Draft Assessment Report

Evaluation of Active Substances

Plant Protection Products

Prepared according to **Retained Regulation (EC) 1107/2009**
as it applies in Great Britain

Prosulfuron

Volume 3 – B.6 (AS)

Toxicology & Metabolism Data

GB Article 7 Amendment

Great Britain

September 2023

Version History

When	What
September 2023	HSE Initial assessment

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B.6. TOXICOLOGY AND METABOLISM DATA

Background information

Prosulfuron was included into Annex I of Council Directive 91/414/EEC in 2002 (Commission Directive 2002/48/EC, 30 May 2002) and it is currently approved under Commission Regulation (EC) 1107/2009 (repealing Commission Directive 91/414/EEC) as specified in Commission Implementing Regulation (EU) No. 540/2011 of 25 May 2011.

Following application for renewal, the Renewal Assessment Report was peer reviewed by EFSA in 2014[1]. A critical area of concern was identified for the potential ground water exposure above the parametric drinking water limit (0.1 µg/L) by the parent prosulfuron in all or the majority of the EU groundwater scenarios, even when use is limited to one application every third year. A data gap was identified for a relevance assessment for the groundwater metabolites CGA150829, CGA300406 and CGA325025. For CGA150829, genotoxic potential could not be ruled out, based on available data.

Implementing Regulation (EU) No 2017/375 of 2 March 2017, renewing the approval of prosulfuron, set out a restriction limiting the use of prosulfuron containing products to one application every three years on the same field at a maximum dose of 20 g active substance per hectare. The same regulation stated that the applicant shall provide further information confirming that the metabolite triazine-amine (CGA150829) does not have genotoxic potential and is not relevant for risk assessment. Shortly before the Implementing Regulation was published, Syngenta Crop Protection AG submitted an Art. 7 application to the EU Rapporteur Member State (RMS) France, who prepared a revised renewal assessment report (RAR), which was submitted to EFSA in April 2018 and made available for commenting. Further information was requested from the applicant following the commenting

[1] Conclusion on the peer review of the pesticide risk assessment of the active substance prosulfuron, EFSA, 2014. Available: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2014.3815

stage, which was again evaluated by France and a further revised renewal assessment report was prepared (France, 2019[2]).

The Commission presented an addendum to the review report for prosulfuron and a draft Regulation to the Standing Committee on Plants, Animals, Food and Feed on 23 October 2020, establishing "that with respect to one or more representative uses of at least one plant protection product containing prosulfuron, when the plant protection product is applied annually, the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009 are satisfied. It is therefore appropriate to remove the restriction limiting the use of prosulfuron to one application every three years on the same field at a maximum dose of 20 g active substance per hectare."

The current dossier addresses an Art. 7 application for a similar amendment of the approval conditions in GB made by Syngenta Crop Protection AG. The application is supported by relevant data already submitted and evaluated at the EU level (included in the further revised RAR mentioned above; France, 2019[2]), and the EFSA Conclusion[3]. Additional new studies have been submitted by the applicant to address data gaps identified during the EU peer review process and listed by EFSA in their conclusions in 2020. These studies concern groundwater metabolites CGA150829 and CGA325025 and their genotoxic potential, which impacts on the relevance assessment and decision on whether the restriction limiting the use of prosulfuron containing products to one application every three years can be lifted. With regard to the toxicological data on the active substance itself, the summaries of the endpoints have been reproduced below as this information may be important to the relevance assessment of the concerned groundwater metabolites.

[2] France, 2019. Further revisions to the revised Renewal Assessment Report (RAR) on prosulfuron prepared by the Rapporteur Member State France in the framework of Regulation (EC) No 1107/2009, February 2019.
[3] Peer review of the pesticide risk assessment of the active substance prosulfuron. EFSA, June 2020. Available: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2020.6181

B.6.1. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION IN MAMMALS

B.6.1.1. Absorption, distribution, metabolism and excretion by oral route

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.1.2. Metabolism studies in vitro

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.1.3. Toxicokinetic information from Toxicodynamic Studies

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.1.4. Absorption, distribution, metabolism and excretion by other routes

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.1.5. Summary of ADME

The unaltered summary of the ADME properties of prosulfuron in mammals as presented in the original RAR 2014 is provided below as some groundwater metabolites could be major rat metabolites.

Prosulfuron was found to be rapidly absorbed and almost completely excreted in metabolism studies conducted over 7 days using both triazine and phenyl labelled prosulfuron forms of this active substance. Twenty four hours after dosing between 66 and 89% of the administered radioactivity was excreted, and by 48 hours over 89% of the administered radioactivity had been recovered. More radioactivity was excreted in the urine than faeces (about two thirds with the urine and one third with the faeces). After 7 days, 93 to 106% of the administered label was recovered from the excreta.

In a pharmacokinetic study using triazine labelled prosulfuron, peak plasma concentrations were reached 15 minutes and 4 hours following oral doses of 0.5 mg/kg and 400 mg/kg, respectively, independent of sex. Radioactivity in the blood had declined to half these maximum levels by 7 and 4 hours for males and females,

respectively, at the low dose level and 18.5 and 21 hours for males and females, respectively, at the high dose level. Some differences in these parameters may have been affected by the different dosing vehicles used at the low and high dose levels in these studies.

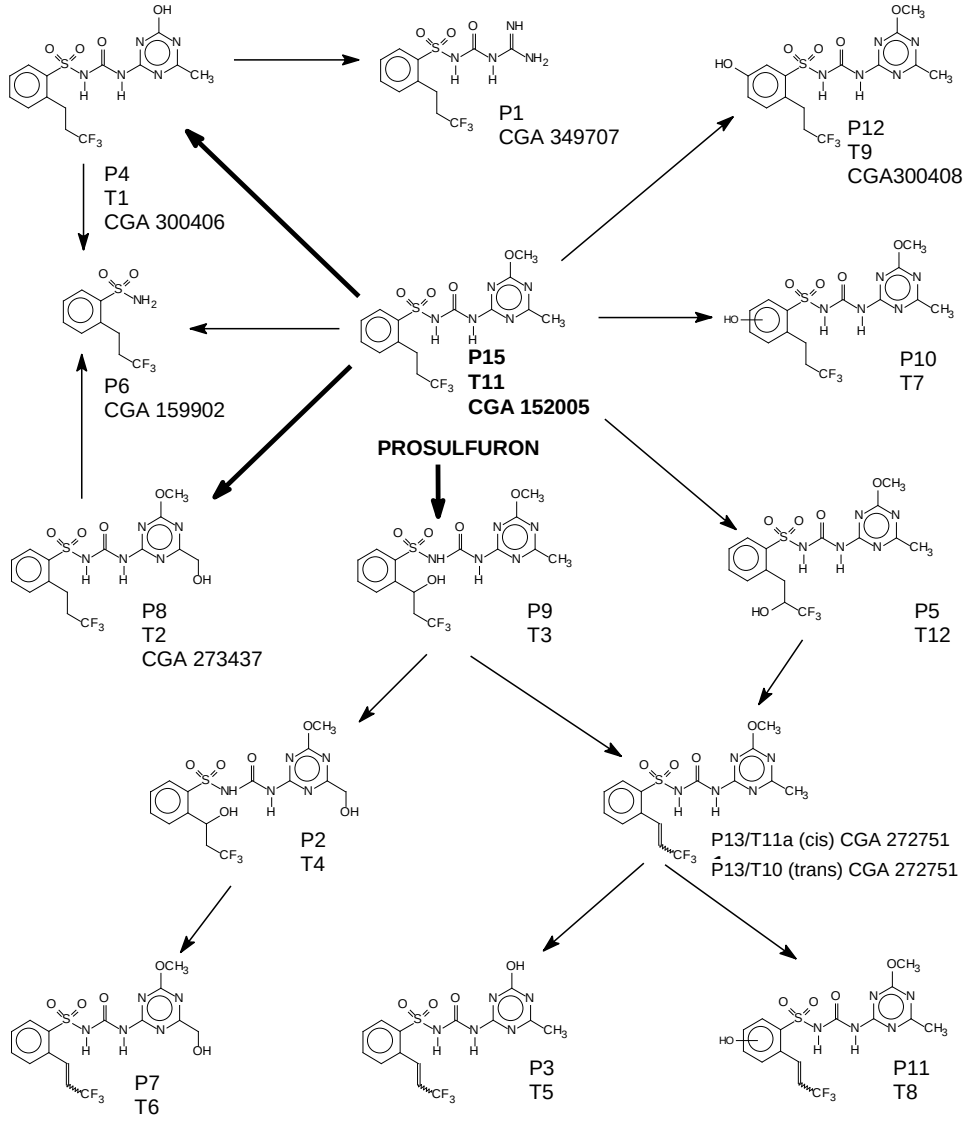
Experiments with bile duct cannulated rats indicate that a small amount (<10%) of prosulfuron or its metabolites is excreted with the bile and correlates with the proportion of the i.v. dose excreted in the faeces. There was no indication of an enterohepatic circulation of biliarily excreted metabolites.

Residual tissue levels were very low after 7 days when a low dose (0.5 mg/kg) was administered, with most measurements of radioactivity being below the limit of quantification. Quantifiable levels of radioactivity were found in the whole blood (<0.0091 to 0.027% of the applied dose), plasma (<0.0045 to 0.018% of the applied dose) and liver (<0.0021 to 0.074% of the applied dose). Higher residues were observed after administration of an 800-fold dose (400 mg/kg). The highest residues were detected in the whole blood (0.014 to 0.050% of the applied dose), liver (0.012 to 0.044% of the applied dose), kidneys (0.0028 to 0.0053% of the applied dose), lungs (0.0018 to 0.0079% of the applied dose), and in the heart (<0.00074 to 0.0020% of the applied dose). Notably high levels of radioactivity were found in the skin, with a wide range of variation between animals (<0.072 to 1.24% of the applied dose). Like the half-life time in whole animals, half-life times in organs and the residual carcass were about 10 hours (range 6.2 to 12.8 hours). There were no notable differences in the distribution of the two labelled forms of prosulfuron.

