissue (cystic dilatation of hindbrain) and skeletal anomalies (related to ribs, sternebrae and vertebrae).	Maternal toxicity was observed at 300 mg/kg bw/day and included decrease in body weight gain at day 7-10 of pregnancy (-12 g vs +33 g in control) and lower food intakes throughout the treatment period when compared to control group. The Maternal NOAEL was set up at 100 mg/kg bw/day. The applicant disagrees with the developmental NOAEL set up by the RSM at 30 mg/kg bw/day based on slight increase in cystic dilatation of hindbrain and skeletal anomalies (see table below).

	0	30	100	300	HCD
Malformed live fetuses (%)	**12.4**	**11.6**	**9.9**	**11.2**	5.4 [1.4-9.2]
Visceral anomalies					
Litter examined	16	16	18	16	13.4 [11-15]
Fetuses examined	122	132	151	148	113.6 [102-119]
Hindbrain, cystic dilatation	2 (2)	2 (2)	**4 (3)**	**5 (4)**	1.2 [0-2] (0.8 [0-2])
Skeletal anomalies					
Litter examined	16	16	18	16	13.4 [11-15]
Fetuses examined	122	132	151	148	113.6 [102-119]
Lumbar vertebrae absent	0 (0)	1 (1)	1 (1)	1 (1)	Not available
Sacral vertebrae absent	0 (0)	0 (0)	1 (1)	0 (0)	Not available
Rib(s) branched	0 (0)	0 (0)	0 (0)	1 (1)	Not available
Rib(s) fused	0 (0)	1 (1)	1 (1)	**2 (2)**	0.2 [0-1] (0.2 [0-1])
26 presacral vertebrae	6 (1)	**11 (3)**	3 **(3)**	**9 (4)**	0.8 [0-3] (0.6 [0-2])

Reproductive toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations

Extra rib (14th) fullsized	51 (12)	**83** (14)	**117*** **(18)**	**128*** **(16)**	37 [20-65] (9.6 [8-15])

*p<0.05

While there is an increase in cystic dilatation in the hindbrain at both 100 and 300 mg/kg bw/day compared to the incidence at 0 and 10 ppm, the increase is not deemed sufficient to support a compound-related effect. Spontaneously occurring cystic dilatations in the brain are also known to occur in rabbits (Julsingha and Bennettt, 1977). Given the spontaneous response in the controls was at least 50% of that seen at ≥100 mg/kg bw/day, the apparent increases are not considered a sensitive or specific indicator of a specific sulcotrione developmental toxicity.

The absence of lumbar vertebrae was noted in a single pup from each treated group. Fused ribs were also observed in treated groups and remained within the HCD at 50 and 100 ppm (one pup each) and slightly above at the top dose (two pups). Additionally, branched ribs (at the top dose) and the absence of sacral vertebrae (at the mid dose) were observed only once.

Among the findings mentioned above, no significant differences were noted in fetal or litter incidences between the control group and any treated groups. Anomalies in the vertebral column, sternebrae, and ribs, although significantly increased at 100 and 300 ppm, were commonly observed in rabbits (Stump *et al.*, 2012; Pitt *et al.*, 2002).

References:

Stump, D. G., Nemec, M. D., Parker, G. A., Coder, P. S., Sloter, E. D., & Varsho, B. (2012). Significance, reliability, and interpretation of developmental and

Reproductive toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
			reproductive toxicity study findings. In R. D. Hood (Ed.), Developmental and reproductive toxicology: A practical approach (3rd ed., Chapter 9, pp. 244–316). London: Informa Healthcare. Pitt, J. A., Snyder, L. T., Smith, B. L., Varsho, B. J., Nemec, M. D., & Holson, J. F. (2002). An x-ray study of the incidence of supernumerary ribs and presacral vertebrae in adult rats, mice and rabbits. Teratology, 65, 328. Van Julsingha, E. B. and Bennet, C. G. (1977). A dissecting procedure for the detection of anomalies in the rabbit fetal head. In: Methods in Prenantal Toxicology.
2 (34)	Vol 1 Table 2.6-37 raw 5 (2 gen, rats)	Applicant: agrees with the Parental NOAEL set up at 10 ppm. See point 28.	Corneal keratitis and kidneys are considered by the RMS as target organs. Specificity of the kidney effects to rats means the can be excluded or downgraded from being a primary driver of the NOAEL.
2 (35)	Vol 1 Table 2.6-37 raw 5 (2 gen, rats)	Applicant: according to the RMS target organs: urinary tract and cornea are identified in offspring, leading to a LOAEL of 225 ppm. See point 28	
2 (36)	Vol 1 Table 2.6-37 Row 6 (2 gen, rats)	Applicant: from the RMS point of view a reproductive NOAEL can be set at 225 ppm, and an offspring one at 1 ppm based on effects on urinary tract and delay in eye opining. See point 30	
2 (37)	Vol 1 Table 2.6-51 Row 1 (2 gen, rats)	Same comments as point 34 (Vol 1 Table 2.6-37 raw 5)	
2 (38)	Vol 1 Table 2.6-51 Row 1 (2 gen, rats)	Same comments as point 35 (Vol 1 Table 2.6-37 raw 5)	
2 (39)	Vol 1 Table 2.6-51 Row 2 (2 gen, rats)	Same comments as point 36 (Vol 1 Table 2.6-37 raw 6)	

