REGISTRATION REPORT

Part B

Section 6
Mammalian Toxicology

Detailed summary of the risk assessment

Product code: 102000007779

Product name(s): Flufenacet SC 508.8 G
(Active substance(s)): Flufenacet 508.8 g/L

Central Zone
Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(Authorization)

Applicant: Bayer Crop Science Division
Submission date: 30 June 2021
MS Finalisation date: February 2022 (initial Core Assessment)
June 2023 (final Core Assessment)

Version history

When	What
June 2021	Original Bayer Crop Science Division submission
February 2022	Initial assessment by the zRMS The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through and shaded for transparency~~.
June 2023	Final report (Core Assessment updated following the commenting period) Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Information no longer relevant ~~is struck through and shaded~~.

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Reviewer comments:

This part of dossier summarizes data related to the toxicological assessment and exposure data for the plant protection product FFA SC 508.8 G/Flufenacet SC 508.8 G and has been submitted to support registration according art. art. 33 of 1107/2009 in Poland.
Product was not a representative formulation reviewed during the Annex I inclusion/renewal of active substance(s).
Product has been previously evaluated in Germany, authorization number: 005908-00; date of approval: 12 October 2006, Austria, authorisation number: 3941, date of approval: 11 March 2019 and Czech Republic authorisation number: 5818-0, date of approval: 11 July 2020; according to the Uniform Principles (refer dRR part B0).
For the currently registered product (Spec. No.102000007779 ver.03), Applicant provided an *in vivo* toxicity studies which were performed using previous composition (Spec. No.102000007779 ver.02 -registered). Comparison of both formulations are presented in the confidential Part C of this dossier. Difference in the composition has been considered as admissible, parameters of both formulations are sufficiently comparable. Thus studies has been accepted as relevant data to prediction of toxicological potential of the product FFA SC 508.8 G/Flufenacet SC 508.8 G (Spec. No.102000007779 ver.03).
Testing strategy takes into account methods compliant with the 3R concept for refinement, reduction and replacement of animal testing where applicable and acceptable (please refer Appendix 2 to this dossier).
ZRMS accepted already existing *in vivo* studies and do not request for the new one. Since there are *in vivo* tests already exist the information gained on animal studies are more than just a classification. Existing animal studies allow to identify of effects following a single exposure to the plant protection product can be established. The data is sufficient to indicate the time course and characteristics of the effect with full details of behavioral changes and possible gross pathological findings at post-mortem. These studies are valid for hazard classification and toxicological risk assessment.
NDE assessment and combined exposure calculations provided for operator, workers and B&R resulting from use of FFA SC 508.8 G/Flufenacet SC 508.8 G (*FFA SC 508.8 G is an aqueous suspension concentrate containing 508.8 g/L flufenacet for use as a herbicide; refer dRR part B0*) considering critical use(s), identify safe applications of the product FFA SC 508.8 G/Flufenacet SC 508.8 G.

6 Mammalian Toxicology (KCP 7)

6.1 Summary

Table 6.1-1: Information on FFA SC 508.8 G *

Product name and code	Flufenacet SC 508.8 G Cadou SC 102000007779
Formulation type	SC (suspension concentrate)
Active substance(s) (incl. content)	508.8 g/L flufenacet
Function	Herbicide
Product already evaluated as the 'representative formulation' during the approval of the active substance(s)	No
Product previously evaluated in another MS according to Uniform Principles	Yes (Evaluation in Germany, authorization number: 005908-00; date of approval: 12 October 2006; Evaluation in Austria, authorisation number: 3941)

* Information on the detailed composition of FFA SC 508.8 G can be found in the confidential dRR Part C.

Justified proposals for classification and labelling

According to the criteria given in Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008, the following classification and labelling with regard to toxicological data is proposed for the preparation:

Table 6.1-2: Justified proposals for classification and labelling for FFA SC 508.8 G according to Regulation (EC) No 1272/2008

Hazard class(es), categories:	Acute oral toxicity: Category 4 Specific target organ toxicity - repeated exposure: Category 2
Hazard pictograms or Code(s) for hazard pictogram(s):	 GHS07  GHS08
Signal word:	Warning
Hazard statement(s):	H302 Harmful if swallowed H373 May cause damage to organs (Nervous system) through prolonged or repeated exposure if swallowed. EUH208 Contains Flufenacet, 1,2-benzisothiazolin-3-one, reaction mass of 5-chloro-2- methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3- one (3:1). May produce an allergic reaction. EUH401 To avoid risks to man and the environment, comply with the instructions for use.
Precautionary statement(s):	P260 Do not breathe gas/ mist/ vapours/ spray. P280 Wear protective gloves ~~protective clothing/ eye protection/ face protection.~~ P308 + P311 IF exposed or concerned: Call a POISON CENTER/ doctor/ physician. P391 Collect spillage. P501 Dispose of contents/container in accordance with local regulation.
Additional labelling phrases:	

Table 6.1-3: Summary of risk assessment for operators, workers, bystanders and residents for FFA SC 508.8 G

	Result	PPE / Risk mitigation measures
Operators	Acceptable	None
Workers	Acceptable	None
Bystanders	Acceptable	None
Residents	Acceptable	None

No unacceptable risk for operators, workers, bystanders and residents was identified when the product is used as intended. No specific PPE is necessary

A summary of the critical uses and the overall conclusion regarding exposure for operators, workers and bystanders/residents is presented in the following table.

Table 6.1-4 Critical uses and overall conclusion of exposure assessment

1	2	3	4	5	6	7	8	9	10			
Use-No.*	**Crops and situation (e.g. growth stage of crop)**	**F, Fn, Fpn G, Gn, Gpn or I ****	**Application**		**Application rate**		**PHI (d)**	**Remarks:** **(e.g. safener/synergist (L/ha))** **critical gap for operator, worker, bystander or resident exposure based on [Exposure model]**	**Acceptability of exposure assessment**			
			Method / Kind (incl. application technique ***| **kg or L product / ha a) max. rate per appl. b) max. total rate per crop/season ~~Max. number (min. interval between applications) a) per use b) per crop/ season~~**	**Max. application rate ~~kg as/ha~~ g as/ha a) a.s. 1**	**Water L/ha min / max**				**Operator**	**Worker**	**Bystander**	**Residents**
29, 53, 65,	Wheat, winter	F	Spraying	a) 0.48	a) FFA	100-400	as per	BBCH 00-09				

1	2	3	4	5	6	7	8	9	10			
77, 33, 57, 69, 81, 37, 61, 73, 85, 89, 97, 105, 113, 93, 101, 109, 117, 129, 133, 137, 141	(TRZAW), Triticale winter (TTLWI), Barley winter (HORVW) Rye (SECCW), Durum wheat (TRZDW), Spelt (TRZSP)		(broadcas, overall)	b) 0.48	244.2 b) FFA 244.2		growth stage					
30, 54, 66, 78, 34, 58, 70, 82, 38, 62, 74, 86, 90, 98, 106, 114, 94, 102, 110, 118, 130, 134, 138, 142	Wheat, winter (TRZAW), Triticale winter (TTLWI), Barley winter (HORVW) Rye (SECCW), Durum wheat (TRZDW), Spelt (TRZSP)	F	Spraying (broadcas, overall)	a) 0.48 b) 0.48	a) FFA 244.2 b) FFA 244.2	100-400	as per growth stage	BBCH 10-13				
31, 55, 67, 79, 35, 59, 71, 83, 39, 63, 75, 87, 91, 99, 107, 115, 95, 103, 111, 119, 131, 135, 139, 143	Wheat, winter (TRZAW), Triticale winter (TTLWI), Barley winter (HORVW) Rye (SECCW), Durum wheat (TRZDW), Spelt (TRZSP)	F	Spraying (broadcas, overall)	a) 0.24 b) 0.24	a) FFA 122.1 b) FFA 122.1	100-400	as per growth stage	BBCH 00-09				
32, 56, 68, 80, 36, 60, 72, 84, 40, 64, 76, 88, 92, 100, 108, 116, 96, 104, 112, 120, 132, 136, 140, 144	Wheat, winter (TRZAW), Triticale winter (TTLWI), Barley winter (HORVW) Rye (SECCW), Durum wheat (TRZDW), Spelt (TRZSP)	F	Spraying (broadcas, overall)	a) 0.24 b) 0.24	a) FFA 122.1 b) FFA 122.1	100-400	as per growth stage	BBCH 10-13				

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1

** F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application

*** e.g. LC: low crops, HC: high crop, TM: tractor-mounted, HH: hand-held

Explanation for column 10 “Acceptability of exposure assessment”

A	Exposure acceptable without PPE / risk mitigation measures
R	Further refinement and/or risk mitigation measures required
N	Exposure not acceptable/ Evaluation not possible

Data gaps

Noticed data gaps are:

- None

6.2 Toxicological Information on Active Substance(s)

Information regarding classification of the active substances and on EU endpoints and critical areas of concern identified during the EU review are given in Table 6.2-1.

Table 6.2-1: Information on active substance flufenacet

Common Name	Flufenacet
CAS-No.	CAS No 142459-58-3
Classification and proposed labelling	
With regard to toxicological endpoints (according to the criteria in Reg. 1272/2008, as amended)	Hazard classes, categories: Acute Tox 4, Skin Sens 1, STOT RE 2 Codes for hazard pictograms: GHS07, GHS08 Signal word: Warning Hazard statements: H302, H317, H373 Precautionary statements: P273, P280, P308+P311
Additional C&L proposal	None
Agreed EU endpoints	
AOEL systemic	0.017 mg/kg bw/d
Reference	EU Review report (7469/VI/98-Final – 3rd July 2003)
Conditions to take into account/critical areas of concern with regard to toxicology	
Review Report/EFSA Conclusion for active substance	Member States should pay particular attention to the protection of operators. Risk mitigation measures should be applied where appropriate

6.3 Toxicological Evaluation of Plant Protection Product

A summary of the toxicological evaluation for FFA SC 508.8 G is given in the following tables. Full summaries of studies on the product that have not been previously considered within an EU peer review process are described in detail in Appendix 2.

Table 6.3-1: Summary of evaluation of the studies on acute toxicity including irritancy and skin sensitisation for FFA SC 508.8 G

Type of test, species, model system (Guideline)	Result	Acceptability	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
LD_{50} oral, rat (OECD 423)	>500 - < 1000 mg/kg bw	Yes	H302: Harmful if swallowed	xxx(1998) M-005460-01-1
LD_{50} dermal, rat (OECD 402)	>4000 mg/kg bw	Yes	None	xxx (1998) M-004746-01-1
LC_{50} inhalation, rat (OECD 403)	>2172 mg/m³ air	Yes	None	xxx (1999) M-009812-01-1
Skin irritation, model system (OECD 404)	Non-irritant	Yes	None	xxx (2001) M-004806-02-1
Eye irritation, model system (OECD 405)	Non-irritant	Yes	None	xxx (2001) M-004807-02-1
Skin sensitisation,/mouse (OECD 406, LLNA)	Non-sensitising	Yes	None	xxx (2005) M-258556-01-1
Supplementary studies for combinations of plant protection products	No data – not required	-	-	-

Table 6.3-2: Additional toxicological information relevant for classification/labelling of product 102000007779 / FFA SC 508.8 G

	Substance (Concentration in product, % w/w)	Classification of the substance (acc. to the criteria in Reg. 1272/2008)	Reference	Classification of product (acc. to the criteria in Reg. 1272/2008)
Toxicological properties of active substance(s) (relevant for classification of product)	Flufenacet 42.4% CAS No. 142459-58-3	Acute Tox. 4, H302 Skin Sens 1, H317 STOT RE 2, H373	Regulation (EC) No. 1272/2008 MSDS	Acute oral toxicity: Category 4 H302 Harmful if swallowed H373 May cause damage to organs (Nervous system) through prolonged or repeated exposure if swallowed.
Non-active substance(s) (relevant for classification of product)	1,2-Benzisothiazol-3(2H)-one > 0.005 – < 0.05% CAS No. 2634-33-5	Eye Dam. 1, H318 Acute Tox. 4, H302 Skin Sens. 1, H317 Skin Irrit. 2, H315	Regulation (EC) No. 1272/2008 MSDS	
Non-active substance(s) (relevant for classification of product)	Reaction mass of 5-chloro-2- methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3- one (3:1) > 0.0002 – < 0.0015% CAS No. 55965-84-9	Acute Tox. 3, H301 Acute Tox. 2, H310 Acute Tox. 2, H330 Skin Corr. 1C, H314 Eye Dam.1, H318 Skin Sens. 1A, H317	Regulation (EC) No. 1272/2008 MSDS	
Non-active substance(s) (relevant for classification of product)	Glycerine > 1% CAS No. 56-81-5	Not classified	Regulation (EC) No. 1272/2008 MSDS	
Further toxicological information	No data – not required			

* Please use concentration range or concentration limit (e.g. 1-10 % or > 1 %) as provided in MSDS.
** Material safety data sheet by the applicant

6.4 Toxicological Evaluation of Groundwater Metabolites

The following data on metabolites with the potential to reach the groundwater in concentrations above 0.1 µg/L and requiring relevance assessment were submitted. Note that the relevance assessment of the metabolites is reported in Part B.10; the submitted toxicological studies are summarized in this document.

6.4.1 Flufenacet metabolite: FOE-oxalate (M01)

The relevance of the groundwater metabolite FOE oxalate (M01) has already been assessed and the assessment agreed at EU level (see Addendum to DAR (Monograph), January 2003). Although the relevance assessment is not applicable for the GAP and groundwater scenarios considered in this dRR, FOE oxalate is not considered relevant according to the criteria laid down in the EC guidance document SANCO/221/2000 –rev.10.

An overview of the results of the accepted toxicological studies for groundwater metabolite FOE-oxalate is given in the following table. Full summaries of studies on the metabolite that have not previously been considered within an EU peer review process are described in detail in Appendix 2 (A 2.11 Other/Special Studies).

Table 6.4-1: Summary of the results of toxicity studies for FOE oxalate

Type of test, species (Guideline)	Concentration	Result	Acceptability	Reference*
Bacterial reverse mutation assay (S. typhimurium, TA1535,	16 - 5000 µg/plate (+/- S9 mix)	Negative (+/- S9 mix)	Yes	Herbold, 2009 A 2.11.1

Type of test, species (Guideline)	Concentration	Result	Acceptability	Reference*
TA1537, TA98, TA100, TA102) (OECD 471)				M-358953-01-1 (KCA 5.8.1) Appendix 2
Mammalian cell gene mutation test (Chinese hamster V79 cells) OECD 476)	150 - 2400 µg/mL (+/- S9 mix)	Negative (+/- S9 mix)	Yes	Wollny, 2010 A 2.11.2 M-361724-01-1 (KCA 5.8.1) Appendix 2
Mammalian chromosome aberration test (Chinese hamster V79 cells) (OECD 473)	600 - 2400 µg/mL (+/- S9 mix)	Negative (+/- S9 mix)	Yes	Nern, 2009 A 2.11.3 M-358043-01-1 (KCA 5.8.1) Appendix 2
Bioavailability study in rats [Fluorophenyl-UL-^{14}C] FOE5043-oxalate	1 mg/kg bw	Excretion of unchanged FOE-oxalate 70% faeces, 28% urine	Yes	Krolski, Bosnak, 1995* M-002278-01-1

* indicates that a study was reviewed at EU level

6.4.2 Flufenacet, FOE-sulfonic acid (M02)

An overview of the results of the accepted toxicological studies for groundwater metabolite metabolite 1 is given in the following table. Full summaries of studies on the metabolite that have not previously been considered within an EU peer review process are described in detail in Appendix 2 (A 2.11 Other/Special Studies).