Fourteen metabolites were identified in urine and faeces after administration of [triazine-4-^{14}C] or [phenyl-^{14}C]prosulfuron. In animals dosed with triazine labelled prosulfuron the identified urinary metabolites comprised 79 to 90% of the total activity in the urine, in the faeces the corresponding figure was 33 to 72%. In phenyl prosulfuron dosed animals the identified urinary metabolites comprised 90 to 99% of the total activity in the urine, in the faeces the corresponding figure was 75 to 90%. Metabolism was almost independent of the administered dose but there were some differences between the sexes in the amount of individual metabolites. The predominant metabolic reactions included the hydroxylation at the side chains and

the phenyl ring, O-demethylation of the triazine methoxy-group, and generation of a double bond on the trifluoropropyl group. There was some evidence for the cleavage of the sulfonylurea bridge. However, this appears to be a minor metabolic pathway.

Figure B.6.1.5-1 Proposed metabolic pathway of prosulfuron in rat



B.6.2. ACUTE TOXICITY

B.6.2.1. Oral

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.2.2. Dermal

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.2.3. Inhalation

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.2.4. Skin irritation

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.2.5. Eye irritation

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.2.6. Skin sensitization

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.2.7. Phototoxicity

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.2.8. Summary of acute toxicity

Table B.6.2.8-1 Summary of the acute toxicity of prosulfuron (from RAR, 2014)

Type of Study	Result		Classification	References
Acute oral LD_{50} in rats	LD_{50} (male)	949 mg/kg	Harmful if swallowed: R22 H302, Acute Tox. Cat. 4	[redacted] 1991e
	LD_{50} (female)	546 mg/kg		
	LD_{50} (both)	986 mg/kg		
Acute oral LD_{50} in mice	LD_{50} (male)	1208 mg/kg		[redacted] 1991d
	LD_{50} (female)	1262 mg/kg		
	LD_{50} (both)	1247 mg/kg		
Acute dermal LD_{50} in rabbits	LD_{50}> 2000 mg/kg		None	[redacted] 1991c

Acute inhalation LC_{50} in rats	LC_{50}> 5.4 mg/L	None	[redacted] 1991
Primary skin irritation in rabbits	Non irritant	None	[redacted] 1991b
Primary eye irritation in rabbits	Slight irritant	None	[redacted] 1991a
Skin sensitisation (Buehler 3 applications) in Guinea pigs	Non sensitizing	None	[redacted] 1991f
Skin sensitization (M&K) in Guinea pigs	Non sensitizing	None	[redacted] 1992a

B.6.3. SHORT-TERM TOXICITY

B.6.3.1. Oral 28-day study

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.3.2. Oral 90- day study

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.3.3. Other routes

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.3.4. Summary of short-term toxicity

The following information as presented in the RAR 2014 has been provided below for completeness as applicable to GB.

In subchronic studies in rats the liver was shown to be a target organ with weight and clinical chemistry changes, though no histopathological effects. Body weight effects and changes in haematological parameters were seen at higher dose levels.

In mice the liver was also a target organ with increased weights, hepatocyte hypertrophy, and changes of liver-associated clinical chemistry parameters. The heart also appeared to be a target organ with multifocal vacuolative degeneration

(precise location not stated). Additionally, some changes of red blood cell parameters were observed.

At high doses administration of prosulfuron to dogs caused decreased body weight gain or even body weight loss. The main effects were noted in the liver (increased weights, changes in clinical chemistry parameters and pigment accumulation, possibly lipofuscin), the heart (myocardial necrosis and degeneration), kidney (pigment accumulation, renal proximal tubular epithelial fatty change) and in the hematopoietic system.

Table B.6.3.4-1 Summary of adverse effects in short-term toxicity studies (from RAR, 2014)

Studies	NOAEL approx. mg/kg bw/day	Dose/ adverse effects approx. mg/kg bw/day	References
28-day rat Gavage	100	300: Liver effects (weights/clinical chemistry), decreases in red blood cell counts/hematocrit	[redacted], 1992
28-day rat Diet	107	209: Decreased body weight gains (changes in clinical chemistry indicative of possible liver effects)	[redacted] ad [redacted], 1991
28-day mouse Diet	14	169: Liver effects (weights/clinical chemistry)	[redacted], 1991a
28-day dog Diet	None*	19 (lowest dose): Liver weight effects	[redacted], 1991b
28-day dog Diet	None*	32 (lowest dose): Liver, adrenal and spleen weight effects	[redacted], 1992
90–day rat Diet	3	33: Reduced body weight and body weight gain, liver weights 255: Liver effects (decreased triglycerides and 5'-nucleotidase activities in males)	[redacted] and [redacted], 1991
90–day mouse Diet	69	264: Liver effects (increased liver weights, hepatocyte hypertrophy, and changes of liver-associated clinical chemistry parameters), some changes of red blood cell parameters 504: heart effects (vacuolative degeneration)	[redacted] and [redacted], 1991
90–day dog Diet	5.9	56: Decreased body weight gain/ body weight loss, liver effects (increased liver weights, and changes clinical chemistry parameters), hematopoietic system (decreased RBC counts, decreased hematocrit, decreased haemoglobin concentration, abnormal erythrocyte	[redacted], 1991

		morphology, thrombocytopenia and changes in leukocyte counts, erythroid hyperplasia of the bone marrow) 110: heart effects (myocardial necrosis and degeneration)	
21-day rabbit Dermal	None	No valid study	[redacted], 1992

* Due to the limited nature of these studies, it is not appropriate to set a NOAEL.

The inconsistency between NOAEL in mice after 28 days (14 mg/kg bw/day) and after 90 days (69 mg/kg bw/day) is only apparent. Both studies were performed in the same lab, with the same strains. The slight effect on the liver, target organ for all species, was considered as non adaptative in the 28-day assay (due to the lack of histopathological data in this assay, thus setting of a conservative NOAEL) and adaptative in the 90-day assay. In fact, if the same criteria are used, NOAEL could be respectively 1000 ppm (155 mg/kg bw/day) and 500 ppm (69 mg/kg bw/day) after 28 and 90 days.

The NOAEL of the 90-day rat study was set at 3 mg/kg bw/day based on marginal but clear effects on body weight and body weight gain seen at 33 mg/kg bw/day. Nevertheless, a clear NOAEL for the rat was seen in the 2-year study at 8.6 mg/kg bw/day. The lower NOAEL set in the 90-day study compared to the 2-year study is due to an inadequation between ranges of doses used in these studies.

Consequently, the relevant lowest oral NOAEL for short term toxicity is 5.9 mg/kg bw/day based on the 90-day dog study.

B.6.4. GENOTOXICITY

B.6.4.1. In vitro studies

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.4.2. In vivo studies in somatic cells

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.4.3. In vivo studies in germ cells

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.4.4. No new data have been submitted. Please refer to original EU RAR 2014.Summary of genotoxicity

The following information as presented in the RAR 2014 has been provided below for completeness as applicable to GB.

When tested in vitro, prosulfuron was negative in an Ames test (with *Salmonella typhimurium* and *Escherichia coli*), a mammalian cell gene mutation assay with V79 cells, a cytogenetic test in Chinese hamster ovary cells, and an autoradiographic DNA-repair test in rat hepatocytes. Prosulfuron was also negative in two recent Ames tests performed due to new manufacturing specification for prosulfuron and submitted during the renewal process of prosulfuron.

When tested in vivo, prosulfuron did not induce micronuclei in a bone marrow micronucleus test in the mouse, although no evidence of bone marrow toxicity data was presented, the test was conducted at or close to the maximum tolerated dose and distribution data (in the rat) indicate that prosulfuron appears to reach the bone.

Table B.6.4.4-1 Summary of genotoxicity studies with prosulfuron (from RAR, 2014)

Test system	Test species/cells	Prosulfuron concentration	Result	Reference
In vitro assays				
In vitro bacterial reverse mutation assay (Ames)	*Salmonella typhimurium* (TA1535, TA1537, TA98, TA100) and *Escherichia coli* (WP2 uvrA)	2.4 to 39.1 µg/plate (*S. typhimurium*), 312.5 to 5000 µg/plate (*E. coli*)	Negative (with and without S9)	[redacted], 1991
In vitro bacterial reverse mutation assay (Ames)	*Salmonella typhimurium* (TA1535, TA1537, TA98, TA100) and *Escherichia coli* (WP2 uvrA pKM101, WP2 pKM101)	Up to 5000 µg/plate	Negative (with and without S9)	[redacted], 2011
In vitro	*Salmonella*	Up to 5000	Negative (with	[redacted]

bacterial reverse mutation assay (Ames)	*typhimurium* (TA1535, TA1537, TA98, TA100) and *Escherichia coli* (WP2 uvrA pKM101, WP2 pKM101)	µg/plate	and without S9)	[redacted], 2011a
In vitro mammalian cell gene mutation assay	Chinese hamster V79 cells	Up to 1400 µg/ml	Negative (with and without S9)	[redacted], 1991
In vitro cytogenetic test	Chinese hamster ovary cells	Up to 500 µg/ml	No increased number of cell chromosome aberrations (with and without S9)	[redacted], 1991
In vitro autoradiographic DNA-repair test	Rat hepatocytes	Up to 1000 µg/ml	No increase in unscheduled DNA-repair	[redacted], 1991
In vivo assays				
In vivo micronucleus test	Mouse bone marrow cells	Up to 1600 mg/kg bw (single dose)	Negative	[redacted], 1991

B.6.5. LONG-TERM TOXICITY AND CARCINOGENESIS

B.6.5.1. Long-term toxicity and carcinogenicity in rats

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.5.2. Long-term toxicity and carcinogenicity in mice

No new study has been submitted. Please refer to original EU RAR 2014.

B.6.5.3. Summary of long-term toxicity and carcinogenicity

The following information as presented in the RAR 2014 has been provided below for completeness as applicable to GB.

In a lifetime study in the rat there was a slightly increased incidence of testicular interstitial cell tumours in males, and a small increase in the incidence of mammary

gland adenocarcinomas in females at the same dose levels, however the changes were not significant when the increased survival in these groups was considered. There was also an apparent earlier onset of mammary gland adenocarcinomas in females, although no significant difference existed for adenocarcinoma onset time between the control and any treatment group. There also was an increased incidence of uterine endometrial hyperplasia and uterine horn dilation in females as well as an increased incidence of acinar atrophy of the mammary gland in males.

