Document Title

Summary of the toxicological and metabolism studies for Amidosulfuron

Data Requirements

EU Regulation 1107/2009 & EU Regulation 283/2013
Document MCA
Section 5: Toxicological and metabolism studies

According to the guidance document SANCO/10181/2013 for preparing dossiers for the approval of a chemical active substance

Date

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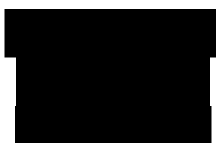
Author(s)



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Version history

Date	Data points containing amendments or additions[1] and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 5 TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

This document provides detailed summaries of new toxicological studies which were not available at the time of the first EU review of amidosulfuron and were therefore not evaluated for the Annex I inclusion of this active substance. Existing studies already submitted for the first EU review are found evaluated in the Draft assessment report (DAR) or its Addenda; in the present document these studies are therefore only briefly referenced, marked in grey shade. In exemption from this, upon specific request by the RMS expressed at the pre-application meeting, studies that have been submitted as part of the confirmatory data post Annex I are summarised and discussed as 'new information', even though they have undergone review for the EU by former RMS AGES Austria and are found summarised in the 'Addendum to monograph prepared in the context of post Annex I procedure (new Annex II data)', December 2010 (rev. 1 Feb. 2011) and are reflected in the updated EU List of Endpoints of December 2010.

Complete reports to all studies are included in the electronic dossier provided by Bayer CropScience. The numbering and the headlines correspond to latest EU requirements.

For transparent overall data interpretation and risk assessment, key endpoints derived from both old and new studies are listed in overview tables, where applicable. For easy discrimination, new information is printed black, whilst existing information is repeated in grey shaded font.

CA 5.1 Studies on absorption, distribution, metabolism and excretion in mammals

The absorption, distribution (including blood and plasma kinetics) metabolism and excretion of amidosulfuron was investigated in Wistar rats using oral doses of 10, 100 and 500 mg/kg bw, oral uptake from diet at a nominal concentration of 10000 ppm and an intravenous low dose of 10 mg/kg bw. The ADME characteristics following repeated oral dosing of radiolabelled amidosulfuron were investigated using the dose rate of 10 mg/kg bw. The molecule was radiolabelled on position 2 of the pyrimidine ring. The metabolite pattern was investigated in liver, blood, urine and faeces by adequate analytical methods.

The test substance was rapidly absorbed and rapidly excreted in rats following oral administration. Radioactivity in major organs was low seven days post dose. Elimination *via* urine ranged between 79.5 and 84.7% of the administered radioactivity after single oral low dose and 87.2 to 90.9% of the administered radioactivity after single oral high dose. Faecal excretion was 10.7 to 13.2% (oral low dose) and 5 to 8% (oral high dose). The main part of the radioactivity was eliminated within 24 hours and elimination was nearly complete within the study duration. Based on mean values from the urinary plus cage wash results for the single oral 10, 100 and 500 mg/kg test groups the oral absorption levels were *ca* 87% for the males and *ca* 89% for the females. The results from the repeated oral dosing showed a similar excretion pattern with most of the radioactivity being excreted *via* the urine within the first 24 hours after multiple dosing.

