REGISTRATION REPORT

Part B

Section 6

Mammalian Toxicology

Detailed summary of the risk assessment

Product code: ADM.4651.H.1.A (former A18032E)

Product name(s): NIKITA

Chemical active substances:

Dicamba, 312.5 g/kg
Mesotrione, 150 g/kg
Nicosulfuron, 100 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Sponsor: ADAMA

Applicant: ADAMA

Submission date: June 2020

MS Finalisation date: December 2021 (initial Core Assessment)

June 2022 (final Core Assessment)

Version history

When	What
June 2020	Applicant initial dRR
December 2021	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 2022	Final report (Core Assessment updated following the commenting period). Additional information/assessments included by the zRMS in the report in response to comments recieved from the cMS and the Applicant are highlighted in yellow. Information no longer relevant ~~is struck through and shaded~~.

ADAMA use the code ADM.4651.H.1.A for the formulation but for consistency the former Syngenta code A18032E is used throughout the dRR.

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Reviewer comments:

This part of dossier summarizes data related to the toxicological studies and exposure data for the plant protection product 'NIKITA' (A18032E) and has been submitted to support registration according art. art. 33 of 1107/2009 in Poland.
The application has been done for 'NIKITA' (A18032E), a water dispersible granule (WG) product containing 312.5 g/kg dicamba, 150 g/kg mesotrione and 100 g/kg nicosulfuron for use as herbicide in maize to control annual and perennial broad leaved weeds and grass weeds. (Note: The product ownership was transferred to ADAMA from Syngenta in 2018.)

For the purposes of the current product registration, APPL provided an assessment of the toxicological potential based on *in vivo* tests. ZRMS points out that 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 classification and toxicological potential assessemt.

All exposure calculations used for estimation of operator, workers and B&R resulting from use of PPP considering all tasks according to the critical use(s), identify safe use of the product NIKITA' (A18032E).

6 Mammalian Toxicology (KCP 7)

6.1 Summary

Table 6.1-1: Information on A18032E *

Product name and code	A18032E / /NIKITA
Formulation type	Water Dispersible Granule (WG)
Active substance(s) (incl. content)	Dicamba; 312.5 g/kg Mesotrione; 150 g/kg Nicosulfuron; 100 g/kg
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 (please refer to Document B.0 Table 0.1-4)

* Information on the detailed composition of A18032E 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 A18032E according to Regulation (EC) No 1272/2008

Hazard class(es), categories:	Eye irritation Category 2; Reproductive toxicity Category 2*; STOT RE 2*
Hazard pictograms or Code(s) for hazard pictogram(s):	 GHS07  GHS08 *
Signal word:	Warning
Hazard statement(s):	H319 Causes serious eye irritation. H361d Suspected of damaging the unborn child* H373 May cause damage to organs (eyes; nervous system)*
Precautionary statement(s):	P102 Keep out of reach of children. P280 Wear protective gloves/ protective clothing/ eye protection~~/face protection.~~ P260 Do not breathe spray P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P337 + P313 If eye irritation persists: Get medical advice/ attention. P308+P313 IF exposed or concerned: Get medical advice/attention.
Additional labelling phrases:	EUH401 To avoid risks to human health and the environment, comply with the instructions for use. EUH208 Contains disodium maleate. May produce an allergic reaction

*adjusted labeling proposal reflects harmonised classification of mesotrion; Annex VI Reg. EC 1272/2008; ATP15, Refer ECHA webside https://echa.europa.eu/pl/information-on-chemicals/cl-inventory-database/-/discli/details/26466

Table 6.1-3: Summary of risk assessment for operators, workers, bystanders and residents for A18032E

	Result	PPE / Risk mitigation measures
Operators	Acceptable	None Reviewer comment: According AOEM Model (EFSA calculator) operator wearing working garment (long-sleeved shirt, long trousers “permeable”) but no protective gloves as PPE
Workers	Acceptable	None Reviewer comment: According AOEM Model (EFSA calculator) worker wearing working garment (long-sleeved shirt, long trousers “permeable”) but no protective gloves as PPE
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.

Several risk assessments of this dRR are based on the worst case GAP for C-EU (Table 6.1-4) with a higher application rate and are therefore more conservative compared to the applied GAP in Poland (B0).

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 *****	**Max. number (min. interval between applications)** **a) per use** **b) per crop/ season**	**Max. application rate kg as/ha** **a) mesotrione** **b) nicosulfuron** **c) dicamba**	**Water L/ha** **min / max**			**Operator**	**Worker**	**Bystander**	**Residents**
3,6.7	Maize (BBCH 12-19)	F	Spraying, LCTM	a) 1; b) 1	a) 0.090; b) 0.060; c) 0.1875	80-400	-	Operators, workers, residents [EFSA Guidance];	A	A	A	A

* 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

Application rate proposed in the GAP (refer point 2.6 B0 p.11) are lower than tested one in the NDE assessment:
1) Mesotrione 0.060 kg a.s./ha;
2) Nicosulfuron 0.040 kg a.s./ha;
3) Dicamba 0.125 kg a.s./ha;
Water 80-400L/ha.
Therefore, accepted NDE calculation is a worse scenario in relation to the proposed dose in the GAP.

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:

- N/A

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(s)

	Dicamba	**Mesotrione***	**Nicosulfuron**
Common Name	Dicamba	Mesotrione	Nicosulfuron
CAS-No.	1982-69-0	104206-82-8	111991-09-4
Classification and proposed labelling			
With regard to toxicological endpoints (according to the criteria in Reg. 1272/2008, as amended)	**Hazard classes (s), categories:** Acute toxicity, Category 4 Acute toxicity, Category 4 Serious eye damage, Category 1 **Code(s) for hazard pictogram(s):** GHS05, GHS07 **Signal word:** Danger **Hazard statement(s):** H302 + H332 Harmful if swallowed or if inhaled H318 Causes serious eye damage. **Precautionary statement(s):** **Prevention:** P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/spray. P280 Wear eye protection/ face protection. **Response:** P304 + P340 + P312 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell. P305 + P351 + P338 + P310 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact enses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor. P391 Collect spillage.	**Hazard classes (s), categories:** ~~n/a~~ Repr. Cat. 2 STOT RE 2 **Code(s) for hazard pictogram(s):** ~~n/a~~  GHS08 **Signal word:** ~~n/a~~ Warning **Hazard statement(s):** ~~n/a~~ **H361d** Suspected of damaging the unborn child* **H373** May cause damage to organs (eyes; nervous system)* **Precautionary statement(s):** **Prevention:** ~~None related to toxicology~~ P201 P202 P280 P260 (reflecting H373) **Response:** ~~None related to toxicology~~ P308+P313 P314 (reflecting H373) *Reviewer comment: ~~There is availabe~~ see agreed harmonised classification – Annex VI Reg. EC 1272/2008; ATP15 Refer ECHA webside	**Hazard classes (s), categories: :** n/a **Code(s) for hazard pictogram(s): :** n/a **Signal word: :** n/a **Hazard statement(s): :** n/a **Precautionary statement(s):** None related to toxicology.
Additional C&L proposal	None	~~None~~ Seveso E1	None
Agreed EU endpoints			
AOEL systemic	0.3 mg/kg bw/d (no correction for oral absorption necessary)	0.005 mg/kg bw/d (corrected for 50 % oral absorption)	0.8 mg/kg bw/d (corrected for 40 % oral absorption)
Reference	EFSA Journal 2011;9(1):1965	EFSA Journal 2016:14(3):4419	*EFSA Scientific Report* (2007) 120, 1-91, Conclusion on the peer review of nicosulfuron
Conditions to take into account/critical areas of concern with regard to toxicology			
Review Report/EFSA	Dicamba has the potential for	A genotoxic potential of	None.

	Dicamba	**Mesotrione***	**Nicosulfuron**
Conclusion for active substance	long-range transport through the atmosphere.	AMBA could not be ruled out due to positive results obtained in an *in vitro* cytogenetic assay, and no *in vivo* genotoxicity follow up testing: A detailed summary of the recently conducted rodent micronucleus assay on AMBA is included in Appendix 2 of this document. AMBA is not genotoxic in this study. Mesotrione is proposed to be classified as Repr. 2 for development by the peer review (in contrast with the harmonised classification in the CLP Regulation.). Studies on the developmental and reproductive toxicity for the groundwater metabolite MNBA are available and detailed study summaries are included in Appendix 2. MNBA in not a reproductive toxin.	

6.3 Toxicological Evaluation of Plant Protection Product

A summary of the toxicological evaluation for A18032E 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 A18032E

Type of test, species, model system (Guideline)	Result	Acceptability	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
LD_{50} oral, rat (OECD 425)	> 2000 mg/kg bw	Yes	None	xxxxxxxxxx 2013
LD_{50} dermal, rat (OECD 402)	> 2000 mg/kg bw	Yes	None	xxxxxxx, 2012
LC_{50} inhalation, rat (OECD 403)	5.05 mg/L air	Yes	None	xxxxxxxxx 2013
Skin irritation, rabbit (OECD 404)	Non-irritant	Yes	None	xxxxxxxxxx, 2012
Eye irritation, rabbit (OECD 405)	Irritant	Yes	H319	xxxxxxxx 2012
Skin sensitisation, mouse (OECD 429, LLNA)	Non-sensitising	Yes	None	xxxxxxxxx J, 2013
Supplementary studies for combinations of plant protection products	No data – not required	-		

Table 6.3-2: Additional toxicological information relevant for classification/labelling of A18032E

	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)	Dicamba ** (31.25 % (w/w))	Hazard statement(s) Acute Tox 4; H302 Acute Tox 4; H332 Eye Dam 1; H318	Reg. 1272/2008	Hazard statement(s) H319 Causes serious eye irritation. H361d Suspected of damaging the unborn child H373 May cause damage to organs (eyes; nervous system)* *ZRMS Reviewer comment: There is availabe harmonised mesotrione classification – Annex VI Reg. EC 1272/2008; ATP15 Refer ECHA webside
	Mesotrione (15 % (w/w))	Hazard statement(s) * ~~n/a~~ Repr. Cat. 2 STOT RE 2 H361d H373 *ZRMS Reviewer comment: There is availabe harmonised classification – Annex VI Reg. EC 1272/2008; ATP15 Refer ECHA webside		
	Nicosulfuron (10 % (w/w))	Hazard statement(s) n/a		
Toxicological properties of non-active substance(s) (relevant for classification of product)	Naphthalenesulfonic acid, dimethyl-, polymer with formaldehyde and methylnaphthalenesulfonic acid, sodium salt	Hazard statement(s) Skin Irrit.2; H315 Eye Irrit.2; H319		

	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)
	(CAS No 9008-63-3, 5 – 10 % (w/w))*			
	Sodium dibutylnaphthalenesulphonate (CAS No. 25417-20-3, 1 - 5 % (w/w))*	Hazard statement(s) Acute Tox.4; H302 Acute Tox.4; H332 Skin Irrit.2; H315 Eye Irrit.2; H319		
	Citric acid (CAS No. 77-92-9, < 5 % (w/w))*	Hazard statement(s) Eye Irrit.2; H319		
Further toxicological information	No data – not required			

* Please use concentration range or concentration limit (e.g. 1-10 % or > 1 %) as provided in MSDS.
** Classification in material safety data sheet (3.2) is for dicamba sodium salt

6.4 Toxicological Evaluation of Groundwater Metabolites

For dicamba, all metabolite concentrations are predicted to stay below 0.1 µg/L – no groundwater assessment is required.

For mesotrione and nicosulfuron, the following data on metabolites with the potential to reach the groundwater in concentrations above 0.1 µg/L and requiring relevance assessments 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 Mesotrione - MNBA

An overview of the results of the accepted toxicological studies for groundwater metabolite MNBA is given in the following table. Genotoxicity, systemic toxicity, ADME and HPPD inhibition studies have been considered within the EU review process for mesotrione and full study summaries are not provided. In addition to MNBA being a potential groundwater metabolite it is also a manufacturing intermediate of mesotrione and a developmental toxicity study and a two generation reproduction study in the rat were conducted as part of the registration requirements for this use. Full summaries of these studies, which have not previously been considered within an EU peer review process, are provided in Appendix 2.

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

Type of test, species (Guideline)	Result	Acceptability	Reference
Acute oral toxicity	LD_{50} >5000mg/kg	Yes	xxxxxxxxxxxxxx
28 day oral toxicity study in the rat (gavage)	NOAEL >1000mg/kg	Yes	xxxxxxxxx(1998)*
90 day dietary toxicity study in the rat	NOAEL 650ppm (50.6 mg/kg) males 3000ppm (263.7 mg/kg) females	Yes	xxxxxxxxxxx (2000)*
Bacterial Reverse Mutation	Not genotoxic	Yes	Callander (1996)*
In vitro Cytogenetics	Not genotoxic	Yes	Fox (2000)*
Unscheduled DNA Synthesis (UDS) *in vivo*	Not genotoxic	Yes	xxxxxxxx)*
Rat Bone Marrow Micronucleus test *in vivo*	Not genotoxic	Yes	xxxxxxxxxxxxxx (2000)*
Developmental toxicity study in the rat	~~NOAEL >1000mg/kg~~	Yes	xxxxxxxxxxx (2016)

Type of test, species (Guideline)	Result	Acceptability	Reference
	Maternal NOAEL 1000 mg/kg bw/d **Developmental NOAEL** 300 mg/kg bw/d		
Two generation reproduction study in the rat	~~NOAEL >1000mg/kg~~ **Maternal NOAEL:** 300 mg/kg bw/d **Offspring NOAEL:** 1000 mg/kg bw/d **Reproductive NOAEL:** 1000 mg/kg bw/d	Yes	xxxxxxxxxxx (2016)
Effects of MNBA on HPPD	MNBA does not inhibit HPPD	Yes	xxxxxxxxxxxx (1998)*
MNBA: Biotransformation in the rat	MNBA is quantitatively metabolised to AMBA	Yes	xxxxxxxxxxxx (2000)*

* indicates that a study was reviewed at EU level

6.4.2 Mesotrione - AMBA

An overview of the results of the accepted toxicological studies for groundwater metabolite AMBA is given in the following table. Genotoxicity (with the exception of the *in vivo* rat micronucleus assay), acute toxicity, ADME and HPPD inhibition studies have been considered within the EU review process for mesotrione and full study summaries are not provided. An *in vivo* rodent micronucleus assay was requested as part of the EU review process and a full study summary is provided in Appendix 2.

Table 6.4-2: Summary of the results of toxicity studies for AMBA

Type of test, species (Guideline)	Result	Acceptability	Reference
Acute oral toxicity	LD50 >5000mg/kg	Yes	xxxxxxxx(1996)*
Bacterial Reverse Mutation	Not genotoxic	Yes	Callander (1996)*
In vitro Cytogenetics	Weakly Positive –S9 at 20 hrs	Yes	Fox (2000)*
Rat Bone Marrow Micronucleus test *in vivo*	Not genotoxic	Yes	xxxxxxxxxxxx(2016)

* indicates that a study was reviewed at EU level

6.4.3 Nicosulfuron – ASDM

An overview of the results of the toxicological studies for ASDM is given below. All studies have been considered within the EU review process for nicosulfuron and full study summaries are not provided.

Table 6.4-3: Summary of the results of toxicity studies for ASDM

Type of test, species (Guideline)	Result	Acceptability	Reference
Acute oral toxicity in the rat	LD50>2000mg/kg	Yes	xxxxxxx, 1993*
Acute oral toxicity in the mouse	LD50>5000mg/kg	Yes	xxxxxxxxxx 1992*
28 day study in the rat	NOAEL 1000mg/kg bw/d	Yes	xxxxxxxxx 1993*
90 day study in the rat	NOAEL 1000mg/kg bw/d	Yes	xxxxxxxxxx 1998*
In vitro bacterial reverse mutation assay *S. typhimurium* and *E. coli*	non-genotoxic	Yes	Seki, 1988*
In vitro bacterial reverse mutation assay *S. typhimurium*	non-genotoxic	Yes	May, 1993*
In vitro chromosome aberration test Human lymphocytes	genotoxic	Yes	Dance, 1993*

Type of test, species (Guideline)	Result	Acceptability	Reference
In vitro mammalian cell gene mutation test Mouse lymphoma L5178Y cells	non-genotoxic	Yes	Wollny, 2003a*
In vivo micronucleus test Mouse	non-genotoxic	Yes	xxxxxxxxxxx 1995*
One generation reproduction study	NOAEL maternal and off-spring 1000 mg/kg	Yes	xxxxxxxxxx(1999)*
Developmental toxicity study in the rat	NOAEL maternal 1000 mg/kg NOAEL developmental 200 mg/kg	Yes	xxxxxxxxxxxxxxx (1998)*

* indicates that a study was reviewed at EU level

6.4.4 Nicosulfuron - AUSN

An overview of the results of the toxicological studies for AUSN is given below. All studies have been considered within the EU review process for nicosulfuron and full study summaries are not provided.

Table 6.4-4: Summary of the results of toxicity studies for AUSN

Type of test, species (Guideline)	Result	Acceptability	Reference*
Acute oral toxicity in the rat	LD50 >2000mg/kg	Yes	xxxxxxxx 1996a*
Bacterial Reverse Mutation Assay *S. typhimurium and E. coli*	non-genotoxic	Yes	Wollny, 1995a*
In vitro mammalian cell gene mutation test Mouse lymphoma L5178Y cells	non-genotoxic	Yes	Wollny, 2003b*
In vitro chromosome aberration test Chinese Hamster V79 cells	non-genotoxic	Yes	Schulz, 2003a*

* indicates that a study was reviewed at EU level

6.4.5 Nicosulfuron – UCSN

Table 6.4-5: Summary of the results of toxicity studies for UCSN

An overview of the results of the toxicological studies for UCSN is given below. All studies have been considered within the EU review process for nicosulfuron and full study summaries are not provided.

Type of test, species (Guideline)	Result	Acceptability	Reference*
Acute oral toxicity in the rat	LD50>2000mg/kg	Yes	xxxxxxxx 1996*
Bacterial Reverse Mutation Assay *S. typhimurium* and *E. coli*	non-genotoxic	Yes	Wollny, 1995b*
In vitro mammalian cell gene mutation test Mouse lymphoma L5178Y cells	non-genotoxic	Yes	Wollny, 2003c*
In vitro chromosome aberration test Chinese Hamster V79 cells	non-genotoxic	Yes	Schulz, 2003b*

* indicates that a study was reviewed at EU level

6.4.6 Nicosulfuron - MU-466

An overview of the results of the toxicological studies for MU-466 is given below. All studies have been considered within the EU review process for nicosulfuron and full study summaries are not provided.

Table 6.4-6: Summary of the results of toxicity studies for MU-466

Type of test, species (Guideline)	Result	Acceptability	Reference*
Acute oral toxicity in the rat	LD50>2000mg/kg	Yes	xxxxxxxx 1996*
Bacterial Reverse Mutation Assay *S. typhimurium* and *E. coli*	non-genotoxic	Yes	Wollny, 1996*
In vitro mammalian cell gene mutation test Mouse lymphoma L5178Y cells	non-genotoxic	Yes	Wollny, 2003d*
In vitro chromosome aberration test Chinese Hamster V79 cells	non-genotoxic	Yes	Schulz, 2003c*

* indicates that a study was reviewed at EU level

6.4.7 Nicosulfuron - HMUD

An overview of the results of the toxicological studies for HMUD is given below. All studies have been considered within the EU review process for nicosulfuron and full study summaries are not provided.

Table 6.4-7: Summary of the results of toxicity studies for HMUD

Type of test, species (Guideline)	Result	Acceptability	Reference*
Bacterial Reverse Mutation Assay *S. typhimurium* and *E. coli*	non-genotoxic	Yes	Matsumoto, 2004a*
In vitro mammalian cell gene mutation test Mouse lymphoma L5178Y cells	non-genotoxic	Yes	Matsumoto, 2004b*
In vitro chromosome aberration test Human lymphocytes	non-genotoxic	Yes	Matsumoto, 2004c*

* indicates that a study was reviewed at EU level

6.4.8 Nicosulfuron - ADMP

An overview of the results of the toxicological studies for ADMP is given below. All studies have been considered within the EU review process for nicosulfuron and full study summaries are not provided.

Table 6.4-8: Summary of the results of toxicity studies for ADMP

Type of test, species (Guideline)	Result	Acceptability	Reference
Bacterial Reverse Mutation	Not mutagenic	Yes	Seki (1988)*

* indicates that a study was reviewed at EU level

6.5 Dermal Absorption (KCP 7.3)

A summary of the dermal absorption rates for the active substances in A18032E are presented in the following table.

Table 6.5-1: Dermal absorption rates for active substances in A18032E

	Dicamba		Mesotrione		Nicosulfuron	
	Value	**Reference**	**Value**	**Reference**	**Value**	**Reference**
Concentrate	2%	study reported in Appendix 2	0.2%	New study reported in Appendix 2	25%	EFSA default value
Dilution (1:66)	75%	EFSA default value	0.8 % without adjuvant	New study reported in	75%	EFSA default value

	Dicamba		Mesotrione		Nicosulfuron	
	Value	**Reference**	**Value**	**Reference**	**Value**	**Reference**
			1% with adjuvant	Appendix 2		
Dilution (1:326)	75%	EFSA default value	0.7% without adjuvant 1% with adjuvant	New study reported in Appendix 2	75%	EFSA default value

6.5.1 Justification for proposed values - dicamba

Proposed dermal absorption rates for dicamba concentrate are based on a dermal absorption study conducted with the current product/formulation . The study results are summarized in the following table. A full summary of the study on the dermal absorption of dicamba concentrate in A18032E is provided in Appendix 2 and details of the derivation of the dermal absorption values used for the concentrate is given in Table 6.5-3. The dermal absorption values for the proposed dilution rates used are default values based on the EFSA guidance document.

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

Test	Concentrate	Spray dilutions EFSA default	Formulation in study	Acceptability of study	Justification provided on representativity of study formulation for current product	Acceptability of justification	Reference*
In vitro (human)	2%	75%	A18032E	Yes, endpoint can be used for current product	Not required	N/A	Blackstock 2013

Table 6.5-3: EFSA derivation of dermal absorption based on the human *in vitro* study for dicamba in A18032E

Active Ingredient	Dicamba
Dicamba 312.5 g/kg in A18032E Dose Concentration (g/L)	Concentrate
Meets EFSA criteria for exclusion of all tape strips (% absorbed in first 12h)	N 61
Meets EFSA criteria for mass balance (Recovery (%) of applied dose)	Y 98.30
Tape strips 3-20 (%) Exposed Skin (%) Receptor Fluid Chamber Wash (%) Total (%)	0.48 0.53 0.21 0.01 1.23
Meets EFSA criteria for addition of Standard Deviation (SD) (SD (%) of mean)	Y 46
SD Mean + SD	0.58 1.83
Dermal Penetration (%) to be used in risk assessment (ESFA Rounding)	**2**

6.5.2 Justification for proposed values - mesotrione

Proposed dermal absorption rates for mesotrione are based on a dermal absorption studies conducted with the current product/formulation . The study results are summarized in the following table.A full summaries of the study on the dermal absorption of mesotrione in A18032E provided in Appendix 2 and justification for the numbers used is given in Table 6.5-5.