Table 6.4-2: Summary of the results of toxicity studies for FOE-sulfonic acid (M02)

Type of test, species (Guideline)	Dose	Result	Acceptability	Reference
Bacterial reverse mutation assay (S. typhimurium, TA1535, TA1537, TA98, TA100, TA102) (OECD 471)	16 - 5000 µg/plate (+/- S9 mix)	Negative (+/– S9 mix)	Yes	xxx, 2000* M-019064-01-1
Mammalian cell gene mutation test (Chinese hamster V79 cells) (OECD 476)	202 - 3230 µg/mL (+ S9 mix) 101 - 808 µg/mL (– S9 mix)	Negative (+/– S9 mix)	Yes **	xxx, 2009 A 2.11.4 M-361158-01-1 (KCA 5.8.1) Appendix 2
Mammalian chromosome aberration test (Chinese hamster V79 cells) (OECD 473)	250 - 3000 µg/mL (+ S9 mix) 200 - 1000 µg/mL (– S9 mix)	Negative (+ S9 mix) positive (–S9)	Yes	xxx, 2010 A 2.11.5 M-366380-01-1 (KCA 5.8.1) Appendix 2
In vivo Micronucleus test (Mouse bone marrow) (OECD 474)	500-2000 mg/kg bw (2x intraperitoneal)	Negative	Yes	xxx, 2010 A 2.11.6 M-368627-01-1 (KCA 5.8.1) Appendix 2
In vivo Unscheduled DNA synthesis (UDS) assay (rat primary hepatocytes) (OECD 486)	1000-2000 mg/kg bw (oral)	Negative	Yes	xxx, 2010 A 2.11.8 M-397810-01-1 (KCA 5.8.1) Appendix 2)
Rat Acute oral (fasted) (OECD 401)	500-2000 mg/kg bw	> 2000 mg/kg bw	Yes	xxx, 1998* M-004749-01-1

Rat Plasma kinetics and excretion (OECD 423)	1 x 100 mg/kg bw (intravenous) 1 x 1000 mg/kg bw (oral)	Low oral absorption (<10%) rapid renal clearance (i.v: t1/2 ≈ 30 min)	Yes	xxxx, 2000* M-042251-01-1
Mouse whole body autoradiography	1 x 500 mg/kg bw i.p.	Test substance distributed to the bone marrow	Yes	xxx, 2017 A 2.11.7 M-580054-01-1 (KCA 5.8.1) Appendix 2

* indicates that a study was reviewed at EU level
** study is considered reliable, with restrictions due to the deficiencies observed compared to OECD 473 (2016). For details refer our comments point A 2.11.5 (xxx, 2010).

6.5 Dermal Absorption (KCP 7.3)

A summary of the dermal absorption rates for flufenacet in FFA SC 508.8 G are presented in the following table.

Table 6.5-1: Dermal absorption rates for active substances in FFA SC 508.8 G

	Flufenacet	
	Value	**Reference**
Concentrate	0.083 %	New study reported in Appendix 2
Dilution (dilution factor)	2.5% @ 2.0 g/L 14 % @ 0.06 g/L	New study reported in Appendix 2

6.5.1 Justification for proposed values - flufenacet

Proposed dermal absorption rates for flufenacet are based on dermal absorption studies on a formulation similar to product 102000007779-03/FFA SC 508.8 G. The study results are summarized in the following table. Full summaries of studies on the dermal absorption of flufenacet in a FFA SC 508.8 G formulation that have not previously been evaluated within an EU peer review process are described in detail in Appendix 2.

Table 6.5-2: Summary of the results of submitted dermal absorption studies for flufenacet

Test	**Concentrate**	**Spray dilution (dilution factor)**	**Formulation in study**	**Acceptability of study**	**Justification provided on representativity of study formulation for current product**	**Acceptability of justification**	**Reference***
In vitro (human)	0.083 %	2.5 % (1 in 200) 14 % (1 in 8000)	102000007779-02/Flufenacet SC 508.8 G	Yes	Yes (see Appendix 0)	Justification accepted. Endpoint can be used for current product	xxx, W. 2020

* indicates that a study was reviewed at EU level

6.6 Exposure Assessment of Plant Protection Product (KCP 7.2)

Table 6.6-1: Product information and toxicological reference values used for exposure assessment

Product name and code	Flufenacet SC 508.8 FFA SC 508.8 G Cadou SC
Formulation type	SC
Category	Herbicide
Container size(s), short description	1-15 L bottles of HDPE with 45-60 mm opening
Active substance(s) (incl. content)	**Flufenacet** 508.8 g/L
AOEL systemic	0.017 mg/kg bw/d
Inhalation absorption	100 %
Oral absorption	100 %
Dermal absorption	Concentrate: 0.083 % Dilution: 14 % (Dilution rate: 1 in 8000)

6.6.1 Selection of critical use(s) and justification

The critical GAP used for the exposure assessment of the plant protection product is shown in Table 6.1-4. A list of all intended uses within the zone is given in Part B, Section 0.

Justification
The intended use of refers to weed control in cereals (winter wheat, winter barley, winter triticale and durum wheat). Application is achieved via vehicle mounted ground boom spray application.
The critical GAP covers the maximum intended application rate as well as the maximum in use concentration. Accordingly, that GAP represents the worst case in terms of operator-, worker- and resident-/bystander exposure. Furthermore, regarding dermal absorption from the spray dilution the value covering the overall minimum concentration of the spray dilution is used for the calculations.

6.6.2 Operator exposure (KCP 7.2.1)

For the active substances to be considered in this evaluation an AAOEL was not derived. Therefore, an acute non-dietary risk assessment is not included in this submission. Lack of scientific guidance or methodology is an acceptable reason for waiving according to Guidance of the European Commission[1]. The absence of such guidance on derivation of an appropriate reference dose ("AAOEL") was recognized by the European Food Safety Authority[2], and the European Commission Standing Committee[3].
Therefore, this waiver is presented in line with the Guidance of the European Commission.
This applies for the same degree with regard to acute operator exposure estimates.

6.6.2.1 Estimation of operator exposure

A summary of the exposure models used for estimation of operator exposure to the active substances

[1] Guidance Document for applicants on preparing dossiers for the approval of a chemical new active substance and for the renewal of approval of a chemical active substance according to Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013. SANCO/10181/2013, May 2013
[2] Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014;12(10):3874
[3] Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. SANTE-10832-2015

during application of 102000007779/FFA SC 508.8 G according to the critical use is presented in Table 6.6-2. Outcome of the estimation is presented in Table 6.6-3. Detailed calculations are in Appendix 3.

Table 6.6-2: Exposure models for intended uses

Critical use(s)	Cereals (max. 0.48 L product/ha)
Model(s)	All exposure calculations are in accordance with the *EFSA guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products.(2014)*[4]

Table 6.6-3: Estimated operator exposure

		Flufenacet	
Model data	**Level of PPE**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**[1]
Tractor mounted boom spray application outdoors to low crops Application rate: 0.2442 kg a.s./ha			
EFSA Operator Model (75^{th} quantile regression) Body weight: 60 kg	no PPE[1]	0.00507	29.8
	with PPE[2]	0.000766	4.51

1 AOEL (RVNAS) of Flufenacet: 0.017 mg/kg bw/day
2 no PPE: Work wear - arms, body and legs covered
3 with PPE: Work wear - arms, body and legs covered. In addtion gloves during mixing and loading as well as when handling contaminated surfaces

6.6.3 Measurement of operator exposure

Since the operator exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) will not be exceeded under conditions of intended uses and considering above mentioned personal protective equipment (PPE), a study to provide measurements of operator exposure was not necessary and was therefore not performed.

6.6.4 Worker exposure (KCP 7.2.3)

6.6.4.1 Estimation of worker exposure

Table 6.6-4 shows the exposure model(s) used for estimation of worker exposure after entry into a previously treated area or handling a crop treated with 102000007779/FFA SC 508.8 G according to the critical use(s). Outcome of the estimation is presented in Table 6.6-6. Detailed calculations are in Appendix 3.

Table 6.6-4: Exposure models for intended uses

Critical use(s)	Cereals (max. 0.48 x 1 L product/ha)
Model	All exposure calculations are in accordance with the *EFSA guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products.(2014)*[5]

The following table shows the crop groups with their respective transfer coefficients (TC) and task duration relevant for the estimation of worker exposure after the intended use of 102000007779/FFA SC

[4] EFSA (European Food Safety Authority), 2014. Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014;12(10):3874, 55 pp., doi:10.2903/j.efsa.2014.3874
[5] EFSA (European Food Safety Authority), 2014. Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014;12(10):3874, 55 pp., doi:10.2903/j.efsa.2014.3874

508.8 G. Worker exposures for all intended uses within the zone/ EU given in Part B, Section 0 are covered by that.

Table 6.6-5: Relevant parameters used for the worker exposure assessment

Crop / Crop Group	**Nº of applications**	**Interval** (Days)	**TC[1]** (cm²/hour)	**Task Duration** (hours)
Cereals	1	365	1400[2]	2

[1] TC = transfer coefficients
[2] TC assuming arms, body and legs covered.
[3] TC assuming hands, arms, body and legs covered.

Table 6.6-6: Estimated worker exposure

Active substance	**Application rate** (kg a.s./ha)	**Total absorbed dose[2] (mg/kg/day)**	**% of systemic AOEL[1] (RVNAS)**
FFA	0.244	0.00479	28.2

[1] AOEL (RVNAS) of flufenacet: 0.017 mg/kg bw/day
[2] Assuming arms, body and legs covered (workwear, bare hands)

6.6.4.2 Refinement of generic DFR value (KCP 7.2)

Using the generic DFR value the worker exposure estimations carried out indicated that the Acceptable Operator Exposure Level (AOEL/RVNAS) will not be exceeded under conditions of intended uses. Therefore, refinement of the generic DFR value is not required and was therefore not performed.

6.6.4.3 Measurement of worker exposure

Since the worker exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) will not be exceeded under conditions of intended uses and considering above mention PPE, a study to provide measurements of worker exposure was not necessary and was therefore not performed.

6.6.5 Bystander and resident exposure (KCP 7.2.2)

For the active substances to be considered in this evaluation an AAOEL was not derived. Therefore, an acute non-dietary risk assessment is not included in this submission. Lack of scientific guidance or methodology is an acceptable reason for waiving according to Guidance of the European Commission[6]. The absence of such guidance on derivation of an appropriate reference dose ("AAOEL") was recognized by the European Food Safety Authority[7], and the European Commission Standing Committee[8].
Therefore, this waiver is presented in line with the Guidance of the European Commission.
According to EFSA longer term exposure of bystanders is covered by the resident scenario.

6.6.5.1 Estimation of bystander and resident exposure

Table 6.6-7 shows the exposure model(s) used for estimation of bystander and resident exposure to 102000007779/FFA SC 508.8 G. Outcome of the estimation is presented in Table 6.6-8. Detailed calculations are in Appendix 3.

[6] Guidance Document for applicants on preparing dossiers for the approval of a chemical new active substance and for the renewal of approval of a chemical active substance according to Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013. SANCO/10181/2013, May 2013
[7] Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014;12(10):3874
[8] Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. SANTE-10832-2015

Table 6.6-7: Exposure models for intended uses

Critical use(s)	Cereals (max. 0.48 x 1 L product/ha)
Model	All exposure calculations are in accordance with the *EFSA guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products.(2014)*[9]

Table 6.6-8: Estimated bystander and resident exposure

	Adult[2]			Child[2]		
Outdoor, Downward spraying, Vehicle-mounted Application rate: 1 x 0.2442 kg a.s./ha, 365 days interval, Minimum water volume: 100 L/ha						
Routes of exposure	**75th centile (mg/kg bw/day)**	**in % of AOEL[1] (RVNAS)**	**Mean (mg/kg bw/day)**	**75th centile (mg/kg bw/day)**	**in % of AOEL[1] (RVNAS)**	**Mean (mg/kg bw/day)**
Spray drift[3]	0.0022	12.9	0.00105	0.00922	54.2	0.00509
Vapour	0.00023	1.35	0.00023	0.00107	6.29	0.00107
Surface deposits	0.000233	1.37	0.000171	0.000696	4.09	0.00051
Entry into treated crops[4]	0.00321	18.9	0.00256	0.00577	33.9	0.0046
Sum of all pathways: default DFR [mg/kg bw/day] of AOEL (RVNAS)			0.004 (23.5%)			0.0113 (66.3%)

[1] AOEL (RVNAS) of FFA: 0.017 mg/kg bw/day
[2] Considered bodyweight: adult = 60 kg, child = 10 kg
[3] Exposure at 2-3 m distance
[4] Default DFR = 3
[5] Measured DFR = -

6.6.5.2 Measurement of bystander and/or resident exposure

Since the bystander and/or resident exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) for flufenacet will not be exceeded under conditions of intended uses and considering above mentioned risk mitigation measures, a study to provide measurements of bystander/resident exposure was not necessary and was therefore not performed.

6.6.6 Combined exposure

Not relevant. The product contains only one active substance.