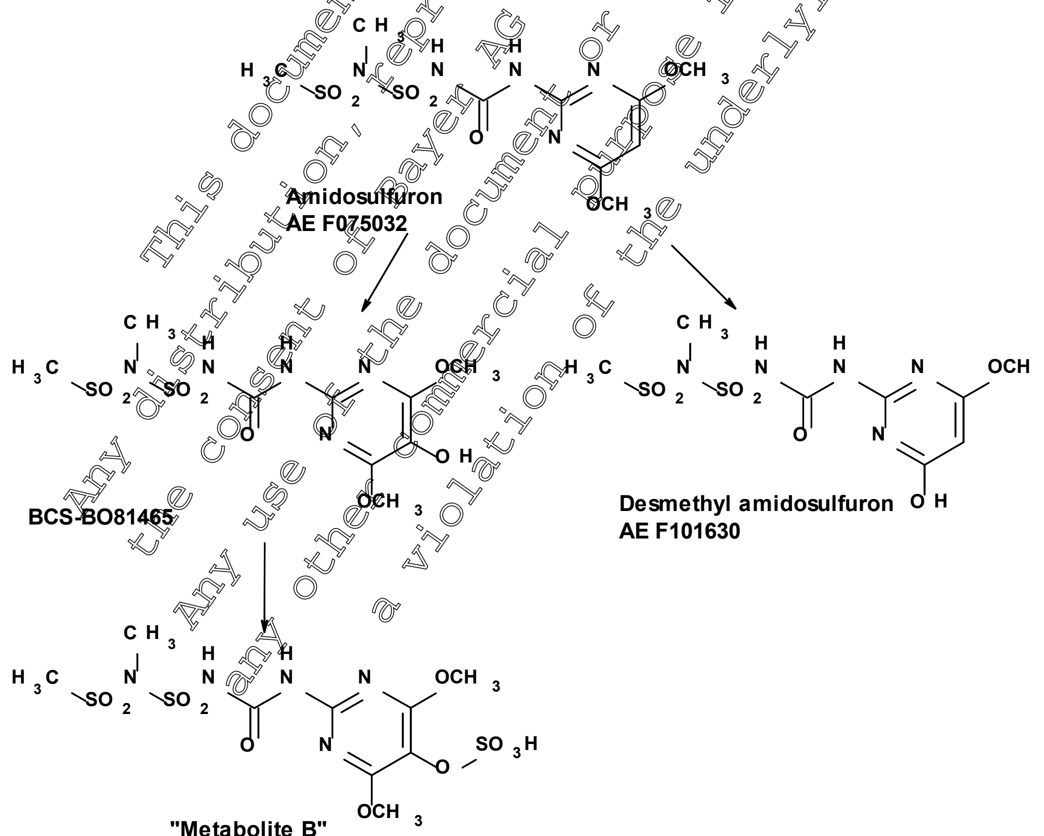
The levels of radioactivity distributed in the tissues were found to be very low for both male and female rats following single oral administration at all dose levels (10, 100 and 500 mg/kg body weight). The results showed mean concentrations that ranged between 0.001 - 0.004 µg equivalents/g for the low dose study and 0.01 - 0.59 µg equivalents/g at the high dose level. The levels of radioactivity following intravenous administration and oral administration at 10 mg/kg were very similar thus, after intravenous administration the concentration mean values in tissues also ranged between 0.001 -0.004 µg equivalents/g. The multiple dosing did not have any impact on the tissue disposition of amidosulfuron. Thus, all radioactive residues were cleared during the depuration phase (eight days after the last of the 14 daily oral administrations) leaving only low levels in cardiac blood,

plasma, subcutaneous fat, kidneys and residual carcass for male and/or female rats. All other tissues presented non-detectable levels.

The pharmacokinetics of amidosulfuron in the plasma of rats indicated a biphasic elimination with a fast initial elimination (half-lives ranged between 1 and 2 hours) in which the main part of the radioactivity was excreted and a second slow elimination of a minor part of the radioactivity (>95% - 24 hours). Maximum plasma concentrations of 809.3 ± 189.8 and 877.2 ± 25.7 µg equivalents/g were attained 0.45 ± 0.11 and 1.0 ± 0 hours after single oral administration of 500 mg/kg bw to female and male rats, respectively. After a single oral administration of 10 mg/kg bw, plasma concentrations of 29.2 ± 7.1 and 57.9 ± 2.5 µg equivalents/g in male and female rats respectively occurred at 0.80 ± 0.74 and 0.30 ± 0.11 hours after dosing.

In the rat metabolism studies the major part of the radioactivity (>60% of the administered radioactivity) was excreted as parent compound. Amidosulfuron was metabolised in rats by O-demethylation on the pyrimidine ring leading to AE F101630, which was excreted in urine and faeces. The results from the repeated oral dosing showed that the multiple dosing did not have any impact on the metabolic pathway. Hence, 24 hours after 14 daily oral administrations, the unchanged amidosulfuron was the main metabolite eliminated in excreta samples and the main metabolic route was the *O*-demethylation of amidosulfuron leading to AE F101630, which was also excreted in urine and faeces. No breakdown of the sulfonylurea bridge was observed after single or multiple dosing.
Hydroxylation of the parent compound was identified to be a minor pathway of metabolism. The corresponding metabolite (ring hydroxylated amidosulfuron) was found in urine only and with less than 1% of administered radioactivity. Furthermore, in one metabolism study the sulphate conjugate of hydroxylated amidosulfuron has been detected.
Overall the studies showed no significant difference in the metabolic profile between sexes or dose levels. The proposed metabolic pathway in rat is given in figure 5.1-1.