Table 6.5-4: Summary of the results of submitted dermal absorption studies for mesotrione

Test	Concentrate	Spray dilution (1:66)	Spray dilution (1:326)	Formulation in study	Acceptability of study	Justification provided on representativity of study formulation for current product	Acceptability of justification	Reference*
In vitro (human)	0.2%	0.8 % without adjuvant 1% with adjuvant	0.7% without adjuvant 1% with adjuvant	A18032E	Yes, endpoint can be used for current product	Not required	N/A	Blackstock 2015 Kerin 2017

* indicates that a study was reviewed at EU level

Table 6.5-5: EFSA derivation of dermal absorption based on the human *in vitro* study for mesotrione in A18032E

Active Ingredient	Mesotrione				
Mesotrione 150g/kg in A18032E	Concentrate	Spray Dilution 1 1.13 g/L		Spray Dilution 2 0.23 g/L	
Dose Concentration (g/L)		+ adj.	- adj	+ adj	- adj
Meets EFSA criteria for exclusion of all tape strips (% absorbed in first 12h)	N 71	Y 79	Y 88	N 65	Y 80
Meets EFSA criteria for mass balance (Recovery (%) of applied dose)	N 94.29	Y 99.78	Y 95.45	Y 98.82	Y 97.76
Tape strips 3-20 (%)	0.05	n/a	n/a	0.37	n/a
Exposed Skin (%)	0.06	0.88	0.48	0.69	0.38
Receptor Fluid	0.01	0.04	0.15	0.04	0.12
Chamber Wash (%)	0.00	0.00	0.01	0.00	0.02
Total (%)	0.12	0.92	0.64	1.11	0.52
Meets EFSA criteria for addition of Standard Deviation (SD) (SD (%) of mean)	Y 54	Y 60	Y 27	N 22	Y 35
SD	0.07	0.55	0.17	0.24	0.18
Mean + SD	0.2	1	0.8	n/a	0.7
Dermal Penetration (%) to be used in risk assessment (ESFA Rounding)	**0.2**	**1**	**0.8**	**1**	**0.7**

6.5.3 Justification for proposed values – nicosulfuron

No data on dermal absorption for nicosulfuron in A18032E are available. Justifications for default values according to Guidance on Dermal Absorption (EFSA Journal 2012; 10(4):2665) are presented in the following table.

Table 6.5-6: Default dermal absorption data for nicosulfuron

	Value	Justification for value	Acceptability of justification
Concentrate	25%	Default value in accordance with EFSA journal 2012; 10(4):2665)	Yes
Dilution	75%	Default value in accordance with EFSA journal 2012; 10(4):2665	Yes

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	A18032E		
Formulation type	WG		
Category	Herbicide		
Container size(s), short description	250g, HDPE, 45 mm diameter screw cap closure 500g, HDPE, 63 mm diameter screw cap closure 1kg, HDPE, 63 mm diameter screw cap closure 5kg, HDPE, 85 mm diameter screw cap closure		
Active substance(s) (incl. content)	**Dicamba** 312.5 g/kg	**Mesotrione** 150 g/kg	**Nicosulfuron** 100 g/kg
AOEL systemic	0.3 mg/kg bw/d	0.005 mg/kg bw/d	0.8 mg/kg bw/d
Inhalation absorption	100%	100%	100%
Oral absorption	100%	50%	40%
Dermal absorption	Concentrate: 2% (156.25 g a.s./L) (Based on product (A18032E)) Dilution: 75% (Default)	Concentrate: 0.2% (75 g a.s./L) Dilution 1: 0.8% (1.13 g a.s./L) Dilution 2: 1% (1.13 g a.s./L + adjuvant) Dilution 3: 0.7% (0.23 g a.s./L) Dilution 4: 1% (0.23 g a.s./L + adjuvant) (Based on product (A18032E))	Concentrate: 25% Dilution: 75% (Default)

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 critical GAP has been defined following evaluation of the individual GAPs for each crop in each relevant Member State.

6.6.2 Operator exposure (KCP 7.2.1)

6.6.2.1 Estimation of operator exposure

A summary of the exposure model used for estimation of operator exposure to the active substances during application of A18032E 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.

As guidance on the derivation of acute endpoints for non-dietary human exposure has not yet been published, it is not possible to carry out an acute risk assessment for operators at this time.

Table 6.6-2: Exposure models for intended uses

Critical use	Maize (max. 0.6 kg product/ha)
Model	EFSA 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.]

Table 6.6-3: Estimated operator exposure

		Dicamba		**Mesotrione**		**Nicosulfuron**	
Model data	**Level of PPE**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL[#]**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL[#]**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL[#]**
Tractor-mounted boom spray application outdoors to low crops[&]							
Application rate: (without the use of adjuvant)		0.1875 kg a.s./ha		0.09 kg a.s./ha		0.06 kg a.s./ha	
EFSA model (75th percentile) Application volume: 80 L/ha** Body weight: 60 kg	no PPE*	0.0219	7.29	0.0012	24.91	0.0197	2.46
Tractor-mounted boom spray application outdoors to low crops[&]							
Application rate: (with the use of adjuvant)		0.1875 kg a.s./ha		0.09 kg a.s./ha		0.06 kg a.s./ha	
EFSA model (75th percentile) Application volume: 80 L/ha** Body weight: 60 kg	no PPE*	0.0219	7.29	0.0013	25.37	0.0197	2.46

[#] Reference Value Non Acutely Toxic Active Substance (RVNAS) for EFSA Guidance

[&] As a worst case, in the EFSA Guidance calculator the crop type “cereals” was chosen in order to present the corresponding operator exposure scenario.

* no PPE: Operator wearing long-sleeved shirt, long trousers (“permeable”) but no gloves

** The application rate of 0.6 kg A18032E/ha (0.09 kg mesotrione/ha; 0.06 kg nicosulfuron/ha; 0.1875 kg dicamba/ha) with the application volume of 80 L water/ha presents the worst case exposure estimation compared to increased water volume and consequently adjusted dermal absorption value.

6.6.2.2 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.

6.6.3 Worker exposure (KCP 7.2.3)

6.6.3.1 Estimation of worker exposure

Table 6.6-4 shows the exposure model used for estimation of worker exposure after entry into a previously treated area or handling a crop treated with A18032E according to the critical use. Outcome of the estimation is presented in Table 6.6-5. Detailed calculations are in Appendix 3.

Table 6.6-4: Exposure models for intended uses

Critical use	Maize (max. 1 x 0.6 kg product/ha)
Model	EFSA 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.]

Table 6.6-5: Estimated worker exposure

		Dicamba		**Mesotrione**		**Nicosulfuron**	
Model data	**Level of PPE**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL[#]**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL[#]**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL[#]**
Number of applications and application rate:		1 x 0.1875 kg a.s./ha		1 x 0.09 kg a.s./ha		1 x 0.06 kg a.s./ha	
2 hours/day (1), Body weight: 60 kg DT50: 30 days DFR: 3 µg/cm^2/kg a.s./ha Dermal absorption: 1% for dilution**	no PPE(3) TC: 1400 cm^2/person/h (2)	0.0197	6.56	0.0001	2.52	0.0063	0.79

[#] Reference Value Non Acutely Toxic Active Substance (RVNAS) for EFSA Guidance

[&] As a worst case, in the EFSA Guidance calculator the crop type "cereals" was chosen in order to present the corresponding worker exposure scenario.

** As a conservative approach, the dermal absorption value used corresponds to the higher of the values between the undiluted product and the in-use dilution. Dermal absorption of 1% corresponding to the use of an adjuvant is thus used in the worker exposure estimate.

(1) 2 h/day for professional applications for maintenance, inspection or irrigation activities etc.

(2) EFSA 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.]

(3) no PPE: Worker wearing long sleeved shirt, long trousers ("permeable") but no gloves

6.6.3.2 Refinement of generic DFR value (KCP 7.2)

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 mentioned PPE, exposure estimates using dislodgeable residue data are considered to be not necessary.

6.6.3.3 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 mentioned PPE, a study to provide measurements of worker exposure was not necessary and was therefore not performed.

6.6.4 Bystander and resident exposure (KCP 7.2.2)

6.6.4.1 Estimation of bystander and resident exposure

Table 6.6-6 shows the exposure model used for estimation of resident exposure to mesotrione, nicosulfuron and dicamba. Outcome of the estimation is presented in Table 6.6-7. Detailed calculations are in Appendix 3.

According to EC guidance document SANTE-10832-2015, the (*EFSA Guidance*) risk assessment on residents and bystanders cannot be fully considered until a procedure for the derivation of the AAOEL and higher risk assessment schemes, identified as missing by the Standing Committee, are available.

Consequently, this evaluation provides a first tier assessment based on the EFSA guidance for longer term exposures to residents only, using 75th percentile data and comparing with the relevant AOEL. This assessment is equally applicable to longer term exposures for bystanders (see Table 6.6-6).

Table 6.6-6: Exposure models for intended uses

Critical use	Maize (max. 1 x 0.6 kg product/ha)
Models	Resident: EFSA 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.]

Table 6.6-7: Estimated resident exposure (EFSA guidance)

		Dicamba		Mesotrione		Nicosulfuron	
Model data		**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL[#]**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL[#]**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL[#]**
Tractor-mounted boom spray application outdoors to low crops[&] Buffer: 2-3 m Drift reduction technology: no DT50: 30 days DFR: 3 µg/cm^2/kg a.s./ha							
Application rate:		1 x 0.1875 kg a.s./ha		1 x 0.09 kg a.s./ha		1 x 0.06 kg a.s./ha	
Vapour pressure		1.67 x 10^{-3} Pa at 25°C		<5.7 x 10^{-6} Pa at 20°C		8.1 x 10^{-10} Pa at 25°C	
Resident (child) Application volume: 80 L/ha** Dermal absorption: 1% for dilution** Body weight: 10 kg	Drift	0.0472	15.73	0.0003	6.53	0.0151	1.89
	Vapour	0.0011	0.36	0.0011	21.40	0.0011	0.13
	Deposits	0.0022	0.73	0.0000	0.99	0.0007	0.08
	Re-entry	0.0237	7.91	0.0002	3.04	0.0076	0.95
	SUM	0.0476	15.86	0.0014	28.25	0.0159	1.99
Resident (adult) Application volume: 80 L/ha** Dermal absorption: 1% for dilution** Body weight:60 kg	Drift	0.0113	3.76	0.0001	1.48	0.0036	0.45
	Vapour	0.0002	0.08	0.0002	4.60	0.0002	0.03
	Deposits	0.0010	0.32	0.0000	0.12	0.0003	0.04
	Re-entry	0.0132	4.39	0.0001	1.69	0.0042	0.53
	SUM	0.0168	5.60	0.0003	6.76	0.0055	0.69

[#] Reference Value Non Acutely Toxic Active Substance (RVNAS) for EFSA Guidance

[&] As a worst case, in the EFSA Guidance calculator the crop type "cereals" was chosen in order to present the corresponding resident exposure scenario.

** As a conservative approach, the dermal absorption value used corresponds to the higher of the values between the undiluted product and the in-use dilution. Dermal absorption of 1% corresponding to the use of an adjuvant is thus used in the worker exposure estimate.

6.6.4.2 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 mesotrione, nicosulfuron and dicamba 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.

6.6.5 Combined exposure

~~The product is a mixture of three active substances. From a scientific point of view it is regarded necessary to take into account potential combination effects. However, the evaluation of cumulative or synergistic effects as requested by Art. 4 (3b) of Regulation (EC) No. 1107/2009 should only be performed when harmonised "scientific methods accepted by the Authority to assess such effects are available."~~

6.6.6 Combined exposure assessment of the active substances (312.5 g/kg dicamba,

150 g/kg mesotrione and 100 g/kg nicosulfuron) in A18032E/NIKITA.

Reviewer comment:
ZRMS PL supports using Hazard Index as efficient approach to assess combined exposure, thus below we summarized exposure to all a.s. resulting from the application PPP A18032E/NIKITA.
At the first tier, combined exposure is calculated as the sum of the component exposures without regard to the mode of action or mechanism/target of toxicity. Initially, the individual Hazard Quotients (HQ) are calculated for all active substances in the PPP by assessing the exposure according to appropriate models and dividing the individual exposure levels by the respective systemic AOEL/RVNAS. This is equivalent to the predicted exposure as % of systemic AOEL/RVNAS to decimal. The Hazard Index (HI) is the sum of the individual HQs.

Table 6.6-2: Acute risk assessment from combined exposure

Application scenario	Active Substance	Estimated exposure / AOEL (RVNAS) (HQ)[3]
Operators, with no PPE. For details please refer to 6.6.2. Only the worst case scenario *as herbicide in maize* is presented	dicamba	0.073
	mesotrione	0.24
	nicosulfuron	0.025
	Cumulative risk Operators (HI)[2]	**0.34**
Workers For details please refer to 6.6.3. Only the worst case scenario *as herbicide in maize* is presented	dicamba	0.06
	mesotrione	0.02
	nicosulfuron	0.008
	Cumulative risk Workers (HI)[2]	**0.088**
Bystander– Adult[1] For details please refer to 6.6.5. Only the worst case scenario *as herbicide in maize* is presented	Since no AAOEL has been determined for a.s. included in the product, estimated exposure for residents cover, exposure for bystanders. Refer Refer point 4.1 table 2 p. 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.,*	
Bystander – Child[1] For details please refer to 6.6.5. Only the worst case scenario *as herbicide in maize* is presented	Since no AAOEL has been determined for a.s. included in the product, estimated exposure for residents cover, exposure for bystanders. Refer point 4.1 table 2 p. 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.,*	
Resident – Adult[1] For details please refer to 6.6.5. Only the worst case scenario *as herbicide in maize* is presented	dicamba	0.056
	mesotrione	0.06
	nicosulfuron	0.0068
	Cumulative risk Resident – Adult (HI)[2]	**0.12**
Resident – Child[1] For details please refer to 6.6.5. Only the worst case scenario *as herbicide in maize* is presented	dicamba	0.15
	mesotrione	0.28
	nicosulfuron	0.02
	Cumulative risk Resident – Child (HI)[2]	**0.45**

[1] The higher exposure value either from the 75th percentile of each of the four pathways (spray drift, vapour, surface deposits, entry into treated crops) or the sum of the mean exposure values is taken into consideration
[2] HI =Hazard Index
[3] HQ = Hazard Quotient

The Hazard Index is < 1. Thus combined exposure to all active substances in A18032E/NIKITA is not expected to present a risk for operators, workers, bystanders and residents. No further refinement of the assessment is required.

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 (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 7.1.1 / 01	xxxxxxxxxx	2013	Mesotrione/Dicamba/Nicosulfuron WG (A18032E) - Acute Oral Toxicity Study in the Rat (Up and Down Procedure) Syngenta xxxxxxxx, GLP not published Syngenta File No A18032E_10018	Y	Syngenta (ADAMA has LoA)
KCP 7.1.2 / 01	xxxxxxxxxxxxxxx	2012	Mesotrione/Dicamba/Nicosulfuron WG (A18032E) - Acute Dermal Toxicity Study in Rats Syngenta, xxxxxxxxxxxx GLP not published Syngenta File No A18032E_10006	Y	Syngenta (ADAMA has LoA)
KCP 7.1.3 / 01	xxxxxxxxxxx	2013	Mesotrione/Dicamba/Nicosulfuron WG (A18032E) - Acute Inhalation Toxicity Study (Nose-Only) in the Rat Syngenta xxxxxxxxxxxx GLP not published Syngenta File No A18032E_10019	Y	Syngenta (ADAMA has LoA)
KCP 7.1.4 / 01	xxxxxxxxxxxxx	2013	Mesotrione/Dicamba/Nicosulfuron WG (A18032E) - Primary Skin Irritation Study in Rabbits Syngenta xxxxxxxxxxxxxxxxx GLP not published Syngenta File No A18032E_10016	Y	Syngenta (ADAMA has LoA)
KCP 7.1.5 / 01	xxxxxxxxxxxxxxxx	2012	Mesotrione/Dicamba/Nicosulfuron WG (A18032E) - Acute Eye Irritation Study in Rabbits Syngenta xxxxxxxxxxxxxxxxxxxxxxxxxxxxxx GLP	Y	Syngenta (ADAMA has LoA)

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**
			not published Syngenta File No A18032E_10013		
KCP 7.1.6 / 01	xxxxxxxxxxx	2013	Mesotrione/Dicamba/Nicosulfuron WG (A18032E) - Local Lymph Node Assay in the Mouse Syngenta xxxxxxxxxxxxxxxxxxxx GLP not published Syngenta File No A18032E_10023	Y	Syngenta (ADAMA has LoA)
KCP 7.3 / 01	Blackstock C., Vinall J.	2013	Mesotrione/Nicosulfuron/Dicamba WG (A18032E) - The In Vitro Percutaneous Absorption of Radiolabelled Dicamba in Concentrate Formulation and Two In-Use Dilutions Through Human Split-Thickness Skin Syngenta Charles River Laboratories, Edinburgh, United Kingdom, 34224 GLP not published Syngenta File No A18032E_10281	N	Syngenta (ADAMA has LoA)
KCP 7.3 / 02	Blackstock C., Haldane C.	2015	Mesotrione/Nicosulfuron/Dicamba WG (A18032E) - The In Vitro Percutaneous Absorption of Radiolabelled Mesotrione in Concentrate Formulation and Two In-Use Dilutions Through Human Split-Thickness Skin Syngenta Charles River Laboratories, Edinburgh, United Kingdom, 36743 GLP not published Syngenta File No A18032E_10320	N	Syngenta (ADAMA has LoA)
KCP 7.3 / 03	Kerin T., Wilinska I.	2017	Mesotrione/Nicosulfuron/Dicamba WG (A18032E) - The In Vitro Percutaneous Absorption of Radiolabelled Mesotrione in Two In Use Dilutions Through Human Skin Syngenta Charles River Laboratories, Edinburgh, United Kingdom, 38101 GLP not published Syngenta File No A18032E_10373	N	Syngenta (ADAMA has LoA)

Mesotrione

KCA2 5.8.1 / 01	xzxxxx	2016	CA3511 - Oral (Gavage) Prenatal Developmental Toxicity Study in the Rat Syngenta xxxxxxxxxxxxxxxxx GLP not published Syngenta File No CA3511_10024	Y	Syngenta (ADAMA has LoA)
KCA2 5.8.1 / 02	xxxxxxxxxxxxx	2016	CA3511 - Oral (Gavage) Two-Generation Reproduction Toxicity Study in the Rat Syngenta xxxxxxxxxxxxxx GLP not published Syngenta File No CA3511_10030	Y	Syngenta (ADAMA has LoA)
KCA2 5.8.1 / 03	xxxxxxxxxxxxxxx	2016	AMBA - Oral (Gavage) Rat Micronucleus Test Syngenta xxxxxxxxxxxxxxxxx GLP not published Syngenta File No R044276_10010	Y	Syngenta (ADAMA has LoA)

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

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:	Accepted. No bridging necessary as all studies were performed with considered formulation

A 2.2 Acute oral toxicity (KCP 7.1.1)

Comments of zRMS:	Study has been reviewed for compliance with the current guidelines, resulting from scientific progress. There is no deviation from studies protocol. The OECD 425 procedure implements the 3R rules thus study is in line with the suggestions of point 5 of Regulation 284/2013. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

Reference:	KCP 7.1.1/01
Report	Mesotrione/Dicamba/Nicosulfuron WG (A18032E) – Acute Oral Toxicity Study in the Rat (Up and Down Procedure). xxxxxxxxxxxx 2013 12/309-001P A18032E_10018
Guideline(s):	Acute Oral Toxicity (rat): OECD Test Guideline 425 (2008): EPA OPPTS 870.1100 (2002)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	A18032E (SMU2BP001)
Species	Rat, CRL:(WI)
No. of animals (group size)	5 rats (female)
Dose(s)	2000 mg/kg bw
Exposure	Once by gavage
Vehicle/Dilution	Distilled water
Post exposure observation period	14 days
Remarks	None

Results and discussions

Table A 1: Results of acute oral toxicity study in rats of A18032E

Dose (mg/kg bw)	Toxicological results *	Duration of signs	Time of death	LD50 (mg/kg bw) (14 days)
Female rats				
2000	0/5/5	2 days	Day 14	> 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 A18032E

Mortality:	No mortality occurred.
Clinical signs:	Treatment with A18032E at the dose level of 2000 mg/kg bw caused decreased activity, vocalisation, prone position, incoordination, increased respiratory rate, limited use of hind limbs and forelimbs all animals. Additionally, hunched back was observed in 4 animals, clonic convulsion was observed in 3 animals and irritability was observed in two animals. All animals were symptom free from 48 hours after the treatment.
Body weight:	Body weight gain was considered to be normal.
Macroscopic examination:	The necropsies performed at the end of the study revealed no apparent findings

Conclusion

Under the experimental conditions, the oral LD50 of A18032E is higher than 2000 mg/kg bw in rats. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

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

Comments of zRMS:	Study has been reviewed for compliance with the current guidelines, resulting from scientific progress.There is no deviation from studies protocol, the OECD 402 procedure is still valid and acceptable. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

Reference:	KCP 7.1.2/01
Report	Mesotrione/Dicamba/Nicosulfuron WG (A18032E) - Acute Dermal Toxicity Study in Rats. xxxxxxxxxxx 2012 12/309-002P A18032E_10006
Guideline(s):	Acute Dermal Toxicity (rat) OECD 402 (1987): OPPTS 870.1200 (1998); EC 440/2008 (2008)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	A18032E (SMU2BP001)
Species	Rat, CRL:(WI) Wistar
No. of animals (group size)	10 rats (5 male & 5 female)
Dose(s)	2000 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 A18032E

Dose (mg/kg bw)	Toxicological results *	Duration of signs	Time of death	LD50 (mg/kg bw) (14 days)
Male rats				
2000	0/0/5	-	Day 14	> 2000
Female rats				
2000	0/0/5	-	Day 14	> 2000

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

Table A 4: Summary of findings of acute dermal toxicity study in rats of A18032E

Mortality:	No mortality occurred.
Clinical signs:	No clinical signs of toxicity were observed.

Body weight:	Body weight gain was considered to be normal.
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 A18032E is higher than 2000 mg/kg bw in rats. 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:	Study has been reviewed for compliance with the current guidelines, resulting from scientific progress.There is no deviation from studies protocol, the OECD 403 procedure is still valid and acceptable. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

Reference:	KCP 7.1.3/01
Report	Mesotrione/Dicamba/Nicosulfuron WG (A18032E) - Acute Inhalation Toxicity Study (Nose-Only) in the Rat. xxxxxxxxxxxx 2013 12/309-004P A18032E_10019
Guideline(s):	Acute Inhalation Toxicity Study (Nose-Only) in the Rat: OECD Test Guideline 403 (2009); EPA OPPTS 870.1300 (1998); EC 440/2008, Annex Part B, B.2 (2008)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	A18032E (SMU2BP001)
Species	Rat, CRL: (WI) Wistar rats
No. of animals (group size)	10 rats (5 male & 5 female)
Concentration(s)	5 mg/L air
Exposure	4 hours (nose only)
Vehicle/Dilution	None
Post exposure observation period	14 days
Remarks	None

Results and discussions

Table A 5: Concentration(s) and exposure conditions

Target conc. (mg/L air)	Actual conc. (mg/L air)	MMAD * (µm)	GSD ** (µm)
5.00	5.05 ± 0.10	2.48	2.85

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

Table A 6: Results of acute inhalation toxicity study in rats of A18032E

Concentration (mg/L air)	Toxicological results *	Duration of signs	Time of death	LC_{50} (mg/L air) (14 days)
Male rats				
5.05	0/5/5	Day 4	Day 14	> 5.05

Concentration (mg/L air)	Toxicological results *	Duration of signs	Time of death	LC_{50} (mg/L air) (14 days)
Female rats				
5.05	0/5/5	Day 4	Day 14	> 5.05

* 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 A18032E

Mortality:	No mortality occurred.
Clinical signs:	Wet fur and fur staining were commonly recorded on the day of exposure and/or several days after exposure. These observations were considered to be related to the restraint and exposure procedures and, in isolation, were considered not to be treatment related. Laboured and noisy respiration, hunched posture and decreased activity were noted for exposed animals on the day of exposure. In addition, weak condition was noted from the following day after exposure, however no significant clinical signs were recorded from Day 4 of the observation period.
Body weight:	Normal bodyweight gain was noted for all animals during the observation period with the exception of one male where slight bodyweight loss was recorded on the first week of the observation period.
Macroscopic examination:	Administration of A18032E was not associated with any macroscopic observations.