[9] EFSA (European Food Safety Authority), 2014. Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014;12(10):3874, 55 pp., doi:10.2903/ j.efsa.2014.3874

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data Point	Author(s)	Year	Title Company Report No. Source GLP or GEP status published or not	Vertebrate study Y/N	Owner
KCP 7.1.1 / 01	xxx	1998	FOE 5043 500 SC 04402/0096 (Fluthiamid (prop.)) - Study for acute oral toxicity in rats Report No.: 27271, Edition Number: M-005460-01-1 xxx GLP/GEP: Yes unpublished	Yes	Bayer
KCP 7.1.2 / 01	xxx	1998	FOE 5043 500 SC 04402/0096 (c.n.: Fluthiamid (prop.)) - Study for acute dermal toxicity in rats Report No.: 27416, Edition Number: M-004746-01-1 xxx GLP/GEP: Yes unpublished	Yes	Bayer
KCP 7.1.3 / 01	xxx	1999	FOE 5043 500 SC 04402/0096 (c.n.: Fluthiamid (proposed)) - Study on acute inhalation toxicity in rats according to OECD no. 403 Report No.: 28609, Edition Number: M-009812-01-1 GLP/GEP: Yes unpublished	Yes	Bayer
KCP 7.1.4 / 01	xxx	2001	Acute skin irritation test (patch test) of FOE 5043 500 SC 04402/0096 in rabbits - first revision of report no. R7215 Report No.: R7993, Edition Number: M-004806-02-1 xxx **... amended: 2001-05-28** GLP/GEP: Yes unpublished	Yes	Bayer
KCP 7.1.5 / 01	xxx	2001	Acute eye irritation study of FOE 5043 500 SC 04402/0096 by instillation into the conjunctival sac of rabbits - first revision of report no. R7216 Report No.: R7994, Edition Number: M-004807-02-1 xxx **... amended: 2001-05-28** GLP/GEP: Yes unpublished	Yes	Bayer
KCP 7.1.6 / 01	xxx	2005	Flufenacet SC 500 (Project: Flufenacet (FOE 5043)) - Local lymph node assay in mice (LLNA/IMDS) Report No.: AT02459, Edition Number: M-258556-01-1 xxx GLP/GEP: Yes unpublished	Yes	Bayer

Data Point	Author(s)	Year	**Title** **Company Report No.** **Source** **GLP or GEP status** **published or not**	**Vertebrate study Y/N**	**Owner**
KCP 7.3 / 01	Maas, W.	2020	The in vitro percutaneous absorption of radiolabelled flufenacet in a concentrate formulation (flufenacet SC 508.8) and two in-use dilutions through human split-thickness skin Report No.: 20215140, Edition Number: M-676520-01-1 Charles River Laboratories Den Bosch BV, DD 's-Hertogenbosch, The Netherlands GLP/GEP: Yes unpublished	No	Bayer
KCA 5.8.1 / 01	Herbold, B.	2009	FOE 5043-Oxalate (Project: FOE 5043 (Flufenacet/AE F133402)) - Salmonella/microsome test - Plate incorporation and preincubation method Report No.: AT05640, Edition Number: M-358953-01-1 Bayer HealthCare AG, Wuppertal, Germany GLP/GEP: Yes unpublished	No	Bayer
KCA 5.8.1 / 02	Wollny, H. E.	2010	FOE 5043-Oxalate - Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT) Report No.: 1277301, Edition Number: M-361724-01-1 Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany GLP/GEP: Yes unpublished	No	Bayer
KCA 5.8.1 / 03	Nern, M.	2009	FOE 5043-oxalate (Project: Flufenacet (FOE 5043)) - In vitro chromosome aberration test with Chinese hamster V79 cells Report No.: AT05598, Edition Number: M-358043-01-1 Bayer Schering Pharma AG, Wuppertal, Germany GLP/GEP: Yes unpublished	No	Bayer
KCA 5.8.1 / 04	Wollny, H. E.	2009	FOE 5043-Sulfonic acid Na-salt - Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT) Report No.: 1277302, Edition Number: M-361158-01-1 Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany GLP/GEP: Yes unpublished	No	Bayer
KCA 5.8.1 / 05	Nern, M.	2010	FOE 5043-sulfonic acid Na-salt (Project: Flufenacet (FOE 5043)) - In vitro chromosome aberration test with Chinese hamster V79 cells Report No.: AT05870, Edition Number: M-366380-01-1 Bayer Schering Pharma AG, Wuppertal, Germany GLP/GEP: Yes unpublished	No	Bayer
KCA 5.8.1 / 06	xxx	2010	FOE 5043-sulfonic acid Na-salt - Project: Flufenacet (FOE 5043) - Micronucleus-test on the male mouse Report No.: AT05913, Edition Number: M-368627-01-1 xxx GLP/GEP: Yes unpublished	Yes	Bayer

Data Point	Author(s)	Year	Title Company Report No. Source GLP or GEP status published or not	Vertebrate study Y/N	Owner
KCA 5.8.1 / 07	xxx	2017	[phenyl-UL-14C]Flufenacet, [thiadiazole-5-14C]Flufenacet and [phenyl-UL-14C]BCS-AZ23374: Distribution of radioactivity in the bone marrow of mice by quantitative whole-body autoradiography Report No.: EnSa-16-1016, Edition Number: M-580054-01-1 xxx GLP/GEP: Yes unpublished	Yes	Bayer
KCA 5.8.1 / 08	xxx.	2010	FOE 5043-sulfonic acid Na-salt (Project: Flufenacet (FOE 5043)) - Unscheduled DNA synthesis test with male rat liver cells in vivo Report No.: AT06167, Edition Number: M-397810-01-1 xxx GLP/GEP: Yes unpublished	Yes	Bayer

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

Please note that all data mentioned as part of DAR, RAR, or EFSA journals are considered as relied on.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
-	-	-	-	-	-

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
-	-	-	-	-	-

List of data relied on not submitted by the applicant but necessary for evaluation

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
-	-	-	-	-	-

Appendix 2 Detailed evaluation of the studies relied upon

A 2.1 Statement on bridging possibilities

Comments of zRMS:	In the bridging statement (Hanslik, L. 04.2021; see Confidential part Part C) compositions of the two formulations flufenacet SC 508 (508.8 g/L) Spec. No. 102000007779 ver.02 (registered) and 102000007779 ver.03 (new) has been compared and showed that the parameters of both formulations are sufficiently comparable. The assessment of the composition of the toxicologically tested formulation FFA SC 508 (508.8 g/L) (Spec. No. 1102000007779; Batch Nos. 04402/0110, EFKF000175) reveals that the available toxicological data package is valid to support the new formulation (Spec. No.102000007779-03) and can be used to propose a toxicological classification for FFA SC 508 (508.8 g/L; Spec. No.102000007779-03). Thus, based on available data discussed in the Part C, ZRMS conclude toxicity studies, which were performed with the previous formulation 102000007779 ver. 02 which is similar to 102000007779 ver. 03, as reliable for current registration. Results of these acute toxicity studies: *- oral route exposure (OECD 423), xxx. 1998* *- dermal route exposure (OECD 402), xxx 1998* *- inhalation exposure (OECD 403), xxx 1999* *- skin irritation model (OECD 404), xxx 2001* *- eye irritation model (OECD 405), xxx 2001* *- skin sensitization (OECD 406 LLNA), xxx 2005* can be taken into account for 102000007779-03/Cadou 500SC.

A 2.2 Acute oral toxicity (KCP 7.1.1)

Comments of zRMS:	Data has been reviewed for compliance with the current guidelines, resulting from scientific progress. Study (xxx 1998) implements 3R rules minimizing the number of animals required to estimate the acute oral toxicity of a chemical. Method uses pre-defined doses and the results allow a substance to be ranked and classified according to the CLP for the classification of chemicals which cause acute toxicity Noted deviation has no critical impact on study outcome. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

Reference:	**KCP 7.1.1/01**
Title:	FOE 5043 500 SC 04402/0096 (Fluthiamid (prop.)) - Study for acute oral toxicity in rats
Report:	xxx 1998; 27271; M-005460-01-1
Authority registration No:	
Guideline(s):	OECD 423; Directive 67/548/EC, Annex IVB, Part B.1 tris; US-EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Humans and Domestic Animals, Series 81-1
Deviations:	The test substance is a commercial product (stable/ homogenous undiluted/ready-to-use dilution). Analytical determinations of stability and homogeneity of the aqueous formulations were not performed. This deviation did not limit the assessment of results.
GLP/GEP:	yes
Acceptability:	Yes
Duplication (if vertebrate study):	No

Materials and methods

Test material (Lot/Batch No.)	FOE 5043 500 SC 04402/0096
Species	Wistar rat strain HsdCpb:Wu
No. of animals (group size)	3 rats/sex/group
Dose(s)	200; 500; 2000 mg/kg bw
Exposure	Once by gavage
Vehicle/Dilution	Demineralised water
Post exposure observation period	14 days

Remarks	None

Results and discussions

Table A 1: Results of acute oral toxicity study in rats of product FFA SC 508.8 G

Dose (mg/kg bw)	Toxicological results *	Duration of signs	Time of death	LD50 (mg/kg bw) (14 days)
Male rats				
200	0/3/3	1hour - 5hours	-	
500	0/3/3	45 min – 5 hours	-	
Female rats				
200	0/3/3	7 hours	-	
500	0/3/3	45 mins – 4 days	-	
2000	3/3/3	15 mins – 2 hours	2 hours	> 500 - < 2000

* Number of animals which died/number of animals with clinical signs/number of animals used

Table A 2: Summary of findings of acute oral toxicity study in rats of FFA SC 508.8 G

Mortality:	Yes
Clinical signs:	Doses ≤ 500 mg/kg bw were tolerated by male and female rats without mortalities. 200 mg/kg bw: decreased motility (males, females), staggering gait, tachypnea and temporary convulsions (females). 500 mg/kg bw: decreased reactivity (males, females), staggering gait and labored breathing (males). 2000 mg/kg bw: uncoordinated gait and abdominal positions (females). All three females died 2 hours after application
Body weight:	There were no treatment-related effects on body weight or body weight gain.
Macroscopic examination:	2000 mg/kg bw: Liver: discoloration, pale, distinct lobulation; Lung: slightly collapsed; Spleen: discoloration, pale No gross pathologic changes were observed in animals sacrificed at the end of the study period.

Conclusion

Under the experimental conditions, the oral LD50 of FFA SC 508.8 G is > 500 - < 2000 mg/kg bw in rats. Thus, the following classification is required according to Regulation (EC) No. 1272/2008 Acute tox 4; H302 Harmful if swallowed.

A 2.3 Acute percutaneous (dermal) toxicity (KCP 7.1.2)

Comments of zRMS:	Data has been reviewed for compliance with the current guidelines, resulting from scientific progress. In the study (xxx 1998) tested material has not been administered at doses which cause pain and distress due to potential corrosive or severely irritant actions (note: FFA SC 508.8 G is not classified as skin irritant). Noted deviation has no critical impact on study outcome. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

Reference:	**KCP 7.1.2/01**
Title:	FOE 5043 500 SC 04402/0096 (c.n.: Fluthiamid (prop.)) - Study for acute dermal toxicity in rats
Report:	xxx; 1998; 27416; M-004746-01-1
Authority registration No:	
Guideline(s):	OECD 402; Directive 67/548/EEC, Annex V, Part B.3.; US-EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Humans and Domestic Animals, Series 81-2
Deviations:	The test substance is a commercial product (stable/ homogenous in undiluted form). Analytical determinations of stability/homogeneity of the formulations for administration were not performed. This deviation did not limit the assessment of results.
GLP/GEP:	yes
Acceptability:	Yes
Duplication (if vertebrate study):	No

Materials and methods

Test material (Lot/Batch No.)	FOE 5043 500 SC 04402/0096
Species	Wistar rat strain HsdCpb:Wu
No. of animals (group size)	5 rats/sex/group
Dose(s)	4000 mg/kg bw
Exposure	24 hours (dermal, semi-occlusive)
Vehicle/Dilution	None
Post exposure observation period	14 days
Remarks	None

Results and discussions

Table A 3: Results of acute dermal toxicity study in rats of FFA SC 508.8 G

Dose (mg/kg bw)	Toxicological results *	Duration of signs	Time of death	LD50 (mg/kg bw) (14 days)
Male rats				
4000	0/5#/5	3 days – 8 days	-	> 4000
Female rats				
4000	0/5#/5	3 days – 8 days	-	> 4000

* Number of animals which died/number of animals with clinical signs/number of animals used
\# Only animals with skin reaction

Table A 4: Summary of findings of acute dermal toxicity study in rats of FFA SC 508.8 G

Mortality:	No mortality occurred.
Clinical signs:	No systemic clinical signs were observed. At 4000 mg/kg bw locally, a yellow discoloration of the treatment area which was partly reddened was observed in all animals. These signs started on day 3 and lasted up to day 8.
Body weight:	The body weight and the body weight development of males and females were not affected by the treatment.
Macroscopic examination:	The necropsies performed at the end of the study revealed no apparent findings.

Conclusion

Under the experimental conditions, the dermal LD_{50} of FFA SC 508.8 G is ≦ > 4000 mg/kg bw in rats. in Thus, no classification is required according to Regulation (EC) No. 1272/2008

A 2.4 Acute inhalation toxicity (KCP 7.1.3)

Comments of zRMS:	Data has been reviewed for compliance with the current guidelines, resulting from scientific progress. In the study (xxx 1999) animals are exposed to one limit concentration for a predetermined duration (4 hours) and obtain sufficient information on the acute toxicity of test article to enable its classification and to provide lethality data (LC_{50}) for both sexes as needed for quantitative risk assessments. No deviation from the study TG has been noted. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

Reference:	**KCP 7.1.3/01**
Title:	FOE 5043 500 SC 04402/0096 (c.n.: Fluthiamid (proposed)) - Study on acute inhalation toxicity in rats according to OECD no. 403
Report:	xxx; 1999; 28609; M-009812-01-1
Authority registration No:	
Guideline(s):	OECD 403; Directive 92/69/EEC; US-EPA 712C-98-193, OPPTS 870.1300
Deviations:	none
GLP/GEP:	yes
Acceptability:	Yes
Duplication (if vertebrate study):	No

Materials and methods

Test material (Lot/Batch No.)	FOE 5043 500 SC 04402/0096
Species	Wistar rat strain HsdCpb:Wu
No. of animals (group size)	5 rats/sex/group
Concentration(s)	2.172 mg/L air
Exposure	4 hours (nose only)
Vehicle/Dilution	water
Post exposure observation period	14 days
Remarks	None

Results and discussions

Table A 5: Concentration(s) and exposure conditions

Target conc. (mg/L air)	or	Nominal conc. (mg/L air)	Actual conc. (mg/L air)	MMAD * (µm)	GSD ** (µm)
xxx		13847	2172	3.52	2.11

* MMAD = Mass Median Aerodynamic Diameter
** GSD = Geometric Standard Deviation

Table A 6: Results of acute inhalation toxicity study in rats of FFA SC 508.8 G

Concentration (mg/L air)	Toxicological results *	Duration of signs	Time of death	LC_{50} (mg/L air) (14 days)
Male rats				
0	0/0/5	-	-	-
2172	0/4/5	0 day – 1 day	-	LC50 > 2172 mg/m3 (maximum techn. attainable concentration)
Female rats				
0	0/0/5	-	-	-
2172	0/5/5	0 day – 1 day	-	LC50 > 2172 mg/m3 (maximum techn.

Concentration (mg/L air)	Toxicological results *	Duration of signs	Time of death	LC_{50} (mg/L air) (14 days)
				attainable concentration)

* Number of animals which died/number of animals with clinical signs/number of animals used

Table A 7: Summary of findings of acute inhalation toxicity study in rats of FFA SC 508.8 G

Mortality:	Yes
Clinical signs:	Control group: All rats tolerated the exposure without specific signs. Test substance group: Piloerection, ungroomed hair-coat, reduced motility, bradypnea, irregular and labored breathing pattern were observed. All rats of the test substance exposure group tolerated the exposure without any effect on reflex measurements. Assessment of body temperatures revealed a mild hypothermia in animals of the test substance group, which gained statistical significance in females.
Body weight:	Comparisons between control animals with those exposed to the test substance did not reveal marked effects on body weight gains. As far as statistical significant differences were observed they are considered to be of no toxicological relevance.
Macroscopic examination:	In rats exposed to the test compound a conclusive, concentration-dependent increased incidence of macroscopic findings could not be ascertained. Therefore, the discolorations of lungs observed are not considered to be causally related to the exposure to the test substance. Findings such as mild discolorations of lung and other parenchymatous organs are often observed in control animals euthanized with pentobarbital.