No evidence of carcinogenicity was seen in mice.

Table B.6.5.3-1. Summary of the results of the long-term toxicity and carcinogenicity studies (from RAR, 2014)

Studies	NOAEL approx. mg/kg bw/day	Dose/adverse effects approx. mg/kg bw/day	References
Rat 2-year oral (diet)	8.6	88: Decreased body weights/body weight gain, effects on red blood cell parameters, possible treatment related increased incidence of testicular interstitial cell tumours/early onset mammary gland adenocarcinomas in females 183: Increased incidence of uterine endometrial hyperplasia and uterine horn dilatation in females and increased incidence of acinar atrophy of the mammary gland in males: indication of hormonal disruption (uterus and mammalian gland in rats) at high dose levels	[redacted], [redacted] and [redacted], 1994
Mouse 18-month oral (diet)	1.71	82: increased incidence of centrilobular hepatocyte hypertrophy in males 410: decreased body weight gain, effects on red blood cells (increased hematocrit, decreased MCHC) and on the liver (weights, centrilobular hepatocyte hypertrophy)	[redacted] and [redacted], 1993
Dog 1-year oral (diet)	1.9	19: Decreased red blood cell parameters, liver effects (increased liver weights, and changes clinical chemistry parameters), accumulation of lipofuscin in the liver and kidney	[redacted] and [redacted], 1993

B.6.6. REPRODUCTIVE TOXICITY

B.6.6.1. Generational studies

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.6.2. Developmental toxicity studies

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.6.3. Summary of reproductive toxicity

The following information as presented in the RAR 2014 has been provided below for completeness as applicable to GB.

In a two generation study in the rat the NOAEL was 200 ppm (equivalent to approximately 12 mg/kg bw/day) based on the body weight effects seen in both parental animals and pups. In the P0 generation there was no indication of any treatment-related effects on precoital interval, mating index, parturition index, or gestation length. The pregnancy and gestation indices were comparable between all groups except for the low dose group, where slightly lower indices were obtained. In the P1 generation there was no indication for treatment-related effects on precoital interval, mating index, parturition index, or gestation length. The pregnancy, fertility, and gestation indices were comparable between all groups except for the low and top dose groups, where lower indices were obtained. This is nevertheless considered incidental.

Table B.6.6.3-1 Summary of the results of the multigeneration study (oral route) (from RAR, 2014)

Species	NOAEL approx. mg/kg bw/day	Dose/reproductive effects approx. mg/kg bw/day	References
Rat (diet)	Parental: 12 Offspring: 12 Reproductive: 251	135: Body weight changes in parental animals and pups No effect on reproductive performance at any dose level.	[redacted] and [redacted], 1993

Prosulfuron was not teratogenic in either rats or rabbits. The no effect level for maternal toxicity and developmental toxicity was 200 and 50 mg/kg bw/day respectively in rats, while in rabbits the no effect level for maternal toxicity and developmental toxicity was 10 mg/kg bw/day. It should be noted that although no maternal toxicity was seen in the rat developmental study at 200 mg/kg bw/day, based on other short term studies in the rat it is likely that some maternal toxicity was present.

Table B.6.6.3-2 Summary of the results of the developmental toxicity studies (oral route) (from RAR, 2014)

Species	NOAEL mg/kg bw/day	Dose/developmental effects mg/kg bw/day	References
Rat (gavage, GD 6-15)	Maternal: 200 Developmental: 50	Maternal: 400: reduced body weight gain and food consumption Developmental: 200: slightly increased incidence of skeletal variations (primarily extra-rudimentary ribs and lobed and/or constricted thoracic vertebrae centra)	[redacted], 1992
Rabbit (gavage, GD 7-19)	Maternal: 10 Developmental: 10	Maternal: 100: reduced body weight gain and food consumption Developmental: 100: increased incidence of resorptions	[redacted], 1992

Prosulfuron was shown not to have endocrine disruptive effects in vivo, and it is considered unlikely that in vitro tests would add any relevant information. Therefore, prosulfuron is unlikely to be an endocrine disruptor (EFSA, 2014).

B.6.7. NEUROTOXICITY

B.6.7.1. Neurotoxicity studies in rodents

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.7.2. Delayed polyneuropathy studies

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.7.3. Summary of neurotoxicity

The following information as presented in the RAR 2014 has been provided below for completeness as applicable to GB.

Table 6.7.3-1 Summary of the results of the neurotoxicity studies (oral route) (from RAR, 2014)

Species	NOAEL mg/kg bw/day	Dose/neurotoxic effects mg/kg bw/day	References
Rat Acute (gavage)	10	250: slight effects on neurological parameters 3 hours after dosing, apparently reversible, in presence of general toxicity 1000: decreased survival in females, decreased bw gain in males	[redacted] and [redacted], 1994
Rat 90-day (diet)	12	152: decreased body weight gain and decreased forelimb grip strength, likely due to general toxicity	[redacted] and [redacted], 1994

B.6.8. OTHER TOXICOLOGICAL STUDIES

B.6.8.1. Toxicity studies on metabolites and relevant impurities

Toxicity data on potential groundwater metabolites CGA349707, CGA159902, CGA150829, SYN542604, CGA325025, SYN547308 and CGA300406 were provided by the applicant and considered in the EU RAR (2014 and Article 7 revised RAR, 2019). Short summaries of these studies are presented in Tables B.6.8.1-1 to B.6.8.1-8 and sections below for completeness.

CGA349707 and CGA159902 are minor rat metabolites, while CGA300406 is considered a major rat metabolite. CGA150829, SYN542604, CGA325025 and SYN547308 are not rat metabolites.

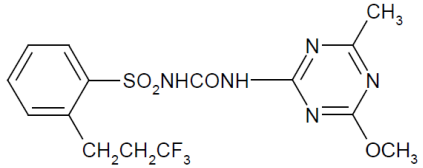
An assessment of the relevance of the metabolites in groundwater in line with SANCO guidance (Sanco/221/2000 – rev.10 - final) is provided in Volume 1.

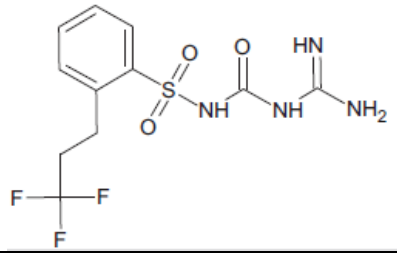
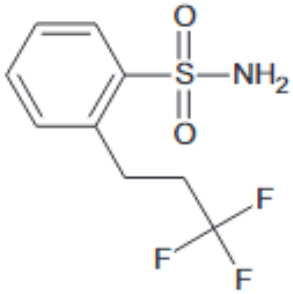
A toxicological relevance assessment was triggered for several metabolites exceeding 0.1 µg/L in groundwater. In their conclusions, EFSA (2020) stated that the genotoxic potential of CGA150829 and CGA325025 could not be determined without further in vitro micronucleus studies. In addition, the groundwater exposure assessment and consumer risk assessment for CGA150829, CGA159902, CGA325025 and SYN547308 could not be finalised.

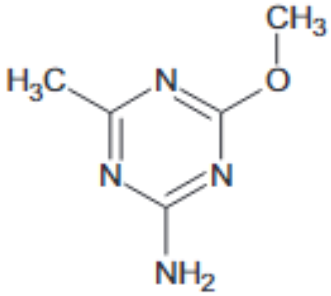
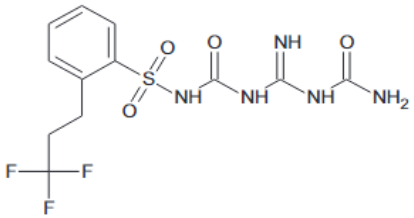
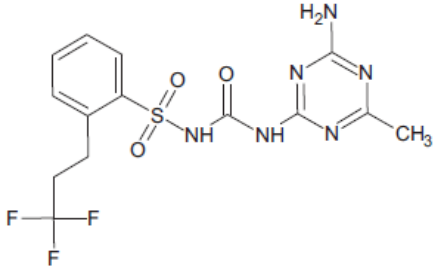
This GB Article 7 amendment application addresses these concerns with new data ([redacted], 2020; [redacted], 2021). HSE's evaluation of these micronucleus studies with CGA150829 and CGA325025 is presented below. Additionally, a repeated dose 28-day study on CGA150829 in rat ([redacted], 2014) and its benchmark dose analysis ([redacted], 2015), both considered at the renewal of another sulfonyl urea herbicide tribenuron-methyl (RAR, 2017), are also mentioned.

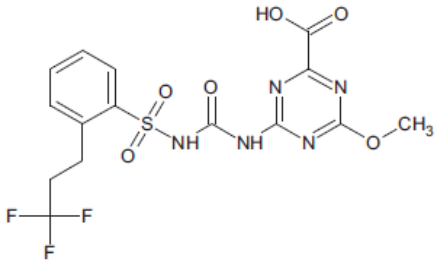
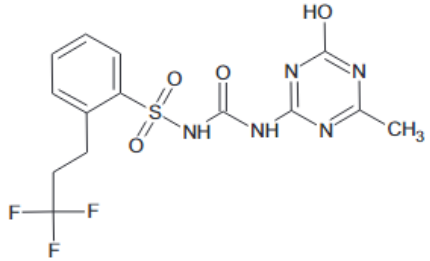
A data gap identified by EFSA (2014) concerning the toxicological relevance of CGA300406 was addressed with genotoxicity studies. However, the data gap was found by EFSA (2020) to be no longer applicable as its relevance assessment was no longer triggered (level of this metabolite < 0.1 µg/L).