Conclusion

Under the experimental conditions, the inhalation LC_{50} of A18032E is greater than 5.05 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 has been reviewed for compliance with the current guidelines, resulting from scientific progress.There is no deviation from studies protocol, the OECD 404 procedure is still valid and acceptable. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

Reference:	KCP 7.1.4/01
Report	Mesotrione/Dicamba/Nicosulfuron WG (A18032E) – Primary Skin Irritation Study in Rabbits. xxxxxxxxxxxx 2013 12/309-006N A18032E_10016
Guideline(s):	Acute Skin Irritation (rabbit) OECD 404 (2002): OPPTS 870.2500 (1998); EC No 440/2008, B.4 (2008)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	A18032E (SMU2BP001)
Species	Rabbit, New Zealand White
No. of animals (group size)	3 (male)
Initial test using one animal	Yes
Exposure	0.5 g (4 hours, semi-occlusive)
Vehicle/Dilution	None
Post exposure observation period	3 days
Remarks	None

Results and discussions

Table A 8: Skin irritation of A18032E

Animal No.		Scores after treatment *				Mean scores (24-72 h)	Reversible (day)
		1 h	24 h	48 h	72 h		
2795	Erythema Oedema	1 1	0 0	0 0	0 0	0 0	0 0
2800	Erythema Oedema	1 0	1 0	0 0	0 0	0 0	0.33 0
2755	Erythema Oedema	1 0	1 0	0 0	0 0	0 0	0.33 0

* scores in the range of 0 to 4

Clinical signs:	No clinical signs of toxicity were observed.

Conclusion

Under the experimental conditions, A18032E 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 has been reviewed for compliance with the current guidelines, resulting from scientific progress.There is no deviation from studies protocol, the OECD 405 procedure is still valid and acceptable. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted.

Reference:	KCP 7.1.5/01
Report	Mesotrione/Dicamba/Nicosulfuron WG (A18032E) - Acute Eye Irritation Study in Rabbits. xxxxxxxxxxxxxxxx 2012 12/309-005N A18032E_10013
Guideline(s):	Acute Eye Irritation (rabbit) OECD 405 (2002): EPA OPPTS 870.2400 (1998): EC No 440/2008, B.5 (2008): Directive 2004/73/EC B.5 (L 152 2004 29th April)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	A18032E (SMU2BP001)
Species	Rabbit, New Zealand White
No. of animals (group size)	3 (male)
Initial test using one animal	Yes
Exposure	0.1 g (single instillation in conjunctival sac)
Irrigation (time point)	The eyes were further examined using 2% fluorescein solution at least 24 hours before treatment and then 1, 24, 48, 72 hours, 1, 2 and 3 weeks after treatment.
Vehicle/Dilution	None
Post exposure observation period	21 days
Remarks	None

Results and discussions

Table A 9: Eye irritation of A18032E

Animal No.		Scores after treatment *				Mean scores (24-72 h)	Reversible (day)
		1 h	24 h	48 h	72 h		
2605	Corneal opacity	0	0	0	0	0.00	-
	Iritis	0	0	0	0	0.00	-
	Redness conjunctivae	2	2	2	2	2.00	14
	Chemosis conjunctivae	1	1	1	1	1.00	14
2611	Corneal opacity	0	0	0	0	0.00	-
	Iritis	0	0	0	0	0.00	-
	Redness conjunctivae	2	2	2	2	2.00	21

	Chemosis conjunctivae	2	2	3	3	2.67	7
2609	Corneal opacity Iritis Redness conjunctivae Chemosis conjunctivae	1 0 2 1	1 0 2 3	1 0 2 3	1 0 2 3	1.00 0.00 2.00 3.00	21 - 21 14

* scores in the range of 0 to 4 for cornea opacity and chemosis, 0 to 3 for redness of conjunctivae and 0 to 2 for iritis

Clinical signs:	No clinical signs of toxicity were observed.

Conclusion

Under the experimental conditions, A18032E is an eye irritant. Thus, H319 classification is required according to Regulation (EC) No. 1272/2008.

A 2.7 Skin sensitisation (KCP 7.1.6)

Comments of zRMS:	Study has been evaluated and reviewed by the evaluators for compliance with the current guidelines, resulting from scientific progress. There is no deviation from studies protocol, the OECD 429 procedure is valid and acceptable. Study is in line with the suggestions of point 5 of Regulation 284/2013 and Annex VII to REACH REG (EC) No 1907/2006. Results of the study and conclusions are adequate for risk assessment and classification purpose. Study accepted. Note: *In vivo* skin sensitization studies that were carried out or initiated before 11 October 2016, and that meet the requirements set out in Article 13(3), first subparagraph, and Article 13(4) shall be considered appropriate to address this standard information requirement. (REG (EC) No 1907/2006 PE&EC. The murine local lymph node assay (LLNA) is the first-choice method for *in vivo* testing.

Reference:	KCP 7.1.6/01
Report	Mesotrione/Dicamba/Nicosulfuron WG (A18032E) - Local Lymph Node Assay in the Mouse. xxxxxxxxxxxxxxxxx 2013 12/309-037E A18032E_10023
Guideline(s):	Dermal Sensitisation Local Lymph Node Assay OECD 429 (2010):EC No 440/2008 of 30 May 2008, B.42
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	A18032E (SMU2BP001)
Species	Guinea pig, CBA/J Rj
No. of animals (group size)	Irritation/Toxicity Test: 4 female mice Test substance group: 4 per group, 3 groups female mice Vehicle control goup: 4 female mice
Range finding:	No
Exposure (concentration(s), no. of applications)	50%, 25% and 10% test substance, 3 applications
Vehicle	The vehicle for the test substance was 1% Pluronic (1 % aqueous Pluronic PE9200).
Pretreatment prior to topical application	No
Reliability check	The sensitivity and reliability of the experimental procedure is assessed in previous studies using α-Hexylcinnamaldehyde as positive control substance (25% (w/v) solution dissolved in 1% Pluronic) which is known to have moderate skin sensitization properties. A significant lympho-proliferative response was noted for HCA in some previous studies performed at the Test Facility within 6 months using the same vehicle (CiToxLAB study codes: 12/071-037E (SI: 3.2); 12/176-037E (SI: 8.1); 12/177-037E (SI: 3.3)).
Remarks	None

Results and discussions

Table A 10: Results of skin sensitisation study of A18032E

Concentration of test substance (%w/v)	Number of lymph nodes assayed	Disintegrations per minute (dpm)	dpm per lymph node	Test : control ratio (SI)
0 (1 % Pluronic)	2	38.5	19.3	N/A
	2	248.5	124.3	
	2	157.5	78.8	
	2	107.5	53.8	
50 % (w/v)	2	460.5	230.3	1.7
in 1 % Pluronic	2	208.5	104.3	
	2	60.5	30.3	
	2	190.5	95.3	
25 % (w/v)	2	139.5	69.8	0.8
in 1 % Pluronic	2	86.5	43.3	
	2	79.5	39.8	
	2	115.5	57.8	
10 % (w/v)	2	25.5	12.8	2.0
in 1 % Pluronic	2	38.5	19.3	
	2	893.5	446.8	
	2	135.5	67.8	

N/A = not applicable

Clinical signs:	Alopecia was observed in the 50 % (w/v) group on Days 3-6. Precipitate was observed on the ears of the one or more animals in the 50 % (w/v) dose group on Days 1-5 and in the 25 % (w/v) group on Day 3.

Conclusion

Under the experimental conditions, A18032E 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)

None.

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)

Comments of zRMS:	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 (EFSA Journal 2012;10(4):2665). Studies are considered to be acceptable and the dermal absorption for mesotrione and dicamba (A18032E/NIKITA) are covered by this studies.

Dicamba

Reference:	KCP 7.3/01
Report	Mesotrione/Nicosulfuron/Dicamba WG (A18032E) - The *In Vitro* Percutaneous Absorption of Radiolabelled Dicamba in Concentrate Formulation and Two In-Use Dilutions Through Human Split-Thickness Skin. Blackstock C, 2013 34224 A18032E_10281
Guideline(s):	OECD 428
Deviations:	No
GLP:	Yes
Acceptability:	Yes ~~/ No / Supplementary~~
Duplication (if vertebrate study)	Not applicable

EXECUTIVE SUMMARY

The absorption of Dicamba as a wettable granule (WG) formulation in Mesotrione/Nicosulfuron/Dicamba WG (A18032E) was measured *in vitro* through human split-thickness skin. The concentration of Dicamba in the concentrate WG formulation was 312.5 g/kg. This was mixed with physiological saline to generate a formulation concentrate paste with 156.25 g/L Dicamba. The highest concentration in-use spray dilution was *ca* 187.5 g/L Dicamba. The lowest concentration in-use spray dilution was *ca* 93.75 g/L Dicamba.

The formulation concentrate in saline paste was applied at 10 mg/cm^2 (equivalent to WG formulation at 5 mg/cm^2) and left unoccluded for an experimental period of 24 h, with an interim wash at 6 h post-application. The dosing procedure was repeated for Spray Dilutions 1 and 2 at 10 µL/cm^2.

The absorption process was followed by taking samples of the receptor fluid (tissue culture medium containing polyoxyethylene 20 oleyl ether (PEG, 6%, w/v), sodium azide (0.01%, w/v), streptomycin (0.1 mg/mL) and penicillin (100 units/mL) at recorded intervals throughout the experimental period. The distribution of Dicamba within the test system and a 24 h absorption profile were determined using liquid scintillation counting.

The mass balance for [^{14}C]-Dicamba Formulation Concentrate, 187.5 g/L Spray Dilution and 93.75 g/L Spray Dilution was 98.30%, 98.45% and 98.76% of the applied dose, respectively. All the mass balances are acceptable according to the OECD guideline criteria (100% ± 10%).

The study demonstrated that the amount of Dicamba absorbed through human split-thickness skin membranes over 24 h (following a 6 h exposure) from the formulation concentrate (156.25 g/L), and the intended in-use concentrations, 187.5 g/L and 93.75 g/L, in Mesotrione/Nicosulfuron/Dicamba WG (A18032E) were 0.22%, 0.19% and 0.23% of the applied dose, respectively, as measured in the receptor fluid and receptor chamber wash.

MATERIALS AND METHODS

Materials:

Test Material:	Dicamba Sodium Tech.
Description:	White solid
Lot/Batch number:	8108B02B1
Purity:	84.7%
Stability of test compound:	Confirmed
Radiolabelled Test Material:	[Phenyl-U-^{14}C]-SAN 837H
Batch number:	RDR-XVI-32
Radiochemical Purity:	97.1%
Specific activity:	78.2 µCi/mg
Stability of test compound:	Confirmed
Formulation:	SAN837/ZA1296/Nicosulfuron WG
Product Design Code:	A18032E
Batch (Lot) Number:	SMU2BP002
Physical Appearance:	Light brown granules
Storage Conditions:	In the dark at ambient temperature

Study Design and Methods:

In-life dates: Start: 08 April 2013 End: 30 April 2013

Diffusion cell: Diffusion of Dicamba into and across the skin to a receptor fluid was measured using glass diffusion cells in which the split-thickness skin formed a horizontal membrane and provided an application area of 3.14 cm^2.

Receptor fluid: The receptor fluid (tissue culture medium containing polyoxyethylene 20 oleyl ether (PEG, 6%, w/v), sodium azide (0.01%, w/v), streptomycin (0.1 mg/mL) and penicillin (100 units/mL)) was chosen to ensure that the Dicamba would freely partition into this from the skin membrane. Dicamba dissolved in this receptor fluid at *ca* 10-fold greater than the total applied dose would in the receptor chamber (*ca* 10 mL).

Skin preparation integrity: The integrity of the membranes was checked by measurement of the electrical resistance across the skin. Only those membranes with an acceptable resistance (>4 kΩ), thereby showing that they were intact, were used on the study.

Test substance: The doses for [^{14}C]-Dicamba were prepared to mimic the commercial formulations (156.25 g/L) and the aqueous spray dilutions (187.5 g/L and 93.75 g/L) using the technical material, [^{14}C]-labelled test item and formulation blank. The doses were prepared as close to the time of application as was practicable and were analysed to confirm their suitability for use in the study.

Application to the skin: Each application was represented by eight replicates from at least four donors at a dose of 10 mg/cm^2 (formulation concentrate) or 10 µL/cm² (spray dilutions 1 and 2) and left unoccluded for the exposure period.

Temperature: Throughout the experiment the receptor fluid was stirred and maintained at a normal skin temperature of 32 ± 1ºC by a circulating water bath.

Duration of exposure and sampling: The skin was exposed to the test preparations for 6 hours and receptor fluid samples were collected at 2, 4 and 6 hours post dose. To allow adequate characterisation of the absorption profile, receptor fluid samples were also collected at 8, 12 and 24 hours post

dose.

Terminal exposure (6 h Post Dose): After dosing the exposure was terminated at 6 h post dose. Commercial hand wash soap (*ca* 50 µL) was applied to the skin. With the soap on, the skin was gently rubbed with a tissue swab. The skin was then rinsed with *ca* 5 mL of a *ca* 2% (v/v) commercial soap solution (Simple Antibacterial Hand Wash/water). The soap solution was applied in 1 mL aliquots and each aliquot was aspirated three times with a pipette. The skin was dried with a tissue swab. The process was repeated and the skin was dried with an additional tissue swab.

The soap solution (skin wash) was pooled into a single pre-weighed vial. Duplicate aliquots (1 mL) were removed from each skin wash sample, mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting. The tissue swabs were pooled into a single vial, mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting. The tip was cut in two, mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting.

Terminal post exposure procedure (24 h Post Dose): After 18 h monitoring period, the 24 h post dose skin was washed again as described above. The sealing clamp and the donor chamber were removed. The donor chamber was transferred to a pre-weighed pot containing Elga water.

Skin was removed from the cell and placed on a piece of tissue to remove any remaining receptor fluid from the underside of the skin. The tissue swabs were placed into a pre-weighed receptor chamber pot.

The stratum corneum was removed with 20 successive tape strips. Each tape strip was placed into an individual vial containing methanol:scintillation fluid (1:10, v/v; 11 mL) and then analysed by liquid scintillation counting. Epidermis was not removed during the process. The skin under the cell flange (unexposed skin) and outside the donor chamber was cut away from the exposed skin. The exposed and unexposed skin samples were placed into separate vials containing Solvable® (3 mL). The skin samples were placed into a waterbath heated to *ca* 60°C to aid solubilisation. When fully dissolved, each sample was mixed with stannous chloride solution (0.2 mg/mL in ethanol, 150 µL) and scintillation fluid (10 mL) and analysed by liquid scintillation counting.

The donor chambers were sonicated for *ca* 10 min then removed from the pots. Duplicate aliquots (1 mL) were taken from donor wash samples. The aliquots were weighed, mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting.

The receptor chamber was rinsed with 4 aliquots (10 mL) of Elga water. The solvent was pooled as a single sample into the receptor wash pot containing the 24 h tissue swab. Duplicate aliquots (1 mL) taken from each receptor wash pot, weighed, mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting.

Analysis: All components of the test system (e.g. receptor fluid, skin, skin wash, donor chamber, tape strips) were analysed by liquid scintillation counting and the recovery determined.

Data: Results of the analysis of the samples of receptor fluid collected in the study were expressed as amounts of Dicamba in the receptor solution in terms of µg equiv./cm^2, percentage of dose absorbed and rates of absorption (µg equiv./cm^2/h). The results of the mass balance and distribution determinations are expressed in terms of amount (µg equiv./cm^2) and 'percentage of applied dose'.

Definition of absorbed test material: The absorbed (systemically available) dose is considered to be the test material detected in the receptor fluid and receptor chamber wash. Material removed from the surface of the epidermis by the washing procedure is regarded as unabsorbed. The test material recovered from the skin at the end of the exposure is also considered to be unabsorbed, although it is recognised that a proportion of this material may be absorbed beyond the duration of the exposure investigated in this study.

RESULTS AND DISCUSSION

[^{14}C]-Dicamba Concentrate Formulation (312.5 g/kg) paste in physiological saline (156.25 g/kg), in human split–thickness skin membranes

A total of 8 samples of human split-thickness skin membranes obtained from 4 different donors were dosed topically with [^{14}C]-Dicamba Concentrate Formulation (312.5 g/kg) in physiological saline (156.25 g/kg). Overall, the absorption profiles looked similar for all samples. All cells increased to 24 h post dose. The mass balance for all individual samples was within 100 ± 10%. The following results are provided as mean values (n = 8).

The mean absorption rate of Dicamba from [^{14}C]-Dicamba Concentrate Formulation through human split-thickness skin was 0.14 µg equiv./cm^2/h during the 24 h experimental period. The amount penetrated at 24 h, as measured in the receptor fluid, was 3.29 µg equiv./cm^2 (0.21% of the applied dose).

Following the skin wash at 6 h, 96.42% of the applied dose of Dicamba was washed off. At 24 h post dose, a further 0.61% was removed by the skin washing procedure and donor chamber wash. A proportion of the dose applied was recovered from the exposed skin (0.53%) and 0.01% was recovered from the receptor chamber wash. The mean total recovery was 98.30% of the applied dose.

[^{14}C]-Dicamba Spray Dilution 1 (Test Preparation 2, 187.5 g/L g/L) in human split-thickness skin membranes

A total of 8 samples of human split-thickness skin membranes obtained from 4 different donors were dosed topically with [^{14}C]-Dicamba Spray Dilution 1 (187.5 g/L). Overall, the absorption profiles looked similar for 7 of the samples, which all increased to 24 h post dose. Cell 31 was an outlier for absorbed dose and dermal delivery (mean ± 2SD), therefore, was excluded from subsequent calculations. The mass balance for all other samples was within 100 ± 10%. Therefore, the following results are provided as mean values (n = 7).

The mean absorption rate of Dicamba from Spray Dilution 1 through human split-thickness skin was 0.14 µg equiv./cm^2/h during the 24 h experimental period. The amount penetrated at 24 h, as measured in the receptor fluid, was 3.44 µg equiv./cm^2 (0.18% of the applied dose).

Following the skin wash at 6 h, 96.60% of the applied dose of Dicamba was washed off. At 24 h post dose, a further 0.62% was removed by the skin washing procedure and donor chamber wash. A proportion of the dose applied was recovered from the exposed skin (0.60%) and 0.01% was recovered from the receptor chamber wash. The mean total recovery was 98.45% of the applied dose.

[^{14}C]-Dicamba Spray Dilution 2 (Test Preparation 3, 93.75 g/L) in human split-thickness skin membranes

A total of 8 samples of human split-thickness skin membranes obtained from 4 different donors were dosed topically with [^{14}C]-Dicamba Spray Dilution 2 (93.75 g/L). The absorption profiles looked similar for 7 of the samples, which all increased to 24 h post dose. The mass balance for all individual samples was within 100 ± 10%. However, Cell 40 was rejected from the mean ± SD due to leakage. Therefore, the following results are provided as mean values (n = 7).

The mean absorption rate of Dicamba from Spray Dilution 2 through human split-thickness skin was 0.08 µg equiv./cm^2/h during the 24 h experimental period. The amount penetrated at 24 h, as measured in the receptor fluid, was 1.98 µg equiv./cm^2 (0.22% of the applied dose).

Following the skin wash at 6 h, 94.37% of the applied dose of Dicamba was washed off. At 24 h post dose, a further 2.41% was removed by the skin washing procedure and donor chamber wash. A proportion of the dose applied was recovered from the exposed skin (0.90%) and 0.01% was recovered from the receptor chamber wash. The mean total recovery was 98.76% of the applied dose.

Table A 11: Summary of Dicamba Distribution in the Test System

Test Preparation	[^{14}C]-Dicamba Formulation Concentrate	[^{14}C]-Dicamba Spray Dilution 1	[^{14}C]-Dicamba Spray Dilution 2
Target Test Item Concentration	312.5 g/kg	187.5 g/L	93.75 g/L
Actual Test Item Concentration by Radioactivity	330.99 g/kg	192.64 g/L	91.03 g/L
Target Test Item Concentration in Saline	156.25 g/kg	N/A	N/A
Actual Test Item Concentration by Radioactivity in Saline	156.48 g/kg	N/A	N/A
Application Rate of Test Preparation	10.09 mg/cm^2	10 µL/cm^2	10 µL/cm^2
Application Rate of Test Item	1578.31 µg equiv./cm^2	1926.43 µg equiv./cm^2	910.34 µg equiv./cm^2
Distribution	% Applied Dose		
Dislodgeable Dose (6 h)*	96.42	96.59	94.37
Dislodgeable Dose (24 h)*	0.61	0.62	2.42
Tape Strips 1-2	0.03	0.02	0.06
Tape Strips 3-20	0.49	0.42	0.72
Unexposed Skin	0.00	0.00	0.06
Exposed Skin	0.53	0.60	0.90
Receptor Fluid	0.21	0.18	0.22
Receptor Chamber Wash	0.01	0.01	0.01
Mass Balance	98.30	98.45	98.76
Distribution	µg equiv./cm^2		
Dislodgeable Dose (6 h)*	1521.75	1860.94	859.13
Dislodgeable Dose (24 h)*	9.58	11.97	21.99
Tape Strips 1-2	0.45	0.44	0.54
Tape Strips 3-20	7.68	8.01	6.56
Unexposed Skin	0.06	0.04	0.52
Exposed Skin	8.43	11.64	8.20
Receptor Fluid	3.29	3.44	1.98
Receptor Chamber Wash	0.20	0.14	0.13
Mass Balance	1551.42	1896.62	899.5

N/A = Not Applicable
*Dislodgeable dose = Skin wash + Tissue swab + Pipette tip + Donor Wash
Absorbed dose = cumulative receptor fluid + receptor wash
Mass balance = unabsorbed dose + dermal delivery

Table A 12: Summary of Dicamba Absorption through Human Split-Thickness Membranes

Application of Test Materials and Actual Concentration of Dose Preparation	**Mean Absorption Rates**	
	Time Period (h)	**Absorption rate (µg equiv./cm^2/h ± SEM)**
Formulation Concentrate	0-2	0.05 ± 0.02
(312.5 g/kg Dicamba)	2-6	0.15 ± 0.04
10 mg/cm^2 (1578.31 µg equiv./cm^2)	6-24	0.14 ± 0.06
Duration of experiment: 24 h, n = 8	0-24	0.14 ± 0.05
Spray Dilution 1	0-2	0.03 ± 0.02
(187.5 g/L Dicamba)	2-6	0.12 ± 0.03
10 µL/cm^2 (1926.43 µg equiv./cm^2)	6-24	0.16 ± 0.05
Duration of experiment: 24 h, n = 7	0-24	0.14 ± 0.04
Spray Dilution 2	0-2	0.06 ± 0.02
(93.75 g/L Dicamba)	2-6	0.14 ± 0.03
10 µL/cm^2 (910.34 µg equiv./cm^2)	6-24	0.07 ± 0.03
Duration of experiment: 24 h, n = 7	0-24	0.08 ± 0.02

CONCLUSION

The study demonstrated that the amount of Dicamba absorbed through human split-thickness skin membranes over 24 h (following a 6 h exposure) from the formulation concentrate (156.25 g/kg), and the intended in-use concentrations, 187.5 g/L and 93.75 g/L, in Mesotrione/Nicosulfuron/Dicamba WG (A18032E) were 0.22%, 0.19% and 0.23% of the applied dose, respectively, as measured in the receptor fluid and receptor chamber wash.

(Blackstock C, 2013)

Mesotrione:

Reference:	KCP 7.3/02
Report	Mesotrione/Nicosulfuron/Dicamba WG (A18032E) - The *In Vitro* Percutaneous Absorption of Radiolabelled Mesotrione in Concentrate Formulation and Two In-Use Dilutions Through Human Split-Thickness Skin. Blackstock C, 2015 36743 A18032E_10320
Guideline(s):	OECD 428
Deviations:	No
GLP:	Yes
Acceptability:	Yes ~~/ No / Supplementary~~
Duplication (if vertebrate study)	Not applicable

EXECUTIVE SUMMARY

The rate and extent of absorption of mesotrione following topical application as a wettable granule (WG) formulation (A18032E) through human split-thickness skin was measured *in vitro*. The concentration of mesotrione in the undiluted WG formulation was *ca* 150 g/kg. The formulation concentrate was mixed with physiological saline (*ca* 1:1, w/w) to generate a paste. A paste was generated as wettable granules cannot be applied accurately to the skin. This is equivalent to an operator becoming exposed to the wettable granule, which in turn mixes with sweat. Therefore, the concentration of mesotrione in the concentrate WG formulation in saline was *ca* 75 g/kg. The highest concentration in-use spray dilution was *ca* 1.13 g/L. The lowest concentration in-use spray dilution was *ca* 0.23 g/L.