Conclusion

Under the experimental conditions, the inhalation LC_{50} of FFA SC 508.8 G is >2172 mg/L air in rats. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

A 2.5 Skin irritation (KCP 7.1.4)

Comments of zRMS:	Study (xxx 2001) has been reviewed for compliance with the current guidelines, resulting from scientific progress. As we mentioned and explained in the our general comment already existed *in vivo* study has been accepted and considered by the ZRMS as reliable for the hazard assessment. Test product was applied in a single dose to the skin of an experimental animal; untreated skin areas of the test animal serve as the control. The degree of irritation/corrosion was read and scored at specified intervals in order to provide a complete evaluation of the effects. The duration of the study was sufficient to evaluate the reversibility or irreversibility of the effects observed. No deviation from the study TG has been noted. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

Reference:	**KCP 7.1.4/01**
Title:	Acute skin irritation test (patch test) of FOE 5043 500 SC 04402/0096 in rabbits - first revision of report no. R7215
Report:	xxx 2001; R7993; M-004806-02-1
Authority registration No:	
Guideline(s):	OECD 404 (1992); EC guideline B.4 (1992)
Deviations:	none
GLP/GEP:	yes
Acceptability:	Yes
Duplication (if vertebrate study):	No

Materials and methods

Test material (Lot/Batch No.)	FOE 5043 500 SC 04402/0096
Species	Rabbit, Himalayan
No. of animals (group size)	3 males
Initial test using one animal	No
Exposure	0.5 mL (4 hours, semi-occlusive)
Vehicle/Dilution	None
Post exposure observation period	14 days
Remarks	None

Results and discussions

Table A 8: Skin irritation of FFA SC 508.8 G

Animal No.		Scores after treatment *				Mean scores (24-72 h)	Reversible (day)
		1 h	24 h	48 h	72 h		
1	Erythema (redness) Oedema	-	0/0	0/0	0/0	0	na
2	Erythema (redness) Oedema	-	0/0	0/0	0/0	0	na
3	Erythema (redness) Oedema	-	0/0	0/0	0/0	0	na

na = not applicable

Response:	-- = negative for mean scores	<1.5	(GHS)
		<2.3	(Regulation (EC) No 1272/2008)
	(+) = mild irritant for mean scores	≥1.5 - <2.3	(GHS category 3)
	+ = irritant for mean scores	≥2.3	(Regulation (EC) No 1272/2008 and GHS category 2

Clinical signs:	No clinical signs of toxicity were observed.

Conclusion

Under the experimental conditions, FFA SC 508.8 G is not a skin irritant. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

A 2.6 Eye irritation (KCP 7.1.5)

Comments of zRMS:	Study (FFA SC 508.8 G) has been reviewed for compliance with the current guidelines, resulting from scientific progress. As we mentioned and explained in the our general comment already existed *in vivo* study (note: FFA SC 508.8 G is not classified as skin irritant) has been accepted and considered by the ZRMS as reliable for the hazard assessment. In the mentioned study degree of eye irritation/serious eye damage were evaluated by scoring lesions of conjunctiva, cornea, and iris, at specific intervals. Duration of the study was sufficient to evaluate the reversibility or irreversibility of the effects. No deviation from the study TG has been noted. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

Reference:	**KCP 7.1.5/01**
Title:	Acute eye irritation study of FOE 5043 500 SC 04402/0096 by instillation into the conjunctival sac of rabbits - first revision of report no. R7216
Report:	xxx 2001; R7994; M-004807-02-1
Authority registration No:	
Guideline(s):	OECD 405 (1987); EC guideline B.5 (1992)
Deviations:	none
GLP/GEP:	yes
Acceptability:	Yes
Duplication (if vertebrate study):	No

Materials and methods

Test material (Lot/Batch No.)	FOE 5043 500 SC 04402/0096
Species	Rabbit, Himalayan
No. of animals (group size)	3 males
Initial test using one animal	No
Exposure	0.1 mL (single instillation in conjunctival sac)
Irrigation (time point)	No
Vehicle/Dilution	None
Post exposure observation period	72 hours
Remarks	None

Results and discussions

Table A 9: Eye irritation of FFA SC 508.8 G

Animal No.		Scores after treatment *				Mean scores (24-72 h)	Reversible (day)
		1 h	24 h	48 h	72 h		
1	Corneal opacity	0	0	0	0	0	na
	Iritis	0	0	0	0	0	na
	Redness conjunctivae	0	0	0	0	0	na
	Chemosis conjunctivae	0	0	0	0	0	na
2	Corneal opacity	0	0	0	0	0	na

	Iritis Redness conjunctivae Chemosis conjunctivae	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	na na na
3	Corneal opacity Iritis Redness conjunctivae Chemosis conjunctivae	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	na na na na

* scores in the range of 0 to 4 for cornea opacity and chemosis, 0 to 2 for redness of conjunctivae and 0 to 2 for iritis

Clinical signs:	No clinical signs of toxicity were observed.

Conclusion
Under the experimental conditions, FFA SC 508.8 G is not an eye irritant. Thus, no classification is required according to Regulation (EC) No. 1272/2008

A 2.7 Skin sensitisation (KCP 7.1.6)

Comments of zRMS:	Study (xxx 2005) has been reviewed for compliance with the current guidelines, resulting from scientific progress. As we mentioned and explained in the our general comment already existed *in vivo* study has been accepted and considered by the ZRMS as reliable for the hazard assessment. Noted deviation has no critical impact on study outcome. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

Reference:	**KCP 7.1.6/01**
Title:	Flufenacet SC 500 (Project: Flufenacet (FOE 5043)) - Local lymph node assay in mice (LLNA/IMDS)
Report:	xxx 2005; AT02459; M-258556-01-1
Authority registration No:	
Guideline(s):	OECD 406, OECD 429; Directive 67/548/EEC, Annex V, Method B.6,B.42; US-EPA 712-C-03-197, OPPTS 870.2600
Deviations:	The test item contains commercial products known to be stable and homogenous both undiluted and in ready-to-use dilution with water. Therefore, analytical determinations of the stability and homogeneity of the formulations in Pluconic/NaCl solution for administration were not performed. This deviations does not limit the assessment of the results.
GLP/GEP:	yes
Acceptability:	
Duplication (if vertebrate study):	No

Materials and methods

Test material (Lot/Batch No.)	AE F133402 00 SC42 A102 (batch no.: EFKF000175)
Species	24 NMRI mice strain: HsdWin:NMRI (SPF)
No. of animals (group size)	Test substance group: 6/concentration 2%, 10%, 50% Vehicle control goup: 6 female mice
Range finding:	Yes / No
Exposure (concentration(s), no. of applications)	Epicutaneously onto the dorsal part of both ears; 25 µL; application on three consecutive days
Vehicle	Pluronic PE 9200 / 0.9 % NaCl solution, 1 % v/v Positive control: Alpha Hexyl Cinnamic Aldehyde (3% - 10% - 30%) (Test methodology was checked for reliability in a separate study on female NMRI. A similar check is done in regular intervals to confirm the reliability of the methods.
Pre-treatment prior to topical application	No
Reliability check	None

Remarks	e.g. None

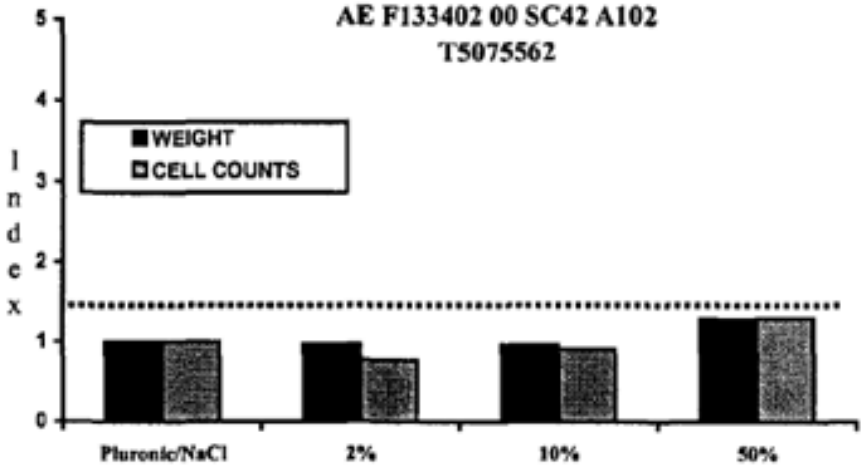
Results and discussions

Clinical signs:	No clinical signs of toxicity were observed.

The "positive level" of ear swelling, which is $2xl0^2$ mm increase, i.e. about 10% of the control values, has not been exceeded or reached in any dose group. No substance specific effects were determined for ear weights, too.

The NMRI mice did not show an increase in the stimulation indices for cell counts or for weights of the draining lymph nodes after application of the test item AE F133402 00 SC42 A102.
The "positive level" which is 1.4 for the cell count index was never reached or exceeded in any dose group.
It has to be clarified that the "positive levels" mentioned above are exclusively defined for the NMRI outbred mice used for this study. Such positive limits have to be calculated for each strain of mice individually.

The body weights of the animals were not affected by any treatment.



Conclusion
Under the experimental conditions, FFA SC 508.8 G is not a skin sensitiser. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

A 2.8 Supplementary studies for combinations of plant protection products (KCP 7.1.7)

No supplemental studies submitted.

A 2.9 Data on co-formulants (KCP 7.4)

A 2.9.1 Material safety data sheet for each co- formulant

Information regarding material safety data sheets of the co-formulants can be found in the confidential dossier of this submission (Registration Report - Part C).

A 2.9.2 Available toxicological data for each co-formulant

Available toxicological data for each co-formulant can be found in the confidential dossier of this

submission (Registration Report - Part C).

A 2.10 Studies on dermal absorption (KCP 7.3)

Dermal absorption of flufenacet was tested under *in vitro* conditions using human skin. The study was conducted using the previous FFA SC 508.8 G formulation (Product code: 102000007779-02). However, as far as the composition is concerned the formulation is regarded to be similar to the current FFA SC 508.8 formulation (Product code102000007779-03). For details please refer to the report: "Bridging Statement, Explaining the changes in the recipe of the product Flufenacet SC 508.8 (508.8 g/L); Company Codes Spec. No. 102000007779-02 and Spec. No. 102000007779-03; L. Hanslik, 2020 (M-759471-01-1) presented in the confidential dossier of this submission (Registration Report – Part C). A summary of the study is presented in the following.

Comments of zRMS:	Cross reading aproache is accepted. Comparison of both formulations are presented in the confidential Part C of this dossier. Difference in the composition has been concluded as admissible, thus study (Maas, W.; 2020) has been considered as relevant to assess dermal absorption of radiolabelled flufenacet in the product FFA SC 508.8 G. Followig studies were conducted according to OECD Guideline 428 and in compliance with GLP. All the recoveries where between the recovery boundaries mentioned in the dermal absorption guidance (Guidance on Dermal Absorption (EFSA Journal, 2017, 15(6): 4873). Studies are considered to be acceptable and the dermal absorption for FFA (FFA SC 508.8 G) are covered by this studies.

Reference:	**KCP 7.3/01**
Title:	The in vitro percutaneous absorption of radiolabelled flufenacet in a concentrate formulation (flufenacet SC 508.8) and two in-use dilutions through human split-thickness skin
Report:	Maas, W.; 2020; 20215140; M-676520-01-1
Authority registration No:	
Guideline(s):	OECD Guideline for Testing of Chemicals, Guideline 428: Skin Absorption: In Vitro Method (2004). OECD Environmental Health and Safety Publications Series onnTesting and Assessment No. 28. Guidance Document for the Conduct of Skin Absorption Studies (2004). European Commission Guidance Document on Dermal Absorption - Sanco/222/2000/Rev. 7 (19 March 2004). Guidance on Dermal Absorption (EFSA Journal, 2017, 15(6): 4873).
Deviations:	None
GLP/GEP:	yes
Acceptability:	Yes
Duplication (if vertebrate study):	Not aplicable

Material and methods

Human skin:
Source: Alphenyx.
Number and sex: 6 female donors.
Anatomical region: 3 Abdomen & 3 breast.
Thickness: 270 to 400 µm.

Test Material:

Non-radiolabelled:
Batch: K664072.
Purity = 97.8% w/w.

Radiolabelled:
[phenyl-UL-^{14}C]-Flufenacet.
Batch: KML10597.
Specific activity: 3.74 MBq/mg.
Radiopurity: >99%.

Formulation:
The formulation used in this experiment was the FFA SC 508.8 formulation (specification number 102000007779-02) flufenacet (508.8 g/L). It was used at three nominal concentrations of flufenacet: neat; 508.8 g/L and two

representative spray dilutions of 2.0 g/L and 0.06 g/L.

Test system: An automated flow-through diffusion system was used consisting of a 16-channel peristaltic pump, PermeGear flow-through cells (diameter: 11.28 mm, area: 1 cm^2), cell warmers, C204 fraction collectors and an ISCO Retriever IV fraction collector.
The flow-through diffusion cells were positioned in a manifold heated via a circulating water bath to maintain a skin surface temperature of 32 ± 1 °C. The cells were connected to a multichannel peristaltic pump from their afferent ports. Effluent from the cells dropped into vials on a fraction collector via tubing. The surface area of exposed skin within the cells was 1 cm^2, with a receptor chamber of 0.25 mL. The peristaltic pump speed was adjusted to obtain a standard flow rate of 1.5 mL/h.
The receptor fluid chosen for use in this study was phosphate buffered saline (PBS) containing polyoxyethylene 20 oleyl ether (6%, w/v), sodium azide (0.01%, w/v), streptomycin (0.1 mg/mL) and penicillin G (100 units/mL). The pH was checked and adjusted to pH 7.4.
In order to confirm adequate solubility of Flufenacet in the receptor fluid, non-radiolabelled Flufenacet and [^{14}C]Flufenacet, both dissolved in ACN, were mixed, added to glass vials (in triplicate) and the solvent was evaporated. Subsequently, receptor fluid (see section 4.9) was added to a final concentration of *ca.* 100 µg/mL. The solution was gently mixed for *ca.* 1 h at 32 °C and then centrifuged at 2000 g for 5 min. From the supernatant, three samples from the top, the middle and the bottom of the vial were analysed using liquid scintillation counting (LSC) to determine of the amount of radioactivity.

Skin integrity: **Static system**:
Sections of split-thickness skin membranes were thawed, mounted in the diffusion cells between the donor and receptor chamber and the skin integrity was tested by permeation of tritiated water. Tritiated water (250 µL, 4.88 kBq (Exp. 1) or 4.89 kBq (Exp. 2)) was applied to the skin and the donor compartment occluded of the flow-through cells. The absorption of tritiated water was assessed over 1 hour by collecting a single 1 hour fraction and measuring the amount of radioactivity associated with the sample. Tritiated water absorption (% applied dose) was calculated for each skin sample. The skin samples should exhibit absorption < 1.6% of the applied dose. At the end of the 1 h period, residual tritiated water remaining at the donor compartment was removed with a pipette and the skin was dried with two cotton swabs, washed with 500 µL water and dried with one cotton swab. The washing procedure was repeated once. The receptor fluid samples were analyzed by LSC.