Table B.6.8.1-1: Toxicological studies available on prosulfuron and its metabolites

Substance	Structure	Available studies	New studies submitted with this GB Art. 7 application
Prosulfuron		In vitro: - Ames test – [redacted], 1991; [redacted], 2011 and 2011a - Gene mutation assay – [redacted], 1991 - Cytogenetic test – [redacted], 1991 - Unscheduled DNA repair test – [redacted], 1991 In vivo: - Bone marrow MN test (mouse) – [redacted], 1991 - Acute oral (rats/mice) – [redacted], 1991e and 1991d - Acute dermal (rabbits) – [redacted], 1991c - Acute inhalation (rats) – [redacted], 1991 - Skin/eye irritation (rabbits) – [redacted], 1991b and 1991a - Skin sensitisation (guinea pigs) – [redacted], 1991f and [redacted], 1992a - 28 day studies (rat/mouse/dog) – [redacted], 1992; [redacted] and [redacted], 1991; [redacted], 1991a,b and 1992 - 90 day studies (rat/mouse/dog) – [redacted] and [redacted], 1991; Chow and [redacted], 1991; [redacted], 1991 - 21 day study (rabbit) – [redacted], 1992 - 1 year study (dog) – [redacted] and [redacted], [redacted], 1993 - 18 month study (mouse) – [redacted] and [redacted], 1993 - 2 year study (rat) – [redacted], [redacted] and [redacted], 1994 - Multigeneration study (rat) – [redacted] and [redacted], 1993 - Developmental studies (rat/rabbit) – [redacted], 1992 and [redacted], 1992 - Neurotoxicity studies (rat) – [redacted] and [redacted], 1994; Chow and [redacted], 1994	n/a

CGA349707		<u>In vitro:</u> - Ames test – [redacted], 2005 - Gene mutation assay – [redacted], 2005 - Cytogenetic test – [redacted], 2005	n/a
CGA159902 (=CA1118A; prosulfuron phenyl sulfonamide)		<u>In vitro:</u> - Ames test – [redacted], 1993 - Gene mutation assay – [redacted], 2005a - Cytogenetic test – [redacted], 2005a <u>In vivo:</u> - Unscheduled DNA repair test – [redacted], 2005b - Bone marrow MN test (mouse) – [redacted], 2005c - Acute oral (rat) – [redacted], 1993 - Acute dermal (rat) – [redacted], 1993a - Skin/eye irritation (rabbit) – [redacted], 1993 and 1993a - Skin sensitisation (guinea pig) – [redacted], 1993b	n/a

CGA150829 (=IN-A4098 or AE F059411 or prosulfuron triazine amine)		<u>In vitro:</u> - Ames test – [redacted], 1991; [redacted], 1998; [redacted], 2009 - Gene mutation assays – [redacted], 2009; [redacted], 2015; [redacted] and [redacted], 2019 - Cytogenetic test – [redacted], 1991; [redacted], 1987; [redacted], 2009 - Unscheduled DNA repair test – [redacted], 1988 and [redacted], 1988 <u>In vivo:</u> - Chromosome aberration test (hamster) – [redacted], 1988 - Acute oral (rat) – [redacted], 1991a - Acute dermal (rat) – [redacted], 1993a - Acute inhalation (rat) – [redacted], 1991b - Skin/eye irritation (rabbit) – [redacted], 1991 and 1991a - Skin sensitisation (guinea pig) – [redacted], 1991b - 28-day study (rat) – [redacted], 2014 (RAR – tribenuron-methyl, 2017) - Benchmark dose modelling based on [redacted], 2014 – [redacted], 2015 (RAR - tribenuron-methyl, 2017)	<u>In vitro:</u> - Mammalian cell MN test – [redacted], 2020
SYN542604 (M5)		<u>In vitro:</u> - Ames test – [redacted], 2010 - Gene mutation assays – [redacted], 2010 - Cytogenetic test – [redacted], 2010	n/a
CGA325025 (=demethoxy amino-prosulfuron)		<u>In vitro:</u> - Ames test – [redacted], 2013 - Gene mutation assays – [redacted], 2013 - Cytogenetic test – [redacted], 2013	<u>In vitro:</u> - Mammalian cell MN test – [redacted], 2021

SYN547308 (M18)		<u>In vitro:</u> - Ames test – [redacted], 2014 - Gene mutation assays – [redacted], 2014 - Cytogenetic test – [redacted], 2014 <u>In vivo:</u> - Bone marrow MN test (mouse) – [redacted], 2014	n/a
CGA300406 (O-desmethyl-prosulfuron)		<u>In vitro:</u> - Ames test – [redacted], 2015a - Gene mutation assays – [redacted], 2015 - Cytogenetic test – [redacted], 2015b <u>In vivo:</u> - Bone marrow MN test (mouse) – [redacted], 2015	n/a

CGA349707

The potential genotoxicity of CGA349707 was investigated in an Ames test, in an in vitro gene mutation assay (MLA/TK) and in an in vitro chromosomal aberration test using human lymphocytes. Negative results were obtained in these tests. It was concluded that CGA349707 has no genotoxic activity.

Table B.6.8.1-2: Summary of genotoxicity data for metabolite CGA349707

Parameter	Result	Reference
In vitro tests		
Bacterial reverse mutation (Ames) assay with *S. typhimurium* and *E. coli*	Negative	[redacted], 2005
L5178Y $TK^{+/-}$ mouse lymphoma mutation assay	Negative	[redacted], 2005
In vitro cytogenetics assay in human lymphocytes	Negative	[redacted], 2005

CGA159902

CGA159902 is not acutely toxic via the oral and dermal route, is not a skin or eye irritant but is a skin sensitiser.

The potential genotoxicity of CGA159902 was investigated in a battery of in vitro and higher tier in vivo genotoxicity tests. Negative results were obtained in an Ames test and in an in vivo unscheduled DNA synthesis assay. Positive results were obtained in in vitro tests (cytogenetics assay in human lymphocytes and MLA/TK assay showing increase in small colonies) and are indicative of an in vitro clastogenic effect of this compound. Nevertheless, the in vivo mouse bone marrow micronucleus test showed negative results. It was concluded that CGA159902 is not an in vivo genotoxicant.

Table B.6.8.1-3: Summary of toxicity data for metabolite CGA159902 (=CA1118A)

Genotoxicity			
In vitro tests			
Parameter		**Result**	**Reference**
Bacterial reverse mutation (Ames) assay with *S. typhimurium* and *E. coli*		Negative	[redacted], 1993*
L5178Y $TK^{+/-}$ mouse lymphoma mutation assay		(-S9) Negative (+S9) Positive (small colonies)	[redacted], 2005a
In vitro cytogenetic assay in human lymphocytes		(-S9) Positive (+S9) Negative	[redacted], 2005a
In vivo tests			
Parameter	**Species**	**Result**	**Reference**
Rat liver unscheduled DNA synthesis assay	Rat	Negative	[redacted], 2005b
Mouse bone marrow micronucleus test	Mouse	Negative	[redacted], 2005c
Acute toxicity			
Parameter	**Species**	**Result (mg/kg or effect)**	**Reference**
Acute oral LD_{50}	Rat	> 2000 mg/kg	[redacted], 1993*
Acute dermal LD_{50}	Rat	> 2000 mg/kg	[redacted], 1993a*
Skin irritation	Rabbit	Non-irritant	[redacted], 1993*
Eye irritation	Rabbit	Non-irritant	[redacted], 1993a*
Skin Sensitization (Maximization Test)	Guinea pig	Sensitizer – H317	[redacted], 1993b*

* Although these studies were already submitted during the first evaluation process of prosulfuron, they were not cited in the original draft assessment report. They are thus reported here for a better understanding of the full toxicology package of the metabolite CGA159902.

CGA150829 – triazine amine [evaluated in the EU RAR, 2014, Art 7 amended RAR, 2019 and presented here for completeness + one new study ([redacted], 2020) and one 28-day study ([redacted], 2014) and its benchmark dose modelling ([redacted], 2015) considered in the EU RAR of tribenuron-methyl, 2017]

CGA150829, also named IN-A4098 or AE F059411 or triazine amine, is a common metabolite to several sulfonyl urea herbicides (e.g. metsulfuron-methyl, thifensulfuron-methyl, triasulfuron, tribenuron-methyl, iodosulfuron-methyl).

CGA150829 is harmful if swallowed, not acutely toxic via the dermal route and via inhalation, not a skin or eye irritant and not a skin sensitiser.

Gene mutation:

CGA150829 is negative in three Ames tests ([redacted] 1991 and two newly submitted tests: [redacted] 2009 and [redacted] 1998). Two other Ames assays were found negative in other sulfonyl urea herbicides dossiers.

CGA150829 was considered equivocal in an in vitro gene mutation assay using Chinese hamster cells in the absence of metabolic activation (HPRT test, [redacted] 2009) and negative in an in vitro mouse lymphoma gene mutation assay ([redacted] 2015). Further in vitro gene mutation assays in other sulfonyl urea dossiers showed negative results (V79/HPRT mutation assay) and equivocal results in the absence of metabolic activation (mouse lymphoma assay). It is noteworthy that, in the two mouse lymphoma assays available, the highest concentration tested in the negative study from [redacted] (40 µg/mL) was in the range of the lowest dose from the equivocal study ([redacted] 2009). Consequently, it is considered that the results with lower doses might not be appropriate to clarify the mutagenic potential of CGA150829. It should also be noted that the two equivocal results were both observed in the absence of metabolic activation. As a conclusion, a mutagenic potential of the metabolite CGA150829 could not be ruled out based on the available data.

This conclusion is in line with the recent EFSA conclusions on tribenuron-methyl (EFSA Journal 2017;15(7):4912).

Clastogenicity:

Three in vitro chromosomal aberration assays were available in the dossier prosulfuron: one negative in Chinese hamster cells ([redacted] 1991), as well as one positive ([redacted] 1987) and one negative ([redacted] 2009, newly submitted) in human lymphocytes. From other sulfonyl ureas dossiers, two other in vitro chromosomal aberration assays showed negative results in human lymphocytes. One study out of the five available studies showed positive results. This study is the oldest study conducted on this metabolite and was performed with the lowest dose levels and the lowest purity of metabolite. Furthermore, due to the poor quality of this study, the results are considered as not reliable. Therefore, by a weight of evidence analysis, it can be concluded that CGA150829 does not induce chromosomal aberrations in vitro. Furthermore, an in vivo chromosomal aberration assay conducted in Chinese hamster showed clear negative results. Although the exposure of the bone marrow was not demonstrated in this study, it should be noted that the tested dose was the highest applicable dose (3200 mg/kg bw) which exceeded the limit dose recommended in the OECD guideline. Therefore, it is concluded that CGA150829 is unlikely to be clastogenic.