The formulation concentrate in saline was applied at *ca* 10 mg/cm^2 and left unoccluded for an experimental period of 24 h, with an interim wash at 6 h post-application. The spray dilutions were applied at 10 µL/cm^2 and treated in the same manner as the formulation concentrate in saline.

The absorption process was followed by taking samples of the receptor fluid (phosphate buffered saline containing polyoxyethylene 20 oleyl ether (PEG, *ca* 6%, w/v), sodium azide (*ca* 0.01%, w/v), streptomycin (0.1 mg/mL) and penicillin G (100 units/mL)) at recorded intervals throughout the experimental period.

The distribution of mesotrione within the test system and a 24 h absorption profile were determined using liquid scintillation counting. Before conducting the main study, stability and solubility assessments were carried out.

The study demonstrated that the amount of mesotrione absorbed through human split-thickness skin membranes over 24 h (following a 6 h exposure) from the formulation concentrate in saline (*ca* 75 g/kg) and the intended in-use concentrations, *ca* 1.13 g/L and *ca* 0.23 g/L, was 0.01%, 0.10% and 0.14% of the applied dose, respectively, as measured in the receptor fluid and receptor chamber wash.

MATERIALS AND METHODS

Materials:

Test Material:	Mesotrione Technical
Description:	Brown solid
Batch Number:	SMO0H028
Purity:	82.3%
Storage Conditions:	<30°C
Radiolabelled Test Material:	[cyclohexanedione-2-^{14}C]-ZA01296 [cyclohexanedione-2-^{14}C]-CSAA587961 [^{14}C]-Mesotrione
Batch Number:	RDR-XXIII-28
Radiochemical Purity:	97.8%
Specific Activity:	78.2 µCi/mg
Stability of Test Compound:	Confirmed
Storage Conditions:	-80°C, protected from light
Commercial Formulation:	Mesotrione/Nicosulfuron/Dicamba WG (A18032E)
Design Code:	A18032E
Batch Number:	SMU4AP005
Physical Appearance:	Solid
Storage Conditions:	<20°C
Blank Formulation Excipient:	Premix for A18032E
Batch Number:	PHA001-051-001
Physical Appearance:	Solid
Storage Conditions:	<20ºC
Blank Formulation Excipient:	CA116V
Batch Number:	LWM1060101
Physical Appearance:	Solid
Storage Conditions:	<20ºC

Study Design and Methods:

In-life dates: Start: 01 June 2015 End: 14 July 2015

Diffusion cell: Diffusion of [^{14}C]-Mesotrione into and across the skin to receptor fluid was measured using glass diffusion cells in which the split-thickness skin formed a horizontal membrane and provided an application area of 0.64 cm^2.

Receptor fluid: The receptor fluid chosen was: phosphate buffered saline containing polyoxyethylene 20 oleyl ether (PEG, *ca* 6%, w/v), sodium azide (*ca* 0.01%, w/v), streptomycin (0.1 mg/mL) and penicillin (100 units/mL). The pH was determined to be 7.33. It was degassed and stored in a

refrigerator set to maintain a temperature of +4ºC prior to use on the study.

Skin preparation integrity: The integrity of the membranes was checked by measurement of the electrical resistance across the skin. Only those membranes with an acceptable resistance (>10.9 kΩ), thereby showing that they were intact, were used on the study.

Test substance: The doses for [^{14}C]-Mesotrione were prepared to mimic the commercial formulation and their aqueous spray dilutions using the technical material, [^{14}C]-labelled test item, blank formulation excipients and CIPAC D water.

Application to the skin: [^{14}C]-Mesotrione Formulation Concentrate in Saline, [^{14}C]-Mesotrione Spray Dilution 1 or [^{14}C]-Mesotrione Spray Dilution 2 were each applied over the exposed stratum corneum area of 8 split-thickness skin samples each, using an MR25 Rainin positive displacement pipette set to deliver *ca* 6.4 mg or *ca* 6.4 µL (10 mg/cm^2 for [^{14}C]-Mesotrione Formulation Concentrate in Saline, or 10 µL/cm^2 for [^{14}C]-Mesotrione Spray Dilution 1 and [^{14}C]-Mesotrione Spray Dilution 2). Following application of the test preparation, the test system remained unoccluded for an experimental period of 24 h, with a wash at 6 h and 24h post-application.

Temperature: Throughout the experiment the receptor fluid was stirred and maintained at a normal skin temperature of 32 ± 1ºC by a circulating water bath.

Duration of exposure and sampling: The skin was exposed to the test preparations for 6 hours and receptor fluid samples were collected at 2, 4 and 6 hours post dose. To allow adequate characterisation of the absorption profile, receptor fluid samples were also collected at 8 hours and 12 hours post dose.

Terminal exposure (6 h Post Dose): At 6 h post dose, the exposure was terminated. Commercial hand wash soap (50 µL) was applied to the skin and gently rubbed in with a tissue swab. The skin was then rinsed with *ca* 5 mL of a *ca* 2% (v/v) commercial soap solution (Simple Antibacterial Hand wash/ultra pure water). The soap solution was applied in aliquots (0.5 mL), and each aliquot was added and removed with a pipette. The skin was dried with a tissue swab. The process was repeated and the skin was dried with an additional tissue swab.

The soap solution (skin wash) was pooled into a single pre-weighed vial for each cell. Samples were weighed, and duplicate weighed aliquots (1 mL) were taken from all skin wash samples, mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting. The tissue swab and tip was retained separately, mixed with methanol:scintillation fluid (1:5, v/v; 12 mL) and analysed by liquid scintillation counting.

Terminal post exposure procedure (24 h Post Dose): After an 18 h monitoring period, *i.e.* at 24 h post dose, the skin was washed and all samples analysed as described above. The donor chambers were transferred to a pre-weighed pot containing acetonitrile. Donor wash pots were left to extract for at least 30 min, sonicated for 10 min and then the apparatus was removed for washing. Duplicate weighed aliquots (1 mL) were taken from each donor wash pot, mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting. The skin was removed from each cell and placed on a piece of tissue to remove any remaining receptor fluid from the underside of the skin. This tissue was placed into the receptor chamber wash pot for that particular cell.

The stratum corneum was removed with 20 successive tape strips. The skin sample was rotated 90° after each tape strip unless any epidermis was removed. If epidermis was removed, rotation was stopped and details of epidermis removal documented. Each tape strip was placed into an individual vial containing methanol:scintillation fluid (1:5, v/v; 12 mL) and then analysed by liquid scintillation counting. The skin under the cell flange (unexposed skin) was cut away from the exposed skin. The exposed and unexposed skin samples were placed into separate vials containing Solvable® (1 mL). The skin samples were placed into a waterbath set to 60°C to aid solubilisation. Additional Solvable® was added to the unexposed skin samples to aid solubilisation. When fully dissolved, stannous chloride solution (0.2 g/mL in ethanol; 150 µL) and scintillation fluid (10 mL) was added to the skin samples and analysed by liquid scintillation counting.

Bulk receptor fluid was removed from each receptor chamber and retained in a vial. This was then split into two vials. Scintillation fluid (10 mL) was added to the new vial and the original bulk receptor fluid vial. All receptor fluid samples were then analysed by liquid scintillation counting.

The receptor chambers were rinsed with acetonitrile (20 mL). The solvent was pooled as a single sample into a pre-weighed receptor wash pot. Duplicate weighed aliquots (1 mL) of the solvent were mixed with scintillation fluid (10 mL) and analysed by liquid scintillation counting.

Analysis: All components of the test system (e.g. receptor fluid, skin wash, donor chamber, tape strips) were analysed by liquid scintillation counting and the recovery determined.

Data: Results of the analysis of the samples of receptor fluid collected in the study were expressed as amounts of [^{14}C]-Mesotrione in the receptor solution in terms of µg equiv./cm^2 or ng equiv./cm^2, 'percentage of dose absorbed' and rates of absorption (µg/ equiv./cm^2/h or ng equiv./cm^2/h). The results of the mass balance and distribution determinations are expressed in terms of amount (µg equiv./cm^2 or ng equiv./cm^2) and 'percentage of applied dose'.

Definition of absorbed test material: The absorbed (systemically available) dose is considered to be the test material detected in the receptor fluid and receptor chamber wash. Material removed from the surface of the skin by the washing procedure is regarded as unabsorbed. The test material recovered from the skin at the end of the exposure is also considered to be unabsorbed, although it is recognised that a proportion of this material may be absorbed beyond the duration of the exposure investigated in this study.

RESULTS AND DISCUSSION

[^{14}C]-Mesotrione Formulation Concentrate in Saline (*ca* 75 g/kg) in human split-thickness skin membranes

A total of 8 samples of human split-thickness skin membranes obtained from 4 different donors were dosed topically with [^{14}C]-Mesotrione Formulation Concentrate in Saline (*ca* 75 g/kg). Overall, the absorption profiles looked similar for all samples. The mass balance for all individual samples was within 100 ± 10%. The following results are provided as mean values (n = 8).

The mean absorption rate of [^{14}C]-Mesotrione from the Formulation Concentrate in Saline through human split-thickness skin was 0.003 µg equiv./cm^2/h during the 24 h experimental period. The amount penetrated at 24 h, as measured in the receptor fluid, was 0.08 µg equiv./cm^2 (0.01% of the applied dose).

Following the skin wash at 6 h, 93.50% of the applied dose of [^{14}C]-Mesotrione was washed off. At 24 h post dose, a further 0.47% was removed during the wash. A proportion of the dose applied was recovered from the donor chamber (0.16%), exposed skin (0.06%) and receptor chamber wash (<0.01%). The mean total recovery was 94.29% of the applied dose.

The total recovery, with the exception of Cell 2, Cell 4 and Cell 6, was below 95%. Therefore, the data for all of the samples in the data set were normalised to 100%. This was based on Section 5.2 of Scientific Opinion on Dermal Absorption (EFSA Journal, 2012, 10(4): 2665). Only the original data has been reported in the text.

[14C]-Mesotrione Spray Dilution 1 (ca 1.13 g/L) in human split-thickness skin membranes

A total of 8 samples of human split-thickness skin membranes obtained from 4 different donors were dosed topically with [^{14}C]-Mesotrione Spray Dilution 1 (*ca* 1.13 g/L). Overall, the absorption profiles looked similar for all samples, with the exception of Cell 13 (Donor 0572). This cell was excluded as a statistical outlier based on its receptor fluid profile (mean + 2 x SD). This is further supported by comparison of this cell versus all other 7 cells; including its donor partner Cell 14 (Donor 0572). The mass balance for all individual samples was within 100 ± 10%. The following results are provided as mean values (n = 7).

The mean absorption rate of [^{14}C]-Mesotrione from Spray Dilution 1 through human split-thickness skin was 0.47 ng equiv./cm^2/h during the 24 h experimental period. The amount penetrated at 24 h, as measured in the receptor fluid, was 11.37 ng equiv./cm^2 (0.09% of the applied dose).

Following the skin wash at 6 h, 92.93% of the applied dose of [^{14}C]-Mesotrione was washed off. At 24 h post dose, a further 1.11% was removed with the wash. A proportion of the dose applied was recovered from the donor chamber (0.08%), exposed skin (0.52%) and receptor chamber wash (0.01%). The mean total recovery was 95.41% of the applied dose.

[^{14}C]-Mesotrione Spray Dilution 2 (*ca* 0.23 g/L) in human split-thickness skin membranes

A total of 8 samples of human split-thickness skin membranes obtained from 4 different donors were dosed topically with [^{14}C]-Mesotrione Spray Dilution 2 (*ca* 0.23 g/L). Overall, the absorption profiles looked similar for all samples. The mass balance for all individual samples was within 100 ± 10%. The following results are provided as mean values (n = 8).

The mean absorption rate of [^{14}C]-Mesotrione from Spray Dilution 2 through human split-thickness skin was 0.13 ng equiv./cm^2/h during the 24 h experimental period. The amount penetrated at 24 h, as measured in the receptor fluid, was 3.02 ng equiv./cm^2 (0.12% of the applied dose).

Following the skin wash at 6 h, 95.35% of the applied dose of [^{14}C]-Mesotrione was washed off. At 24 h post dose, a further 1.10% was removed with the wash. A proportion of the dose applied was recovered from the donor chamber (0.15%), exposed skin (0.38%) and receptor chamber wash (0.02%). The mean total recovery was 97.76% of the applied dose.

Table A 13: Summary of Mesotrione Distribution Through Human Split Thickness Membranes

Test Preparation	[^{14}C]-Mesotrione Formulation Concentrate in Saline (75 g/kg)	[^{14}C]-Mesotrione Spray Dilution 1 (1.13 g/L)	[^{14}C]-Mesotrione Spray Dilution 2 (0.23 g/L)
Test Item	mesotrione		
Distribution	% Applied Dose	% Applied Dose	% Applied Dose
Dislodgeable Dose 6 h*	93.50	92.93	95.35
Total Dislodgeable Dose**	94.14	94.12	96.60
Donor Chamber Wash	0.16	0.08	0.15
Tape Strips 1-2	0.03	0.09	0.09
Tape Strips 3-20	0.05	0.57	0.53
Unexposed Skin	0.01	0.01	0.02
Exposed Skin	0.06	0.52	0.38
Receptor Fluid	0.01	0.09	0.12
Receptor Chamber Wash	<0.01	0.01	0.02
Mass Balance	94.29	95.41	97.76
Distribution	µg equiv./cm^2	ng equiv./cm^2	ng equiv./cm^2
Dislodgeable Dose 6 h*	718	11149	2352
Total Dislodgeable Dose**	722	11292	2383
Donor Chamber Wash	1.21	9.33	3.59
Tape Strips 1-2	0.22	10.51	2.26
Tape Strips 3-20	0.36	68.18	13.01
Unexposed Skin	0.05	1.72	0.41
Exposed Skin	0.47	61.86	9.45
Receptor Fluid	0.08	11.37	3.02
Receptor Chamber Wash	0.03	0.83	0.42
Mass Balance	724	11447	2412

* Dislodgeable Dose 6 h = Skin Wash 6 h + Tissue Swab 6 h + Pipette Tip 6 h

** Total Dislodgeable Dose = Dislodgeable Dose 6 h + Skin Wash 24 h + Tissue Swab 24 h + Pipette Tip 24 h + Donor Chamber Wash

Table A 14: Summary of Mesotrione Absorption through Human Split-Thickness Membranes

Application of Test Materials and Actual Concentration of Dose Preparation	Mean Absorption Rates	
	Time Period (h)	Absorption Rate
[^{14}C]-Mesotrione Formulation Concentrate in Saline (76.9 g/kg mesotrione) 9.991 mg/cm^2 (767.90 µg ai/cm^2) Unoccluded Duration of experiment: 24 h, n = 8	 0-2 2-6 6-24 0-24	µg equiv./cm^2/h ± SEM 0.015 ± 0.005 0.005 ± 0.002 0.002 ± 0.001 0.003 ± 0.000
[^{14}C]-Mesotrione Spray Dilution 1 (1.19 g/L mesotrione) 10.05 µL/cm^2 (12.0 µg ai/cm^2) Unoccluded Duration of experiment: 24 h, n = 7	 0-2 2-6 6-24 0-24	ng equiv./cm^2/h ± SEM 1.06 ± 0.42 0.75 ± 0.15 0.35 ± 0.08 0.47 ± 0.11
[^{14}C]-Mesotrione Spray Dilution 2 (0.25 g/L mesotrione) 10.05 µL/cm^2 (2.47 µg ai/cm^2) Unoccluded Duration of experiment: 24 h, n = 8	 0-2 2-6 6-24 0-24	ng equiv./cm^2/h ± SEM 0.60 ± 0.14 0.15 ± 0.06 0.08 ± 0.02 0.13 ± 0.03

CONCLUSION

The study demonstrated that the amount of mesotrione absorbed through human split-thickness skin membranes over 24 h (following a 6 h exposure) from the formulation concentrate in saline (*ca* 75 g/kg) and the intended in-use concentrations, *ca* 1.13 g/L and *ca* 0.23 g/L, was 0.01%, 0.10% and 0.14% of the applied dose, respectively, as measured in the receptor fluid and receptor chamber wash.

(Blackstock, C, 2015)

Mesotrione – In use dilutions with adjuvant included:

Reference:	KCP 7.3/03
Report	Mesotrione/Nicosulfuron/Dicamba WG (A18032E) - The *In Vitro* Percutaneous Absorption of Radiolabelled Dicamba in Concentrate Formulation and Two In-Use Dilutions Through Human Split-Thickness Skin. Kerin T, 2017 38101 A18032E_10373
Guideline(s):	OECD 428
Deviations:	No
GLP:	Yes
Acceptability:	Yes ~~/ No / Supplementary~~
Duplication (if vertebrate study)	Not applicable

This study is a repeat of the study reported in Charles River Report number 36743 using dilutions of the test material which contain the adjuvant A12127R.

Executive Summary

The rate and extent of absorption of Mesotrione following topical application as a water dispersible

granule (WG) formulation (A18032E) was measured *in vitro* through human split-thickness skin. The in-use dilutions were produced by mixing the Mesotrione stock solution with blank formulation, water and A12127R to generate final test item concentrations of *ca* 1.109 g/L and *ca* 0.229 g/L. The doses were applied at 10 µL/cm2 and left unoccluded for an experimental period of 24 h, with an interim wash at 6 h post-application.

The mean absorption rate of [14C]-Mesotrione from Spray Dilution 1 through human split-thickness skin was 0.19 ng equiv./cm2/h during the 24 h experimental period. The amount penetrated at 24 h, as measured in the receptor fluid, was 4.58 ng equiv./cm2 (0.04% of the applied dose).

Following the wash at 6 h, 97.54% of the applied dose of [14C]-Mesotrione was washed off. At 24 h post dose, a further 0.96% was removed with the wash. A proportion of the dose applied was recovered from the donor chamber wash (0.03%), exposed skin (0.88%) and receptor chamber wash (<0.01%). The mean total recovery was 99.78% of the applied dose. The mean absorption rate of [14C]-Mesotrione from Spray Dilution 2 through human split-thickness skin was 0.04 ng equiv./cm2/h during the 24 h experimental period. The amount penetrated at 24 h, as measured in the receptor fluid, was 1.03 ng equiv./cm2 (0.04% of the applied dose).

Following the wash at 6 h, 96.52% of the applied dose of [14C]-Mesotrione was washed off. At 24 h post dose, a further 1.07% was removed with the wash. A proportion of the dose applied was recovered from the donor chamber wash (0.04%), exposed skin (0.69%) and receptor chamber wash (<0.01%). The mean total recovery was 98.82% of the applied dose. The study demonstrated that the amount of [14C]-Mesotrione absorbed through human split-thickness skin membranes over 24 h (following a 6 h exposure) from the in-use dilutions (1.109 g/L and 0.229 g/L) was 0.05% and 0.05% of the applied dose, respectively, as measured in the receptor fluid and receptor chamber wash.

Materials and methods

Test material	Name (Lot/Batch No.)	[cyclohexanedione-2-14C]-ZA01296 (NP-I-12)
	Test preparation	Radioformulation
	Specific activity	104.3 µCi/mg
	Radiochemical purity	97.8%
	Name (Lot/Batch No.)	Mesotrione Technical (SMO0H028)
	Company code	ZA1296B
Product	Name (Lot/Batch No.)	A18032E (SAN837/ZA1296/nicosulfuron WG (31.25/15/10)) (SMU4AP005)
	Company code	A18032E
	Concentration a.s.	150 [g/kg]
	Formulation type	Water dispersible granule (WG) formulation
Blank product	Name (Lot/Batch No.)	A18032E (SAN837/ZA1296/nicosulfuron WG (31.25/15/10)) (PHA001-051-002)
	Concentration a.s.	0 [g/kg]
	Name (Lot/Batch No.)	CA116V (LWM1060101)
	Name (Lot/Batch No.)	A12127R (UNI6A87543)
	Name (Lot/Batch No.)	CIPAC D water (SMU2BP003)

Test system		
Diffusion cell	Cell type	Static
	(if dynamic) Flow rate	N/A
	Exposed skin area	0.64 cm²
	Cover	Unoccluded
Membrane	Skin type	Dermatomed
	Skin thickness range	390-400 µm
	Skin donors age	19-63
	Skin donors sex	F
	Location	abdomen / breast
	Source	ex vivo
	Integrity test	Electrical resistance

Receptor	Receptor medium	Phosphate buffered saline containing polyoxyethylene 20 oleyl ether (PEG, *ca* 6%, w/v), sodium azide (*ca* 0.01%, w/v), streptomycin (*ca* 0.1 mg/mL) and penicillin (*ca* 100 units/mL) at pH 7.43.
	Solubility in receptor medium	Yes
Sample Time	Exposure time	6 h
	Observation time	24 h
Sampling	Sample intervals	Receptor fluid samples were collected at 0, 2, 4, 6, 8, 12 and 24 h.
Washing		Post exposure and post observation
Final Procedure	Tape stripping	Yes
	TS1-2 analysed separately	Yes
Remarks:		

Tested doses	Spray dilution 1	Spray dilution 2
Target concentration [mg/ml]	1.109	0.229
Area dose [µL/cm²]	10	10
Total dose [µL/cell]	6.4	6.4
Specific activity [µCi/ml]	104.3	104.3
No. of donors	4	4
No of cells used/valid cells*	8/8	8/8

Results and discussions

Table A 15: In-vitro dermal penetration of active substance 1 formulated as product code/name through human skin - Recovery data

Dose group		Mid dose (Spray dilution 1)		Low dose (Spray dilution 2)	
Target concentration	[mg/mL]	1.109		0.229	
Target dose	[µL/cm²]	10		10	
Mean actual applied dose	[µL/cm²]	10		10	
		Recovery [%]		Recovery [%]	
		Mean	S.D.	Mean	S.D.
Dislodgeable dose		98.54	0.86	97.63	1.24
e.g. Skin washing after 6 h		97.54	0.92	96.52	1.02
e.g. Skin washing after 24 h		0.96	0.41	1.07	0.52
Donor chamber wash		0.03	0.03	0.04	0.03
Dose associated to skin		N/A	N/A	N/A	N/A
Tape strips: 1 – 2		0.05	0.03	0.08	0.06
Tape strips: 3 - 20		0.26	0.14	0.37	0.22
Exposed skin		0.88	0.53	0.69	0.14
Absorbed dose		0.05	0.03	0.05	0.03
Receptor fluid		0.04	0.03	0.04	0.03
Receptor chamber wash		0.00	0.00	0.00	0.00
Total recovery[1]		99.78	0.98	98.82	1.29
Absorption essentially complete at end of study (>75% absorption within half the study duration) [%Absorption at $t_{0.5}$]		Yes [79.48% absorbed at 12 h]		No [65.05% absorbed at 12h]	
If no: Absorption estimates = absorbed dose + skin preparation + tape strips 3-20)[2]		N/A	N/A	1.11	0.24
If yes: Absorption estimates = absorbed dose + exposed skin		0.92	0.55	N/A	N/A
Absorption estimate normalised[3]		N/A		N/A	
Relevant absorption estimate[4]		1.47%		N/A	
Absorption estimates used for risk assessment[5]		**1%**		**1%**	

[1] Values may not calculate exactly due to rounding of figures

[2] In accordance with the EFSA Guidance on Dermal Absorption (EFSA Journal 2012;10(4):2665) the radioactivity in the second tape-strip pool (3rd to nth tape strip) is considered potentially absorbable if less than 75% of the absorption occurred in the first half of the study (see Table 7.6.2-1) Finally, the skin preparation is also considered potentially absorbable.