Treatment: A single 10 µL dose (10 µL/cm^2) of each of the test preparations was applied evenly over the surface of 8 split-thickness human skin membranes using a positive displacement pipette. The donor chambers of the cells were left non-occluded. Seven representative aliquots (10 µL) of each of the [^{14}C]Flufenacet containing test preparations were dispensed into vials at the time of dosing, mixed with scintillation cocktail and analyzed by LSC.

Sampling: The exposure period was terminated at 8 h post dose. Commercial hand wash soap (50 µL) was applied to the skin and the soap was gently rubbed onto the skin with a cotton swab. The skin was then rinsed with approximately 5 mL of a 2% (v/v) commercial soap solution. The soap solution was applied in aliquots (1 mL) and each aliquot was aspirated with a pipette. The skin was dried with a cotton swab. This process was repeated once.
The soap solution (skin wash) and cotton swabs samples were mixed with scintillation cocktail and analyzed using LSC.
At 24 hours post dose, each diffusion cell was dismantled and the skin

removed. The receptor compartment was rinsed twice with *ca*. 1 mL of receptor fluid followed by air to remove all remaining receptor fluid in the compartment, and collected in a LSC vial. The underside of the skin was dried using a piece of tissue paper and this same piece of paper was used to dry the receptor compartment. The tissue was then added to a second LSC vail. The receptor compartment was further cleaned using a cotton swab wetted with ethanol and the swab added to the vial containing the tissue paper. These receptor wash samples were then mixed with scintillation cocktail and analyzed by LSC. The receptor rinses represented the absorbed test item, which was in the receptor chamber, but had not been collected into the 22 to 24 h receptor fluid fraction. The donor chamber was transferred into separate tube (donor compartment wash) and extracted using 10 mL EtOH. After sonication, the donor compartment was removed from the tube while removing any adhering solution as much as possible. The extraction volume was split in two, mixed with scintillation cocktail and analyzed by LSC.
The stratum corneum was removed with a maximum of 20 successive tape strips (CuDerm). The skin sample was rotated 90° after each tape strip. Rotation was stopped if the epidermis/dermis junction became fragile or if the epidermis was removed. Where all of the epidermis was removed tape stripping was stopped. Tape strips were extracted in liquid scintillant individually prior to analysis by LSC.
The skin under the cell flange (unexposed skin) was cut away from the exposed skin. The exposed and non-exposed skin samples were digested individually in Solvable® (2 mL). All skin samples were placed in a water bath set to *ca* 60°C to aid solubilization. Stannous chloride solution (0.2 g/mL in ethanol; 150 µL) and scintillation fluid were then added and total radioactivity was determined by LSC.

Radioassay: All radioactivity measurements were performed by LSC using a PerkinElmer Tri-Carb 2910TR scintillation counter (PerkinElmer Life and Analytical Sciences, Boston, MA, USA). Up until an applied radioactive dose of *ca*. 200.000 DPM/membrane, samples were counted to a statistical precision of ± 0.2% with a maximum counting time of 5 minutes. For higher radioactive doses, the counting time was set at 2 minutes. The PerkinElmer Tri-Carb 2910TR was programmed to convert counts per minute to degradations per minute. The background was subtracted manually.

Findings:
Flufenacet was demonstrated to be sufficiently soluble in the receptor fluid to avoid any risk of back diffusion.
Measurements of the homogeneity of the three concentrations of formulation applied indicated that it was acceptable.
No cells were excluded.
The study results are presented in the following Table.

Table A 10: **Mean distribution of radioactivity at 24 hours after dose application of [14C]-flufenacet in an SC 508.8 formulation at the rates of 508.8 g/L, 2.0 g/L and 0.06 g/L to human skin samples.** ***Results expressed in terms of percentage of applied radioactivity.***

	Distribution of radioactivity (% dose)					
Dose Levels	Neat formulation: High dose (508.8 g/L)		Dilution: Intermediate dose (2.0 g/L)		Dilution: Low dose (0.06 g/L)	
Species	Human: 4 donors (n = 8, K N° = 0.84)		Human: 4 donors (n = 8, K N° = 0.84)		Human: 4 donors (n = 8, K N° = 0.84)	
	Mean	SD	Mean	SD	Mean	SD
SURFACE COMPARTMENT						
Dislodgeable dose (8h)	99.63	1.70	96.46	3.74	83.95	6.67
Donor chamber	0.009	0.007	0.306	0.192	0.548	0.251
Surface Dose (1st two tape-strips)	0.013	0.015	0.764	0.800	1.548	1.348
Total Non-absorbable	99.65	1.70	97.53	2.78	86.05	7.35
SKIN COMPARTMENT						
Skin [a]	0.003	0.003	0.365	0.467	1.315	1.157
Surrounding skin	0.003	0.005	0.041	0.037	0.022	0.009
Total skin	0.006	0.007	0.406	0.491	1.337	1.157
Stratum corneum (SC 3-20) [b]	0.026	0.020	1.878	2.309	3.079	3.042
Total % at dose site	**0.032**	**0.023**	**2.284**	**2.798**	**4.416**	**4.062**
RECEPTOR COMPARTMENT						
Receptor fluid (0-12 hr)	0.014	0.005	1.133	0.419	**7.339**	**5.953**
Receptor fluid (0-24 hr)	0.024	0.007	1.379	0.493	**8.073**	**6.059**
Receptor Wash + Rinse	0.003	0.000	0.033	0.024	**0.092**	**0.094**
Total % directly absorbed	**0.027**	**0.007**	**1.411**	**0.509**	**8.164**	**6.097**
STUDY: Total % Potentially Absorbable A (%skin + %direct)	0.033	0.010	1.818	0.851	9.501	5.941
STUDY: Total % Potentially Absorbable B (%dose site + %direct)	0.059	0.028	3.695	3.065	12.58	6.32
TOTAL % RECOVERY	**99.71**	**1.68**	**101.20**	**0.56**	**98.64**	**11.83**
Evaluation according to EFSA Guidance						
Absorption >75% within half of study duration	No (include SC values except SC1 & SC2)		Yes (exclude SC values)		Yes (exclude SC values)	
Recovery <95%	No correction needed		No correction needed		No correction needed	
Total % Potentially Absorbable adjusted according to EFSA (2017)	Mean %dose site +%directly absorbed + SD*0.84		Mean %skin+%directly absorbed + SD*0.84		Mean %skin+%directly absorbed + SD*0.84	
	0.083		**2.5**		**14**	

[a]: Radioactivity found in skin after tape-stripping procedure.
[b]: tape-strips excluding numbers 1 & 2 which are considered to be non-absorbed dose.
SD: standard deviation
n: number of skin cells used for calculation
In the above table, the presented means do not always calculate exactly from the presented individual data.
This is due to rounding-up differences resulting from the use of the spreadsheet program.

Conclusion:
The dermal penetration through human dermatomed skin of [^{14}C]-flufenacet in the SC 508.8 formulation was investigated at three concentrations corresponding to the neat product (508.8 g /L) and to two representative dilutions of 2.0 g/L and 0.06 g/L.
Concentrate
The mean percentage of flufenacet in the SC 508.8 formulation that was considered to be potentially absorbable *(directly absorbed plus total remaining at dose site)* over a period of 24 hours for the neat formulation applying the EFSA guidance (2017) is 0.083%.
Intermediate Dose level (Spray dilution)
The mean percentage of flufenacet in the SC 508.8 formulation that was considered to be potentially absorbable *(directly absorbed plus total skin)* over a period of 24 hours for the intermediate dose rate was applying the EFSA guidance (2017) was 2.5%.
Low Dose level (Spray dilution)
The mean percentage of flufenacet in the SC 508.8 formulation that was considered to be potentially absorbable *(directly absorbed plus total skin)* over a period of 24 hours for the low dose rate applying the EFSA guidance (2017) was 14%.
Therefore, the following dermal absorption value can be proposed for use in the non-dietary risk assessments for flufenacet in the FFA SC 508.8 formulation:

- 0.083% for the neat formulation (508.8 g/L)
- 2.5% for the low dose (2.0 g/L).
- 14% for the low dose (0.06 g/L).

A 2.11 Other/Special Studies

A 2.11.1 Study AT05640

Comments of zRMS:	Genotoxicity study (Herbold, B.; 2009) has been peer reviewed and accepted as reliable during renewal of FFA in 2018 (refer DRAR Vol. 3CA B6, point B.6.8.1 Toxicity studies of metabolites). Outcome of the study confirm lack of genotoxic potential of FFA metabolite M01 FOE 5043-Oxalate.

Reference:	**KCA 5.8.1/01**
Title:	FOE 5043-Oxalate (Project: FOE 5043 (Flufenacet/AE F133402)) - Salmonel-la/microsome test - Plate incorporation and preincubation method
Report:	Herbold, B.; 2009; AT05640; M-358953-01-1
Authority registration No:	
Guideline(s):	OECD 471; Council Regulation 440/2008/EEC, Method B.13/14.; US-EPA712-C-98-247, OPPTS 870.5100
Deviations:	none
GLP/GEP:	yes
Acceptability:	Yes
Duplication (if vertebrate study):	Not aplicable

Materials and methods

Test material (Lot/Batch No.)	FOE 5043 oxalate Batch No. SES10564-3-1	
Test system	*Salmonella typhimurium* strains TA1535, TA1537, TA100, TA98, TA102	
Metabolic activation	S9-mix	
Vehicle	DMSO Deionized water (only for MMC)	
Positive control (without metabolic activation)	TA98: 4-nitro-1,2-phenylene diamine (4-NPDA) TA100: Nitrofurantoin (NF) TA102: mitomycin C (MMC) Cumene TA1535: Sodium azide (Na-azide)	0.5-1 µg/plate 0.2-0.4 µg/plate 0.2-0.4 µg/plate 50-75 µg/plate 10-20 µg/plate

	TA1537: 4-nitro-1,2-phenylene diamine (4-NPDA)	10-20 µg/plate
Positive control (with metabolic activation)	TA98, TA100, TA102, TA1535, TA1537: 2-aminoanthracene (2-AA)	3-6 µg/plate
Test substance Dose	0-16-50-158-500-1581-5000 µg/plate	
Application volume	0.1 mL/plate	
Incubation time	48 hours, 37°C	

Results and discussions

Table A 11: Summary of findings

Observations:	Doses up to and including 5000 µg per plate FOE 5043-oxalate produced weak bacteriotoxic effects, starting at 158 µg per plate in the plate incorporation trial only. Evaluation of individual dose groups, with respect to relevant assessment parameters (dose effect, reproducibility) revealed no biologically relevant variations from the respective negative controls. In spite of the low doses used, positive controls increased the mutant counts significantly compared with negative controls, and thus demonstrated the system's high sensitivity. Despite this sensitivity, no indications of mutagenic effects of FOE 5043-oxalate could be found at assessable doses of up to 5000 µg per plate in any of the Salmonella typhimurium strains used.

Table A 12: Summary of results FOE 5043-oxalate bacterial reverse mutation test

Mean revertants per plate							
Substance	**Dose (µg/plate)**	**S9 mix**	**Strain**				
			TA1535	**TA100**	**TA1537**	**TA98**	**TA102**
Plate incorporation							
FOE 5043-oxalate	0	–	9	106	6	17	191
	16	–	7	101	5	19	204
	50	–	9	115	7	21	207
	158	–	8	93	6	19	200
	500	–	9	109	7	19	193
	1581	–	7	108	6	21	202
	5000	–	8	118	2	22	229
Na-azide	10	–	877				
	20	–	1085				
NF	0.2	–		302			
	0.4	–		541			
4-NPDA	10	–			32		
	20	–			54		
	0.5	–				63	
	1	–				92	
MMC	0.2	–					705
	0.4	–					896
FOE 5043-oxalate	0	+	9	177	10	30	254
	16	+	10	170	8	29	248
	50	+	10	150	8	35	279
	158	+	9	176	9	31	289
	500	+	9	167	9	30	247
	1581	+	9	187	6	30	245
	5000	+	8	150	8	30	236
2-AA	3	+	135	2270	283	1527	601
	6	+	103	1598	80	1748	1112
Pre-incubation							
FOE 5043-oxalate	0	–	9	99	6	21	149
	16	–	10	112	6	17	164
	50	–	8	123	7	21	159
	158	–	9	118	7	17	182
	500	–	9	101	6	18	158
	1581	–	8	112	5	19	176
	5000	–	10	103	5	21	180
Na-azide	10	–	761				
	20	–	884				
NF	0.2	–		428			

Mean revertants per plate							
Substance Dose (µg/plate)		S9 mix	Strain TA1535	TA100	TA1537	TA98	TA102
	0.4			775			
4-NPDA	10	–			34		
	20	–			77		
	0.5	–				71	
	1	–				100	
Cumene	50	–					335
	75	–					335
FOE 5043-oxalate	0	+	10	141	11	30	244
	16	+	10	125	9	27	264
	50	+	8	135	10	31	247
	158	+	12	145	9	26	254
	500	+	9	120	9	31	238
	1581	+	10	144	8	28	221
	5000	+	10	123	8	31	224
2-AA	3	+	100	2254	275	1534	488
	6	+	62	2023	196	2180	801

Conclusion
Under the experimental conditions, FOE 5043-oxalate has to be regarded as non-mutagenic.

A 2.11.2 Study 1277301

Comments of zRMS:	FFA metabolite FOE 5043-Oxalate genotoxicity study (Wollny, H. E.; 2010) has been peer reviewed and accepted as reliable during renewal of FFA in 2018 (refer DRAR Vol. 3CA B6, point B.6.8.1 Toxicity studies of metabolites; Completion of metabolite data package). Under the experimental conditions test item did not induce gene mutations at the HPRT locus in V79 cells. Therefore, FOE 5043-oxalate is considered to be non-mutagenic in this HPRT assay. Outcome of the study confirm lack of genotoxic potential of FFA metabolite M01 FOE 5043-Oxalate.