It is noteworthy that, in the framework of confirmatory data request to address the triazine amine genotoxic potential for several sulfonyl ureas active substances, a Triazine-amine Task Force has been created in order to jointly submit all the available data to a unique Rapporteur Member State at the end of December 2017. Therefore, the Aminotriazine Task Force (originally Bayer AG, Syngenta AG and DuPont, later DuPont transferred the agreement to FMC Corporation), submitted a weight of evidence assessment in December 2017, which was evaluated by the designated rapporteur Member State (RMS) Sweden (RMS for iodosulfuron), and co-RMS France (RMS for prosulfuron) in the form of an addendum to the draft assessment report. The outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for iodosulfuron and prosulfuron in light of confirmatory data (EFSA Supporting publication 2018:EN-1470) concluded: "*There was general agreement that triazine amine does not induce gene mutations in bacteria in vitro and chromosome aberration in vitro. However, no firm conclusion could be drawn regarding the gene mutation potential of triazine amine*

on the basis of the confirmatory information submitted, since some issues were identified with regard to the quality and the interpretation of the results of two in vitro gene mutation studies". Consequently, the genotoxic potential of triazine amine (CGA150829) was assessed by the EFSA PPR Panel (2020)[4]. Two additional GLP and OECD test guideline compliant in vitro studies not included in the EU RAR: mammalian cell gene mutation assays in mouse lymphoma L5178Y cells ([redacted], 2019) and Chinese hamster ovary (CHO) cells ([redacted] and [redacted], 2019), were considered by the PPR Panel, which, concluded that there was no concern for the potential of CGA150829 to induce gene mutations and clastogenicity. However, it was agreed that an additional in vitro micronucleus test would be needed to conclude on its aneugenic potential.

In response to this, in their Article 7 GB application, the applicant provided an in vitro micronucleus assay in human lymphocytes ([redacted], 2020), which was GLP and OECD compliant and provided clear negative results. The evaluation of this study is presented below.

Report:	[redacted] (2020), IN-A4098: *In Vitro* Mammalian Cell Micronucleus Test in Human Peripheral Lymphocytes. Covance Laboratories Limited, Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, UK. Laboratory Report No. 8441503, Sponsor No.: FMC-54579. Unpublished. FMC Corporation.

Author(s)	[redacted]
Study title	IN-A4098: In Vitro Mammalian Cell Micronucleus Test in Human Peripheral Lymphocytes.
Study reference	[redacted], 2020, Unpublished CRO Study No: 8441503 Sponsor Report No.: FMC-54579
Laboratory	Covance Laboratories Limited, Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, UK.
Test substance	IN-A4098 CAS Name (uninverted): 4-Methoxy-6-methyl-1,3,5-triazin-2-amine
Purity (%)	98.7%

[4] Scientific Opinion of the Scientific Panel on Plant Protection Products and their Residues (PPR Panel) on the genotoxic potential of triazine amine (metabolite common to several sulfonylurea active substances). EFSA Journal, February 2020. Available: https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2020.6053

Batch no.	050942-015
Test organisms	Human peripheral blood lymphocytes Preliminary test: 2 male donors (aged 21 and 23) Main test: 2 female donors (aged 27 and 35)
GLP	Compliant
Guideline	OECD TG 487 (2016 – this is the current guideline),
Deviation	none
Impact of deviations	n/a
Acceptable	Yes.
Conclusion	IN-A4098 was non-mutagenic in the in vitro micronucleus test.

Methods

IN-A4098 was tested for its potential to induce micronuclei in cultured human peripheral blood lymphocytes in vitro in the absence and presence of metabolic activation (S9 mix from the livers of rats, pre-treated with β-naphthoflavone/phenobarbital) in an OECD test guideline and GLP compliant study. The study consisted of a preliminary toxicity test and a main micronucleus test.

A preliminary test was performed in single cultures (only vehicle controls were duplicates) with 10 concentrations (0.59 – 300 µg/mL) to determine the concentrations to be used in the main experiment. The highest concentration for the main experiment 300 µg/mL was limited by the maximum achievable concentration in a suitable vehicle.

The main test was performed in duplicate cultures (only vehicle controls and untreated controls, if included, were quadruplicates). In both, the preliminary and the main tests the cells were incubated for 3 h (in the presence or absence of metabolic activation;) or 20 h (in the absence of metabolic activation) with the test substance at concentrations in the range of 37.5 – 300 µg/mL; three concentrations 75, 150 and 300 µg/mL were chosen for evaluation (see above table, test concentrations in bold and results in table below).

The vehicle HML (containing RPMI 1640, supplemented with 10% fetal calf serum) served as negative control, for positive controls mitomycin C and colchicine (in the absence of metabolic activation) and cyclophosphamide (in the presence of metabolic activation) were used. Treatment was started after a 48 hour stimulation

period with phytohemeagglutinine. Thereafter, cytochalasin B was added to the cultures to arrent cell cycle and the cultures were fixed and stained after another 17 hours. Cytokinesis-block proliferation index (CBPI) and cytostasis determined in 1000 binucleated cells/culture served as cytotoxicity parameters and the number of micronucleated cells was determined in 2000 binucleated cells for evaluation of mutagenicity. To support and additionally verify the results of experiments with 3 hour exposure (with and without S9-mix) in the experiment with 20 hour treatment an additional number of binucleated cells was determined: 2000 binucleated cells/culture (4000 in total per tested concentration/positive control).
A validated method of analysis for in vitro genotoxicity studies is not required.

Results

Cytotoxicity, precipitation and osmolarity

No test item precipitation in the culture medium was observed at the end of treatment in the preliminary and the main test at 300 µg/mL.

Micronucleus assay

There were no significant reductions in the CBPI compared with vehicle control values at any concentrations tested in all exposure conditions.

Concentrations of the test item selected for micronucleus analysis were 75, 150 and 300 µg/mL.
In both experiments (3 and 20 hour treatment), in the absence and presence of metabolic activation, no statistically significant and biologically-relevant increase in the number of binucleated cells carrying micronuclei was observed. No concentration-dependant relationship was observed. The mean number of micronuclei was within the range of the historical control data for the vehicle control.

In both experiments, either MMC, colchicine or CPA were used as positive controls and showed distinct increases in cells with micronuclei, so demonstrating the sensitivity of the test system. All positive control values were compatible with the laboratory historical positive control data demonstrating the validity of the study.

Summary of results of the in vitro micronucleus test in human lymphocytes with IN-A4098 (=CGA150829)

Exp.	Exposure period [h]	Test item concentration [µg/mL]	Proliferation index (CBPI)	Cytostasis in %[a]	Micronucleated cells[b] Mean	HCD	
Without S9 mix							
I	3	Vehicle control[1]	1.68	0.0	7.3	No. experiments	57
						Mean	0.62
						95 % ctrl limit	3.2 – 10.6
						SD	1.8
						Min	3.3
						Max	12.0
		IN-A4098 (75)	1.77	0.0[c]	5.5		
		IN-A4098 (150)	1.86	0.0[c]	5.0		
		IN-A4098 (300)	1.81	0.0[c]	6.5		
		MMC (0.3)	1.39	43.1	**41.0***	No. experiments	57
						Mean	37.3
						95 % ctrl limit	23.4 – 61.2
						SD	11.9
						Min	14.5
						Max	76.0
		Colchicine (0.06-0.07)	1.30 – 1.49	27.3 – 55.5	**33.0***	No. experiments	57
						Mean	26.8
						95 % ctrl limit	12.4 – 41.2
						SD	7.2
						Min	16.0
						Max	48.0
II	20	Vehicle control[1]	1.72	0.0	6.8	No. experiments	53
						Mean	7.0
						95 % ctrl limit	3.4 – 10.6
						SD	1.8
						Min	3.0
						Max	10.5
		IN-A4098 (75)	1.73	0.0[c]	7.0		
		IN-A4098 (150)	1.83	0.0[c]	8.0		
		IN-A4098 (300)	1.83	0.0[c]	9.5		

Exp.	Exposure period [h]	Test item concentration [µg/mL]	Proliferation index (CBPI)	Cytostasis in %[a]	Micronucleated cells[b] Mean	HCD	
		MMC (0.1)	1.53	27.1	**17.0***	No. experiments	53
						Mean	26.2
						95 % ctrl limit	17.6 – 39.3
						SD	6.5
						Min	15.5
						Max	39.5
		Colchicine (0.015-0.02)	1.36 – 1.55	23.7 – 50.5	**21.0***	No. experiments	53
						Mean	17.0
						95 % ctrl limit	12.4 – 21.5
						SD	2.3
						Min	13.5
						Max	23.5
With S9 mix							
I	3	Vehicle control[1]	1.78	0.0	7.8	No. experiments	62
						Mean	6.2
						95 % ctrl limit	3.2 – 9.3
						SD	1.5
						Min	3.3
						Max	10.5
		IN-A4098 (75)	1.82	0.0[c]	8.5		
		IN-A4098 (150)	1.77	0.6	8.5		
		IN-A4098 (300)	1.78	0.5	8.0		
		CPA (10)	1.32	59.4	**22.5***	No. experiments	62
						Mean	23.8
						95 % ctrl limit	13.4 – 34.3
						SD	5.2
						Min	14.0
						Max	36.0

Exp.	Exposure period [h]	Test item concentration [µg/mL]	Proliferation index (CBPI)	Cytostasis in %[a]	Micronucleated cells[b] Mean	HCD
a: For the positive control groups and the test item treatment groups the values are related to the solvent controls b: The number of micronucleated cells was determined in a sample of 2000 binucleated cell (4000 for vehicle and the 20 h treatment) c: Cytostasis reported as 0.0 as the CBPI value is equal to or higher than the vehicle control value CBPI: cytokinesis-block proliferation index HCD: historical control data of the performing laboratory (collected 2018 – 2020) *: The number of micronucleated cells is statistically significantly higher than corresponding control values (p≤0.001) 1 HML medium 50% (v/v)						

Conclusion

In conclusion, under the conditions of this GLP and OECD test guideline compliant study, IN-A4098 did not induce micronuclei. Therefore, IN-A4098 is considered by HSE to be non-mutagenic in this in vitro micronucleus test when tested up to cytotoxic concentrations with and without metabolic activation.