[3] According to the EFSA Guidance on Dermal Absorption, cells with insufficient recovery (< 95%) can be corrected by normalisation of absorption estimate to 100% recovery; explanation should be included.

[4] In accordance with the EFSA Guidance on Dermal Absorption, one standard deviation was added to the mean% dermal penetration in cases where the standard deviation was ≥ 25% of the mean value.

[5] Relevant absorption estimate was rounded to the required number of significant figures.

N/A: not applicable

Remarks

No cells were excluded or normalisation undertaken.

Conclusion/endpoint:

The dermal penetration of Mesotrione formulated as A18032E through human dermatomed skin was determined *in vitro*. The amount of applied dose penetrating to the receptor fluid within 24 hours was determined to be 0.05% ± 0.03 (mean ± standard deviation) and 0.05% ± 0.03 for the Spray Dilution 1

and Spray Dilution 2, respectively. The dermal penetration estimates to be used for risk assessment were set at 1% for both Spray Dilution 1 and Spray Dilution 2, based on the EFSA (2012) guidance criteria.

A 2.11 Other/Special Studies

Comments of zRMS:	Reviewer has not identified any limitations and deviations from TG according to recent guidelines (OECD 414). Study has been considered as acceptable. Information is relevant to assess prenatal developmental toxicity effect of groundwater MNBA metabolite. Considering observed effects reported in the foetuses at 1000 mg/kg: ↓litter weight, ↑dilated ureter, ↑supra-occipital incomplete cartilage, ↑misshaped/ misaligned sternebrae, and ↑unossified forelimb metacarpals (only foetal incidence) we proposed following endpoints for developmental toxicity study: **Maternal NOAEL** 1000 mg/kg bw/d **Developmenta**l **NOAEL** 300 mg/kg bw/d Both in terms of classification and labelling, and in terms of determination of the relevant NOAEL's, it was demonstrated that the metabolite MNBA was less toxic than the mother compound mesotrione. The metabolite does not share the same characteristics as mesotrione itself, and if found in the groundwater, is thus not considered a substance of higher concern than mesotrione.

Reference:	KCA2 5.8.1/01
Report	CA3511 - Oral (Gavage) Prenatal Developmental Toxicity Study in the Rat. xxxxxxxxxxx 2016 BFI0417 CA3511_10024
Guideline(s):	Prenatal Developmental Study (rat) OECD 414 (2001): OPPTS 870.3700 (1998): 2004/73/EC B.31 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes / No / Supplementary
Duplication (if vertebrate study)	No

EXECUTIVE SUMMARY

Eighty-eight female rats of the Crl:WI(Han) strain were allocated to the study. Groups of 22 females were dosed orally, by gavage, with 0 (vehicle), 100, 300 or 1000 mg/kg/day CA3511 once daily from Day 6 to Day 19 of gestation, inclusive.

The following were assessed during the course of the study: clinical observations, body weights and food intake. On Day 20 of gestation, the females were killed, the live foetuses were removed from the uterus, weighed, the sex determined and they were examined for external, visceral, skeletal and cartilage abnormalities. Placenta and gravid uterus weights were also recorded.

There were no deaths during the study and no clinical observations considered related to CA3511.

Overall mean body weight gain was slightly lower in the groups given the test item, particularly Groups 3 and 4, when compared with the Controls although there was no clear dose-relationship. There was no effect of CA3511 on food intake.

There were six non-pregnant females, two in each of the groups given 100, 300 and 1000 mg/kg/day, respectively. The uterine and foetal data were unaffected by CA3511 administration.

There was no adverse effect of CA3511 on the incidence of external, visceral, skeletal or

cartilaginous major or minor foetal abnormalities or variants.

Administration of CA3511 at dose levels of 100, 300 or 1000 mg/kg/day once daily, by oral gavage, to the Crl:WI(Han) rat from Days 6 to 19 of gestation inclusive, was well tolerated, with slight reductions in maternal body weight gains and no effect on embryonic or foetal development.

~~Based on the above findings the No Observed Adverse Effect Level (NOAEL) for maternal toxicity and embryo-foetal development was considered to be 1000 mg/kg/day.~~

zRMS PL proposal regarding NOAEL values:
Based on the following findings reported in the foetuses at 1000 mg/kg (↓litter weight, ↑dilated ureter, ↑supra-occipital incomplete cartilage, ↑misshaped/ misaligned sternebrae, and ↑unossified forelimb metacarpals (only foetal incidence) the No Observed Adverse Effect Level (NOAEL) for maternal toxicity was considered to be 1000 mg/kg/day and for developmental was considered 300 mg/kg/day

MATERIALS AND METHODS

Materials:

Test Material:	CA3511
Description:	Yellow powder
Lot/Batch number:	SMO3C0689
Purity:	99.8 % (w/w)
Stability of test compound:	Homogeneity and stability of test item formulations prepared at concentrations between 1 and 100 mg/mL, spanning those used in this study (10 to 100 mg/mL), were examined in an earlier formulation validation study. These formulations, prepared at a similar scale to those prepared in this study, were found to be accurately prepared, homogeneous and stable for six days when stored at room temperature, 12 days refrigerated and one month frozen (at approximately -18 °C).

Vehicle: The test substance was administered as a suspension in 1 % (w/v) aqueous carboxymethylcellulose.

Test Animals:

Species	Rat
Strain	Crl:WI(Han)
Age/weight at dosing	9 to 10 weeks/ 193 to 259 g
Source	Charles River (UK) Limited, Margate, Kent, CT9 4LT, England.
Housing	Females were housed individually in grid-floor cages over paper lined trays.
Acclimatisation period	Four days.
Diet	VRF1 (manufactured by SDS) *ad libitum*
Water	Mains tap water (in bottles) *ad libitum*
Environmental conditions	Temperature: 18 to 23°C Humidity: 40 to 70%* Photoperiod: Alternating 12 hour light and dark cycles.

* The humidity on one day was found to be 25 %; however this was an isolated incident and humidity readings on all other days were within the range 40 to 70 %.

Study Design and Methods:

In-life dates: Start: 12 November 2015End: 04 December 2015

Mating procedure: - The females were obtained from the supplier time-mated. The day on which mating was detected was designated Day 0 of gestation.

Animal assignment: Animals were allocated to groups using a stratified body weight randomisation procedure based on individual body weights recorded on Day 0 of gestation (making sure that females mated with the same male were spread across the groups). The cages were positioned in the battery using a randomised cage allocation procedure, starting at the top left-hand corner of the rack and then working from left to right, top to bottom. All groups were allocated to each rack. Each animal was uniquely identified by a subcutaneously implanted micro-identification device.

Table A 16: Animal numbers and treatment groups

Females	0 (control)	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
88	22	22	22	22

Dose selection rationale: CA3511 is a manufacturing intermediate and the oral (gavage) route of administration was used in accordance with OECD Test Guideline 414 as it is considered a possible route of human exposure. The dose levels were selected in consultation with the Sponsor on the basis of a 28 day study in rats, a pilot one generation reproductive toxicity study in rats and a two generation reproductive toxicity study in rats, where CA3511 was administered up to 1000 mg/kg/day and was well tolerated with no evidence of systemic toxicity. Based on these existing data, a high dose level of 1000 mg/kg/day was expected to elicit minimal or no adverse effects and was the limit dose for this study type. An intermediate dose level of 300 mg/kg/day would provide an understanding of the dose response if effects were seen at the high dose. The low dose level of 100 mg/kg/day was considered to provide a clear no effect level.

Dosage preparation and analysis: The test item was formulated at approximately weekly intervals (within known stability), for each group separately, as a suspension in 1 % (w/v) aqueous carboxymethylcellulose.

A weighed quantity of test item was added to a mortar, wetted with a small quantity of vehicle and initially made into a smooth paste using a pestle. After further addition of vehicle and mixing, the resultant suspension was transferred into a tared beaker on a balance. The mortar was thoroughly rinsed out with vehicle and these rinsings were added to the suspension which was then made up to final weight with vehicle and mixed with a laboratory homogeniser.

Formulations were divided into daily aliquots and were stored refrigerated (2 °C to 8 °C). They were stirred from 15 minutes before the start of dosing until the completion of dosing, to ensure thorough resuspension and homogeneity.

Test item formulations from the first preparation occasion and those used on the last day of dosing were considered to have been accurately prepared and homogeneous (where assessed) since the mean measured concentrations of CA3511 were within 4 % of their nominal values with coefficients of variation no greater than 3.3 % which fulfilled the acceptance criteria.

No CA3511 was detected in vehicle used to dose animals in Group 1.

Dosage administration: Animals were dosed once daily from Day 6 to Day 19 of gestation inclusive, by gavage, using a rubber catheter and disposable syringe at a constant dose volume of 10 mL/kg body weight. Individual doses were adjusted according to the most recent body weight.

Observations:

Maternal observations: Animals were examined twice daily for mortality and morbidity and were given a detailed clinical examination daily.

Foetal observations: On Day 20 of gestation, live foetuses were killed by rapid cooling, weighed, sexed and examined for external abnormalities.

Approximately 50 % of the live foetuses were fixed in Bouin's fluid for subsequent examination for visceral abnormalities using a combined sectioning/dissection technique. The remaining foetuses were placed in 70 % alcohol. Later in the day the viscera were examined and the foetuses eviscerated. A coronal section was made through the head along the frontal parietal suture and the brain examined. The carcasses were then cleared in potassium hydroxide, stained with Alizarin red S and Alcian blue to visualise the ossified skeleton and cartilage and examined.

Structural congenital abnormalities that impair, or potentially impair, the survival or constitution of the foetus were classified as major abnormalities. Other defects were classified as minor abnormalities or variants.

Foetuses with major external or visceral abnormalities were photographed.

For archiving, all foetuses fixed in Bouin's fluid were stored in neutral buffered formaldehyde and all skeletal specimens were stored in aqueous glycerol with thymol crystals (to prevent fungal growth).

Statistical analyses: Data were processed to give group mean values and standard deviations, where appropriate. Where the data allowed, the following methods were used for statistical analysis comparing Groups 2, 3 and 4 against Group 1.

General Approach: All statistical tests were two-sided with minimum significance levels of 5 % and 1 %. Non-parametric statistics were not routinely conducted. The litter, rather than the foetus, was considered as the experimental unit. When used, Dunnett's test was conducted regardless of the outcome of the analysis of variance (ANOVA) or analysis of covariance (ANCOVA).

Data were examined for unusually high or low values which could influence the statistical analysis and interpretation (possible outliers). After examining for any outliers, if the variances were clearly heterogeneous, transformations (e.g. log, double arcsine or square root) were used in an attempt to stabilise the variances. If the transformations failed, the data set was examined and a decision taken on further action.

For Quantitative Data: Body weight, cumulative body weight gain from the start of dosing, food intake, terminal body weight, numbers of corpora lutea, implants, live foetuses, dead foetuses, early deaths, late deaths, gravid uterus weight, total litter weight, placental weight and mean foetal weight (sexes separately and combined) were analysed using a parametric ANOVA.

For Percentages: Pre-implantation loss, post-implantation loss, sex ratios (% male foetuses) and litter based mean percentages were analysed using a parametric ANOVA, following a double arcsine transformation (Freeman and Tukey, 1950).

Maternal Performance: (e.g. the proportion of females with live foetuses at termination, abortions, total resorptions) were analysed by a two-tailed Fisher's Exact Text (Steel and Torrie, 1980), comparing each treated group to the control group.

Foetal Morphology Data: The incidence of foetal malformations and developmental variations (external, visceral and skeletal) were summarised as the proportion of foetuses affected, the proportion of litters affected and the proportion of foetuses affected within each litter. The proportions of litters affected were analysed by the exact version of the Cochran-Armitage Test. The percentages of foetuses affected within each litter were analysed by the exact version of the Jonckheere Trend Test. In both cases the tests were performed in a step-wise manner, where, when a test was significant at the 5 % level, the test was repeated after removing the then top dose, until only the control group was left. Tests were one-sided looking for increase in treated groups versus the control group.

Outliers: Exclusion of outlier values was considered where this was deemed appropriate. If a particular value was excluded from statistical analyses of a group because of known mitigating circumstances (e.g.

missing value, instrument malfunction), the reason for exclusion was clearly stated in the final report data tables. For outliers that were biologically plausible, statistical results were presented both with and without influential values if they affected the interpretation of the parameter. In these rare cases, the results were presented including all values and a secondary table provided after excluding the outlier. The interpretation discussed the influence of the outlier.

Dunnett's Test: For all of the parameters evaluated initially by ANOVA or ANCOVA, Dunnett's Test was used to compare the control and treated groups, based on the error mean square in the ANOVA or ANCOVA. The Dunnett's Test was performed for all continuous data parameters, regardless of whether the initial ANOVA or ANCOVA was statistically significant, and statistical flags were presented in the tables of results in the final report.

Indices:

$$\text{Pre-implantation loss (\%)} = \frac{\text{(no. of corpora lutea – no. of implantation sites)}}{\text{no. of corpora lutea}} \times 100$$

$$\text{Post-implantation loss (\%)} = \frac{\text{(no. of implantation sites – no. of live foetuses)}}{\text{no. of implantation sites}} \times 100$$

Mean pre- and post-implantation losses were calculated on a proportional litter basis.

Mean foetal body weights were calculated separately by sex for each litter and group means were calculated from the litter means.

The percentage of foetuses in each litter exhibiting each classification of abnormality was calculated; group mean percentages were calculated from the litter percentages.

The percentage of male foetuses, out of the total number of foetuses, was calculated for each litter.

RESULTS

Maternal toxicity:

Mortality and clinical signs: There were no deaths during the study and no clinical observations related to the test item.

Body weight: Up to Day 16 of gestation, mean body weight gains were comparable in all groups. Between Days 17 and 20 of gestation however, body weight gains were slightly lower in groups given CA3511, particularly groups 3 and 4, when compared with the Controls although there was no dose-relationship. The body weight data are summarised in the tables below:

Table A 17: Intergroup comparison of body weight gain (spurious weight excluded)

Sex: Female		**Body Weight Gain (Day of Gestation)**					
Animal ID		**0 to 5 (#)**	**0 to 6 (#)**	**6 to 7 (#)**	**6 to 8 (#)**	**6 to 9 (#)**	**6 to 10 (#)**
Group: 1 Control 0 mg/kg/day	Mean	16.3	20.1	1.0	4.0	4.0	9.8
	SD	14.2	13.5	6.6	4.2	5.9	7.0
	N	22	22	22	22	22	22
Group: 2 CA3511 100 mg/kg/day	Mean	14.0	16.2	0.7	2.5	4.5	7.7
	SD	9.2	9.0	4.0	4.8	5.1	7.5
	N	20	20	20	20	20	20
Group: 3 CA3511 300 mg/kg/day	Mean	10.7	13.4	1.3	2.5	3.8	8.7
	SD	7.7	7.9	3.5	5.7	5.1	5.8
	N	20	20	20	20	20	20
Group: 4 CA3511 1000 mg/kg/day	Mean	18.3	21.4	1.3	2.1	5.1	8.8
	SD	18.4	18.6	3.7	4.9	5.0	7.5
	N	20	20	20	20	20	20

[Statistically Analysed]

On Day 18, Animal 15 (Control) had a spurious bodyweight recorded. The single spurious body weight has therefore been excluded from the group means.

Sex: Female		**Body Weight Gain (Day of Gestation)**					
Animal ID		**6 to 11 (#)**	**6 to 12 (#)**	**6 to 13 (#)**	**6 to 14 (#)**	**6 to 15 (#)**	**6 to 16 (#)**
Group: 1 Control 0 mg/kg/day	Mean	15.1	20.1	23.9	29.7	34.9	44.5
	SD	8.1	5.5	6.4	5.6	7.9	9.0
	N	22	22	22	22	22	22
Group: 2 CA3511 100 mg/kg/day	Mean	14.0	18.0	22.3	27.8	32.9	40.5
	SD	7.4	7.9	5.7	7.0	8.0	10.5
	N	20	20	20	20	20	20
Group: 3 CA3511 300 mg/kg/day	Mean	13.8	17.1	20.4	26.2	30.0	37.6
	SD	5.5	5.1	7.1	4.5	7.4	7.0
	N	20	20	20	20	20	20
Group: 4 CA3511 1000 mg/kg/day	Mean	15.5	18.8	22.6	28.6	32.6	38.7
	SD	6.3	6.6	7.6	9.0	9.4	11.8
	N	20	20	20	20	20	20

[Statistically Analysed]

Sex: Female		**Body Weight Gain (Day of Gestation)**			
Animal ID		**6 to 17 (#)**	**6 to 18 (#)**	**6 to 19 (#)**	**6 to 20 (#)**
Group: 1 Control 0 mg/kg/day	Mean	56.0	68.8	80.7	95.4
	SD	9.0	10.9	11.1	12.2
	N	22	21	22	22
Group: 2 CA3511 100 mg/kg/day	Mean	52.2	63.6	74.6	87.7
	SD	11.0	14.2	15.2	16.8
	N	20	20	20	20
Group: 3 CA3511 300 mg/kg/day	Mean	49.2	60.5	72.0	84.5
	SD	7.3	10.1	12.1	15.5
	N	20	20	20	20
Group: 4 CA3511 1000 mg/kg/day	Mean	49.8	62.7	74.0	85.9
	SD	11.0	13.2	14.4	15.4
	N	20	20	20	20

[Statistically Analysed]

On Day 18, Animal 15 (Control) had a spurious bodyweight recorded. The single spurious body weight has therefore been excluded from the group means.

Table A 18: Intergroup comparison of body weight gain (spurious weight included)

Sex: Female		Body Weight Gain (Day of Gestation)					
Animal ID		**0 to 5 (#)**	**0 to 6 (#)**	**6 to 7 (#)**	**6 to 8 (#)**	**6 to 9 (#)**	**6 to 10 (#)**
Group: 1 Control 0 mg/kg/day	Mean	16.3	20.1	1.0	4.0	4.0	9.8
	SD	14.2	13.5	6.6	4.2	5.9	7.0
	N	22	22	22	22	22	22
Group: 2 CA3511 100 mg/kg/day	Mean	14.0	16.2	0.7	2.5	4.5	7.7
	SD	9.2	9.0	4.0	4.8	5.1	7.5
	N	20	20	20	20	20	20
Group: 3 CA3511 300 mg/kg/day	Mean	10.7	13.4	1.3	2.5	3.8	8.7
	SD	7.7	7.9	3.5	5.7	5.1	5.8
	N	20	20	20	20	20	20
Group: 4 CA3511 1000 mg/kg/day	Mean	18.3	21.4	1.3	2.1	5.1	8.8
	SD	18.4	18.6	3.7	4.9	5.0	7.5
	N	20	20	20	20	20	20

[Statistically Analysed]

On Day 18, Animal 15 (Control) had a spurious bodyweight recorded. The single spurious body weight is included in the group means.

Sex: Female		Body Weight Gain (Day of Gestation)					
Animal ID		**6 to 11 (#)**	**6 to 12 (#)**	**6 to 13 (#)**	**6 to 14 (#)**	**6 to 15 (#)**	**6 to 16 (#)**
Group: 1 Control 0 mg/kg/day	Mean	15.1	20.1	23.9	29.7	34.9	44.5
	SD	8.1	5.5	6.4	5.6	7.9	9.0
	N	22	22	22	22	22	22
Group: 2 CA3511 100	Mean	14.0	18.0	22.3	27.8	32.9	40.5
	SD	7.4	7.9	5.7	7.0	8.0	10.5
	N	20	20	20	20	20	20
Group: 3 CA3511 300	Mean	13.8	17.1	20.4	26.2	30.0	37.6
	SD	5.5	5.1	7.1	4.5	7.4	7.0
	N	20	20	20	20	20	20
Group: 4 CA3511 1000 mg/kg/da	Mean	15.5	18.8	22.6	28.6	32.6	38.7
	SD	6.3	6.6	7.6	9.0	9.4	11.8
	N	20	20	20	20	20	20

[Statistically Analysed]

Sex: Female		Body Weight Gain (Day of Gestation)			
Animal ID		**6 to 17 (#)**	**6 to 18 (#)**	**6 to 19 (#)**	**6 to 20 (#)**
Group: 1 Control 0 mg/kg/day	Mean	56.0	70.8	80.7	95.4
	SD	9.0	14.2	11.1	12.2
	N	22	22	22	22
Group: 2 CA3511 100 mg/kg/day	Mean	52.2	63.6	74.6	87.7
	SD	11.0	14.2	15.2	16.8
	N	20	20	20	20
Group: 3 CA3511 300 mg/kg/day	Mean	49.2	60.5 *	72.0	84.5
	SD	7.3	10.1	12.1	15.5
	N	20	20	20	20
Group: 4 CA3511 1000 mg/kg/day	Mean	49.8	62.7	74.0	85.9
	SD	11.0	13.2	14.4	15.4
	N	20	20	20	20

[Statistically Analysed]

* Statistically significant difference from control group mean $p<0.05$ (Dunnett's 2-sided test)

On Day 18, Animal 15 (Control) had a spurious bodyweight recorded. The single spurious body weight is included in the group means.

Food consumption: Food intake was not affected by CA3511 at any dose level. The Food consumption data are summarised in the table below:

Table A 19: Intergroup comparison of food consumption

Sex: Female		**Mean Food Intake (Day of Gestation)**					**Overall Mean Food Intake (Day of Gestation)**
Group		**6 to 9#**	**9 to 12#**	**12 to 15#**	**15 to 18#**	**18 to 20#**	**6 to 20#**
Group: 1 Control 0 mg/kg/day	Mean	19.7	21.8	22.7	25.1	22.9	22.4
	SD	2.0	2.3	2.1	2.8	3.1	1.7
	N	22	22	22	22	22	22
Group: 2 CA3511 100 mg/kg/day	Mean	19.6	21.5	22.0	23.5	22.4	21.8
	SD	2.3	2.8	2.4	2.9	4.2	1.9
	N	20	20	20	20	20	20
Group: 3 CA3511 300 mg/kg/day	Mean	19.5	20.6	20.7 *	22.7 **	21.2	20.9 *
	SD	2.2	2.6	2.3	1.7	3.1	1.7
	N	20	20	20	20	20	20
Group: 4 CA3511 1000 mg/kg/day	Mean	18.5	21.7	21.3	23.2 *	22.9	21.4
	SD	5.2	4.6	2.8	2.5	2.7	1.9
	N	20	20	20	20	20	20

[Statistically Analysed]
* Statistically significant difference from control group mean $p<0.05$ (Dunnett's 2-sided test)
** Statistically significant difference from control group mean $p<0.01$ (Dunnett's 2-sided test)

Sacrifice and pathology:

Gross pathology: There were no findings at necropsy considered to be related to the test item.

Caesarean section data: Six females were not pregnant, two in each of the groups given 100, 300 or 1000 mg/kg/day. There was no adverse effect of CA3511 on the mean numbers of implantations, the incidence of pre- or post-implantation loss or on the number of live foetuses. Caesarean section data is shown in the table below:

Table A 20: Caesarean section observations for all pregnant females

Observation	**CA3511 (mg/kg/day)**			
	0 (control)	**100#**	**300#**	**1000#**
# Animals Assigned (Mated)	22	22	22	22
# Animals Pregnant	22	20	20	20
# Non-pregnant	0	2	2	2
#Intercurrent deaths	0	0	0	0
# Died Pregnant	0	0	0	0
# Died Non-pregnant	0	0	0	0
# Totally resorbed	0	0	0	0
Corpora Lutea/Dam	13.6	13.1	13.2	12.9
Implantations/Dam	12.9	12.3	12.3	12.4
Total # Litters (viable)	22	20	20	20
Live Foetuses/Dam	12.2	11.3	12	11.6
% Intra-uterine deaths/Dam				
Early (Proportion of litters affected)	45.5	50.0	25.0	45.0
Late (Proportion of litters affected)	0.0	5.0	0.0	5.0
Litter Weight (g)	44.83	42.24	43.14	41.84

Observation	CA3511 (mg/kg/day)			
	0 (control)	100#	300#	1000#
Mean Foetal Weight (g)	3.71	3.77	3.65	3.64
Males (g)	3.78	3.85	3.74	3.73
Females (g)	3.63	3.69	3.57	3.54
Sex Ratio (% Males per litter)	51.9	51.1	44.4	55.2
Pre-implantation Loss (%)	5.2	6.1	7.8	4.6
Post-implantation Loss (%)	6.0	8.0	2.4	6.5

Developmental Toxicity: One foetus from each group was noted to have major abnormalities (see below).