Figure 5.1-1: Proposed metabolic pathway of amidosulfuron in the rat



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CA 5.1.1 Absorption, distribution, metabolism and excretion by oral exposure

Report:	KCA 5.1.1/01 ,; 1988; M-124446-01-1
Title:	Hoe 075032-14-C Metabolism and Pharmacokinetics in Male and Female Rats after a Single Oral Administration of 10mg/kg Body weight
Report No.:	A41330
Document No.:	M-124446-01-1
Guideline(s):	USEPA (=EPA): § 85-1
Guideline deviation(s):	--
GLP/GEP:	**no**

Report:	KCA 5.1.1/02 ,; 1988; M-121292-01-1
Title:	Hoe 075032-14-C Metabolism and Pharmacokinetics in Male and Female Rats after a Single Oral Administration of 100 mg/kg Body Weight
Report No.:	A39717
Document No.:	M-121292-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Report:	KCA 5.1.1/03 ,; 1989; M-124590-01-1
Title:	Hoe 075032-14-C Metabolism and Pharmacokinetics in Male and Female Rats after a Single Oral Administration of 500 mg/kg Body weight
Report No.:	A41501
Document No.:	M-124590-01-1
Guideline(s):	USEPA (=EPA): § 85-1
Guideline deviation(s):	--
GLP/GEP:	**yes**

Report:	KCA 5.1.1/04 ,; 1992; M-138078-01-1
Title:	Hoe 075032-14-C Metabolism in rats following single oral administration of test substance at a dose level of 500 mg/kg-1 body weight
Report No.:	A48996
Document No.:	M-138078-01-1
Guideline(s):	USEPA (=EPA): § 85-1
Guideline deviation(s):	--
GLP/GEP:	**yes**

Report:	KCA 5.1.1/05 ,; 2002; M-215171-01-1
Title:	Amidosulfuron (14C-AE F075032) - Repeat Oral Low Dose ADME Study in the Rat
Report No.:	C028393
Document No.:	M-215171-01-1
Guideline(s):	EU (=EEC): 94/79/EC Dec1994; MAFF: 12 NohSan N° 8147, 2000; USEPA (=EPA): OPPTS 870.7485, 1998
Guideline deviation(s):	--
GLP/GEP:	**yes**

Report:	KCA 5.1.1/06 ,; 1989; M-125121-01-1
Title:	Hoe 075032-14-C Metabolism and pharmacokinetics in male rats following oral administration of test substance at a concentration of 10,000 ppm in food
Report No.:	A42082
Document No.:	M-125121-01-1
Guideline(s):	USEPA (=EPA): § 85-1
Guideline deviation(s):	--
GLP/GEP:	**yes**

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Report:	KCA 5.1.1/07 ,; 2015; M-504340-02-1
Title:	[Pyrimidyl-2-14C]amidosulfuron: Metabolic stability and profiling in liver microsomes from rats and humans for inter-species comparison
Report No.:	EnSa-14-0235
Document No.:	M-504340-02-1
Guideline(s):	Regulation (EC) No 1107/2009 amended by the Commission Regulation (EU) No. 283/2013 (Europe) US EPA OCSPP Not Applicable
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

According to the new data requirements (Commission regulation (EU) No 283/2013), an *in vitro* metabolism study was performed and is summarised below.

Materials and Methods

The comparative metabolism of [pyrimidine-2-^{14}C]-amidosulfuron (^{14}C-amidosulfuron) was investigated in animal in-vitro systems by incubating the test item with liver microsomes from male Wistar rats (RLM) and humans (HLM) in the presence of NADPH cofactor. The 10 µM test item concentration was chosen in order to have enough sample material for possible identification of metabolites by chromatographic or spectroscopic methods. The sampling times were 0, 0.5 and 1 hour after test start. The metabolic activity of the microsomes was demonstrated by determining 6β-hydroxy-testosterone that was formed from testosterone by testosterone 6β-hydroxylase. This biochemical reaction is well known for the CYP3A microsomal enzyme.

The test duration of 1 hour for the test item was considered as reasonable because positive results were obtained from the enzymatic reaction of Testosterone to hydroxy-testosterone already after 10 minutes. Samples were analyzed following protein precipitation by reversed phase HPLC with radiochemical detection (HPLC-RAD).

Results

The recovery of radioactivity was measured in both microsome incubations and amounted to ≥95.0% for the 0.5 and 1 hour samples.
The results of the tests with ^{14}C-amidosulfuron demonstrated that the test item was highly stable after incubation with RLM and HLM.
No metabolites were detected after incubation of ^{14}C-Amidosulfuron with HLM and RLM during 0.5 and 1 hour, leading to the conclusion that phase I metabolism enzymes are unlikely to play a significant role in the biotransformation of amidosulfuron in rat and human liver microsomes. Consequently, no differences with respect to the metabolic pattern were found in both *in-vitro* systems.

Conclusion

Overall, the results suggest that phase I metabolism is not involved in the biotransformation of amidosulfuron-methyl in rat and human liver microsomes. Consequently, no differences with respect to the metabolic pattern were found in both *in-vitro* systems.