Reference:	**KCA 5.8.1/02**
Title:	FOE 5043-Oxalate - Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT)
Report:	Wollny, H. E.; 2010; 1277301; M-361724-01-1
Authority registration No:	
Guideline(s):	OECD 476; Commission Regulation (EC) No. 440/2008, B17; US-EPA 712-C-98-221, OPPTS870.5300
Deviations:	none
GLP/GEP:	yes
Acceptability:	
Duplication (if vertebrate study):	

Materials and methods

Test material (Lot/Batch No.)	FOE 5043 oxalate Batch No. SES 10564-3-1	
Test system	Chinese hamster V79 cells	
Metabolic activation	S9-mix	
Vehicle	DMSO	
Positive control	Without metabolic activation ethylmethane sulfonate (EMS) With metabolic activation: 7,12-dimethylbenz(a)anthracene (DMBA)	 0.15 mg/mL 1.1 µg/mL
Test substance Dose	0-300-600-1200-2400 µg/mL	

Treatment duration	5 hours

Results and discussions

Table A 13: Summary of findings

Observations:	No precipitation of the test item was observed up to the maximal concentration in all experimental parts. No relevant cytotoxic effects occurred up to the maximal concentration of 2400 µg/mL. No relevant and reproducible increase in mutant colony numbers/10^6 cells was observed in the main experiments up to the maximal concentration. The mutation frequency generally remained within the historical range of solvent controls; the induction factor did not reach or exceed the threshold of 3.0. A linear regression analysis (least squares) was performed to assess a possible dose dependent increase of mutant frequencies. No significant dose dependent trend of the mutation frequency indicated by a probability value of <0.05 was determined in any of the experimental groups. A significant trend detected in the first culture of the first experiment with metabolic activation was judged as irrelevant since it actually was reciprocal, going down versus increasing concentrations. In both experiments of this study (with and without S9 mix) the range of the solvent controls was from 13.2 up to 34.6 mutant colonies per 10^6 cells; the range of the groups treated with the test item was from 5.7 up to 26.5 mutant colonies per 10^6 cells. The highest solvent controls (32.2 and 34.6 colonies per 10^6 cells) of the first experiment with metabolic activation slightly exceeded the historical range of solvent controls (0.8 – 31.3 colonies per 10^6 cells). However, this effect was judged as irrelevant since it is very minor and the solvent controls of the second experiment with metabolic activation remained well within the range of historical controls. The solvent control of the second culture of the first experiment slightly exceeded the historical range but the solvent control of the parallel culture was completely acceptable. EMS (0.15 mg/mL) and DMBA (1.1 µg/mL) were used as positive controls and showed a distinct increase in induced mutant colonies.

Table A 14: Summary of results FOE 5043-oxalate mammalian gene mutation test

	concentration µg/mL	S9	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10^6 cells	induction factor	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10^6 cells	induction factor
Experiment I / 5 h treatment			culture I				culture II			
Solvent control DMSO		–	100.0	100.0	14.7	1.0	100.0	100.0	34.5	1.0
Positive control EMS	150.0	–	109.8	60.3	96.7	6.6	85.9	74.9	187.7	5.4
FOE 5043-oxalate	150.0	–	87.5	culture was not continued#			96.6	culture was not continued#		
	300.0	–	106.1	98.4	6.9	0.5	94.8	83.1	15.0	0.4
	600.0	–	123.5	99.6	11.3	0.8	97.2	77.8	12.4	0.4
	1200.0	–	108.8	79.0	7.8	0.5	106.0	77.4	16.8	0.5
	1800.0	–	63.3	78.0	16.1	1.1	91.5	71.3	23.8	0.7
	2400.0	–	10.6	59.2	24.2	1.6	82.5	75.6	18.4	0.5
Solvent control DMSO		+	100.0	100.0	32.2	1.0	100.0	100.0	34.6	1.0
Positive control DMBA	1.1	+	63.2	71.1	1103.2	34.2	47.5	74.0	1265.3	36.6
FOE 5043-oxalate	150.0	+	94.9	culture was not continued#			91.6	culture was not continued#		
	300.0	+	106.1	83.7	21.1	0.7	86.1	105.5	21.7	0.6
	600.0	+	102.0	86.2	20.6	0.6	87.0	107.1	20.5	0.6
	1200.0	+	96.5	126.1	19.4	0.6	84.1	103.0	13.3	0.4
	1800.0	+	97.8	107.2	16.4	0.5	82.9	102.5	17.5	0.5
	2400.0	+	99.7	110.3	14.8	0.5	74.8	74.1	14.8	0.4

	concentration µg/mL	S9	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10^6 cells	induction factor	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10^6 cells	induction factor
Experiment II / 5 h treatment			culture I				culture II			
Solvent control DMSO		–	100.0	100.0	17.6	1.0	100.0	100.0	18.5	1.0
Positive control EMS	150.0	–	70.9	82.4	108.5	6.2	74.2	96.3	96.9	5.2
FOE 5043-Oxalate	150.0	–	82.3	culture was not continued#			98.9	culture was not continued#		
	300.0	–	77.9	90.5	21.1	1.2	93.8	86.0	8.1	0.4
	600.0	–	88.1	78.2	18.7	1.1	99.0	87.9	8.3	0.4
	1200.0	–	76.9	71.6	5.7	0.3	99.0	85.2	12.3	0.7
	1800.0	–	35.7	77.4	26.5	1.5	39.0	97.4	16.4	0.9
	2400.0	–	3.1	81.1	22.5	1.3	12.0	86.8	11.4	0.6
Solvent control DMSO		+	100.0	100.0	13.2	1.0	100.0	100.0	1511	1.0
Positive control DMBA	1.1	+	28.1	54.0	1104.7	83.9	52.3	70.8	617.5	41.0
FOE 5043-Oxalate	150.0	+	68.0	culture was not continued#			89.9	culture was not continued#		
	300.0	+	68.8	78.4	15.3	1.2	87.8	89.6	12.1	0.8
	600.0	+	75.3	83.1	17.6	1.3	89.7	98.7	6.3	0.4
	1200.0	+	85.1	70.0	11.9	0.9	86.7	64.2	24.0	1.6
	1800.0	+	83.0	95.0	15.5	1.2	89.1	67.9	10.9	0.7
	2400.0	+	75.0	92.9	7.5	0.6	92.2	72.2	15.5	1.0

Conc. = concentration
# Culture was discontinued since a minimum of only four analyzable concentrations is required

Conclusion
It can be stated that under the experimental conditions reported the test item did not induce gene mutations at the HPRT locus in V79 cells. Therefore, FOE 5043-oxalate is considered to be non-mutagenic in this HPRT assay.

A 2.11.3 Study AT05598

Comments of zRMS:	FFA metabolite FOE 5043-Oxalate genotoxicity study (Nern, M.; 2009) has been peer reviewed and accepted as reliable during renewal of FFA in 2018 (refer DRAR Vol. 3CA B6, point B.6.8.1 Toxicity studies of metabolites; Completion of metabolite data package). Outcome of the study confirm that FOE 5043-oxalate is not to be clastogenic for mammalian cells *in vitro*.

Reference:	**KCA 5.8.1/03**
Title:	FOE 5043-oxalate (Project: Flufenacet (FOE 5043)) - In vitro chromosome aberration test with Chinese hamster V79 cells
Report:	Nern, M.; 2009; AT05598; M-358043-01-1
Authority registration No:	
Guideline(s):	Directive 2000/32/EC, Method B.10; OECD 473; US-EPA 712-C-98-223, OPPTS 870.5375
Deviations:	none
GLP/GEP:	yes
Acceptability:	Yes
Duplication (if vertebrate study):	Not aplicable

Materials and methods

Test material (Lot/Batch No.)	FOE 5043 oxalate Batch No. SES 10564-3-1	
Test system	Chinese hamster V79 cells	
Metabolic activation	S9-mix	
Vehicle	DMSO (FOE 5043-oxalate) Hanks' balanced salt solution (positive control: mitomycin C, cyclophosphamide)	
Positive control	Without metabolic activation Mitomycin C (MMC) 4 / 18 hour treatment With metabolic activation: Cyclophosphamide (CP)	 0.1 / 0.03 µg/mL 2 µg/mL
Test substance Dose	0-150-300-600-1200-2400 mg/mL (+/– S9 mix)	
Treatment duration	With S9 mix: 4 hours Without S9 mix: 4 and 18 hours	
Harvest	18 and 30 hours	
Incubation temperature	37°C	
Replicates	2/culture	

Results and discussions

Table A 15: Summary of findings

Observations:	Chinese hamster V79 cells were treated with FOE 5043-oxalate concentrations of 600, 1200 and 2400 µg/ml for 4 hours without and with S9 mix for assessment of the clastogenic potential of FOE 5043-oxalate. In addition, after 18 hours treatment with FOE 5043-oxalate concentrations of 600, 1200 and 2400 µg/ml were read without S9 mix. None of these cultures treated with FOE 5043-oxalate in the absence or presence of S9 mix showed statistically significant or biologically relevant increases of numbers of metaphases with aberrations. The positive controls mitomycin C and cyclophosphamide induced clear clastogenic effects and demonstrated the sensitivity of the test system and in the case of cyclophosphamide the activity of the

	used S9 mix.

Table A 16: Summary of cells with structural aberrations

Substance Dose (µg/mL)		+/- S9	Cells scored	Metaphases with aberrations (%) Including gaps	 Excluding gaps	Mitotic Index (%)
Experiment 1 (4 hour treatment + 18 hour harvest, +/- S9)						
Solvent (DMSO)		–	200	3.5	3.5	100.0
FOE 5043-oxalate	600	–	200	3.0	2.5	97.9
	1200	–	200	3.5	3.5	105.7
	2400	–	200	3.0	3.0	94.3
Mitomycin C	0.1	–	168	38.5	37.0[a]	134.2
Solvent (DMSO)		+	200	5.0	4.0	100.0
FOE 5043-oxalate	600	+	200	4.0	3.5	106.3
	1200	+	200	3.0	2.5	127.7
	2400	+	200	4.0	3.5	110.7
Cyclophosphamide	2	+	186	75.5	75.5[a]	39.9[a]
Experiment 2 (4 hour treatment + 30 hour recovery, +/- S9)						
Solvent (DMSO)		–	200	1.5	1.5	100.0
FOE 5043-oxalate	2400	–	200	2.5	2.5	99.4
Solvent (DMSO)		+	200	3.5	3.0	100.0
FOE 5043-oxalate	2400	+	200	4.5	4.0	106.4

[a] statistical significant at $p \leq 0.01$

Table A 17: Additionally observed polyploid metaphases

Substance Concentration [µg/mL]		Harvest time [h]	Polyploid Metaphases without metabolic activation	 with metabolic activation
4 hours Treatment				
Solvent (DMSO)	0	18	12 7	13 10
FOA 5043-oxalate	600	18	9 7	13 12
FOA 5043-oxalate	1200	18	11 13	7 14
FOA 5043-oxalate	2400	18	12 12	8 15
Mitomycin C	0.1	18	7	--
			5	
Cyclophosphamide	2.0	18	--	9 10
Solvent (DMSO)	0	30	2 7	7 7
FOA 5043-oxalate	2400	30	8 15	11 5
18 hours Treatment				
Solvent (DMSO)	0	18	3 8	
FOA 5043-oxalate	600	18	8 9	
FOA 5043-oxalate	1200	18	6 7	
FOA 5043-oxalate	2400	18	5 5	
Mitomycin C	0.03	18	7 8	

Conclusion

Under the experimental conditions, FOE 5043-oxalate is considered not to be clastogenic for mammalian cells *in vitro*.

FOE 5043-sulfonic acid (M02)

A 2.11.4 Study 1277302

Comments of zRMS:	FFA metabolite FOE 5043-Sulfonic acid (M02) genotoxicity study (Wollny, H. E.; 2009) has been peer reviewed and accepted as reliable during renewal of FFA in 2018 (refer DRAR Vol. 3CA B6, point B.6.8.1 Toxicity studies of metabolites). Outcome of the study confirm that FOE 5043-sulfonic acid Na-salt is non-mutagenic in this HPRT assay.

Reference:	**KCA 5.8.1/04**
Title:	FOE 5043-Sulfonic acid Na-salt - Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT)
Report:	Wollny, H. E.; 2009; 1277302; M-361158-01-1
Authority registration No:	
Guideline(s):	OECD 476; Commission Regulation 440/2008/EC, Method B.17; US-EPA 712-C-98-221, OPPTS 870.5300
Deviations:	none
GLP/GEP:	yes
Acceptability:	Yes
Duplication (if vertebrate study):	Not aplicable

Materials and methods

Test material (Lot/Batch No.)	FOE 5043-sulfonic acid Na-salt Batch No. SES 10294-2-2	
Test system	Chinese hamster V79 cells (V79/HPRT)	
Metabolic activation	S9-mix	
Vehicle	Deionized water (FOE 5043-sulfonic acid Na-salt) DMSO (positive controls)	
Positive control	Without metabolic activation ethylmethane sulfonate (EMS) With metabolic activation: 7,12-dimethylbenz(a)anthracene (DMBA)	 0.15 mg/mL 1.1 µg/mL
Test substance Dose	Experiment I and II: 0-201.9-403.8-807.5-1615.0-3230 µg/mL (+ S9 mix) 0-101.0-201.9-403.8-604.8-807.5 µg/mL (– S9 mix) (highest applied conc. equal to approximately 10 mM)	
Treatment duration	5 hours	
Incubation time and temperature	8 days, 37°C	

Results and discussions

Table A 18: Summary of findings

Observations:	No precipitation of the test item was observed up to the maximal concentration in all experimental parts. Relevant cytotoxic effects defined as a reduction of the relative cloning efficiency I to values below 50% in both parallel cultures were noted in the first experiment without metabolic activation at 604.8 µg/mL and above. In the second experiment cytotoxic effects as described above occurred at 807.5 µg/mL. The recommended toxic range of the relative cloning efficiency of approximately 10-20% was covered without metabolic activation. No relevant cytotoxic effects were observed in the presence of metabolic activation up to the maximum concentration. No substantial and reproducible dose dependent increase of the mutation frequency was observed in both main experiments. Appropriate reference mutagens, used as positive controls, induced a distinct increase in mutant colonies and thus, showed the sensitivity of the test item and the activity of the metabolic activation system. Under the experimental conditions the test item did not induce gene mutations at the HPRT locus in V79 cells.