An additional repeated dose 28-day study on CGA150829 in rat ([redacted], 2014) and its benchmark dose analysis ([redacted], 2015), both considered at the renewal of another sulfonyl urea herbicide tribenuron-methyl (RAR, 2017) and relevant for this Art. 7 application, are shortly summarised below.

Reports:	[redacted] (2014), Repeated-dose oral toxicity 28-day feeding study in rats. [redacted] [redacted]. (2015), Benchmark dose modelling of IN-A4098 Repeated-dose oral toxicity 28-day feeding study in rats ([redacted], 2014). Position paper. [redacted].

During the renewal of tribenuron-methyl (RAR, 2017) a GLP and OECD 407 compliant repeat dose 28-day study in rats ([redacted], 2014) was considered. The NOAEL for males was <50 ppm (3.6 mg/kg bw/day) and for females was 150 ppm (11 mg/kg bw/day). This NOAEL was based on lower body weight and nutritional parameter in males at all dose groups. The NOAEL for female rats was based on lower body weight and nutritional parameters at the LOAEL of 500 ppm. No adverse

effects were observed at any concentration on neurobehavioral, clinical pathology, organ weights, gross or microscopic pathology in rats fed up to 1000 ppm.

As the NOAEL was determined to be less than the lowest tested dose (<3.6 mg/kg bw/day), benchmark dose analysis of the 28-day oral study was performed to identify a departure point for risk assessment ([redacted], 2015).

The BMD analysis used an exponential model and a benchmark response (BMR) of 10% relative deviation from the control mean, consistent with U.S. EPA Office of Pesticide Program (OPP) policy (U.S. EPA, 2006). The good-fitting models derived a BMD_{10} (benchmark dose resulting in 10% deviation of response compared to negative control) value of 1.8 mg/kg bw/day. The 95% lower confidence limit ($BMDL_{10}$) was 0.7 mg/kg bw/day and this value was regarded as the NOAEL in the 28-day study.
From the $BMDL_{10}$ value concluded in the renewal of tribenuron-methyl, an acceptable daily intake (ADI) of the metabolite IN-A4098 can be derived by applying a standard uncertainty factor of 100 (10 for inter species and 10 for intra species variation), plus an additional factor of 10 for extrapolation from sub-acute to chronic exposure. Therefore, the ADI of IN-A4098 from this study is concluded to be 0.0007 mg/kg bw/day.

For a refined risk assessment for IN-A4098, the metabolite specific ADI of 0.0007 mg/kg bw/day should be used (see Volume 1).

Table B.6.8.1-4: Summary of toxicity data for metabolite CGA150829 (includes studies evaluated in the EU RAR, 2014, Art 7 revised EU RAR, 2019; in the EFSA PPR Panel Opinion, 2020; tribenuron-methyl RAR, 2017 and one new study)

Studies evaluated in the EU RAR, 2014, and Art 7 revised EU RAR, 2019 - presented here for completeness as applicable to GB			
Genotoxicity			
In vitro tests			
Parameter		**Result**	**Reference**
Bacterial reverse mutation (Ames) assay with *S. typhimurium and E. coli*		Negative (+/- S9)	[redacted] 1991
Bacterial reverse mutation (Ames) assay with *S. typhimurium and E. coli*		Negative (+/- S9)	[redacted], 2009
Bacterial reverse mutation (Ames) assay with *S. typhimurium and E. coli*		Negative (+/- S9)	[redacted], 1998
L5178Y $TK^{+/-}$ mouse lymphoma gene mutation assay		Negative *Choice of highest tested dose is questionable*	[redacted], 2015
CHO/HGPRT gene mutation assay		Equivocal (-S9) Negative (+S9)	[redacted], 2009
In vitro cytogenetics assay in Chinese Hamster cells		Negative (+/- S9)	[redacted] 1991
In vitro cytogenetics assay in human lymphocytes		Positive (+S9) Negative (-S9)	[redacted] 1987
In vitro cytogenetics assay in human lymphocytes		Negative (+/- S9)	[redacted], 2009
Unscheduled DNA synthesis assay on rat hepatocytes		Negative	[redacted] 1988
Unscheduled DNA synthesis assay on human fibroblasts		Negative	[redacted] 1988
In vivo tests			
Parameter	**Species**	**Result**	**Reference**

Chromosome studies on somatic cells	Chinese Hamster	Negative	[redacted] 1988
Acute toxicity			
Parameter	**Species**	**Result (mg/kg or effect)**	**Reference**
Acute oral LD_{50}	Rat	> 2000 mg/kg bw in males = 1000 mg/kg bw in females (**H302**)	[redacted] 1991
Acute dermal LD_{50}	Rat	> 2000 mg/kg bw	[redacted] 1991a
Acute inhalation LC_{50}	Rat	> 5.2 mg/L	[redacted] 1991b
Skin irritation	Rabbit	Non-irritant	[redacted] 1991
Eye irritation	Rabbit	Non-irritant	[redacted] 1991a
Skin Sensitization (Maximization Test)	Guinea pig	Non sensitizer	[redacted] 1991b
Studies evaluated in the EFSA PPR Panel Opinion, 2020 - presented here for completeness as applicable to GB			
In Vitro Mammalian Cell Forward Gene Mutation (CHO/HPRT) Assay	Chinese hamster ovary (CHO) cells	Negative	[redacted] and [redacted], 2019
In Vitro Mammalian Cell Mutation Assay	Mouse Lymphoma L5178Y Cells	Negative	[redacted], 2019
Studies evaluated in the tribenuron-methyl EU RAR, 2017 - presented here for completeness as applicable to GB			
Repeat dose 28 day study	Rat	NOAEL < the lowest tested dose	[redacted]., 2014
Benchmark dose modelling of IN-A4098 Repeated-dose oral toxicity 28-day feeding study in rats ([redacted], 2014).		$BMDL_{10}$ 0.7 mg/kg bw/day	[redacted], 2015
Study submitted with Article 7 (Reg. 1107/2009) application to support the amendment of approval conditions in GB (October, 2022)			

In Vitro Mammalian Cell Micronucleus Test	Human Peripheral Lymphocytes	Negative	[redacted], 2020

SYN542604

The potential genotoxicity of SYN542604 was investigated in an Ames test, in an in vitro gene mutation assay (MLA/TK) and in an in vitro chromosomal aberration test using human lymphocytes. Negative results were obtained in these tests. It is concluded that SYN542604 has no genotoxic activity.

Table B.6.8.1-5: Summary of toxicity data for metabolite SYN542604

Genotoxicity		
In vitro tests		
Parameter	**Result**	**Reference**
Bacterial reverse mutation (Ames) assay with *S. typhimurium and E. coli*	Negative (+/- S9)	[redacted], 2010
L5178Y $TK^{+/-}$ mouse lymphoma gene mutation assay	Negative (+/- S9)	[redacted], 2010
In vitro cytogenetics assay in human lymphocytes	Negative (+/- S9)	[redacted], 2010

CGA325025 (evaluated in the EU RAR, 2014, - presented here for completeness as applicable to GB + one new study [redacted], 2021)

The potential genotoxicity of CGA325025 has been investigated in an Ames test, in an in vitro gene mutation assay (MLA/TK) and in an in vitro chromosomal aberration test using human lymphocytes. Negative results were obtained in the Ames test and in the in vitro gene mutation assay. It is concluded that CGA325025 is not a mutagenic compound in vitro. Nevertheless, as the in vitro chromosomal aberration assay showed equivocal results, no conclusion can be drawn on the clastogenic properties of CGA325025.

During the commenting period, one comment suggested to "*consider whether the negative results of the L5178Y TK+/- mouse lymphoma gene mutation assay can contribute to the weight of evidence for clastogenicity too and the concern for clastogenicity might be considered low*". The RMS considered that the equivocal results of the in vitro chromosomal aberration assay cannot be completely ruled out by the negative results of the MLA/TK assay. In order to clarify the clastogenic potential of CGA325025, an in vitro micronucleus test may be sufficient in view of the equivocal results of the in vitro chromosomal aberration assay.

The available data were assessed at an EFSA expert meeting and it was noted that the genotoxic potential of metabolite CGA325025 could not be finalised. All experts agreed that CGA325025 did not induce gene mutation however, the chromosome aberration test showed equivocal results and an in vitro micronucleus assay, which could allow conclusion on the genotoxic potential (clastogenicity and aneugenicity) of the metabolite was missing, leading to a data gap (EFSA, 2020).

In response to this, in their Article 7 GB application, the applicant provided an additional GLP and OECD compliant in vitro micronucleus assay in human lymphocytes ([redacted], 2021) which showed clear negative results. The evaluation of this study is presented below.

Report:	[redacted] (2021), CGA325025 - Micronucleus Test in Human Lymphocytes In Vitro. ICCR-Roßdorf GmbH In den Leppsteinswiesen 19 64380 Rossdorf, Germany. Laboratory Report No. 2147800.

Author(s)	[redacted]
Study title	CGA325025 - Micronucleus Test in Human Lymphocytes In Vitro
Study reference	[redacted], 2021
Laboratory	ICCR-Roßdorf GmbH In den Leppsteinswiesen 19 64380 Rossdorf, Germany
Test substance	CGA325025
Purity (%) **Batch no.**	98% MES 240/3
Test organisms	Human peripheral blood lymphocytes Experiment I: 22 year old male

	Experiment II: 32 year old female
GLP	Compliant
Guideline	OECD TG 487 (2016 – this is the current guideline),
Deviation	None
Impact of deviations	-
Acceptable	Yes
Conclusion	CGA325025 was non-mutagenic in the in vitro micronucleus test

Methods

CGA325025 was tested for its potential to induce micronuclei in cultured human peripheral blood lymphocytes in vitro in the absence and presence of metabolic activation (S9 mix from the livers of rats, pre-treated with β-naphthoflavone/phenobarbital) in an OECD test guideline and GLP compliant study. The study consisted of a preliminary toxicity test and a main micronucleus test.

Two independent experiments were performed in duplicate cultures where the cells were incubated for 4 hours (in the presence or absence of metabolic activation, Experiment I) or 20 hours (in the absence of metabolic activation, Experiment II). Experiment I was performed with 10 concentrations (5.2 – 800 µg/mL), and based on the observed precipitation, the concentrations used in Experiment II were in a range between 13.9 µg/mL and 700 µg/mL (the maximum concentration).