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CA 5.1.2 Absorption, distribution, metabolism and excretion by other routes

Studies submitted and evaluated for the first inclusion of amidosulfuron on Annex I:

Report:	KCA 5.1.2/01 ,; 1990; M-127312-01-1
Title:	Hoe 075032-14-C Metabolism and pharmacokinetics in male and female rats after a single intravenous administration of 10 mg/kg body weight
Report No.:	A44437
Document No.:	M-127312-01-1
Guideline(s):	USEPA (=EPA): § 85-1
Guideline deviation(s):	--
GLP/GEP:	**yes**

CA 5.2 Acute toxicity

The acute toxicity of amidosulfuron is very low after oral, dermal and inhalative administration. It is non-irritating to the rabbit skin and only slightly irritating to the rabbit eye. The maximization test according to Magnusson and Kligman did not show a skin sensitization effect of amidosulfuron.

Classification:
No classification and labelling is proposed for amidosulfuron regarding acute toxicity, respiratory tract irritation, skin and eye irritation or skin sensitization.
No specific target organ toxicity after single exposure (STOT-SE) was observed in acute toxicity studies.

Table 5.2-1: Summary of the acute toxicity studies

TYPE OF STUDY	SPECIES	RESULTS	REFERENCE
Acute oral toxicity	Wistar rat	LD50 > 5000 mg/kg bw (m, f)	,; ; 1987; M-121349-01-1
Acute oral toxicity	NMRI mouse	LD50 > 5000 mg/kg bw (m, f)	,; ; 1988; M-120196-01-1
Acute dermal toxicity	Wister rat	LD50 >5000 mg/kg bw (m,f)	,; ; 1987; M-121348-02-1
Acute inhalative toxicity	Wister rat	LC50 > 1.8 mg/l air (4hours, nose only)	Z; ; 1988; M-120198-01-1
Dermal irritation study	New Zealand White Rabbit	No dermal irritation	L; ; 1987; M-121100-01-1
Eye irritation study	New Zealand White Rabbit	Slight eye irritation	,; ; 1987; M-121350-01-1
Dermal sensitization (Maximisation test)	Guinea Pig	No skin sensitization	,; ; 1991; M-120199-02-1
Dermal sensitization (Maximisation test)	Guinea Pig	No skin sensitization	B; 2003; M-216833-01-1

m = males, f = females

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CA 5.2.1 Oral

Studies submitted and evaluated for the first inclusion of amidosulfuron on Annex I:

Report:	KCA 5.2.1/01 [redacted],;[redacted]; 1987; M-121349-01-1
Title:	Hoe 075032 - substance technical (Code: Hoe 075032 OH ZC98 0001) Testing for acute oral toxicity in the male and female Wistar rat
Report No.:	A39775
Document No.:	M-121349-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Report:	KCA 5.2.1/02 [redacted],;[redacted]; 1988; M-120196-01-1
Title:	Hoe 075032 - active ingredient technical (Code: Hoe 075032 OH ZC97 0001) Testing for acute oral toxicity in the male and female NMRI mouse
Report No.:	A38569
Document No.:	M-120196-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

CA 5.2.2 Dermal

Studies submitted and evaluated for the first inclusion of amidosulfuron on Annex I:

Report:	KCA 5.2.2/01 [redacted],;[redacted]; 1987; M-121348-02-1
Title:	Hoe 075032 - substance technical (Code: Hoe 075032 OH ZC98 0001) Testing for acute dermal toxicity in the male and female Wistar rat
Report No.:	A41227
Document No.:	M-121348-02-1
Guideline(s):	USEPA (=EPA): § 81-2
Guideline deviation(s):	--
GLP/GEP:	**yes**

CA 5.2.3 Inhalation

Studies submitted and evaluated for the first inclusion of amidosulfuron on Annex I:

Report:	KCA 5.2.3/01 [redacted]Z;[redacted]; 1988; M-120198-01-1
Title:	Hoe 075032 - active ingredient technical (Code: Hoe 075032 OH ZC97 0001) Testing for acute dust inhalation toxicity in the male and female SPF Wistar rat 4-hour LC50
Report No.:	A38571
Document No.:	M-120198-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

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CA 5.2.4 Skin irritation

Studies submitted and evaluated for the first inclusion of amidosulfuron on Annex I:

Report:	KCA 5.2.4/01 K; ; 1987; M-121100-01-1
Title:	Hoe 075032 - substance technical (Code: Hoe 075032 OH ZC98 0001) Testing for primary dermal irritation in the rabbit
Report No.:	A39521
Document No.:	M-121100-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

CA 5.2.5 Eye irritation

Studies submitted and evaluated for the first inclusion of amidosulfuron on Annex I:

Report:	KCA 5.2.5/01 .; ; 1987; M-121350-01-1
Title:	Hoe 075032 - substance technical (Code: Hoe 075032 OH ZC98 0001) Testing for primary eye irritation in the rabbit
Report No.:	A39776
Document No.:	M-121350-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