Table A 19: Summary of results FOE 5043-sulfonic acid Na-salt mammalian gene mutation test

	concen-tration µg/mL	S9 mix	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10^6 cells	induction factor	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/10^6 cells	induction factor
Experiment I / 5 hr treatment										
Solvent control		–	100.0	100.0	18.9	1.0	100.0	100.0	16.0	1.0
Positive control EMS	150.0	–	66.7	83.8	109.9	5.8	63.0	37.4	608.2	38.1
FOE 5043-sulfonic acid Na-salt	50.5	–		93.0	culture not continued#			90.8	culture not continued#	
	101.0	–	96.6	57.2	10.0	0.5	95.5	101.3	29.1	1.8
	201.9	–	96.1	55.9	27.2	1.4	97.1	112.4	9.5	0.6
	403.8	–	78.9	39.6	35.6	1.9	89.0	128.4	12.0	0.7
	604.8	–	33.7	53.1	13.2	0.7	36.0	120.0	13.7	0.9
	807.5	–	25.5	41.8	45.3	2.4	14.7	124.8	9.5	0.6
Solvent control		+	100.0	100.0	12.7	1.0	100.0	100.0	19.4	1.0
Positive control DMBA	1.1	+	45.3	65.9	1082.2	85.3	55.2	74.5	660.9	34.1
FOE 5043-sulfonic acid Na-salt	101.0	+		100.9	culture not continued#			94.3	culture not continued#	
	201.9	+	100.4	85.7	24.6	1.9	86.4	106.4	18.7	1.0
	403.8	+	101.2	95.1	15.8	1.2	85.7	105.5	17.5	0.9
	807.5	+	102.2	95.9	23.7	1.9	88.3	109.5	9.2	0.5
	1615.0	+	99.1	91.3	7.0	0.5	95.0	99.3	6.0	0.3
	3230.0	+	78.3	94.6	10.4	0.8	55.5	93.4	12.1	0.6
Experiment II/ 5 hr treatment										
Solvent control		--	100.0	100.0	16.7	1.0	100.0	100.0	14.2	1.0
Positive control EMS	150.0	--	70.8	89.0	171.1	10.3	61.5	81.9	139.6	9.8
FOE 5043-sulfonic acid Na-salt	50.0	--	91.5	culture not continued#			91.7	culture not continued#		
	101.0	--	84.5	78.6	17.8	1.1	93.1	105.2	14.2	1.0
	201.9	--	80.4	76.4	18.7	1.1	90.2	98.5	9.4	0.7
	403.8	--	70.3	102.1	14.4	0.9	93.7	100.1	17.0	1.2
	604.8	--	28.9	76.1	11.6	0.7	52.4	99.6	8.0	0.6
	807.5	--	18.8	87.9	7.5	0.5	21.5	103.6	20.6	1.5

	concen-tration µg/mL	**S9 mix**	**relative cloning efficiency I %**	**relative cloning efficiency II %**	**mutant colonies/10^6 cells**	**induction factor**	**relative cloning efficiency I %**	**relative cloning efficiency II %**	**mutant colonies/10^6 cells**	**induction factor**
Experiment II/ 5 hr treatment										
Solvent control		+	100.0	100.0	14.1	1.0	100.0	100.0	9.4	1.0
Positive control DMBA	1.1	+	60.8	48.7	1399.1	99.1	41.7	66.6	879.6	93.2
FOE 5043-sulfonic acid Na-salt	101.0	+	103.5	culture not continued#			105.0	culture not continued#		
	201.9	+	98.8	87.7	18.3	1.3	104.2	91.3	10.3	1.1
	403.8	+	102.8	80.7	11.5	0.8	96.8	80.7	12.9	1.4
	807.5	+	99.9	119.8	10.4	0.7	97.6	92.7	117	1.2
	1615.0	+	94.0	119.2	10.7	0.8	96.3	92.7	13.7	1.4
	3230.0	+	70.4	120.1	19.9	1.4	65.7	92.8	20.0	2.1

Culture was not continued since a minimum of only four analysable concentrations is required

Conclusion
It can be stated that under the experimental conditions reported FOE 5043-sulfonic acid Na-salt did not induce gene mutations at the HPRT locus in V79 cells. Therefore, FOE 5043-sulfonic acid Na-salt is considered to be non-mutagenic in this HPRT assay.

A 2.11.5 Study AT05870

Comments of zRMS:	FFA metabolite FOE 5043-sulfonic acid genotoxicity study (Nern, M.; 2010a) has been peer reviewed during renewal of FFA in 2018 (refer DRAR Vol. 3CA B6, point B.6.8.1 Toxicity studies of metabolite). There are some deficiencies noted in this study conducted in 2010 when compared to the TG OECD 473 rev 2016. Some limitations in toxicity measure could explain the positive of this study result, especially when considered together with the results of other studies for this data point (Chromosome alteration: *In vitro* clastogenicity). The cytotoxicity measures used may have under-estimated the degree of toxicity to the cell cultures, such that excessively toxic concentrations were evaluated for aberrations. This is substantiated by the negative results noted in the other assays for this endpoint, and the higher tier study negative bone marrow micronucleus findings reported in mice (*Nern, M.; 2010b; FOE 5043-sulfonic acid Na-salt - Project: Flufenacet (FOE 5043) - Micronucleus-test on the male mouse*, see A.2.11.6) compared to the weak positive result obtained in this assay. Overall, this study is considered reliable, with restrictions due to the deficiencies observed compared to OECD 473 (2016).

Reference:	**KCA 5.8.1/05**
Title:	FOE 5043-sulfonic acid Na-salt (Project: Flufenacet (FOE 5043)) - In vitro chromosome aberration test with Chinese hamster V79 cells
Report:	Nern, M.; 2010; AT05870; M-366380-01-1
Authority registration No:	
Guideline(s):	OECD 473; Directive 2000/32/EC, Method B.10; US-EPA 712-C-98-223, OPPTS 870.5375
Deviations:	There are some deficiencies noted in this study conducted in 2010 when compared to the TG OECD 473 rev 2016: - no continuous exposure in the absence of S9, - only 200 (instead of 300) metaphases were analysed for aberrations, - no statistical assessment of linear trend was included, - an inappropriate measure of cytotoxicity (mitotic index and % survival, measured by cell count at the end of treatment) was used, - the maximum concentration in experiment 1, without S9 did not achieve the required toxicity limit, - use of the CHO cell line combined with the use of mitotic survival index, rather than relative population doubling (RPD) or relative increase in cell count (RICC)) may be responsible for biologically irrelevant increases in aberrations
GLP/GEP:	yes
Acceptability:	Yes, with restrictions due to the deficiencies observed compared to OECD 473 (2016).
Duplication (if vertebrate study):	

Materials and methods

Test material (Lot/Batch No.)	FOE 5043-sulfonic acid Na-salt Batch No. SES 10294-2-2	
Test system	Chinese hamster V79 cells	
Metabolic activation	S9-mix	
Vehicle	DMSO (FOE 5043-oxalate) Hanks' balanced salt solution (positive control: mitomycin C, cyclophosphamide)	
Positive control	Without metabolic activation Mitomycin C (MMC) With metabolic activation: Cyclophosphamide (CP)	 0.1 µg/mL 2 µg/mL

Test substance Dose	0-200-400-600-700-800-900-1000 µg/mL (– S9 mix) 0-250-500-1000-2000-3000 µg/mL (+ S9 mix)
Treatment duration	4 hours
Harvest	18 and 30 hours
Incubation temperature	37°C
Replicates	At least 2 slides/culture

Results and discussions

Table A 20: Summary of findings

Observations:	Chinese hamster V79 cells were treated with FOE 5043-sulfonic acid Na-salt at concentrations of 200, 400 and 800 µg/ml without S9 mix for assessment of the clastogenic potential of FOE 5043-sulfonic acid Na-salt. In an independent repeat, concentrations of 600, 700 and 800 µg/ml of the test substance were used for assessment. With S9 mix concentrations of 500, 1000 and 3000 µg/ml were employed. Cultures treated with FOE 5043-sulfonic acid Na-salt in the absence of S9 mix showed statistically significant and biologically relevant increases of numbers of metaphases with aberrations, starting at a concentration of 700 µg/ml. In contrast cultures treated in the presence of S9 mix showed no statistically significant or biologically relevant increases of numbers of metaphases with aberrations. The positive controls mitomycin C and cyclophosphamide induced clear clastogenic effects and demonstrated the sensitivity of the test system and in the case of cyclophosphamide the activity of the used S9 mix.

Table A 21: Summary of cells with structural aberrations

Substance Dose (µg/mL)	+/- S9	Cells scored	Metaphases with aberrations (%) Including gaps	 Excluding gaps	Mitotic Index (%)
Experiment 1A (4 hour treatment + 18 hour harvest +/– S9)					
Solvent (water)	–	200	1.5	1.5	100.0
FOE 5043-sulfonic acid Na-salt					
200	–	200	1.5	1.5	96.9
400	–	200	2.0	1.5	109.7
800	–	200	4.5	4.5	93.3
Mitomycin C 0.1	–	168	79.0	79.0**	134.2
Solvent (water)	+	200	3.5	3.0	100.0
FOE 5043-sulfonic acid Na-salt	+				
500		200	5.5	5.0	89.3
1000	+	200	5.0	4.5	102.5
3000	+	200	3.5	3.5	135.8
Cyclophosphamide 2	+	186	69.0	68.0**	44.0**
Experiment 1B (4 hour treatment + 30 hour harvest +/– S9)					
Solvent (water)	–	200	2.0	2.0	100.0
FOE 5043-sulfonic acid Na-salt					
800	–	200	13.5	13.0**	79.8*
Solvent (water)	+	200	3.0	2.5	100.0
FOE 5043-sulfonic acid Na-salt	+				
3000	+	200	1.0	0.5	118.7
Experiment 2 (4 hour treatment + 30 hour recovery, –S9)					
Solvent (water)	–	200	1.5	1.5	100.0
FOE 5043-sulfonic acid Na-salt					
600	–	200	1.5	1.5	119.3
700	–	200	10.0	10.0**	108.4
800	–	200	12.5	12.5**	67.2*
Mitomycin C 0.1	–	200	50.0	50.0**	107.6

* statistical significant at $p < 0.05$ ** statistical significant at $p < 0.01$

Table A 22: Additionally observed polyploid metaphases – 4 hr treatment – experiment 1

without metabolic Activation			with metabolic Activation		
Concentration [µg/mL]	Harvest time [h]	Polyploid Metaphases	Concentration [µg/mL]	Harvest time [h]	Polyploid Metaphases
Control (water)	18	3 8	Control (water)	18	10 11
FOE 5043-sulfonic acid Na-salt 200	18	3 5	FOE 5043-sulfonic acid Na-salt 500	18	9 2
400	18	6 9	1000	18	5 7
800	18	5 9	3000	18	8 7
Mitomycin C 0.1	18	5 3	cyclophosphamide 2	18	7 4
Control (water)	30	5 4	Control (water)	30	4 3
FOE 5043-sulfonic acid Na-salt 800	30	9 8	FOE 5043-sulfonic acid Na-salt 3000	30	3 10

Table A 23: Additionally observed polyploid metaphases – 4 hr treatment – experiment 1

without metabolic Activation		
Concentration [µg/mL]	**Harvest time [h]**	**Polyploid Metaphases**
Control (water)	30	9 8
FOE 5043-sulfonic acid Na-salt 600	30	9 9
700	30	17 14
800	30	12 14
Mitomycin C 0.1	30	12 11

Conclusion
Under the experimental conditions, FOE 5043-sulfonic acid Na-salt is considered to be clastogenic without S9-mix in mammalian cells *in vitro*.

A 2.11.6 Study AT05913

Comments of zRMS:	FFA metabolite FOE 5043-Sulfonic acid (M02) genotoxicity study (Nern, M.; 2010b) has been peer reviewed and accepted as reliable during renewal of FFA in 2018 (refer DRAR Vol. 3CA B6, point B.6.8.1 Toxicity studies of metabolites). The use of the intraperitoneal (IP) route is considered to ensure high systemic exposure and mouse bone marrow exposure has been confirmed by quantitative whole-body autoradiography following a single intraperitoneal injection of [phenyl-UL-^{14}C]-labelled M02 (xxx 2017 see A.2.11.7). Outcome of the study confirm that there was no indication of a clastogenic effect of intraperitoneally administered FOE 5043-sulfonic acid Na-salt in the micronucleus test on the male mouse, i.e. in a somatic test system *in vivo*.

Reference:	**KCA 5.8.1/06**
Title:	FOE 5043-sulfonic acid Na-salt - Project: Flufenacet (FOE 5043) - Micronucleus-test on the male mouse
Report:	xxx; 2010; AT05913; M-368627-01-1
Authority registration No:	
Guideline(s):	OECD 474 (1997); Council Regulation 440/2008, Method B.12. (2008); US-EPA 712-C-98-226, OPPTS 870.5395 (1998)
Deviations:	~~None~~ In the revision to OECD 474 in 2016, the number of PCE required to be analysed was increased from 2000 to 4000. The purpose of this increase was to increase the statistical power such that a minimum 2-fold increase in micronucleated PCE could be detected with 80% power. However, it should be noted that the statistical power is dependent on the spontaneous, background frequency of micronuclei. However, sampling of 2000 PCEs/animal is in line with the OECD 474 (1997) that was valid at the time of study conduct The reduced number of evaluated PCEs as compared to the current OECD 474 does not affect the outcome of the study. The study is still considered to be valid.
GLP/GEP:	yes
Acceptability:	Yes
Duplication (if vertebrate study):	No

Materials and methods

Test material (Lot/Batch No.)	FOE 5043-sulfonic acid Na-salt Batch No. SES 10294-6-2
Vehicle	Deionized water physiological saline solution (positive control)
Positive control	cyclophosphamide
Species	Mouse, NMRI BR
No. of animals (group size)	5 males/group
Dose(s)	0-500-1000-2000 mg/kg bw positive control:
Application route	intraperitoneal
No of applications:	Test substance and negative control: 2 at 24 hours Positive control: 1
Application volume	20 mL/kg bw (test item, negative control); 10 mL/kg bw (positive control)
Sacrifice time	24 h after the last application
Observations	Mortality, clinical signs
Microscopic evaluation	2000 PCEs/animal
Remarks	None

Results and discussions

Table A 24: Summary of findings observed after i.p. application of FOE 5043-sulfonic acid Na-salt

Mortality:	No mortality occurred.
Clinical signs:	After two intraperitoneal administrations of 500, 1000 and 2000 mg/kg bw FOE 5043-sulfonic acid Na-salt treated males showed compound-related symptoms such as apathy, spasm and difficulty in breathing. Symptoms were recorded for up to 4 hours after the second treatment. These symptoms demonstrate relevant systemic exposure of males to FOE 5043-sulfonic acid Na-salt. Thereafter, their external appearance and physical activity remained unaffected. There was no substance-induced mortality. For the control group animals no symptoms were recorded
Microscopic	Normally, cells with micronuclei (Howell-Jolly bodies) occur in polychromatic erythrocytes with an

evaluation:	incidence of up to approximately 6.0/2000. The increase in micronucleated polychromatic erythrocytes, due, for example, to chromosome breaks or spindle disorders, is the criterion for clastogenic effects in this test model. The results with FOE 5043-sulfonic acid Na-salt gave no indications of clastogenic effects for male mice after two intraperitoneal treatments with doses of up to and including 2000 mg/kg bw. The number of micronucleated normochromatic erythrocytes did not increase relevantly in any of the groups. The known mutagen and clastogen cyclophosphamide had a clear clastogenic effect at an intraperitoneal dose of 20 mg/kg. The number of micronucleated polychromatic erythrocytes increased to a biologically relevant degree. Furthermore, the ratio of polychromatic to normochromatic erythrocytes was not altered by treatment in any of the groups.