Reproductive toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
2 (40)	Vol 1. Table 2.6-55 Row 2 (terato, rabbits)	Same comments as point 37 (Vol 3 B.6.6.2.2)	

Neurotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations

Further toxicological studies			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
2 (41)	Vol 3 B.6.8.3 Studies on Endocrine disruption Pubertal study in female rat	Applicant: disagrees with the RMS that the inclusion of females with incomplete cycles and the female remaining in diestrus should have been included in the calculation of cycle length that might have shown treatment-related effect on the oestrous cycle. According to the OPPTS 890.1500 guideline "Cycle length may be defined as either the number of days from one proestrus to the next proestrus, or from one diestrus to the next diestrus. Whichever definition is chosen, it should be applied uniformly to all groups in the study. Incomplete cycles should not be counted in calculating mean cycle length." In this study, cycle length was defined as the number of days from one diestrus to the next diestrus. Therefore, females 741 and 744 with incomplete cycles, and female 737 remaining in diestrus cannot be used for calculating cycle length.	
2 (42)	Vol 3. B.6.8.3.1 Tox Cast Model predictions for sulcotrione	Applicant: The RMS considers that the ToxCast model predictions are still not validated and cannot be used as an alternative of the in vitro	Applicant: New mechanistic studies have been conducted and will be submitted upon request. For E-Modality:

Further toxicological studies			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		mechanistic studies for the evaluation of E- and A- mediated activity. New mechanistic studies have been conducted and will be submitted upon request.	Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists and Antagonist (OECD TG 455) was performed. In this study, Sulcotrione technical did not show any estrogenic agonist or antagonist activity. In addition, an *in vivo* Uterotrophic bioassay in adult ovariectomized Sprague Dawley female rats (OECD TG 440) was conducted, and no positive response for estrogenic activity was observed after administration of Sulcotrione at dose levels up to 700 mg/kg bw/day. For A-modality: Stably Transfected Human Androgen Receptor Transcriptional Activation Assay – OECD TG 458) has been conducted. In this study Sulcotrione technical did not show any androgenic agonist or antagonist activity. In addition, an Hersberger Bioassay in rats (OECD TG 441) was conducted. In this study Sulcotrione had no potential androgenic or anti-androgenic effects in castrated, peripubertal male rats.
2 (43)	Vol 1 2.10.2.1.5 Conclusion of the assessment of T-modality	Applicant: based on the RMS opinion, with the available information, in the MoA analysis, a biologically plausible link between the thyroid adversity (histopathological findings) and the endocrine activity (changes in T4 and TSH levels) cannot be established, and the postulated MoA based on the inhibition of HPPD presents important uncertainties. Additional mechanistic information should be generated to support the postulated MoA along with the pieces of information needed according to the	Applicant: New mechanistic studies have been conducted and will be submitted upon request: - *In vitro* Interaction Studies of Sulcotrione with human NIS (SLC5A5) Sodium Iodide Symporter - *In Vitro* Determination of Transthyretin (TTR) Binding of Sulcotrione Technical Using the TTR-TRβ-CALUX® Assay - *In vitro* direct CYP inhibition in Human liver microsomes by Sulcotrione - Assessment of induction of oxidative stress in vitro using reporter genes Srxn1/Blvrb in a mouse embryonic stem reporter cell line.