CA 5.2.6 Skin sensitization

Studies submitted and evaluated for the first inclusion of amidosulfuron on Annex I:

Report:	KCA 5.2.6/01 .; ; 1991; M-120199-02-1
Title:	Hoe 075032 - active ingredient technical (Code: Hoe 075032 OH ZC96 0001) Testing for sensitizing properties in the Pirbright-White guinea pig in a maximisation test
Report No.:	A38572
Document No.:	M-120199-02-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Report:	KCA 5.2.6/02 B; 2003; M-216833-01-1
Title:	Amidosulfuron (code AE F075032) skin sensitization test in guinea pigs (according to magnusson and kligman).
Report No.:	C029247
Document No.:	M-216833-01-1
Guideline(s):	EU (=EEC): 96/54/EEC; JMAF: 59 NohSan No- 4200; OECD: No.406; USEPA (=EPA): OPPTS 870.2600
Guideline deviation(s):	--
GLP/GEP:	**yes**

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CA 5.2.7 Phototoxicity

According to the new data requirements (Commission Regulation (EU) No. 283/2013 of 1 March 2013; Official Journal of the European Union, L 93/1, 3.4.2013), the conduct of a phototoxicity study is required under certain conditions.

The circumstances in which a phototoxicity study, according to the new data requirements, is required are "where the active substance absorbs electromagnetic radiation in the range 290-700 nm and is liable to reach the eyes or light-exposed areas of the skin, either by direct contact or through systemic distribution. If the Ultraviolet / visible molar extinction / absorption coefficient of the active substance is less than 10 L x mol^{-1} x cm^{-1}, no toxicity testing is required."

As the Ultraviolet / visible molar extinction / absorption coefficient of amidosulfuron exceeds the trigger of 10 L x mol^{-1} x cm^{-1}, a cytotoxicity study has been performed *in vitro* using BALB/c 3T3 cells.

Report:	KCA 5.2.7/01 [redacted]; 2015; M-541941-01-1
Title:	Amidosulfuron, technical: Cytotoxicity assay *in vitro* with BALB/c 3T3 cells: Neutral red (NR) test during simultaneous irradiation with artificial sunlight
Report No.:	1693100
Document No.:	M-541941-01-1
Guideline(s):	Commission Regulation (EC) No. 440/2008 B 41", dated May 30, 2008. Committee for Proprietary Medicinal Products (CPMP) Note for Guidance on Photosafety testing, EMEA, CPMP/SWP/398/01, adopted 27 June 2002, into operation in Dec 2002. OECD Guideline for Testing of Chemicals: Guideline: 432; *In vitro* 3T3 NRU phototoxicity test (Revised and approved by the National Co-ordinators in May 2002, approved by Council April 2004).
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive summary:
The study was performed to assess the phototoxic potential of amidosulfuron, technical. The test was performed using BALB/c 3T3 cells clone 31 with and without irradiation with artificial sunlight.
The experiment was performed twice. The first experiment served as a range finding experiment (RFE), the second one was the main experiment (ME).
The following concentrations (constant dilution factor: 2) of the test item solved in DMSO (final concentration of DMSO in EBSS: 1% (v/v)) were tested in the presence and in the absence of irradiation in both experiments: 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250, 500 µg/mL
As solvent control for the test item EBSS containing 1% (v/v) DMSO was used.
Chlorpromazine was used as positive control.
One test group of cells treated with the test item was irradiated with artificial sunlight for 50 minutes with 1.65 mW/cm^2 UVA, resulting in an irradiation dose of 5 J/cm^2 UVA. Another test group of test item treated cells were kept in the dark for 50 minutes.
Cytotoxic effects were not observed after treatment of the cells with Amidosulfuron, technical, neither in the presence nor in the absence of irradiation with artificial sunlight in both experiments. Therefore, ED_{50}-values or a PIF could not be calculated. The resulting MPE value was -0.020 and -0.018, respectively. Consequently, the test item is classified as not phototoxic (see chapter 3.9).
The acceptance criteria were met.
In conclusion, it can be stated that in this study and under the experimental conditions reported, the test item amidosulfuron, technical does not possess any phototoxic potential.