Dam 7 (Control)	Foetus R5	Malformed cervical vertebral neural arch(es)
Dam 33 (100 mg/kg/day)	Foetus R4	Severely bent scapula(e);
Malformed clavicle(s);		
Bowed femur(s)		
Dam 59 (300 mg/kg/day)	Foetus L3	Absent cartilage rings in the trachea;
		Incomplete interventricular septum
Dam 85 (1000 mg/kg/day)	Foetus R10	Anopthalmia

Since the above major abnormalities were single incidences, each in separate dose groups, they were considered to be consistent with natural variation and not an effect of CA3511. Details of minor abnormalities and variant findings are given below.

External and visceral examinations: The variant findings of dilated ureter and left sided umbilical artery were observed to be higher in the litters of dams given 300 mg/kg/day or 1000 mg/kg/day compared with Controls ($p<0.05$). However, these abnormalities are not clearly dose related and have been seen in Control animals of this strain in these laboratories and as such the incidences here are considered to be spontaneous anomalies and not an effect of CA3511 administration.

Table A 21: External and visceral findings

Observation	Type	CA3511 (mg/kg/day)				Historical control range (%)
		0 (control)	100#	300#	1000#	
Dilated ureter	Variant					
Foetal incidence (percentage)		1 (1.1)	3 (2.3)	3 (2.5)	7 (6.2)	0 – 7.5
Litter incidence		1	3	2	5*	
Left sided umbilical artery	Variant					
Foetal incidence (percentage)		18 (12.7)	18 (16.9)	25 (24.3)	23 (20.8)	11.2 - 23
Litter incidence		12	11	15*	14*	

*Statistically significant $p<0.5$

Skeletal examinations: The number of litters with foetuses showing the minor skeletal abnormality, misshapen or misaligned sternebra(e), was statistically significantly higher ($p<0.05$) in the group given 1000 mg/kg/day compared with Controls. The variant finding, non-ossified 5th sternebra, was also noted to be higher ($p<0.05$) in the groups given 300 or 1000 mg/kg/day, however the incidence was not dose related. These abnormalities have previously been seen in Control animals and as such they were considered not to be related to CA3511.

Table A 22: Skeletal findings

Observation	Type	CA3511 (mg/kg/day)				Historical control range (%)
		0 (control)	100#	300#	1000#	
Misshapen or misaligned sternebra(e)	Minor					
Foetal incidence (percentage)		4 (3.3)	4 (3.3)	7 (5.3)	12 (11.3)	0 – 4.4
Litter incidence		4	4	6	9**	
5th sternebra not ossified	Variant					
Foetal incidence (percentage)		2 (1.1)	2 (1.8)	8 (6.5)	7 (6.3)	1.7 - 10.7
Litter incidence		1	2	7**	4*	

Statistically significant * p<0.5 ** p<0.01

CONCLUSION

Administration of CA3511 at dose levels of 100, 300 or 1000 mg/kg/day once daily, by oral gavage, to the Crl:WI(Han) rat from Days 6 to 19 of gestation inclusive, was well tolerated, with slight reductions in maternal body weight gains, particularly in Groups 3 and 4, and no effect on embryonic or foetal development.

Based on the above findings the No Observed Adverse Effect Level (NOAEL) for maternal toxicity and embryo-foetal development was considered to be 1000 mg/kg/day.

(Pottle C, 2016)

Comments of ZRMS:	Reviewer has not identified any limitations and deviations from TG according to recent guidelines (OECD 416). Study has been considered as acceptable; The information is relevant to assess reproduction toxicity effect of groundwater MNBA metabolite. Considering observed effects reported in P-generation males and in F1-generation adult males and females from the 1000 mg/kg/day dose level (↑kidney weight) we proposed following endpoints for Two-Generation Reproduction Toxicity Study: **Maternal:** 300 mg/kg bw/d **Offspring:** 1000 mg/kg bw/d **Reproductive:** 1000 mg/kg bw/d Both in terms of classification and labelling, and in terms of determination of the relevant NOAEL's, it was demonstrated that the metabolite MNBA was less toxic than the mother compound mesotrione. The metabolite does not share the same characteristics as mesotrione itself, and if found in the groundwater, is thus not considered a substance of higher concern than mesotrione.

Reference:	KCA2 5.8.1/02
Report	CA3511 - Oral (Gavage) Two-Generation Reproduction Toxicity Study in the Rat. xxxxxxxxxxxxxxxx 2016 11214 CA3511_10030
Guideline(s):	Multigeneration Reproduction Study in the rat (oral gavage) OECD 416 (2001): OPPTS 870.3800 (1998): EC No. 440/2008, B.35 (2008): JMAFF, 12 Nousan No. 8147 (2000)

Deviations:	No
GLP:	Yes
Acceptability:	Yes / No / Supplementary
Duplication (if vertebrate study)	No

EXECUTIVE SUMMARY

The purpose of this study was to investigate the potential effects of the test substance, CA3511, when administered via oral gavage, on the integrity and performance of the male and female reproductive systems in the rat, including gonadal function, the oestrous cycle, mating behaviour, conception, gestation, parturition, lactation and weaning, and the growth and development of the offspring over two successive generations. This study also provided information about the effects of the test substance on neonatal morbidity and mortality, and data on pre- and post-natal development toxicity.

A total of 240 rats were allocated to this study. Male and female rats, 30 rats/sex/group were given the vehicle (1 % (w/v) aqueous carboxymethylcellulose) or CA3511 (100, 300, and 1000 mg/kg/day) by oral gavage at a dose volume of 10 mL/kg. P-generation (P-gen) animals were dosed once daily for the entire in-life phase of the study. In-life phases included: Premating phase, 10 weeks; Mating, 14 days; Gestation, approximately 22 days; and Lactation, weaning on Day 21. F1 pups were maintained after weaning with initiation of the second generation occurring once all selected animals had reached the age of postnatal Day (PND) 21. F1 pups were dosed once daily beginning on PND 21 and continuing for the entire in-life phase. Parameters examined included clinical observations, mortality and moribundity checks, body weights, food consumption, gross pathology, organ weights, and various reproductive parameters.

Test substance-related non-adverse findings were limited to increased kidney weights in P-generation males and in F1-generation adult males and females from the 1000 mg/kg/day dose level. More specifically, there were statistical increases in absolute (5%) and relative (8%) left kidney weights and non-statistical increases in absolute (5%) and relative (8%) right kidney weights in 1000 mg/kg/day P-generation males. For the F1-generation, there were statistical increases in absolute (9% and 8%) and relative (8% and 6%) kidney weights (left and right), respectively, in 1000 mg/kg/day males and there were statistical increases (6% and 5%) in absolute kidney (left and right) weights, respectively, in 1000 mg/kg/day females.

Oral (gavage) administration of CA3511 to Crl:WI(Han) rats at dose levels of 100, 300, and 1000 mg/kg/day, continuously for two successive generations, was well tolerated.

There were no effects on mortality, clinical observations, body weights, food consumption, reproductive function or performance, mating behaviour, conception, or pup development at any dose level. In addition, there were no macroscopic findings or histopathology changes related to the test substance at any dose level as evaluated in either adults or offspring.

Test substance-related non-adverse findings were limited to increased kidney weights in

P-generation males and in F1-generation adult males and females from the 1000 mg/kg/day dose level.

The No Observed Adverse Effect Level (NOAEL) for both systemic toxicity and reproductive parameters was considered to be 1000 mg/kg/day, respectively for both males and females and for their offspring.

MATERIALS AND METHODS

Materials:

Test Material:	CA3511
Description:	Yellow powder
Lot/Batch number:	694472
Purity:	99.8% a.i
CAS#:	110964-79-9
Stability of test compound:	Recertification date end of May 2016

Vehicle and/or positive control:

Formulations were prepared by placing the appropriate amounts of CA3511 into 1% (w/v) aqueous carboxymethylcellulose vehicle in appropriate glass containers. The dose formulations were generally prepared twice weekly since stability was confirmed for up to 6 days at room temperature conditions (18–25 °C average daily temperature). Dose formulations were stored at room temperature conditions (18–25 °C average daily temperature). The vehicle was prepared in advance of the dose formulation preparation and brought to room temperature conditions prior to use. The formulations were kept stirring with a magnetic stirrer while in use (dosing and dose analysis).

Test Animals:

Species	Rat
Strain	Wistar: Crl:WI(Han)
Age/weight at dosing	9 weeks at initiation of exposure/Males: 204–308 g and Females: 141–218 g
Source	Charles River Laboratories, Inc. (Raleigh, NC)
Housing	Animals were housed individually (except during the mating phase and as noted below for the F1 pups) in suspended stainless steel cages, with cage board in the bedding trays. During gestation and lactation, individual dams (and their litter) were housed in polycarbonate cages with corn cob bedding. Pups passing vaginal patency and preputial separation were transferred to individual cages.
Acclimatisation period	June 2, 2014 (receipt) through June 9, 2014 (release)
Diet	ad libitum; Purina Mills Certified Rodent Diet 5002M in “meal” form.
Water	ad libitum; free and continuous access to tap water
Environmental conditions	Temperature: 20-26 °C Humidity: 30-70 % Air changes: 13 air changes/hour Photoperiod: 12 hour light/dark cycle

Study Design and Methods:

In-life dates: Start: 18th June 2014 End: 12th March 2015

Animal assignment:

The animals were assigned to dose groups by weight stratification using Provantis™ Software, Version 8.4.1.0, (Instem LSS, Stone, Staffordshire, United Kingdom). The cages were positioned using a randomized cage allocation procedure. Animals were assigned as shown in the following table.

Table A 23: Animal identification

Dose of CA3511 (mg/kg)	**Animal No.**	
	Males	**Females**
0 (vehicle control)	BZ0001 – BZ0030	BZ0101 – BZ0130

100	BZ1001 – BZ1030	BZ1101 – BZ1130
300	BZ2001 – BZ2030	BZ2101 – BZ2130
1000	BZ3001 – BZ3030	BZ3101 – BZ3130

Table A 24: Animal numbers and treatment groups

Group	Animals/group		CA3511 Dose Level (mg/kg/day)	Concentration (mg/mL)	Dose volume (mL/kg)
	P-gen Males	P-gen Females			
0 mg/kg/day (Vehicle Control)	30	30	0	0	10
100 mg/kg/day (Low)	30	30	100	10	10
300 mg/kg/day (Mid)	30	30	300	30	10
1000 mg/kg/day (High)	30	30	1000	100	10

At approximately the same time each day, animals were administered a once-daily dose of either the vehicle [1% (w/v) aqueous carboxymethylcellulose] or CA3511 via oral gavage using disposable polypropylene syringes with plastic feeding tubes at one of three dose levels (100, 300, and 1000 mg/kg/day) at a dosing volume of 10 mL/kg beginning on the first day of premating through LD 20 (males were dosed up to the day prior to necropsy). Doses were administered volumetrically, based on the most recent body weight of the animal

Dose selection rationale:

The dose levels were selected by the sponsor based on existing data from a dose range-finding reproduction pilot study in rats conducted at the test facility (Gilmore R, 2015). In the pilot study, the test substance was administered via oral gavage for approximately 18 weeks to groups of ten male and ten female young adult Wistar Han rats at dose levels of 0, 350, 700, or 1000 mg/kg body weight/day (1% (w/v) aqueous carboxymethylcellulose used as the control substance and vehicle). There was no evidence of toxicity on any of the examined parameters (clinical observations, mortality, body weights, food consumption, gross pathology, organ weights, and various reproductive parameters) at dose levels up to and including the limit dose of 1000 mg/kg bw/day. Based on these results, the dose levels selected for this definitive reproduction toxicity study were 100, 300, and 1000 mg/kg/day (dose volume of 10 mL/kg). The highest dose of 1000 mg/kg/day is a limit dose for this study type.

Dosage preparation and analysis:

Homogeneity and stability

According to the analytical report provided by the sponsor (Sequani Study #BFI0149), formulations in the concentration range 1 to 100 mg/mL remain stable for up to 6 days at room temperature (approximately 25 ºC) and formulations were found homogeneous.

Dose analysis

All analyses were conducted at Xenometrics. Dose formulations prepared the first week of both pre-mating periods (P-gen and F1-gen) and once, each, during P-gen and F1-gen gestation and lactation periods (total of six dose formulation analyses) were analysed using a high performance liquid chromatography (HPLC) with ultraviolet (UV) detection method validated over a concentration range of 1–120 mg/mL. Xenometrics SOPs were followed for the analysis of the test substance formulations. The dose formulation analysis, as well as verification of formulation homogeneity (performed on June 18, 2014, during the first week of premating for P-generation), was performed using a validated method (Xenometrics Study No. 11070).

Homogeneity and concentration verification

Duplicate samples [top, middle, and bottom (0.5 mL each)] of the formulated test substance or vehicle dosing formulations were collected at selected intervals. One set was stored at room temperature and analysed for dose concentration verification (only middle sample) and homogeneity (top, middle, and bottom samples) within 6 days of preparation. The vehicle sample was analysed to confirm the absence of the test substance. The remaining set of dose samples were stored frozen and disposed of after finalization of the dose analysis report. Formulations were considered acceptable as mean results were within ±15% of the theoretical concentration and the relative standard deviation (RSD) was equal to or less than 10%. When

there was a deviation from the given criteria (with respect to the % mean and RSD), a reanalysis was carried out using the retained samples.

Observations:

Parental animals:

Mortality and clinical signs

Mortality checks (cage-side observations) were performed at least once daily (nominally twice daily during the normal workweek and once on weekends and holidays). Cage-side observations characterized mortality, moribundity, behavioural changes, signs of difficult or prolonged delivery, and overt toxicity by viewing the animal in the cage. A detailed evaluation of clinical signs included both observing the animal in the cage and removing the animal to perform a physical examination and was conducted once per week throughout the entire in-life phase of the study.

Body weight and food consumption

Body weight and food consumption were measured once per week for both males and females during the 10-week pre-mating period. During the mating period and until sacrifice, body weights for the males and unmated females were measured once per week. Also during the mating period, fresh feed was provided for both males and unmated females, but food consumption was not measured. During gestation, dam body weight was measured and fresh feed was provided and food consumption measured on Days 0, 6, 13, and 20. During lactation, dam body weight and food consumption was measured on Days 0, 4, 7, 14, and 21. Fresh feed was provided once per week.

Animal mating

Males and females were exposed to the test material for ten weeks prior to mating. Mating was accomplished by co-housing one female with one male for up to 14 consecutive days. During the mating phase, vaginal smears were taken each morning and examined for the presence of sperm and/or internal vaginal plug. Once females were inseminated, they were placed in a polycarbonate nesting cage. The day on which insemination was observed in the vaginal smear was designated Day 0 of gestation for that female. For randomization the pups into the second generation, dam numbers were different when pairing the male and female pups to avoid litter-mate pairing.

Litter observations:

Pup viability and clinical signs

The number of live and stillborn pups (both F1 and F2 generations) were recorded for each litter. Both F1 and F2 pups were observed daily for clinical signs (cage side) from birth until the start of the premating phase (F1 pups) and until weaning (F2 pups). Mortality checks (cage-side observations/pup counts) were performed once daily (a.m.) during the workweek and on weekends and holidays. Cage-side observations characterized mortality, moribundity, behavioural changes, and overt toxicity by viewing the pups in the cage. In the event a possible clinical sign was observed during the cage-side evaluation, the pups were removed from the cage and a detailed assessment conducted. A detailed evaluation of clinical signs included both observing the pups in the cage and removing the animals to perform a physical examination and was conducted daily (Day 0–21). The size of each litter was adjusted on LD 4 to yield, as closely as possible, four males and four females per litter. When the number of male or female pups was less than four, a partial adjustment was made (e.g. three females and five males). No adjustment was made for litters of fewer than eight pups. Adjustments were made by random selection of the pups using software provided by SAS (SAS Institute Inc., Version 6.09 Enhanced, Cary, North Carolina). Culled pups were sacrificed by decapitation and discarded.

Pup body weights

Pups were sexed and their body weights were recorded as soon as possible following parturition (LD 0) and also on LD 4, 7, 14, and 21. Fresh feed was provided at least once/week for the weanlings, from LD 21 until the start of the premating phase of the F1-generation. Due to rapid growth after weaning, body weights were measured on PND 21, 24, 28 and weekly thereafter for F1-generation animals. The dosing volumes

were adjusted on these days as well. (Note: Dosing volumes were not adjusted on days that body weights were taken when the animals pass vaginal patency and preputial separation).

Pup reproductive parameters

The F1 pups retained to produce the next generation were observed for vaginal opening and preputial separation.

Reproductive Parameters

Oestrous cycle staging

The oestrous cycle (determined by examining daily vaginal smears) was characterized for all P and F1-generation females, over a three-week period prior to mating. Additionally, the oestrous cycle stage was determined for all females by histopathological evaluation of the reproductive organs (ovaries, uterus, cervix and/or vagina).

Male reproductive function

For all P- and F1-generation males at termination, sperm was collected from one testis and one epididymis for enumeration of homogenization-resistant spermatids and cauda epididymal sperm reserves, respectively. In addition, an evaluation of the morphology and motility was performed on sperm sampled from the distal portion (closest to the urethra) of the vas deferens. Sperm motility and counts were conducted using IVOS (Integrated Visual Operating System (2005 and 2011), Hamilton Thorne Research; Beverly, MA). Morphology and counts were conducted on the control and highest dose group. Since no findings were attributed to the test substance, the other dose levels were not evaluated.

Investigations *post mortem:*

Parental animals

Gross Pathology – Females

Following the weaning of their respective litters on LD 21, each dam (both P- and F1-generations) was euthanized by carbon dioxide asphyxiation, and a gross external examination performed. Terminal body weights were measured, gross pathologic alterations were recorded. The uterus was excised and the implantation sites were counted. In addition, corpora lutea were counted externally during the gross necropsy.

Females which were sperm positive and/or had an internal vaginal plug but did not deliver were sacrificed near the end of the gestation phase. Females that were never observed as being inseminated and/or with an internal vaginal plug and did not deliver at least 24 days after the completion of the mating phase were sacrificed near the end of the gestation phase and necropsied. A gross necropsy was performed on these animals as described above. In addition, patency of the cervical/uterine os in these females was examined via flushing of the uterine horns with 10% buffered formalin.

Gross Pathology – Males

Near the end of the lactation phase, male rats were either euthanized by carbon dioxide asphyxiation (P-generation), or anesthetized with Isoflorane (<2 minutes) then terminated via decapitation (F1-generation) and a gross external examination was performed. Terminal body weights were measured, gross pathologic alterations were recorded.

Organ Collection and Tissues Weighed and Examined

The following tissues were collected at necropsy. All tissues were preserved in 10% buffered formalin except the ovaries and right testicle, which were collected in Modified Davidson's fixative. The left testicle and epididymide utilized for sperm counts were kept on ice until placed in -80 °C (± 5 °C) freezer pending analysis. Gross lesions were collected with representative tissues from control group for comparison.

Testis[1]
Epididymis[1]
Epididymis Cauda (side not utilized for sperm)
Brain
Kidneys
Uterus (with oviduct and cervix)

Seminal Vesicle (with coagulating glands and fluids)	**Pituitary Gland**
Prostate	Coagulating Gland
Thyroid (**includes parathyroid**)	Physical Identifier
Liver	Vagina
Spleen	**Ovaries**
Adrenal Glands	Gross Lesions

Items in bold were weighed.
Paired organs were weighed individually.
[1] = left side taken for sperm analysis, right side taken for histopathology

The following tissues from P- and F1 adults were evaluated for micropathology:

Coagulating Gland	Seminal Vesicle
Epididymis	Testis
Epididymis Cauda	Uterus (with oviduct and cervix)
Ovaries	Vagina
Prostate	Gross Lesions
Kidneys	

Necropsy of Moribund or Found Dead Animals

Animals found moribund while on study were sacrificed and a gross necropsy performed.
Animals found dead were necropsied as soon as possible.

Histopathology

Adults

Tissues were processed, embedded in paraffin, sectioned, mounted, and stained with hematoxylin and eosin (H & E) and examined microscopically. Processing of tissues and histopathological evaluations were conducted on the control and highest-dose groups, with one exception. The exception is that the reproductive organs were evaluated in any animal demonstrating reduced fertility (e.g., those who failed to mate, conceive, sire, or deliver healthy offspring), or where altered sperm motility was observed. Since no histopathological findings were attributed to treatment, no other evaluations were performed.

A quantitative evaluation of the ovarian follicles (primordial follicles and small follicles) was conducted on the left ovary for all F1 dams from the control and highest dose level. Corpora lutea were only counted externally at gross necropsy. The left ovary from all F1 dams from all dose levels was taken to slide (ovary was bisected before embedding; three levels were sectioned approximately 100 µm apart yielding five levels) for possible ovarian follicle evaluation in the event that histopathological findings were attributed to treatment. Since no histopathological findings were attributed to treatment in the highest dose group, the other dose levels were not evaluated. In addition, the oestrous cycle stage was determined for all females by histopathological evaluation of the reproductive organs (ovaries, uterus, cervix and/or vagina).

Pups

The F1 and F2 pups not culled on LD 4 were maintained with the dam until weaning on LD 21. On lactation Day 21, a sufficient number of F1 pups/sex/litter were maintained to produce the next generation. F1 pups not selected to become parents of the next generation and all F2 pups were sacrificed, examined macroscopically and had organs weighed. One pup/sex/litter for each generation had tissues collected and evaluated for any structural abnormalities or pathological changes, particularly as they may relate to the organs of the reproductive system.

Pups past PND 21, that were not retained to become F1 parents, were subjected to a gross necropsy. Gross lesions were collected with representative tissues from control group for comparison. Histopathology was not performed on gross lesions as none were considered to be test substance-related. Pups found dead underwent a gross necropsy for possible defects and/or to determine the cause of death. A lung flotation test was performed on all PND 0 pups found dead for stillborn determination.

Data analyses:

Statistics:

The data were analysed using applications provided by Provantis™ Software, Version 8.4.1.0. (Instem LSS, Stone, Staffordshire, United Kingdom), SAS (SAS Institute Inc., Version 6.09 Enhanced, Cary, North Carolina), TASC (Toxicology Analysis Systems Customized, 1993, Scientific Computer Consultants, New Jersey), or Excel (Microsoft Office Excel, Version 11, USA 2007, Windows).

Parametric data (including body weight gain and food consumption) were analysed using a univariate Analysis of Variance (ANOVA), and when significant differences were observed, a Dunnett's Test was performed. Nonparametric data (e.g., number of oestrous cycles, litter size, and number of implantation sites) were first analysed by the Kruskal-Wallis Test and then subjected to Dunn's Test when significant differences were identified. Nonparametric dichotomous data (e.g., fertility and gestation indices) were initially analysed by the Chi Square Test and when significance was observed between groups then by the Fisher's Exact Test with the Bonferroni adjustment. To the extent possible, the frequency of gross lesions was first examined visually, then, in the event of questionable distribution, by statistical analysis using the Chi-Square and Fisher's Exact Tests. Differences between the control and test substance-treated groups were considered statistically significant when $p < 0.05$ or $p < 0.01$.

As a general rule for data collected in TASC, the following outlines the statistical analysis performed:

- For data reported as N/%: Fisher Exact test was used
- For data reported as Mean: ANOVA and when significant then Dunnett's test was used
- For data reported as Mean% or Median: Kruskall Wallis and when significant then Dunn's test was used.

For Provantis, the test substance-treated dose groups were compared to the vehicle control group. Mean and standard deviations were calculated for all quantitative data. Continuous group mean data (e.g. organ weights) that were examined statistically were evaluated for equality or homogeneity of variance using the Decision Tree statistical structure (described in Provantis™ Statistical Users Guide, Software Version 8.0.1.6).