Positive controls, mitomycin C and demecolcine (in the absence of metabolic activation) and cyclophosphamide (in the presence of metabolic activation) were used. Treatment was started after a 48 hour stimulation period with phytohemeagglutinine. Thereafter, cytochalasin B was added to the cultures to arrent cell cycle and the cultures were fixed and stained after another 20 hours. Cytokinesis-block proliferation index (CBPI) and cytostasis determined in 500 cells/culture served as cytotoxicity parameters and the number of micronucleated cells was determined in 2000 binucleated cells/test substance concentration for evaluation of mutagenicity. A validated method of analysis for in vitro genotoxicity studies is not required.

Results

Cytotoxicity, precipitation and osmolarity

Phase separation (precipitation) of the test item in the culture medium was observed at the end of treatment in Experiment I at 261 µg/mL and above (-S9 mix) and at 149 µg/mL and above (+S9 mix) and these were the highest concentrations evaluated for micronuclei induction. In Experiment II precipitation was observed from 400 µg/mL. No relevant influence on osmolarity or pH was observed.

No cytotoxicity was observed in any of the experiments up to the highest evaluated concentration showing precipitation.

Micronucleus assay

In both experiments, in the absence and presence of metabolic activation, no statistically significant and biologically-relevant increase in the number of binucleated cells carrying micronuclei was observed (table below). No concentration-dependant relationship was observed. The mean number of micronuclei was within the 95% historical control data limits.

All solvent control values were within the range of the laboratory historical negative control data (Table 6.4-11). In both experiments, either demecolcine (75 ng/mL), MMC (0.8 µg/mL) or CPA (20 µg/mL) were used as positive controls and showed distinct increases in cells with micronuclei, so demonstrating the sensitivity of the test system. All positive control values were within the range of the laboratory historical positive control data demonstrating the validity of the study.

Summary of results of the in vitro micronucleus test in human lymphocytes with CGA325025

Exp.	Exposure period [h]	Test item concentration [µg/mL]	Proliferation index (CBPI)	Cytostasis in %[a]	Micronucleated cells[b] Mean %	HCD
Without S9 mix						

Exp.	Exposure period [h]	Test item concentration [µg/mL]	Proliferation index (CBPI)	Cytostasis in %[a]	Micronucleated cells[b] Mean %	HCD	
I	4	Vehicle control[1]	1.93	-	0.40	No. experiments	95
						Mean	0.49
						95 % ctrl limit	0.00-0.99
						SD	0.25
						Min	0.15
						Max	1.25
		CGA325025 48.7	2.13	n.c.	0.30		
		CGA325025 85.3	2.15	n.c.	0.65		
		CGA325025 149	2.17	n.c.	0.65		
		CGA325025 261	2.09	n.c.	0.40		
		MMC (0.8)	1.71	22.8	12.85*	No. experiments	95
						Mean	11.20
						95 % ctrl limit	2.63-19.78
						SD	4.29
						Min	3.55
						Max	25.95
II	20	Vehicle control[1]	2.00	-	0.60	No. experiments	82
						Mean	0.47
						95 % ctrl limit	0.06 – 0.88
						SD	0.21
						Min	0.10
						Max	1.25
		CGA325025 74.6	1.98	1.7	0.45		
		CGA325025 131	1.98	1.8	0.50		
		CGA325025 229	2.00	0.3	0.80		
		CGA325025 400	1.99	0.9	0.45		
		Demecolcine (0.075)	1.98	1.6	4.85*	No. experiments	82
						Mean	4.55
						95 % ctrl limit	2.10-7.01
						SD	1.23
						Min	2.85
						Max	8.30
With S9 mix							

Exp.	Exposure period [h]	Test item concentration [µg/mL]	Proliferation index (CBPI)	Cytostasis in %[a]	Micronucleated cells[b] Mean %	HCD	
I	4	Vehicle control[1]	2.09	-	0.35	No. experiments	86
						Mean	0.53
						95 % ctrl limit	0.02-1.04
						SD	0.25
						Min	0.10
						Max	1.18
		CGA325025 27.9	2.08	1.0	0.30		
		CGA325025 48.7	1.99	9.1	0.15		
		CGA325025 85.3	2.04	4.8	0.40		
		CGA325025 149	2.05	3.6	0.30		
		CPA (20)	1.66	39.6	2.75*	No. experiments	83
						Mean	4.26
						95 % ctrl limit	1.26-7.26
						SD	1.50
						Min	2.20
						Max	8.70

a: For the positive control groups and the test item treatment groups the values are related to the solvent controls
b: The number of micronucleated cells was determined in a sample of 2000 binucleated cell
n.c: Cytostasis reported as 0.0 as the CBPI value is equal to or higher than the vehicle control value
CBPI: cytokinesis-block proliferation index
HCD: historical control data of the performing laboratory (collected 2020)
*: The number of micronucleated cells is statistically significantly higher than corresponding control values
1 DMSO 1% (v/v)

Conclusion

In conclusion, under the conditions of this GLP and OECD test guideline compliant study, CGA325025 did not induce micronuclei, as determined by the in vitro micronucleus test in human lymphocytes. Therefore, CGA325025 is considered by HSE to be non-mutagenic in this in vitro micronucleus test when tested up to cytotoxic concentrations with and without metabolic activation.

Table B.6.8.1-6: Summary of toxicity data for metabolite CGA325025 (from EU RAR, 2014, Art 7 revised EU RAR, 2019 + one new study)

Studies evaluated in the EU RAR, 2014; Art 7 revised EU RAR, 2019 - presented here for completeness as applicable to GB			
Genotoxicity			
In vitro tests			
Parameter		**Result**	**Reference**
Bacterial reverse mutation (Ames) assay with *S. typhimurium and E. coli*		Negative (+/- S9)	[redacted], 2013
L5178Y $TK^{+/-}$ mouse lymphoma gene mutation assay		Negative (+/- S9)	[redacted], 2013
In vitro cytogenetics assay in human lymphocytes		Equivocal (+/- S9)	[redacted], 2013
Study submitted with Article 7 (Reg. 1107/2009) application to support the amendment of approval conditions in GB (October, 2022)			
In Vitro Mammalian Cell Micronucleus Test	Human Peripheral Lymphocytes	Negative (+/- S9)	[redacted], 2021

SYN547308

The potential genotoxicity of SYN547308 has been investigated in an Ames test, in an in vitro gene mutation assay (MLA/TK) and in an in vitro chromosomal aberration test using human lymphocytes as well as in an in vivo mouse bone marrow micronucleus test. Negative results were obtained in the Ames test and in the in vitro gene mutation assay. As the in vitro chromosomal aberration assay showed positive results without metabolic activation, an in vivo micronucleus assay was conducted and gave negative results. Therefore, SYN547308 can be considered as devoid of genotoxic properties.

Table B.6.8.1-7: Summary of toxicity data for metabolite SYN547308

Genotoxicity			
In vitro tests			
Parameter		**Result**	**Reference**
Bacterial reverse mutation (Ames) assay with *S. typhimurium and E. coli*		Negative (+/- S9)	[redacted], 2014
L5178Y $TK^{+/-}$ mouse lymphoma gene mutation assay		Negative (+/- S9)	[redacted], 2014
In vitro cytogenetics assay in human lymphocytes		(-S9) Positive (+S9) Negative	[redacted], 2014
In vivo tests			
Parameter	**Species**	**Result**	**Reference**
Mouse bone marrow micronucleus test	Mouse	Negative	[redacted], 2014

CGA300406

The potential genotoxicity of CGA300406 has been investigated in an Ames test, in an in vitro gene mutation assay (MLA/TK) and in an in vitro chromosomal aberration test using human lymphocytes as well as in an in vivo mouse bone marrow micronucleus test. Negative results were obtained in the Ames test and in the in vitro gene mutation assay. As the in vitro chromosomal aberration assay showed positive results with and without metabolic activation, an in vivo micronucleus assay was conducted and gave negative results. Therefore, CGA300406 can be considered as devoid of genotoxic properties.

Table B.6.8.1-8: Summary of toxicity data for metabolite CGA300406

Genotoxicity			
In vitro tests			
Parameter		**Result**	**Reference**
Bacterial reverse mutation (Ames) assay with *S. typhimurium and E. coli*		Negative (+/- S9)	[redacted], 2015a
L5178Y $TK^{+/-}$ mouse lymphoma gene mutation assay		Negative (+/- S9)	[redacted], 2015
In vitro cytogenetics assay in human lymphocytes		(-S9) Positive (+S9) Positive	[redacted], 2015b
In vivo tests			
Parameter	**Species**	**Result**	**Reference**
Mouse bone marrow micronucleus test	Mouse	Negative	[redacted], 2015

B.6.8.2. Supplementary studies on the active substance

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.8.3. Studies on endocrine disruption

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.8.4. Endocrine disruption assessment

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.8.5. Immunotoxicity

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.9. MEDICAL DATA AND INFORMATION

B.6.9.1. Medical surveillance on manufacturing plant personnel and monitoring studies

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.9.2. Data collected on humans

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.9.3. Direct observation

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.9.4. Epidemiological studies

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.9.5. Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical test

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.9.6. Proposed treatment: first aid measures, antidotes, medical treatment

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.9.7. Expected effects of poisoning

No new data have been submitted. Please refer to original EU RAR 2014.

B.6.10. SUMMARY OF MAMMALIAN TOXICITY AND OVERALL EVALUATION

B.6.10.1. Summary of mammalian toxicity (from EU RAR, 2014)

Prosulfuron was found to be rapidly absorbed and almost completely excreted in ADME studies conducted over 7 days using both triazine and phenyl labelled prosulfuron. The principal route of excretion was via the urine (about two thirds with the urine and one third with the faeces). A small amount (<10%) of prosulfuron or its

metabolites were excreted with the bile. In a pharmacokinetic study using triazine labelled prosulfuron, peak plasma concentrations were reached 15 minutes and 4 hours following oral doses of 0.5 mg/kg and 400 mg/kg respectively, independent of sex. Pharmacokinetic parameters may have been affected by the different dosing vehicles used at the low and high dose levels in these studies.