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I. Materials and methods

A. Materials

1. Test material:

Identification:	Amidosulfuron technical
Batch Code:	AE F075032-01-05
CAS. No.:	120923-37-7
Purity:	98.1% (w/w) (dose calculation was adjusted to purity)
Appearance:	Solid, powder, white to light fawn
Expiry Date:	04 March 2016
Storage Conditions:	At room temperature, protected from light
Stability in Solvent:	Stable under specified storage conditions

2. Vehicle: EBSS containing 1% (v/v) DMSO

3. Positive control: Chlorpromazine (Sigma) dissolved in EBSS

4. Test system:
Cells: BALB/c 3T3 cells clone

Cell cultures:

Large stocks (Master Cell Stock) of the BALB/c 3T3 c31 cell line (supplied by [redacted] [redacted], Berlin, Germany) are stored in liquid nitrogen in the cell bank of Envigo CCR. The master cell stock has been characterised by Envigo CCR. A working cell stock is produced by multiplying from the master cell stock.
Thawed stock cultures were propagated at 37 ± 1.5 °C in 75 cm^2 plastic flasks. Seeding was done with about 1 x 10^6 cells per flask in 15 mL of Dulbecco's Minimal Essential Medium (DMEM), supplemented with 10 % NCS. The cells were sub-cultured twice weekly. The cell cultures were incubated at 37 ± 1.5 °C in a 7.5 ± 0.5% carbon dioxide atmosphere.

B. Study design and methods

1. Treatments:
The experiment was performed twice. The first experiment served as a range finding experiment (RFE), the second one was the main experiment (ME).

The following concentrations were tested in a range finding as well as in a main experiment (each concentration of the test item was measured in six replicates):

Table 5.2.7-1: Doses

	µg/mL of the test item							
with and without irradiation	3.91	7.81	15.63	31.25	62.5	125	250	500

2. Experimental procedures

Solar Simulator:
The irradiation was performed with a Dr. Hönle Sol 500 solar simulator. The filter H1 was used to keep the UVB irradiation as low as possible. The produced wavelength of the solar simulator with the filter was > 320 nm. Due to the heterogenous distribution of irradiation intensity the UVA intensity

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was measured for the complete area with a UV-meter. The homogeneous irradiation area was marked and the cultures were irradiated in this area. The solar simulator was switched on about 30 minutes prior to the start of experiment.

The absorption spectrum of the test item was determined in the range from 270-800 nm. The test item showed an absorption maximum at 273.1 nm.

Seeding of Cultures:
About 2 x 10^4 cells per well were seeded in 100 µL culture medium (two plates, one was exposed to artificial sun light, one was kept in the dark).

Treatment:
Approximately 24 hours after seeding the cultures were treated with the test item. The treatment was performed according to the OECD guideline as follows:

- the cultures were washed with EBSS
- 8 dilutions of the solved test item were tested on two 96-well plates (100 µL/well)
- both plates were pre-incubated for 1 hour in the dark
- after one hour one 96-well plate was irradiated through the lid at 1.65 mW/cm^2 (5 J/cm^2), for 50 min, the other plate was stored for 50 min in the dark (temperature: 26 °C in the RFE and 27 °C in the ME).
- after irradiation the test item was removed and both plates were washed twice with EBSS.
- fresh culture medium was added and the cells were incubated for 20 - 24 hours at 37 ± 1.5 °C and 7.5 ± 0.5% CO_2.

Cytotoxicity determination:
The medium was removed and 0.1 mL serum free medium containing 50 µg Neutral Red / mL was added to each well. The plates were returned to the incubator for another 3 hours to allow uptake of the vital dye into the lysosomes of viable cells. Thereafter, the medium was removed completely and the cells were washed with EBSS. Then 0.15 mL of a solution of 49% (v/v) deionised water, 50% (v/v) ethanol and 1% (v/v) acetic acid were added to each well to extract the dye. After an additional approximately 10 minutes at room temperature and a brief agitation, the plates were transferred to a microplate reader (Versamax®, Molecular Devices, SoftMax Pro Enterprise (version 4.7.1)) equipped with a 540 nm filter to determine the absorbance of the extracted dye. This absorbance showed a linear relationship with the number of surviving cells.

Data Recording:
The data generated were recorded in the laboratory raw data file. The results are presented in tabular form, including experimental groups with the test item, solvent, and positive control. Arithmetic means ± standard deviation were calculated for every test group.

The ED_{50} values, the Photo-Irritancy-Factor (PIF), as well as the Mean Phototoxic Effect (MPE), were calculated using the software Phototox (Version 2.0) (distributed by ZEBET, 12277 Berlin, Germany, and recommended by the OECD guideline).

The ED_{50} values (effective dose where only 50% of the cells survived) were determined by curve-fitting software.

The PIF is defined by the following equation:

$$PIF = \frac{ED_{50}\ (-UV)}{ED_{50}\ (+UV)}$$

If a chemical is only cytotoxic +UV and is not cytotoxic when tested –UV, the PIF cannot be calculated, although this result indicates a phototoxic potential. In such cases, a > PIF value can be

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calculated if the (-UV) cytotoxicity test is performed up to the highest test concentration (Cmax) and this value is used for calculation of the > PIF:

$$> PIF = \frac{C_{max}\,(-UV)}{ED_{50}\,(+UV)}$$

Since the > PIF is not an exact numerical value, no biostatistical procedure can be applied to determine the optimum cut-off. Consequently, the classification rule as follows: If only a > PIF can be obtained, then any value > 1 predicts a phototoxic potential.