The Decision Tree statistical structure includes analysis of variance (ANOVA) and covariance (ACOVA), nonparametric analysis of variance, pair wise tests by the Dunnett's Test for parametric and nonparametric data, simple t-tests, and the Bartlett's Test for homogeneity of variance. When appropriate, the data were analysed for a dose-related trend using the Williams Test (parametric data) or the Shirley Test (nonparametric data). Nonhomogenous data were analysed using a stepwise Dunnett's Test (parametric data) or a modified Steel Test (nonparametric data). Frequency data (gross pathology observations) were examined statistically using the Chi-Square and/or Fisher's Exact Tests. In general, statistical tests were performed as two-sided tests with results taken as significant with probability (p) levels of < 0.05 or < 0.01, with the exception of trend tests (Williams and Shirley), when only the top dose was analysed using a two-sided test.

For the purpose of data interpretation, statistical significance was not automatically considered to imply toxicological significance. Conversely, the absence of a statistically significant comparison was not considered to imply the lack of a biologically important effect.

Indices:

Reproductive indices:

The following reproductive indices were calculated from breeding and parturition records of animals in the study:

$$\text{Mating Index (\%)} = \frac{\text{\# of inseminated females}^{a}}{\text{\# of females co-housed}} \times 100$$

$$\text{Fertility Index (\%)} = \frac{\text{\# of pregnant females}^{b}}{} \times 100$$

$$\text{Gestation Index (\%)} = \frac{\text{\# of females with live pups}}{\text{\# of inseminated females}} \times 100$$

$$\text{Post Implantation Loss (\%)} = \frac{\text{\# of implantation sites - \# of pups born}}{\text{\# of implantation sites}} \times 100$$

[a] Includes pregnant females not observed sperm positive or with an internal vaginal plug
[b] Includes females, which did not deliver, but had implantation sites

Offspring Viability Indices:

The following viability indices were calculated from lactation records of litters in the study:

$$\text{Birth Index (\%)} = \frac{\text{total \# of pups born per litter}}{\text{total \# of implantation sites per litter}} \times 100$$

$$\text{Livebirth Index (\%)} = \frac{\text{\# of live pups born per litter}}{\text{total \# of pups per litter}} \times 100$$

$$\text{Viability Index 1 (\%)} = \frac{\text{\# of live pups per litter on Day 4 (pre-culling)}}{\text{\# of live pups born per litter}} \times 100$$

$$\text{Viability Index 2 (\%)} = \frac{\text{\# of live pups per litter on Day 7}}{\text{\# of live pups per litter on Day 4 (post-culling)}} \times 100$$

$$\text{Viability Index 3 (\%)} = \frac{\text{\# of live pups per litter on Day 14}}{\text{\# of live pups per litter on Day 7}} \times 100$$

$$\text{Viability Index 4 (\%)} = \frac{\text{\# of live pups per litter on Day 21}}{\text{\# of live pups per litter on Day 14}} \times 100$$

$$\text{Lactation Index (\%)} = \frac{\text{\# of live pups per litter on Day 21}}{\text{\# of live pups per litter on Day 4 (post-culling)}} \times 100$$

$$\text{Cumulative Survival (\%)} = \frac{\text{\# pups Day 21}}{\text{\# pups Day 4 (post-culling)}} \times \frac{\text{\# pups Day 4 (pre-culling)}}{\text{\# pups born}} \times 100$$

Gestation Length = Number of whole days from day on which insemination was observed in the vaginal smear (designated Day 0 of gestation) to Lactation Day (LD) 0 (delivery of pups and entry in computer system).

Historical control data:

Historical control data were obtained from reproduction studies performed in this laboratory in the Wistar rat. Historical control data is provided in this summary where required to contextualise a finding.

RESULTS

Dose Concentration Analysis

There was no quantifiable peak present in 0 mg/mL (vehicle control) sample. The samples were prepared at four concentrations (0, 10, 30, and 100 mg/mL) of CA3511 in 1 % (w/v) aqueous carboxymethylcellulose.

The dose formulations ranged from 89% to 114% of expected concentrations for all samples.

Results of the dose formulation analysis conducted during the first week of both pre-mating periods (P-gen and F1-gen) and once, each, during P-gen and F1-gen gestation and lactation periods (total of six dose

formulation analysis) revealed these formulations met the acceptance criteria for study use. Based on these analyses, animals received the intended dose. The assay results demonstrate that the samples conform to the concentration and homogeneity requirements of ±15 % of target concentration and a %RSD of ≤10.0%.

Mortality and Clinical Signs–Parental Animals

There were no test substance-related mortalities during the course of this study at any dose level.

One 1000 mg/kg/day F1-generation female (BZ3603) was removed from the study on LD 5 due to all pups found dead or missing.

P-generation and F1-generation Males: There were no test substance-related clinical observations during the course of the study.

P-generation Females: There were no test substance-related clinical observations during the course of the study.

F1-generation Females: There were no test substance-related clinical observations during the course of the study. There was a statistical increase in hair thinning and scab formation in mid and high-dose females during the premating period. These findings are not thought to be due to the test substance since generally there was no dose relationship (incidence of hair thinning was greater at the mid-dose and scab formation was generally seen in equal number of mid- and high-dose animals) and this was not seen at any other time point in this study or in males.

Body Weight and Food Consumption–Parental Animals

Males

P-generation: There were no test substance-related effects on body weight, body weight gain, or food consumption at any dose level.

F1-generation: There were no test substance-related effects on body weight, body weight gain, or food consumption at any dose level. There were occasional statistical increases in food consumption on various occasions (g/animal/day: Days 14–21, 28–35, 42–49, 63–70) in males given 1000 mg/kg/day. These few differences from control in food consumption were most likely due to normal variability since they were generally transient.

Females (pre-mating)

P-generation: There were no test substance-related effects on body weight, body weight gain, or food consumption at any dose level during the 10-week pre-mating period.

F1-generation: There were no test substance-related effects on body weight, body weight gain, or food consumption at any dose level during the 10-week pre-mating period.

Gestation

P-generation: There were no test substance-related effects on body weight, body weight gain, or food consumption during gestation at any dose level tested.

F1-generation: There were no test substance-related effects on body weight, body weight gain, or food consumption during gestation at any dose level tested.

Lactation

P-generation: There were no test substance-related effects on body weight or food consumption during lactation at any dose level tested.

F1-generation: There were no test substance-related effects on body weight or food consumption during lactation at any dose level tested. There was a statistical increase in body weights on LD 21 in 1000 mg/kg/day females which was likely due to biological variability since the increase was slight (5%, compared to concurrent controls) and occurred on a single occasion.

Reproductive function:

Oestrous cycle length and periodicity

P-generation: There were no test substance-related effects on oestrous cycle length or periodicity at any dose level tested.

F1-generation: There were no test substance-related effects on oestrous cycle length or periodicity at any dose level tested.

Sperm analysis and evaluation

P-generation: There were no test substance-related effects on sperm motility (% motile or progressive), counts (testis or epididymis), or morphology at any dose level. Sperm counts (testis) were statistically increased (37.0 sperm/gram vs. 32.7 sperm/gram for controls) in 1000 mg/kg/day P-generation males. This increase, compared with the controls, is considered to be incidental and due to several control males (BZ0004, BZ0021, and BZ0030) with lower values for these measurements. See the table below for historical control sperm count (testes) measures.

Table A 25: Historical Control – Sperm Analysis (P Generation)

Study Number	Sperm Count (Testes) – Sperm/Gram
07-R72-IH	30.7
07-R72-MK	37.3
08-R72-MQ	30.9
08-R72-MX	39.8
08-R72-OB	34.6
Range:	30.7-39.8

F1-generation: There were no test substance-related effects on sperm motility (% motile or progressive), counts (testis or epididymis), or morphology at any dose level.

Reproductive Performance

P-generation: There was no test substance-related effect on any reproductive parameter (e.g., mating, fertility, or gestation indices, days to insemination, gestation length, or the median number of implants) at any dose level tested. There was a slight decrease (73.3% vs. 90.0% for controls) in the fertility index in 1000 mg/kg/day females, compared to controls, but this was likely attributed to biological variability since this was not seen in the F1-generation females, there were no other effects on reproduction performance or on sperm analysis, and was only slightly outside the range of historical control. See the table below for historical Fertility Index at this facility.

Table A 26: Summary of Reproduction data (P-generation/F1-pups)

P-generation/F1-pups					
		Dose group (mg/kg/day)			
		0	100	300	1000
No. of Animals Cohoused		30	30	30	30
No. of Animals mated		30	30	30	30
No. of Animals Delivered		27	26	26	22
No. of Animals with Implants		27	26	26	22
Mating Index		100.0	100.0	100.0	100.0
Fertility Index		90.0	86.7	86.7	73.3
Gestation Index		100.0	100.0	100.0	100.0
Number of Oestrous Cycles	Mean	2.7	2.5	2.6	2.4
	S.E.	0.2	0.1	0.1	0.1
	Range	1.0-4.0	1.0-4.0	1.0-4.0	1.0-4.0
Oestrous Cycle Length	Mean	5.1	5.6	5.4	5.3
	S.E.	0.2	0.4	0.2	0.2
	Range	4.0-8. 0	4.0-13.0	4.0-10.0	4.0-8.5
Day to Insemination	Mean	3.0	2.4	2.7	2.4
	S.E.	0.29	0.45	0.43	0.21
	Median	2.5	2.0	2.0	2.0
	Range	1.0 – 6.0	1.0 – 14.0	1.0 – 14.0	1.0 – 4.0
Gestation Length (days):	Mean	22.1	22.0	22.1	22.0
	S.E.	0.14	0.09	0.11	0.14
	Median	22.0	22.0	22.0	22.0
	Range	21.0-24.0	21.0 – 23.0	21.0-24.0	21.0 – 23.0
Total No. of Implan-tations		315	288	289	241
	Mean	11.7	11.1	11.1	11.0
	S.E.	0.50	0.51	0.60	0.47
	Median	13.0	11.5	11.0	11.0
	Range	5.0 – 16.0	6.0 – 15.0	3.0 – 16.0	7.0 – 16.0

Table A 27: Historical Control – Fertility Index (P Generation)

Study Number	**Fertility Index (%)**
03-R72-PT	86.7
04-R72-SJ	90.0
06-R72-DI	93.3
06-R72-DX	100
06-R72-GY	96.6
06-R72-HW	76.7
07-R72-IH	93.3
07-R72-MK	100
08-R72-MQ	92.9
08-R72-MX	93.3
08-R72-OB	100
Range:	76.7-100

F1-generation: There was no test substance-related effect on any reproductive parameter (e.g., mating, fertility, or gestation indices, days to insemination, gestation length, or the median number of implants) at any dose level tested.

Table A 28: Summary of Reproduction data (F1-adults/F2-pups)

F1-adults/F2-pups					
		Dose group (mg/kg/day)			
		0	100	300	1000
No. of Animals Cohoused		30	30	30	30
No. of Animals mated		29	30	30	30
No. of Animals Delivered		29	28	28	28
No. of Animals with Implants		29	28	28	28
Mating Index		96.7	100.0	100.0	100.0
Fertility Index		100.0	93.3	93.3	96.7
Gestation Index		100.0	100.0	100.0	96.6
Number of Oestrous Cycles	Mean	2.7	2.9	2.9	2.7
	S.E.	0.2	0.1	0.2	0.2
	Range	1.0 - 4.0	1.0 - 4.0	1.0 - 4.0	1.0 - 4.0
Oestrous Cycle Length	Mean	4.9	4.9	4.9	4.6
	S.E.	0.2	0.2	0.2	0.2
	Range	4.0-8.0	4.0-8.5	4.0-6.5	4.0-7.0
Day to Insemination	Mean	2.6	3.3	3.1	2.6
	S.E.	0.24	0.42	0.41	0.39
	Median	3.0	3.0	2.5	2.0
	Range	1.0 – 5.0	1.0 – 13.0	1.0 – 12.0	1.0 – 12.0
Gestation Length (days):	Mean	21.9	22.0	21.8	21.9
	S.E.	0.10	0.08	0.09	0.12
	Median	22.0	22.0	22.0	22.0
	Range	21.0 – 23.0	21.0 – 23.0	21.0 – 23.0	21.0 – 23.0
Total No. of Implan-tations		279	299	294	284
	Mean	9.6	10.7	10.5	10.1
	S.E.	0.28	0.49	0.34	0.37
	Median	10.0	11.0	10.0	10.5
	Range	6.0 – 13.0	3.0 – 16.0	7.0 – 14.0	4.0 – 14.0

Table A 29: Summary of Litter Data (P-generation/F1-pups)

P-generation/F1-pups					
		Dose group (mg/kg)			
		Control	100	300	1000
No of litters		27	26	26	22
Total No. of Pups Born		306	275	276	232
Total No. of Pups Missing		1	2	1	2
Litters with Pups Missing		1	1	1	2
Total No. of Pups Found Dead		0	2	4	0
Litters with Pups Found Dead		0	2	4	0
Total No. of Pups Cannibalized		1	0	3	1
Litters with Pups Cannibalized		1	0	2	1
Litter Size:	Mean	11.3	10.6	10.6	10.5
	S.E.	0.47	0.56	0.64	0.45
	Median	11.0	11.0	11.0	10.0
	Range	5.0 – 16.0	5.0 – 15.0	3.0 – 16.0	7.0 – 14.0
Mean Weight Viable Pups (g):	Birth	6.0	5.9	5.9	6.2
	Day 4 (precull)	9.8	9.5	9.9	10.0
	Day 4 (post cull)	9.8	9.5	9.9	10.1
	Day 7	16.1	15.6	15.8	15.8
	Day 14	31.8	30.8	32.2	31.0
	Day 21	48.8	47.5	49.5	48.2
	Gain	42.9	41.6	43.6	42.1
Sex Distribution at Birth (% Males)	Mean	46.4	48.7	48.1	47.8
	S.E.	3.15	2.37	3.53	2.63
	Median	45.5	50.0	50.0	47.7
	Range	10.0 – 77.8	25.0 – 70.0	0.0 – 75.0	28.6 – 77.8
Stillborn Pups:	Number	5	0	1	1
	%	1.6	0.0	0.4	0.4
	Mean	0.2	0.0	0.0	0.0
	S.E.	0.12	0.00	0.04	0.05
	Median	0.0	0.0	0.0	0.0
	Range	0.0 – 3.0	0.0 – 0.0	0.0 – 1.0	0.0 – 1.0
Mean No. of Viable Pups	Birth	11	11	11	11
	Day 4 (precull)	11	10	11	10
	Day 4 (post cull)	8	8	7	8
	Day 21	8	8	7	8
Live Birth Index	Mean	97.6	100.0	99.7	99.5
	S.E.	1.43	0.00	0.26	0.51
	Median	100.0	100.0	100.0	100.0
	Range	64 – 100	100 – 100	93 - 100	89 – 100
Viability Index	Mean	99.4	99.0	99.2	99.7
	S.E.	0.42	0.81	0.77	0.32
	Median	100.0	100.0	100.0	100.0
	Range	90.9 – 100	80.0 – 100	80.0 – 100	92.9 – 100
Lactation Index	Mean	100.0	99.5	97.6	99.4
	S.E.	0.00	0.48	1.96	0.57
	Median	100.0	100.0	100.0	100.0
	Range	100-100	88-100	50-100	88-100
Birth Index	Mean	97.1	95.0	95.7	96.4
	S.E.	1.11	1.92	2.33	1.37
	Median	100.0	100.0	100.0	100.0
	Range	81.8-100	60.0-100	45.5-100	80.0-100

All statistical comparisons were done using the dam as the unit of comparison.

Table A 30: Summary of Litter Data (F1-adults/F2-pups)

F1-adults/F2-pups					
		Dose group (mg/kg)			
		Control	100	300	1000
No of litters		29	28	28	28
Total No. of Pups Born		271	286	288	277
Total No. of Pups Missing		1	2	2	0
Litters with Pups Missing		1	2	2	0
Total No. of Pups Found Dead		0	0	2	0
Litters with Pups Found Dead		0	0	2	0
Total No. of Pups Cannibalized		0	0	0	0
Litters with Pups Cannibalized		0	0	0	0
Litter Size:	Mean	9.3	10.2	10.3	9.9
	S.E.	0.28	0.48	0.33	0.37
	Median	9.0	10.5	10.0	10.0
	Range	6.0 – 12.0	3.0-15.0	7.0-13.0	4.0-14.0
Mean Weight Viable Pups (g):	Birth	6.0	6.0	6.0	6.1
	Day 4 (precull)	10.1	10.1	9.9	10.0
	Day 4 (post cull)	10.1	10.1	9.9	10.0
	Day 7	15.5	15.8	15.4	15.6
	Day 14	30.4	30.9	30.4	30.7
	Day 21	47.0	47.5	47.1	47.4
	Gain	41.0	41.5	41.1	41.3
Sex Distribution at Birth (% Males)	Mean	58.6	54.0	49.3	51.8
	S.E.	3.17	2.91	2.91	2.81
	Median	60.0	54.2	50.0	55.6
	Range	22.2-89	20.0-100	12.5-80	0.0-75
Stillborn Pups:	Number	0	1	0	4
	%	0.0	0.3	0.0	1.4
	Mean	0.0	0.0	0.0	0.1
	S.E.	0.00	0.04	0.00	0.11
	Median	0.0	0.0	0.0	0.0
	Range	0.0-0.0	0.0-1.0	0.0-0.0	0.0-3.0
Mean No. of Viable Pups	Birth	9	10	10	10
	Day 4 (precull)	9	10	10	10
	Day 4 (post cull)	8	8	8	8
	Day 21	8	8	8	8
Live Birth Index	Mean	100.0	99.7	100.0	97.9
	S.E.	0.00	0.32	0.00	1.46
	Median	100.0	100.0	100.0	100.0
	Range	100-100	91-100	100-100	67-100
Viability Index	Mean	99.6	99.2	98.4	100.0
	S.E.	0.38	0.52	0.87	0.00
	Median	100.0	100.0	100.0	100.0
	Range	89-100	89-100	83-100	100-100
Lactation Index	Mean	99.1	100.0	99.6	100.0
	S.E.	0.94	0.00	0.40	0.00
	Median	100.0	100.0	100.0	100.0
	Range	73-100	100-100	89-100	100-100
Birth Index	Mean	97.2	95.8	98.0	97.6
	S.E.	0.99	1.45	0.74	0.93
	Median	100.0	100.0	100.0	100.0
	Range	81.8 - 100	71.4 - 100	88.9 - 100	81.8 - 100

All statistical comparisons were done using the dam as the unit of comparison.

Parental Post Mortem Results

Terminal body weight and organ weights (P- and F1-generation adults)

P-generation: Terminal body weights were not different from control in at any dose level in either sex. Test substance-related nonadverse organ weight changes were noted in kidneys (increase; absolute and relative) at 1000 mg/kg/day males. When compared with the vehicle controls, there were statistical increases (5%

and 8%) in absolute and relative left kidney weights, respectively, in 1000 mg/kg/day males. Also, there were 5% and 8% non-statistical increases in absolute and relative right kidney weights, respectively, in 1000 mg/kg/day males. There were no notable changes in kidney weights in females. There were no corresponding histopathology changes in the kidneys of males or females at 1000 mg/kg/day.

F1-Generation: Terminal body weights were not different from control in at any dose level in either sex. Test substance-related nonadverse organ weight changes were noted in kidneys (statistically significant increase) in 1000 mg/kg/day males and females when compared with the vehicle controls. There were statistical increases in absolute (9% and 8%) and relative (8% and 6%) kidney (left and right) weights, respectively, in 1000 mg/kg/day males. In addition, there were statistical increases (6% and 5%) in absolute kidney (left and right) weights, respectively, in 1000 mg/kg/day females. There were no corresponding histopathology changes in the kidneys of males or females at 1000 mg/kg/day.

Macroscopic examination (P- and F1-generation Adults)

There were no test substance-related gross necropsy findings observed at any dose level for both the P- and F1-generation adults. All gross lesions for P- and F1-generation adults were considered to be incidental/background and not test substance-related.

Microscopic examination (P- and F1-generation Adults)

There were no test substance-related histopathology changes noted in P- and F1-generation males or females. All microscopic changes observed in the P- and F1-generation adults were considered to be incidental and/or background seen in rats of this strain and age. The oestrous cycle stage was determined for all females by microscopic evaluation of the reproductive organs (ovaries, uterus, cervix and/or vagina). There were no notable test substance-related findings in the oestrous cycle staging in any of the treatment groups as compared to the vehicle control group.

Ovarian follicle counts (F1-Generation Females)

None of the mean primordial follicles, small follicle, or growing follicle counts for F1-generation adult females were statistically different from vehicle controls nor were the differences from vehicle controls sufficiently large enough to be considered biologically significant. Ovarian follicular counts; therefore, were not affected by test substance administration.

Offspring

Viability and clinical signs

P-generation (pre-weaning): There were no test substance-related effects observed on the viability of the pups at any dose level. No test substance-related clinical observations were observed at any dose level.

P-generation (post-weaning): No test substance-related clinical observations were observed at any dose level. F1-generation (pre-weaning): There were no test substance-related effects observed on the viability of the pups at any dose level. No test substance-related clinical observations were observed at any dose level.

Pup body weight (PND 0-21)

P-generation: There were no test substance-related effects on pup body weight or body weight gain at any dose level in either sex.

F1-generation: There were no test substance-related effects on pup body weight or body weight gain at any dose level in either sex.

Pup body weight (PND 21-77)

F1-generation: There were no test substance-related effects on pup body weight at any dose level in either sex.

Sexual maturation

There were no effects observed on either vaginal patency or preputial separation for the F1 pups at any dose

level. There were no test substance-related effects observed on body weight at passing either preputial separation or vaginal patency. There were a few instances where there was a statistically higher incidence of females from the 300 mg/kg/day dose level passing vaginal patency (Days 33, 37, 39). In addition, the average age of onset for this group of females was statistically lower (32.5 vs. 36.8 for controls). These differences from control are not considered related to the test substance but rather normal biological variability, since these differences from control were not seen in females from the 1000 mg/kg/day dose level.

Anogenital distance

Anogenital distance measurements were not deemed necessary in this study.

PND 21 pup terminal body weight and organ weights (F1- and F2-generation)

There were no test substance-related effects on final body weights or on organ weights of F1-generation male and female pups from P-generation adults and F2-generation male or female pups from F1-generation adults.

PND 21 macroscopic examination (F1- and F2-generation)

There were no test substance-related gross necropsy findings observed at any dose level. All gross findings for F1-and F2- generation pups were considered to be incidental and not test substance-related.

PND 21 microscopic examination (F1- and F2-generation)

Microscopic evaluation of brain, spleen, thymus, ovaries and testes from PND 21 pups (F1-and F2-generations) was not deemed necessary, since, there were no test substance-related changes noted in P- or F1-generation adults.

CONCLUSION:

Oral (gavage) administration of CA3511 to Crl:WI(Han) rats at dose levels of 100, 300 and 1000 mg/kg/day, continuously for two successive generations, was well tolerated.

There were no effects on mortality, clinical observations, body weights, food consumption, reproductive function or performance, mating behaviour, conception, or pup development. In addition, there were no macroscopic findings or histopathology changes related to the test substance at any dose level in either adults or offspring.

Test substance-related non-adverse findings were limited to increased kidney weights in P-generation males and in F1-generation adult males and females from the 1000 mg/kg/day dose level.

The No Observed Adverse Effect Level (NOAEL) for both systemic toxicity and reproductive parameters was considered to be 1000 mg/kg/day, respectively for both males and females and for their offspring.

REFERENCES

Gilmore R.G. (2015). CA3511 – Pilot One-Generation Reproduction Toxicity Study in the Rat. Xenometrics LLC, Report No. 11069, Unpublished.