Table A 25: Summary of results of the microscopic evaluation

Experimental groups	Number of evaluated PCE	Number of NCE per 2000 PCE	MNNCE per 2000 NCE	MNPCE per 2000 PCE
negative control	10000	2110 ± 557	4.9 ± 2.2	5.0 ± 2.6
FOE 5043-sulfonic acid Na-salt				
500 mg/kg bw	10000	1807 ± 418	4.5 ± 2.3	4.0 ± 2.3
1000 mg/kg bw	10000	1879 ± 493	4.2 ± 2.7	5.2 ± 2.8
2000 mg/kg bw	10000	1725 ± 448	4.6 ± 1.6	5.6 ± 2.1
positive control CPA 20 mg/kg	10000	1990 ± 397	5.2 ± 3.8	26.2* ± 5.1

PCE = polychromatic erythrocytes; NCE = normochromatic erythrocytes; MNNCE = micronucleated NCE;
MNPCE = micronucleated PCE
CPA = cyclophosphamide
*Statistical significant at $p < 0.01$ in non-parametric Wilcoxon ranking test

Conclusion

Under the experimental conditions there was no indication of a clastogenic effect of intraperitoneally administered FOE 5043-sulfonic acid Na-salt in the micronucleus test on the male mouse, i.e. in a somatic test system *in vivo*.

A 2.11.7 Study 8354779

Comments of zRMS:	Study (xxx 2017) regarding distribution of radioactivity in the bone marrow of mice has been peer reviewed and accepted as reliable during renewal of FFA in 2018 (refer DRAR Vol. 3CA B6, point B.6.8.1 Toxicity studies of metabolites). Based on the study results it can be concluded that radioactivity was absorbed and distributed into the bone and bone marrow. Therefore, this studies demonstrates that after oral application of flufenacet or i.p. application of FOE 5043 sulfonic acid Na-salt both substances were distributed to the bone and bone marrow. This results supports outcome of the study (Nern, M.; 2010b; FOE 5043-sulfonic acid Na-salt - Project: Flufenacet (FOE 5043) - Micronucleus-test on the male mouse.

Reference:	**KCA 5.8.1/07**
Title:	[phenyl-UL-14C]Flufenacet, [thiadiazole-5-14C]Flufenacet and [phenyl-UL-14C]BCS-AZ23374: Distribution of radioactivity in the bone marrow of mice by quantitative whole-body autoradiography
Report:	xxx 2017; EnSa-16-1016; M-580054-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council amended by Commission Regulation (EU) No 283/2013 OECD Guideline for Testing Chemicals, 417 US EPA OCSPP 870.7485 Japanese MAFF Test Guideline 12 Nousan 8147
Deviations:	not applicable
GLP/GEP:	yes
Acceptability:	Yes
Duplication (if vertebrate study):	No

Materials and methods

Test material (Lot/Batch No.)	FOE 5043-sulfonic acid Na-salt Batch No. AE 0841914-01-02
Vehicle	water
Radiolabelledt/Batch No.) (Lo test material	[phenyl-UL-^{14}C]BCS-AZ23374 Batch No KML 10223
Species	Mouse, NMRI BR
No. of animals (group size)	1 male
Dose(s)	500 mg/kg bw
Application route	intraperitoneal
No of applications:	1
Application volume	10 mL/kg bw
Sacrifice time	0.5 h after application
Observations	Mortality, clinical signs, whole body autoradiography

Results and discussions

Table A 26: Summary of findings observed after i.p. application of radiolabelled FOE 5043-sulfonic acid Na-salt

Mortality:	No mortality occurred.
Clinical signs:	No clinical signs were observed
Distribution of radioactivity in bone and bone marrow	Following intraperitoneal administration of [phenyl-UL-^{14}C]BCS-AZ23374 to a male albino mouse, radioactivity was absorbed and distributed in the bone and bone marrow at 0.5 hours. The concentrations of radioactivity were 28.3 and 90.8 µg equiv/g in bone and bone marrow, respectively. The results for all dose groups are summarised in the following table.

Table A 27: Concentrations of radioactivity in the bone and bone marrow of a male albino mouse

Test substance	Applied nominal dose (mg/kg bw)	Sampling time* (h)	Mean radioactivity concentration (µ equiv/g tissue)	
			Bone**	Bone marrow**
[phenyl-UL-^{14}C]BCS-AZ23374	500	0.5	28.3	90.8

* Sampling time based on available ADME data for the test substances
** mean of 6 bone and bone marrow regions (Humerus Right, Femur Right, Ileum/Ischium Right, Ileum/Ischium Left, Humerus Left, Femur Left)

Conclusion
Based on the study results it can be concluded that radioactivity was absorbed and distributed into the bone and bone marrow.
Therefore, this study demonstrates that after i.p. application of FOE 5043 sulfonic acid Na-salt the substance was distributed to the bone and bone marrow.

A 2.11.8 Study AT06167

Comments of zRMS:	FFA metabolite FOE 5043-Sulfonic acid (M02) genotoxicity study (Nern, M.; 2010c) has been peer reviewed and accepted as reliable during renewal of FFA in 2018 (refer DRAR Vol. 3CA B6, point B.6.8.1 Toxicity studies of metabolites; Completion of metabolite data package). Outcome of the study confirm that FOE 5043-sulfonic acid Na-salt is considered negative in the *in vivo* UDS Assay with rat liver cells.

Reference:	**KCA 5.8.1/08**
Title:	FOE 5043-sulfonic acid Na-salt (Project: Flufenacet (FOE 5043)) - Unscheduled DNA synthesis test with male rat liver cells in vivo
Report:	xxx 2010; AT06167; M-397810-01-1
Authority registration No:	
Guideline(s):	Council Regulation No. 440/2008, B.39.; OECD 486
Deviations:	none
GLP/GEP:	yes
Acceptability:	Yes
Duplication (if vertebrate study):	No

Materials and methods

Test material (Lot/Batch No.)	FOE 5043-sulfonic acid Na-salt Batch No. SES 10294-6-2
Vehicle	Deionized water, corn oil, physiological saline solution (positive control)
Positive control	2-Acetylaminofluorene (2-AAF), 1,2-Dimethlyhydrazine (DMH)
Species	Wistar rat, Crl:(WI)BR (SPF)
No. of animals (group size)	4 males/group
Dose(s)	0-1000-2000 mg/kg bw positive control: 2-AAF 100 mg/kg bw; DMH 40 mg/kg bw
Application route	Oral gavage
No of applications	1
Application volume	20 mL/kg bw (test item, negative control); 10 mL/kg bw (positive control)
Sacrifice time	Test substance groups: 4 and 16 hours 2-AA-group: 16 hours DMH-group: 4 hours
Observations	Mortality, clinical signs, cytotoxicity after cell isolation
Microscopic evaluation:	Nuclear grains, cytoplasmic grains, nuclear net grain (NNG) count in hepatocytes 3 slides and 50 cells/animal were counted
Remarks	None

Results and discussions

Table A 28: Summary of findings observed after i.p. application of FOE 5043-sulfonic acid Na-salt

Mortality:	No mortality occurred.
Clinical signs:	No clinical signs were observed in any dose group.
Cytotoxicity	No treatment related cytotoxic effects were observed. The availability of a high quality cell population for the in vitro part of the assay was demonstrated.
Microscopic evaluation:	After treatment with FOE 5043-sulfonic acid Na-salt no biologically relevant increase in nuclear labelling was induced. The positive controls (2-AAF, DMH) induced significant increases in NNG (net grain count) and in the percentage of cells ion repair and thus demonstrated the sensitivity of the test system for the detection of induced DNA-damage.

Table A 29: Mean grain values per dose group

Experimental groups	Mean NNG ± SD	Mean NG ± SD	Mean CG ± SD
Sacrifice time point 16 hours			
negative control	–0.71 ± 0.40	1.54 ± 0.93	2.25 ± 0.88
FOE 5043-sulfonic acid Na-salt			
1000 mg/kg bw	–0.55 ± 0.37	1.90 ± 1.25	2.45 ± 1.46
2000 mg/kg bw	–0.60 ± 0.25	1.49 ± 0.63	2.08 ± 0.59
positive control 2-AAF 100 mg/kg	4.15#* ± 0.73	6.69 ± 1.50	2.54 ± 0.85
Sacrifice time point 4 hours			
negative control	–0.60 ± 0.37	3.34 ± 0.42	3.94 ± 0.78
FOE 5043-sulfonic acid Na-salt			
1000 mg/kg bw	–0.67 ± 0.18	2.60 ± 0.31	3.27 ± 0.41
2000 mg/kg bw	–0.81 ± 0.10	2.74 ± 1.08	3.55 ± 1.13
positive control DMH 40 mg/kg	11.28#* ± 2.10	13.55 ± 2.52	2.27 ± 0.46

NNG =nuclear net grains ; NG = nuclear grains ; CG = cytoplasmic grains ; SD = standard deviation
*$p \leq 0.05$ # biologically relevant increase

Conclusion

Under the experimental conditions FOE 5043-sulfonic acid Na-salt is considered negative in the *in vivo* UDS assay with rat liver cells.

Appendix 3 Exposure calculations

A 3.1 Operator exposure calculations (KCP 7.2.1.1)

A 3.1.1 Calculations for Flufenacet

Table A 30: Estimation of operator exposure towards Flufenacet, Cereals, no PPE / with PPE

Substance	Flufenacet	**Formulation =** Soluble concentrates, emulsifiable concentrate, etc.	**Application rate =** 0.244 kg a.s. /ha	**Spray dilution** = 2.44 g a.s./l	**Vapour pressure =** low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Cereals, Outdoor, Downward spraying, Vehicle-mounted			**Buffer =** 2-3 m	**Number of applications =** 1 **Application interval** = 365 days
Percentage Absorption	Dermal for product = 0.083%	**Dermal for in use dilution** = 14%	**Oral =** 100%	**Inhalation =** 100%	
RVNAS[1] (AOEL)	0.017 mg/kg bw/day		RVAAS[2]	- mg/kg bw/day	

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.00765	% of RVNAS[1]	45%
	Acute systemic exposure mg/kg bw/day		-	% of RVAAS[2]	-%
Mixing and Loading		**Gloves = Yes**	Clothing = Work wear - arms, body and legs covered	PPE = None	Soluble bags = No
Application		**Gloves = Yes**	Clothing = Work wear - arms, body and legs covered	PPE = None	Closed cabin = No
Exposure (Workwear)	Longer term systemic exposure mg/kg bw/day		**0.00507**	% of RVNAS[1]	**29.8%**
	Acute systemic exposure mg/kg bw/day		-	% of RVAAS[2]	-%
Exposure (Including PPE options above)	Longer term systemic exposure mg/kg bw/day		**0.000766**	% of RVNAS[1]	**4.51%**
	Acute systemic exposure mg/kg bw/day		-	% of RVAAS[2]	-%

[1] RVNAS = Reference Value Non Acutely toxic active Substance = AOEL
[2] RVAAS = Reference Value Acutely toxic active Substance

A 3.2 Worker exposure calculations (KCP 7.2.3.1)

A 3.2.1 Calculations for Flufenacet

Table A 31: Estimation of worker exposure towards Flufenacet, Cereals, no PPE

Substance	Flufenacet	**Formulation =** Soluble concentrates, emulsifiable concentrate, etc.	**Application rate =** 0.244 kg a.s. /ha	**Spray dilution =** 2.44 g a.s./l	**Vapour pressure =** low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Cereals, Outdoor, Downward spraying, Vehicle-mounted			**Buffer =** 2-3 m	**Number of applications =** 1 **Application interval** = 365 days
Percentage Absorption	Dermal for product = 0.083%	**Dermal for in use dilution** = 14%	**Oral =** 100%	**Inhalation =** 100%	
RVNAS[1] (AOEL)	0.017 mg/kg bw/day		RVAAS[2]	- mg/kg bw/day	
DFR	3 µg a.s./cm² per kg a.s./ha		DT50	30 days	
Worker – Inspection, irrigation	Potential exposure mg/kg bw/day		0.0427	% of RVNAS[1]	251%
	Working clothing mg/kg bw/day		0.00479	% of RVNAS[1]	28.2%
	Working clothing and gloves mg/kg bw/day		-	% of RVNAS[1]	-%

[1] RVNAS = Reference Value Non Acutely toxic active Substance = AOEL
[2] RVAAS = Reference Value Acutely toxic active Substance

A 3.3 Bystander and resident exposure calculations (KCP 7.2.2.1)

A 3.3.1 Calculations for Flufenacet

Table A 32: Estimation of bystander and resident exposure towards Flufenacet, Cereals

Substance	Flufenacet	**Formulation =** Soluble concentrates, emulsifiable concentrate, etc.	**Application rate =** 0.244 kg a.s. /ha	**Spray dilution =** 2.44 g a.s./l	**Vapour pressure =** low volatile substances having a vapour pressure of <5*10-3Pa
Scenario	Cereals, Outdoor, Downward spraying, Vehicle-mounted			**Buffer =** 2-3 m	**Number of applications =** 1 **Application interval =** 365 days
Percentage Absorption	Dermal for product = 0.083%	**Dermal for in use dilution** = 14%	**Oral =** 100%	**Inhalation =** 100%	
RVNAS[1] (AOEL)	0.017 mg/kg bw/day		RVAAS[2]	- mg/kg bw/day	
DFR	3 µg a.s./cm² per kg a.s./ha		DT50	30 days	

Bystander - child	Spray drift (95th percentile) mg/kg bw/day	-	% of RVNAS[1]	-%
	Vapour (95th percentile) mg/kg bw/day	-	% of RVNAS[1]	-%
	Surface deposits (95th percentile) mg/kg bw/day	-	% of RVNAS[1]	-%
	Entry into treated crops (95th percentile) mg/kg bw/day	-	% of RVNAS[1]	-%
Bystander - adult	Spray drift (95th percentile) mg/kg bw/day	-	% of RVNAS[1]	-%
	Vapour (95th percentile) mg/kg bw/day	-	% of RVNAS[1]	-%
	Surface deposits (95th percentile) mg/kg bw/day	-	% of RVNAS[1]	-%
	Entry into treated crops (95th percentile) mg/kg bw/day	-	% of RVNAS[1]	-%

Resident - child	Spray drift (75th percentile) mg/kg bw/day	0.00922	% of RVNAS[1]	54.2%
	Vapour (75th percentile) mg/kg bw/day	0.00107	% of RVNAS[1]	6.29%
	Surface deposits (75th percentile) mg/kg bw/day	0.000696	% of RVNAS[1]	4.09%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.00577	% of RVNAS[1]	33.9%
	All pathways (mean) mg/kg bw/day	0.0113	% of RVNAS[1]	66.3%
Resident - adult	Spray drift (75th percentile) mg/kg bw/day	0.0022	% of RVNAS[1]	12.9%
	Vapour (75th percentile) mg/kg bw/day	0.00023	% of RVNAS[1]	1.35%
	Surface deposits (75th percentile) mg/kg bw/day	0.000233	% of RVNAS[1]	1.37%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.00321	% of RVNAS[1]	18.9%
	All pathways (mean) mg/kg bw/day	0.004	% of RVNAS[1]	23.5%

[1] RVNAS = Reference Value Non Acutely toxic active Substance = AOEL
[2] RVAAS = Reference Value Acutely toxic active Substance

A 3.4 Combined exposure calculations

Not relevant.

Appendix 4 Detailed evaluation of exposure and/or DFR studies relied upon (KCP 7.2, KCP 7.2.1.1, KCP 7.2.2.1, KCP 7.2.3.1)

None.