The Mean Phototoxic Effect (MPE) is based on comparison of the complete concentration response curves. It is defined as the weighted average across a representative set of photo effect values.

The photo effect (PEc) at any concentration (C) is defined as the product of the response effect (REc) and the dose effect (DEc) i.e. PEc = REc x DEc. The response effect (REc) is the difference between the responses observed in the absence and presence of light, i.e., REc = Rc (-UV) – Rc (+UV)).

The calculation of MPE is restricted to the maximum concentration value at which at least one of the two curves still exhibits a response value of at least 10%. If this maximum concentration is higher than the highest concentration used in the +UV experiment the residual part of the +UV curve is set to the response value "0". Depending on whether the MPE value is larger than a properly chosen cut-off value (MPE = 0.15) or not, the chemical is classified as phototoxic.

Evaluation criteria:
Based on the results obtained, the test item is evaluated as follows:

If **PIF < 2 or MPE < 0.1**: no phototoxic potential is predicted.
If **PIF > 2 and < 5 or MPE >0.1 and <0.15** a probable phototoxic potential is predicted.
If **PIF > 5 or MPE > 0.15** a phototoxic potential is predicted.

Acceptability of the Assay:
The assay meets the acceptance criteria:

- if after irradiation with a UVA dose of 5 J/cm² the cell viability of the solvent control is > 80% of non-irradiated cells.
- if for the positive control CPZ the factor (PIF) between the two ED_{50} values is > 6.
- if the mean OD540 of solvent controls is > 0.4.

II. Results and discussion

The study was performed to assess the phototoxic potential of Amidosulfuron, technical. The test was performed using BALB/c 3T3 cells clone 31.

500 µg/mL of the test item, dissolved in DMSO (DMSO = 1% (v/v) in EBSS) was applied as the highest concentration in a range finder as well as in a main experiment.

No cytotoxic effects were observed after treatment of cells with Amidosulfuron, technical in the presence or in the absence of irradiation with artificial sunlight in both experiments. Therefore, ED_{50}-values or a PIF value could not be calculated. The MPE value was -0.020 and -0.018, respectively, and therefore, the test item is classified as not phototoxic.

The acceptance criteria were met:

- The PIF of the positive control was > 6 (47.64 and 10.08).
- The mean OD540 of the solvent controls was > 0.4 (range: 0.8143 to 1.2844)
- The cell viability of the solvent control is > 80% of non-irradiated cells (95.0% and 102.3% for the test item, 92.8% and 83.1% for the positive control).

In conclusion, it can be stated that in this study and under the experimental conditions reported, the test item Amidosulfuron, technical does not possess any phototoxic potential. The results are summarized in table CA 5.2.7-2.

Table CA 5.2.7-2: Summary of Results

	Substance	ED_{50} (+UV) [µg/mL]	ED_{50} (-UV) [µg/mL]	PIF	MPE	% viability of solvent control of irradiated versus non-irradiated plate
RFE	Test Item	-	-	-	-0.020	95.0
	Positive control	0.55	26.24	47.64	0.574	92.8
ME	Test Item	-	-	-	-0.018	102.3
	Positive control	1.03	10.32	10.08	0.434	83.1

CA 5.3 Short-term toxicity

Studies with oral application in rats (28 and 90 days of exposure), a 28-day rat inhalation study and 2 studies with dermal exposure to rats (8 and 30 days), studies in mice (28 and 90 days of exposure) and in dogs (29, 90 days and 1 year of exposure) are available.

In a 28-day dietary study in the rat, a slightly reduced body weight gain and an increase of the relative liver weight at 10000 ppm in males was seen. In the report a NOAEL was set at 2000 ppm for both sexes (equivalent to 215 and 199 mg/kg bw for males and females, respectively).

In a 90-day oral rat toxicity study, no compound-related effects were seen, so that the NOAEL was established at the highest dose of 10000 ppm (equivalent to 792 mg/kg bw for males and 870 mg/kg bw for females).

In a 28-day dietary dose range finding study in mice, changes of some clinical chemistry parameters (bilirubin, cholesterol, inorganic phosphorus, uric acid, triglycerides and AST) were not regarded as dose-related and thus not as treatment-related effect. Thus the highest dose of 8000 ppm represents the NOAEL in this study (equivalent to 1776.1 mg/kg bw for males and to 1882 mg/kg bw for females). In a 90-day dietary study in mice, some changes in organ weights of the liver (increased relative and absolute organ weight of females of the highest dose group and in all male dose groups) were not regarded as treatment-related effects. Therefore, the NOAEL was set at the highest dose of 8000 ppm (equivalent to 1297 mg/kg bw for males and 1385 mg/kg bw for females).