Further toxicological studies			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		Appendix A of the EFSA/ECHA ED guidance (2018) that include the thyroid activity assays (e.g. NIS, TPO). New generated studies are available upon request.	- Inhibition potential of rat microsomal thyroid peroxidase by sulcotrione, Tyrosine, Monoiodotyrosine and diiodotyrosine (Nov 2021) – studies KLC-AD21-01A & B and KLC-AD21-01A+B+D
2 (44)	Vol 1 2.10.2.2.1 Have EAS – mediated parameters been sufficiently investigated?	Applicant: From the RMS point of view the following parameters were not investigated in the two-generation study: - Hormone levels, namely oestradiol, FSH, LH) (EAS in vivo mechanistic parameter) - Anogenital distance - Nipple development Sperm assessment: morphology, motility and number New generated studies are available upon request	Applicant: In addition, to its conclusion the RMS considers the following parameters not sufficiently investigated in the 2-generation study: - Coagulating gland weight and histopathology - Male mammary gland histopathology - Oviduct histopathology - Seminal vesicles weight and histopathology - Vaginal smears (no information of the oestrous cycle length is provided) In the pubertal assay (See point 117) weight of coagulating gland and seminal vesicles was measured as a unit instead of individual organs, making impossible to distinguish the weight difference between these two organs.
2 (45)	Vol 1 2.10.2.2.3 Initial analysis of the evidence and identification of relevant scenario for the ED assessment of EAR-modalities	Applicant: Based on the RMS request, the following studies were performed: - OECD TG 455 (ER binding and transactivation assay) - OECD TG 440 (Uterotrophic bioassay) - OECD TG 458 (AR STTA assays) - OECD TG 456 (H295R Steroidogenesis Assay)	

Further toxicological studies			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		- OPPTS 890.1200 (Aromatase assay) -OECD TG 441 (Hersberger bioassay)	
2 (46)	Vol 1 2.10.2.2.4 Conclusion of the EAS-modalities	Same comment as in point 45 (Vol 1 2.10.2.2.3)	
2 (47)	Vol 1: 2.10.2.3 Overall Conclusion on the ED assessment for humans	Applicant: RMS considers that addition data are necessary to conclude on T-modalities New mechanistic studies have been conducted and could be made available upon request.	
2 (48)	Vol 1: 2.10.2.3 Overall Conclusion on the ED assessment for humans	Same comment as in point 45 (Vol 1 2.10.2.2.3)	

Toxicological data on metabolites			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
2 (49)	Vol 3 B.6.8.1.1 – Eye irritation	Applicant: The applicant agrees with the RMS to classify the metabolite as Eye Dam. 1 (H318) based on the pH.	
2 (50)	Vol 3 B.6.8.1.2 Oral 28-day study, rat	Applicant: The applicant disagrees with the RMS to set a NOEL of 50 mg/kg bw/d based on the dose-dependent increase of salivation in both sexes.	Salivation often a physiological response to unpleasant tasting materials.

Toxicological data on metabolites			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
2 (51)	Vol 3 B.6.8.1.3 – Study 6 – In vitro mammalian cell gene mutation test	Applicant: the applicant agrees with the RMS that the observed exceed of GEF in assay 1 at the highest concentration is not reproducible in assay 5. But the applicant disagrees with the RMS to consider that the CMBA response is positive under the conditions of this test.	Detailed information regarding the non-biological relevance of the observed increase, considering IWGT criteria should be added. According to the study report "It *was concluded that CMBA is not mutagenic in mouse lymphoma L5178Y cells when tested at concentrations extending into the toxic range. Statistically significant, but biologically irrelevant increases in mutant numbers were obtained. CMBA was tested in the absence and presence of S9 mix with a 4 h exposure period to the predetermined maximum concentration of 2346.6 µg/mL (10 mM). CMBA was also tested in the absence of S9 mix with a 24 h exposure period at concentrations extending into the toxic range."* According to the study report, the result observed is not of biological relevance. *"Biological relevance was given to any increase in mutant fraction greater than 126 mutants per million above the concurrent control value. In addition, the results were analysed for comparison of the log mutant fraction between the vehicle controls and each concentration of CMBA. In addition, all the experiments were tested for dose-related trends.* *In 4 of the experiments, the highest concentration of CMBA tested significant (at P<0.05) for increase in log mutant fraction (the exception was the second experiment in the presence of S9 mix). In addition, all 5 experiments gave a significant dose-related trend with CMBA (P = 0.007 to 0.046). However, the only treatment that met the IWGT criteria for a biologically relevant increase was that from the first experiment in the absence of S9 mix (4 h exposure period). The value for the induced mutant fraction of 135 mutants per million was just higher than the threshold value of 126, and this level of response was shown not to be*