Residual tissue levels were low after 7 days when a low dose (0.5 mg/kg) was administered, with most measurements of radioactivity being below the limit of quantification. Quantifiable levels of radioactivity were found in the whole blood (<0.0091 to 0.027% of the administered dose), plasma (<0.0045 to 0.018% of the applied dose) and liver (<0.0021 to 0.074% of the applied dose). Higher residues were observed after administration of an 800-fold dose (400 mg/kg). The highest residues were detected in the whole blood (0.014 to 0.050% of the applied dose), liver (0.012 to 0.044% of the applied dose), kidneys (0.0028 to 0.0053% of the applied dose), lungs (0.0018 to 0.0079% of the applied dose), and in the heart (<0.00074 to 0.0020% of the applied dose).

Prosulfuron was well metabolised (less than ca. 30% parent compound was excreted), 14 metabolites were identified in urine and faeces. Metabolism was almost independent of the administered dose but there were some differences between the sexes in the amount of individual metabolites. The predominant metabolic reactions included the hydroxylation at the side chains and the phenyl ring, O-demethylation of the triazine methoxy-group, and generation of a double bond on the trifluoropropyl group. Cleavage of the sulfonylurea bridge appears to be a minor metabolic pathway.

Prosulfuron was moderately toxic by the oral route. The classification of prosulfuron as harmful if swallowed (**Xn, R22**) was already agreed at European level. Prosulfuron is also classified for acute toxicity by oral route, category 4 (**H302**, harmful if swallowed) according to the criteria of the regulation (EC) n°1272/2008. Although prosulfuron is not a skin sensitiser in two skin sensitization studies, the presence of an impurity classified as Xi, R43 in the technical specifications and not sufficiently tested in these studies triggers the classification **Xi, R43** for prosulfuron (**H317**, may cause an allergic skin reaction).

In subchronic studies in rats the liver was shown to be a target organ with weight and clinical chemistry changes, though no histopathological effects. Body weight effects and changes in haematological parameters were seen at higher dose levels. In mice the liver was also a target organ with increased weights, hepatocyte hypertrophy, and changes of liver-associated clinical chemistry parameters. The heart also appeared to be a target organ with multifocal vacuolative degeneration (precise location not stated). Additionally, some changes of red blood cell parameters were observed. At high doses administration of prosulfuron to dogs caused decreased body weight gain or even body weight loss. The main effects were noted in the liver (increased weights, changes in clinical chemistry parameters and pigment accumulation, possibly lipofuscin), the heart (myocardial necrosis and degeneration), kidney (pigment accumulation, renal proximal tubular epithelial fatty change) and in the hematopoietic system.

When tested in vitro, prosulfuron was negative in an Ames test, a mammalian cell gene mutation assay, a cytogenetic test, and an autoradiographic DNA-repair test. Prosulfuron was also negative in two recent Ames tests. When tested in vivo, prosulfuron did not induce micronuclei in a bone marrow micronucleus test in the mouse.

In a lifetime study in the rat there was a slightly increased incidence of testicular interstitial cell tumours in males, and a small increase in the incidence of mammary gland adenocarcinomas in females at the same dose levels, however the changes were not significant when the increased survival in these groups was considered. There was also an apparent earlier onset of mammary gland adenocarcinomas in females, although no significant difference existed for adenocarcinoma onset time between the control and any treatment group. There was also an increased incidence of uterine endometrial hyperplasia and uterine horn dilation in females as well as an increased incidence of acinar atrophy of the mammary gland in males. No evidence of carcinogenicity was seen in mice.

In a two generation study in the rat body weight effects were seen in both parental animals and pups. In the P0 generation there was no indication of any treatment-related effects on precoital interval, mating index, parturition index, or gestation

length. The pregnancy and gestation indices were reduced in the low dose group. In the P1 generation there was no indication for treatment-related effects on precoital interval, mating index, parturition index, or gestation length. Low indices were obtained for pregnancy, fertility, and gestation in the low and top dose groups. This is nevertheless considered incidental.

Prosulfuron was not teratogenic in either rats or rabbits. Developmental toxicity (a slightly increased incidence of skeletal variations, primarily extra rudimentary ribs and lobed and/or constricted thoracic vertebrae centra) was seen in the rat, at levels where some maternal toxicity was likely to be present, based on other studies. Increased incidence of resorptions was observed in the rabbit in the presence of maternal toxicity.

In neurotoxicity studies in the rat, acute and repeat dose, no neurotoxic potential was demonstrated.

Prosulfuron was shown not to have endocrine disruptive effects in vivo, and it is considered unlikely that in vitro tests would add any relevant information. Therefore, prosulfuron is unlikely to be an endocrine disruptor (EFSA, 2014).

Table B.6.10.1-1 Summary of NOAELs for toxicology studies (from EU RAR, 2014 and Art 7 revised EU RAR, 2019)

Studies	NOAEL approx. mg/kg bw/day	LOAEL and effects approx. mg/kg bw/day	References
Rat, 28-day (gavage)	100	300: Liver effects (weights/clinical chemistry), decreases in red blood cell counts/hematocrit	[redacted], 1992
Rat, 28–day (diet)	107	209: Decreased body weight gains (changes in clinical chemistry indicative of possible liver effects)	[redacted] ad [redacted], 1991
Mouse, 28-day (diet)	14	169: Liver effects (weights/clinical chemistry)	[redacted], 1991a
Dog, 28-day (diet)	None	19 (lowest dose): Liver weight effects	[redacted], 1991b
Dog, 28-day (diet)	None	32 (lowest dose): Liver, adrenal and spleen weight effects	[redacted], 1992

Rat, 90–day (diet)	3	33: Reduced body weight and body weight gain, liver weights	[redacted] and [redacted], 1991
Mouse, 90–day (diet)	69	264: Liver effects (increased liver weights, hepatocyte hypertrophy, and changes of liver-associated clinical chemistry parameters), some changes in red blood cells parameters	[redacted] and [redacted], 1991
Dog, 90-day (diet)	6	56: Decreased body weight gain/ body weight loss, liver effects (increased liver weights, and changes clinical chemistry parameters), hematopoietic system (decreased RBC counts, decreased hematocrit, decreased haemoglobin concentration, abnormal erythrocyte morphology, thrombocytopenia and changes in leukocyte counts, erythroid hyperplasia of the bone marrow)	[redacted], 1991
Rabbit, 21-day dermal	None	No valid study	[redacted], 1992
Rat, 2-year (diet)	8.6	88: Decreased body weights, body weight gain, effects on red blood cell parameters, possible treatment related increased incidence of testicular interstitial cell tumours/early onset mammary gland adenocarcinomas in females	[redacted], [redacted] and [redacted], 1994
Mouse, 18-month (diet)	1.71	82: Increased incidence of centrilobular hepatocyte hypertrophy in males	[redacted] and [redacted], 1993
Dog, 1-year (diet)	1.9	19: Decreased red blood cell parameters, liver effects (increased liver weights, and changes clinical chemistry parameters), accumulation of lipofuscin in the liver and kidney	[redacted] and [redacted], 1993
Rat, multigeneration (diet)	Parental: 12 Offspring: 12 Reproductive: 251	135: Body weight changes in parental animals and pups No effect on reproductive performance at any dose level.	[redacted] and [redacted], 1993
Rat developmental (gavage)	Maternal: 200 Developmental: 50	Maternal: 400: reduced body weight gain and food consumption Developmental: 200: slightly increased incidence of skeletal variations (primarily extra-rudimentary ribs and lobed and/or constricted thoracic vertebrae centra)	[redacted], 1992
Rabbit development	Maternal: 10	Maternal: 100: reduced body weight gain and food consumption	[redacted], 1992

al (gavage)	Developmental: 10	Developmental: 100: increased incidence of resorptions	
Rat, acute neurotoxicity (gavage)	10	250: slight effects on neurological parameters 3 hours after dosing, apparently reversible, in presence of general toxicity	[redacted] and [redacted], 1994
Rat, 90-day neurotoxicity (diet)	12	152: decreased body weight gain and decreased forelimb grip strength	[redacted] and [redacted], 1994

B.6.10.2. Acceptable daily intake (ADI)

The **ADI was set at 0.02 mg/kg bw/d** based on the 1-year dog (NOAEL of 1.9 mg/kg bw/d based on decreased red blood cell parameters and liver effects) and the 18-month mouse (NOAEL of 1.7 mg/kg bw/d based on liver effects) studies and by using a safety factor of 100 (EFSA Conclusions, 2014).

B.6.10.3. Acceptable operator exposure level (AOEL)

The **AOEL was set at 0.06 mg/kg bw/d** based on the 90-day dog study (NOAEL of 5.9 mg/kg bw/d based on liver effects and effects on the hematopoietic system) and by using a safety factor of 100 (EFSA Conclusions, 2014).

B.6.10.4. Acute reference dose (ARfD)

An ARfD was set based on the lowest NOAEL observed in the short-term and teratology toxicity studies. The most relevant study appeared to be the developmental toxicity study performed in rabbits, where the NOAEL was 10 mg/kg bw/day, based on the increased incidence of resorptions observed at the dose level of 100 mg/kg bw/day. Therefore the following ARfD was derived, with a safety factor of 100 (EFSA Conclusion, 2014):

ARfD = NOAEL / SF = 10 mg/kg bw/d / 100 = **0.1 mg/kg bw**

B.6.11. REFERENCES RELIED ON

Studies submitted in the context of an Art. 7 application for the amendment of the approval conditions for prosulfuron in GB:

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	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 7.1.7/13	[redacted]	2020	Title: IN-A4098: In vitro mammalian cell micronucleus test in human peripheral lymphocytes Sub Company : Owner Company : FMC AGRO LIMITED Report No : 8441503 Date : 25/09/2020 GLP Status : yes	N	Y	New data; eligible for data protection according to SANCO/12576/2012	FMC Agro Limited
KCP 9.2.4/01	[redacted]	2021	Title: CGA325025 - Micronucleus test in human lymphocytes in vitro Final Report Sub Company : Owner Company : SYNGENTA UK LIMITED Report No : 2147800 Date : 07/06/2021 GLP Status : yes	N	Y	New data; eligible for data protection according to SANCO/12576/2012	Syngenta