(Gilmore R, 2016)

Comments of zRMS:	Reviewer has not identified any limitations and deviations from TG according to recent guidelines (OECD 474). Study has been accepted; Considering discussion during EFSA PPRM 134 meeting on the mesotrione metabolites MNBA and AMBA: (..) *structure comparison between **AMBA** and **MNBA**, the reduction of MNBA to AMBA and the fact that MNBA was extensively investigated for genotoxicity and found non-genotoxic, it is not very likely that AMBA would indeed prove positive for mutagenicity* (..) and taking into account newly provided data, zRMS PL is of the opinion that in the following study [Dunton J, 2016] it has been shown that the metabolite AMBA reaches the bone marrow. Genotoxicity potential of AMBA has been clarified; AMBA to be neither clastogenic nor aneugenic in the rat bone marrow micronucleus assay.

Reference:	KCA2 5.8.1/03
Report	AMBA – Oral (Gavage) Rat Micronucleus Test. xxxxxxxxxxxxxx 2016 BFI0493 R044276_10010
Guideline(s):	Rat bone marrow micronucleus test OECD 474 (2014)
Deviations:	No
GLP:	Yes
Acceptability:	Yes / No / Supplementary
Duplication (if vertebrate study)	No

EXECUTIVE SUMMARY

AMBA was tested to evaluate its potential to cause damage to chromosomes or cell division apparatus, or to cause cell cycle interference, leading to micronucleus formation in polychromatic erythrocytes in the bone marrow of young adult rats.

In all phases, the dosing of the vehicle and test item was by oral (gavage) administration twice, approximately 24 hours apart.

In the range-finding phase, a group of three male and three female rats was given AMBA at 2000 mg/kg/day, in order to select the highest dose level of AMBA that did not produce mortality or severe signs of clinical toxicity up to the maximum tolerated dose (MTD) or limit-dose level. The MTD was confirmed to be greater than the limit dose of 2000 mg/kg/day in male and female rats, and as there was no inter-sex difference in toxicity, the main study was conducted in males only.

Proof of exposure was conducted as part of the range-finding phase to demonstrate that the bone marrow was exposed to the test item, via LC-MS/MS analysis of test item in the whole blood of animals given AMBA. The presence of AMBA was confirmed by analysis of the study samples using a validated method.

For the main study phase, three groups, each of six male rats were dosed with 500, 1000 or 2000 mg/kg/day AMBA. A group of six male rats (negative Controls) was dosed with the vehicle alone and a positive Control group, also of six male rats, was given a single 15 mg/kg oral (gavage) dose of Cyclophosphamide monohydrate (CPA).

Bone marrow was harvested from all range-finding and main study animals approximately 24 hours after the final dose administration and smears were prepared. The stained slides prepared for the main study were coded and 6000 polychromatic erythrocytes (PCE) per animal were scored for the presence of micronuclei and the group frequencies were statistically analysed.

There were no statistically significant increases in micronucleus frequency in male rats treated at any dose level of AMBA, compared with the negative Control group.

There was no evidence of a statistically significant reduction in the PCE/NCE ratio in male rats treated with

AMBA and, since proof of exposure to the bone marrow was demonstrated in the range finding phase of the study, this indicated a lack of toxicity of AMBA to the bone marrow.

The animals dosed with CPA, the positive Control item, had statistically significant increases in the number of micronucleated cells compared with the concurrent Control group, which demonstrated that the test system was capable of detecting a known clastogen and that the scorers were capable of detecting micronuclei. There was no statistically significant decrease in the PCE/NCE ratio in the positive Control group, indicating a lack of toxicity to the bone marrow.

In conclusion, it can be stated that there was no evidence of clastogenicity or aneugenicity following oral (gavage) administration of AMBA up to the OECD 474 limit dose of 2000 mg/kg/day in male rats. AMBA is considered to be neither clastogenic nor aneugenic in the rat bone marrow micronucleus assay.

MATERIALS AND METHODS

Materials:

Test Material:	AMBA
Description:	Light yellow powder
Lot/Batch number:	924777
Purity:	98.6 % ± 0.5 % (w/w)
Stability of test compound:	Retest date : 31 March 2019

Control Materials:

Negative control (if not vehicle) :	N/A	**Final Volume:** N/A	**Route:** N/A
Vehicle:	1.0 % (w/v) carboxymethylcellulose with 0.1 % (v/v) Tween 80	**Final Volume:** 10 mL/kg	**Route:** oral
Positive control :	Cyclophosphamide monohydrate	**Final Doses:** 15 mg/kg	**Route:** oral

Test Animals:

Species	Rats
Strain	Crl:WI(Han)
Age/weight at dosing	8 – 9 weeks (at start of experiment); Main study: range 249 g to 291 g mean weight 268 g
Source	Charles River (UK) Ltd., Margate, Kent, CT9 4LT, England
Housing	3/cage
Acclimatisation period	At least 5 days
Diet	Pelleted standard diet, *ad libitum*
Water	Tap water, *ad libitum*
Environmental conditions	Temperature: 19-21 °C Humidity: 45 % to 49 % Photoperiod: 12 hours dark/12 hours light

Test compound administration:

	Dose Levels	Final Volume	Route
Preliminary:	Range-finding phase: 2000 mg/kg/day	10 mL/kg b.w.	oral
Main Study:	500, 1000, 2000 mg/kg/day males only	10 mL/kg b.w.	oral

Study Design and Methods:

Study initiation date: 21 March 2016 (study plan issued).
Experimental start date: 24 March 2016 (first animal arrival).
Experimental termination date: 06 June 2016 (last day of slide scoring).

Preliminary Toxicity Assay: Dosing was by oral (gavage) administration twice, separated by approximately 24 hours. Range-finding animals were observed periodically for up to 24 hours after the second dose.

Since bone marrow is well perfused, exposure of the bone marrow to the test item was indirectly assessed by collection of blood and analysis for AMBA. Blood samples were obtained via the lateral tail vein from all animals in the range-finding phase at 1 hour and 4 hours after the second dose and at termination of each group. At each collection, 0.1 mL samples were taken into tubes containing K_2EDTA and gently flicked to mix. Immediately following collection of each sample, 0.05 mL of whole blood was accurately measured into a polypropylene tube containing exactly 0.05 mL of deionised water, gently mixed and placed directly onto dry ice and then was stored frozen (≤ -70 °C), before analysis. The concentration of AMBA was determined by analysis of the study samples using a validated bioanalytical method.

Micronucleus Test:

Table A 31: Experimental Design

Group number	Number of animals	Dose level (mg/kg/day) AMBA
1	6	Negative Control
2	6	500
3	6	1000
4	6	2000
5	6	Positive Control CPA 15 mg/kg

Animals in Groups 1 to 4 were dosed twice, approximately 24 hours apart, with vehicle alone (negative control) or AMBA at a dose volume of 10 mL/kg. Group 5 animals (positive Control) were given a single 15 mg/kg dose of CPA at a dose volume of 5 mL/kg.

Animals were observed periodically for up to 24 hours after the first (all groups) and second (Groups 1 to 4) dose.

Slide Preparation: Range-finder animals were killed after the terminal blood sampling, approximately 24 hours after the second administration of the test item. The main study animals in Groups 1 to 4 were killed approximately 24 hours after the second test item or vehicle administration. Group 5 animals were killed approximately 24 hours after the single administration of the positive Control. A single femur was removed from each animal. The bone marrow cells from the femur were aspirated into labelled tubes and centrifuged. The supernatant was withdrawn and the cells were re-suspended in a minimal volume of foetal bovine serum. One drop of cell suspension was placed on each of two slides and spread. All slides were left to air dry and age overnight before fixing for five minutes in methanol. Fixed slides were stained for 20 to

30 minutes in 11.5 % (v/v) Giemsa in Sorensen's buffer pH 6.0.

Slide Analysis: A unique, unambiguous code was devised for each main study animal. Adhesive labels that covered the animal and group identity were affixed to each slide so that the analyst could see only the study number and the new code.
6000 polychromatic erythrocytes (PCE), including micronucleated PCE (MN-PCE), were counted for each main study animal. The numbers of normochromatic erythrocytes (NCE) and micronucleated NCE (MN-NCE) were also recorded for the first 1000 cells scored. Only areas of slides of good technical quality and appropriate staining characteristics were scored.

RESULTS AND DISCUSSION

Preliminary toxicity assay: Range-finding phase: There were no clinical signs observed in either males or females following administration of AMBA at 2000 mg/kg/day. No significant body weight loss was observed.
Based on the results of this phase, the MTD was considered to exceed the limit dose of 2000 mg/kg/day in males and females.
Exposure to AMBA was confirmed in all range-finder blood samples. Bone marrow smears were not analysed in the range-finding phase since the presence of AMBA was confirmed in the blood samples.

Table A 32: Study Sample Concentration Data – AMBA in blood (ng/mL)

Males Animal no	Time (h)	Concentration AMBA (ng/mL)	Female Animal no	Time (h)	Concentration AMBA (ng/mL)
71	1	126000	74	1	155000
72	1	118000	75	1	117000
73	1	109000	76	1	135000
71	4	84100	74	4	53700
72	4	91800	75	4	24600
73	4	79200	76	4	34600
71	Terminal	745	74	Terminal	714
72	Terminal	756	75	Terminal	1780
73	terminal	1180	76	terminal	1590

Micronucleus test: There were no clinical signs observed following administration of AMBA to male rats at dose levels up to 2000 mg/kg/day, nor were there any adverse clinical observations in Group 1 (negative Control) or Group 5 (positive Control) animals. There were no statistically significant increases in micronucleus frequency in male rats given any dose level of AMBA, compared with the negative Control group. There was no evidence of a statistically significant reduction in the PCE/NCE ratio in male rats given AMBA, and, since proof of exposure to the bone marrow was demonstrated in the range finding phase of the study, this indicated a lack of toxicity of AMBA to the bone marrow. The animals dosed with CPA, the positive Control item, had statistically significant increases in the number of micronucleated cells compared with the concurrent Control group, which demonstrated that the test system was capable of detecting a known clastogen and that the scorers were capable of detecting micronuclei. There was no statistically significant decrease in the PCE/NCE ratio in the positive Control group, indicating a lack of toxicity to the bone marrow.

CONCLUSION

There was no evidence of clastogenicity or aneugenicity following oral (gavage) administration of AMBA up to the OECD 474 limit dose of 2000 mg/kg/day in male rats. AMBA is considered to be neither clastogenic nor aneugenic in the rat bone marrow micronucleus assay.

(Dunton, J. 2016)

Appendix 3 Exposure calculations

A 3.1 Operator exposure calculations (KCP 7.2.1.1)

A 3.1.1 Calculations for dicamba

Table A 33: Input parameters considered for the estimation of operator exposure (EFSA Guidance) (LCTM; no PPE)

Substance	dicamba	Formulation = Wettable granules, soluble granules	Application rate-0.1875 kg a.s. /ha	Spray dilution = 2.34375 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of
Scenario	Cereals / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 1, Application interval = 365 days
Percentage Absoprtion	Dermal for product = 2	Dermal for in use diluation = 75	Oral = 100	Inhalation = 100	
RVNAS	0.3 mg/kg bw/day		RVAAS	mg/kg bw/day	
DFR	3 µg a.s./cm2 per kg a.s./ha		DT50	30 days	

Table A 34: Estimation of operator exposure towards dicamba using the EFSA Guidance (LCTM; no PPE)

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.0333	% of RVNAS	11.09%
	Acute systemic exposure mg/kg bw/day		0.2262	% of RVAAS	
Mixing and Loading		Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application		Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure mg/kg bw/day		0.0219	% of RVNAS	7.29%
	Acute systemic exposure mg/kg bw/day		0.1667	% of RVAAS	

A 3.1.2 Calculations for mesotrione

Table A 35: Input parameters considered for the estimation of operator exposure (EFSA Guidance) (without the use of adjuvant; LCTM; no PPE)

Substance	mesotrione	Formulation = Wettable granules, soluble granules	Application rate-0.09 kg a.s. /ha	Spray dilution = 1.125 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of
Scenario	Cereals / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 1, Application interval = 365 days
Percentage Absoprtion	Dermal for product = 0.2	Dermal for in use diluation = 0.8	Oral = 50	Inhalation = 100	
RVNAS	0.005 mg/kg bw/day		RVAAS	mg/kg bw/day	
DFR	3 µg a.s./cm2 per kg a.s./ha		DT50	30 days	

Table A 36: Estimation of operator exposure towards mesotrione using the EFSA Guidance (without the use of adjuvant; LCTM; no PPE)

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.0014	% of RVNAS	28.20%
	Acute systemic exposure mg/kg bw/day		0.0073	% of RVAAS	
Mixing and Loading		Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application		Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure mg/kg bw/day		0.0012	% of RVNAS	24.91%
	Acute systemic exposure mg/kg bw/day		0.0062	% of RVAAS	

Table A 37: Input parameters considered for the estimation of operator exposure (EFSA Guidance) (with the use of adjuvant; LCTM; no PPE)

Substance	mesotrione (150g/kg)	Formulation = Wettable granules, soluble granules	Application rate-0.09 kg a.s. /ha	Spray dilution = 1.125 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of
Scenario	Cereals / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 1, Application interval = 365 days
Percentage Absoprtion	Dermal for product = 0.2	Dermal for in use diluation = 1	Oral = 50	Inhalation = 100	
RVNAS	0.005 mg/kg bw/day		RVAAS	mg/kg bw/day	
DFR	3 µg a.s./cm2 per kg a.s./ha		DT50	30 days	

Table A 38: Estimation of operator exposure towards mesotrione using the EFSA Guidance (with the use of adjuvant; LCTM; no PPE)

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.0014	% of RVNAS	28.91%
	Acute systemic exposure mg/kg bw/day		0.0076	% of RVAAS	
Mixing and Loading		Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application		Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure mg/kg bw/day		0.0013	% of RVNAS	25.37%
	Acute systemic exposure mg/kg bw/day		0.0065	% of RVAAS	

A 3.1.3 Calculations for nicosulfuron

Table A 39: Input parameters considered for the estimation of operator exposure (EFSA Guidance) (LCTM; no PPE)

Substance	nicosulfuron	Formulation = Wettable granules, soluble granules	Application rate-0.06 kg a.s. /ha	Spray dilution = 0.75 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of
Scenario	Cereals / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 1, Application interval = 365 days
Percentage Absoprtion	Dermal for product = 25	Dermal for in use diluation = 75	Oral = 40	Inhalation = 100	
RVNAS	0.8 mg/kg bw/day		RVAAS	mg/kg bw/day	
DFR	3 µg a.s./cm2 per kg a.s./ha		DT50	30 days	

Table A 40: Estimation of operator exposure towards nicosulfuron using the EFSA Guidance (LCTM; no PPE)

Operator Model		Mixing, loading and application AOEM			
Potential exposure	Longer term systemic exposure mg/kg bw/day		0.0337	% of RVNAS	4.21%
	Acute systemic exposure mg/kg bw/day		0.2396	% of RVAAS	
Mixing and Loading		Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Soluble bags = No
Application		Gloves = No	Clothing = Work wear - arms, body and legs covered	RPE = None	Closed cabin = No
Exposure (including PPE options above)	Longer term systemic exposure mg/kg bw/day		0.0197	% of RVNAS	2.46%
	Acute systemic exposure mg/kg bw/day		0.1324	% of RVAAS	

A 3.2 Worker exposure calculations (KCP 7.2.3.1)

A 3.2.1 Calculations for dicamba

Table A 41: Input parameters considered for the estimation of worker exposure (EFSA Guidance) (Crop inspection)

Substance	dicamba	Formulation = Wettable granules, soluble granules	Application rate-0.1875 kg a.s. /ha	Spray dilution = 2.34375 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of
Scenario	Cereals / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 1, Application interval = 365 days
Percentage Absoprtion	Dermal for product = 2	Dermal for in use diluation = 75	Oral = 100	Inhalation = 100	
RVNAS	0.3 mg/kg bw/day		RVAAS	mg/kg bw/day	
DFR	3 µg a.s./cm2 per kg a.s./ha		DT50	30 days	

Table A 42: Estimation of worker exposure towards dicamba using the EFSA Guidance (Crop inspection)

Worker - Inspection, irrigation	Potential exposure mg/kg bw/day	0.1758	% of RVNAS	58.59%
	Working clothing mg/kg bw/day	0.0197	% of RVNAS	6.56%
	Working clothing and gloves mg/kg bw/day		% of RVNAS	

A 3.2.2 Calculations for mesotrione

Table A 43: Input parameters considered for the estimation of worker exposure (EFSA Guidance) (Crop inspection)

Substance	mesotrione (150g/kg)	Formulation = Wettable granules, soluble granules	Application rate-0.09 kg a.s. /ha	Spray dilution = 1.125 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of
Scenario	Cereals / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 1, Application interval = 365 days
Percentage Absoprtion	Dermal for product = 0.2	Dermal for in use diluation = 1	Oral = 50	Inhalation = 100	
RVNAS	0.005 mg/kg bw/day		RVAAS	mg/kg bw/day	
DFR	3 µg a.s./cm2 per kg a.s./ha		DT50	30 days	

Table A 44: Estimation of worker exposure towards mesotrione using the EFSA Guidance (Crop inspection)

Worker - Inspection, irrigation	Potential exposure mg/kg bw/day	0.0011	% of RVNAS	22.50%
	Working clothing mg/kg bw/day	0.0001	% of RVNAS	2.52%
	Working clothing and gloves mg/kg bw/day		% of RVNAS	

A 3.2.3 Calculations for nicosulfuron

Table A 45: Input parameters considered for the estimation of worker exposure (EFSA Guidance) (Crop inspection)

Substance	nicosulfuron	Formulation = Wettable granules, soluble granules	Application rate-0.06 kg a.s. /ha	Spray dilution = 0.75 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of
Scenario	Cereals / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 1, Application interval = 365 days
Percentage Absoprtion	Dermal for product = 25	Dermal for in use diluation = 75	Oral = 40	Inhalation = 100	
RVNAS	0.8 mg/kg bw/day		RVAAS	mg/kg bw/day	
DFR	3 µg a.s./cm2 per kg a.s./ha		DT50	30 days	

Table A 46: Estimation of worker exposure towards nicosulfuron using the EFSA Guidance (Crop inspection)

Worker - Inspection, irrigation	Potential exposure mg/kg bw/day	0.0563	% of RVNAS	7.03%
	Working clothing mg/kg bw/day	0.0063	% of RVNAS	0.79%
	Working clothing and gloves mg/kg bw/day		% of RVNAS	

A 3.3 Bystander and resident exposure calculations (KCP 7.2.2.1)

A 3.3.1 Calculations for dicamba

Table A 47: Input parameters considered for the estimation of bystander exposure (EFSA Guidance) (LCTM)

At this time, bystander exposure cannot be calculated.

Table A 48: Input parameters considered for the estimation of resident exposure (EFSA Guidance) (LCTM)

Substance	dicamba	Formulation = Wettable granules, soluble granules	Application rate-0.1875 kg a.s. /ha	Spray dilution = 2.34375 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of
Scenario	Cereals / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 1, Application interval = 365 days
Percentage Absoprtion	Dermal for product = 2	Dermal for in use diluation = 75	Oral = 100	Inhalation = 100	
RVNAS	0.3 mg/kg bw/day		RVAAS	mg/kg bw/day	
DFR	3 µg a.s./cm2 per kg a.s./ha		DT50	30 days	

Table A 49: Estimation of resident exposure towards dicamba (EFSA Guidance) (LCTM)

Resident - child	Spray drift (75th percentile) mg/kg bw/day	0.0472	% of RVNAS	15.73%
	Vapour (75th percentile) mg/kg bw/day	0.0011	% of RVNAS	0.36%
	Surface deposits (75th percentile) mg/kg bw/day	0.0022	% of RVNAS	0.73%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0237	% of RVNAS	7.91%
	All pathways (mean) mg/kg bw/day	0.0476	% of RVNAS	15.86%
Resident - adult	Spray drift (75th percentile) mg/kg bw/day	0.0113	% of RVNAS	3.76%
	Vapour (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	0.08%
	Surface deposits (75th percentile) mg/kg bw/day	0.0010	% of RVNAS	0.32%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0132	% of RVNAS	4.39%
	All pathways (mean) mg/kg bw/day	0.0168	% of RVNAS	5.60%

A 3.3.2 Calculations for mesotrione

Table A 50: Input parameters considered for the estimation of bystander exposure (EFSA Guidance) (LCTM)

At this time, bystander exposure cannot be calculated.

Table A 51: Input parameters considered for the estimation of resident exposure (EFSA Guidance) (LCTM)

Substance	mesotrione (150g/kg)	Formulation = Wettable granules, soluble granules	Application rate-0.09 kg a.s. /ha	Spray dilution = 1.125 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of
Scenario	Cereals / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 1, Application interval = 365 days
Percentage Absoprtion	Dermal for product = 0.2	Dermal for in use diluation = 1	Oral = 50	Inhalation = 100	
RVNAS	0.005 mg/kg bw/day		RVAAS	mg/kg bw/day	
DFR	3 µg a.s./cm2 per kg a.s./ha		DT50	30 days	

Table A 52: Estimation of resident exposure towards mesotrione (EFSA Guidance) (LCTM)

Resident - child	Spray drift (75th percentile) mg/kg bw/day	0.0003	% of RVNAS	6.53%
	Vapour (75th percentile) mg/kg bw/day	0.0011	% of RVNAS	21.40%
	Surface deposits (75th percentile) mg/kg bw/day	0.0000	% of RVNAS	0.99%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	3.04%
	All pathways (mean) mg/kg bw/day	0.0014	% of RVNAS	28.25%
Resident - adult	Spray drift (75th percentile) mg/kg bw/day	0.0001	% of RVNAS	1.48%
	Vapour (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	4.60%
	Surface deposits (75th percentile) mg/kg bw/day	0.0000	% of RVNAS	0.12%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0001	% of RVNAS	1.69%
	All pathways (mean) mg/kg bw/day	0.0003	% of RVNAS	6.76%

A 3.3.3 Calculations for nicosulfuron

Table A 53: Input parameters considered for the estimation of bystander exposure (EFSA Guidance) (LCTM)

At this time, bystander exposure cannot be calculated.

Table A 54: Input parameters considered for the estimation of resident exposure (EFSA Guidance) (LCTM)

Substance	nicosulfuron	Formulation = Wettable granules, soluble granules	Application rate-0.06 kg a.s. /ha	Spray dilution = 0.75 g a.s./l	Vapour pressure = low volatile substances having a vapour pressure of
Scenario	Cereals / Outdoor / Downward spraying / Vehicle-mounted			Buffer = 2-3	Number applications = 1, Application interval = 365 days
Percentage Absoprtion	Dermal for product = 25	Dermal for in use diluation = 75	Oral = 40	Inhalation = 100	
RVNAS	0.8 mg/kg bw/day		RVAAS	mg/kg bw/day	
DFR	3 µg a.s./cm2 per kg a.s./ha		DT50	30 days	

Table A 55: Estimation of resident exposure towards nicosulfuron (EFSA Guidance) (LCTM)

Resident - child	Spray drift (75th percentile) mg/kg bw/day	0.0151	% of RVNAS	1.89%
	Vapour (75th percentile) mg/kg bw/day	0.0011	% of RVNAS	0.13%
	Surface deposits (75th percentile) mg/kg bw/day	0.0007	% of RVNAS	0.08%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0076	% of RVNAS	0.95%
	All pathways (mean) mg/kg bw/day	0.0159	% of RVNAS	1.99%
Resident - adult	Spray drift (75th percentile) mg/kg bw/day	0.0036	% of RVNAS	0.45%
	Vapour (75th percentile) mg/kg bw/day	0.0002	% of RVNAS	0.03%
	Surface deposits (75th percentile) mg/kg bw/day	0.0003	% of RVNAS	0.04%
	Entry into treated crops (75th percentile) mg/kg bw/day	0.0042	% of RVNAS	0.53%
	All pathways (mean) mg/kg bw/day	0.0055	% of RVNAS	0.69%

A 3.4 Combined exposure calculations for mesotrione, nicosulfuron and dicamba

Not required.

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)

Not required.