Toxicological data on metabolites			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
			reproducible in the confirmatory experiment (Assay 5). It is therefore concluded that CMBA does not induce biologically relevant increases in mutant fraction in mouse lymphoma cells, and is therefore not a mammalian cell mutagen."
2 (52)	Vol 1. Table 2.6.-66– Raw 6	the applicant agrees with the RMS that the observed exceed of GEF in assay 1 at the highest concentration is not reproducible in assay 5. But the applicant disagrees with the RMS to consider that the CMBA response is positive under the conditions of this test. See point 52 Vol 3 B.6.8.1.3 – Study 6	
2 (53)	Vol 1. 2.6.8.1.3 Toxicological evaluation of CMBA- genotoxicity	Applicant: new genotoxicity study with CMBA will be provided as requested by the RMS. A new *in vitro* micronucleus test (OECD 487) has been performed.	
2 (54)	Vol 1. 2.6.8.1.3 Toxicological evaluation of CMBA- genotoxicity	Applicant: based on the study report of the *in vitro* mammalian gene mutation (Riach, 2009, ASB2020-12476), the conclusion of the study is negative See point 53	

Medical data and information			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
2 (55)	Vol 3. B.6.9.2.1	Applicant: The applicant disagrees with the RMS to consider that sulcotrione is at the origin of the I observed effect (erythematous rash after dermal	

Medical data and information			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		exposure, solved by use of corticosteroid and antihistamine). The effect is observed following a contact with a commercial product (Mikado) which composition is not available to the applicant. Therefore in the absence of other information (patch test), it cannot be excluded that the reaction is due to a co-formulant.	

Toxicological end points: ADI, ARfD, AOEL			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations

Product exposure and risk assessment, including dermal absorption			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations

Other comments, proposals for classification			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations

Appendix to Section 2

Distribution of Sulcotrione and metabolites to the bone marrow in rats:

Of all the vascular endothelia, discontinuous endothelium, as found in in the liver spleen and bone marrow has the greatest permeability and is characterized by a poorly formed basement membrane, large fenestrations without diaphragms, and pores within individual cells. Molecules less than 5 nm can freely diffuse in and out of the myeloid bone marrow. In contrast that of the brain is the most resistant to transport and movement is restricted to water and solutes with a radius less than 3 nm. See Tibbitts et al 2016 and Sarin, 2010

Using a notional atom-to-atom bond length of 0.15nm (with the exception of 0.17-0.19 for C-Cl and C-S bond lengths) sulcotrione is estimated at its longest point to be no more than 1.5 nm in length. Its non-conjugated metabolites would be similar in size or marginally smaller.

Average Bond lengths as can be readily found in organic chemistry text books e.g. Harrison, R. D. (1984). Revised Nuffield Advanced Science Book of Data.

Bond	Bond Length (Å)	Bond	Bond Length (Å)
C—C	1.54	N—N	1.47
C=C	1.34	N=N	1.24
C≡C	1.20	N≡N	1.10
C—N	1.43	N—O	1.36
C=N	1.38	N=O	1.22
C≡N	1.16		
		O—O	1.48
C—O	1.43	O=O	1.21
C=O	1.23		
C≡O	1.13		

Therefore sulcotrione and metabolites would only be preferentially excluded from bone marrow if they were strongly bound to plasma proteins / macromolecules which cannot freely enter the bone marrow. The plasma :whole blood ratios in Table B.6.1.3 in the RAR would indicate considerable free sulcontrione / metabolites are available to diffuse freely in to the bone marrow. Significant concentration of sulcotrione-derived radioactivity found in the brain of one animal at 96 hours (MCA5 Tables CA 6.1.3) also corroborated that significant permeation of bone marrow by chlorotouron and its metabolites would occur, not least because brain vascular endothelium is less permeable than that of bone marrow. Given that bone marrow is more permeable than brain to sulcotrione and metabolites, is likely that concentrations in the bone marrow would exceed those of the brain at plasma Cmax.

Tibbitts, J., Canter, D., Graff, R., Smith, A., & Khawli, L. A. (2016, February). Key factors influencing ADME properties of therapeutic proteins: A need for ADME characterization in drug discovery and development. In *MAbs* (Vol. 8, No. 2, pp. 229-245). Taylor & Francis.

Sarin, H. (2010). Physiologic upper limits of pore size of different blood capillary types and another perspective on the dual pore theory of microvascular permeability. *Journal of angiogenesis research*, *2*, 1-19.