In a 1-month dietary study in dogs, macroscopic (spleen: white pulp; discoloration of kidneys) and histological findings (follicular hypertrophy and hyperplasia of thyroid and spleen) were seen in animals at 2000 and 10000 ppm. A NOAEL was set at 400 ppm (equivalent to 25.6 mg/kg bw for males and 23.7 mg/kg bw for females). The findings in the spleen, kidneys and thyroid were not confirmed in the 3-month or 52-week dog studies.

A 3-month dietary study in dogs showed some body weight effects which however were not dose-related so that the highest dose of 2000 ppm can be regarded as NOAEL (equivalent to 175.4 mg/kg bw for males and 144.1 mg/kg bw for females).

In a 52-week dietary toxicity dog study, at 8000 ppm decreased plasma glucose and increased magnesium concentrations in the females, and decreased urinary specific gravity and osmolality in both sexes were seen. These effects were not severe and of low biological relevance so that the NOAEL was established at 8000 ppm according to the study report.

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A 29-day inhalation study in rats, with 21 days of treatment, did not show any treatment-related effects. The NOAEL could be established at the highest test concentration of 1 mg/L.

In a study with dermal application of 1000 mg/kg bw (5 times over a period of 8 days) to rats an increased absolute liver weight in females was seen which was regarded as adaptive effect and therefore not as adverse. The NOAEL is therefore 1000 mg/kg bw for both sexes.

In another study with dermal administration to rats (21 treatments in 30 days), no treatment related effects were seen. Changes of some clinical chemistry parameters were not regarded as substance-related or were regarded as adaptive effects. The NOAEL was established at the highest dose of 1000 mg/kg bw for both sexes.

Classification:
The effects observed in the oral subchronic studies in rat, mouse and dog, like liver weight and slight changes in some clinical chemistry parameters would not trigger classification and labelling for repeated dose toxicity and were anyway above the cut-off values for classification and labelling for repeated dose toxicity (28 days rodent studies: 300 mg/kg bw/d; 90 days rodent studies: 100 mg/kg bw/d; no cut-off values for dog studies). The results of the subchronic inhalation study show no effects at 1.0 mg/L air, which is above the cut-off value of 0.6 mg/l. The results of the subchronic dermal studies do not show effects at 1000 mg/kg bw/d, which is above the cut-off value of 600 mg/kg bw/d. Therefore, effects observed in the subchronic (oral, inhalative and dermal) studies in rat, mouse and dog do not trigger the criteria for classification and labelling for repeated dose toxicity according to CLP.

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Table CA 5.3-1: Summary of the short-term studies

STUDY/ SPECIES	DOSES TESTED	NOEL/ NOAEL	LOEL/LOAEL EFFECTS	REFERENCE
28-day oral , rat (diet)	0, 80, 400, 2000, 10000 ppm M: 8.1, 43, 215, 1068.3 mg/kg bw F: 7.9, 41.3, 199, 1028.5 mg/kg bw	2000 ppm M: 215 mg/kg bw, F: 199 mg/kg bw	10000 ppm Body weight effects (m) Bilirubin and uric acid decrease (f)	,; ; 1988; M-123241-01-1
Subchronic oral toxicity (13-week feeding study) in the Wistar rat	0, 400, 2000, 5000, 10000 ppm M: 30.9, 153.9, 387.8, 792.1 mg/ kg bw F: 34.2, 169.4, 433.5, 870 mg/kg bw	10000 ppm M: 792.1 mg/ kg bw/day F: 870 mg/kg bw/day	10000 ppm No treatment-related adverse effects	,; ; 1989; M-123312-01-1
29-day inhalation toxicity (21 applications within 29 days) in Wistar rats	0.04, 0.2, 1.0 mg/L	1.0 mg/L	> 1.0 mg/L No treatment-related adverse effects	,; ; 1992; M-136551-01-1
Dermal toxicity study (5 treatments in 8 days) in Wistar rats	0, 1000 mg/kg bw	1000 mg/kg bw	1000 mg/kg bw No treatment-related adverse effects	,; ; 1989; M-123306-01-1
Dermal toxicity study (21 treatments in 30 days) in Wistar rats	0, 500, 1000 mg/kg bw	1000 mg/kg bw	1000 mg/kg bw No treatment-related adverse effects	,; ; 1990; M-127700-01-1
Subchronic oral toxicity - dose range finding - (28-day feeding study) in the NMRI mouse	0, 64, 320, 1600, 8000 ppm M: 13.8, 70.5, 335.7, 1771.6 mg/kg bw F: 15.9, 67.8, 394.4, 1881.5 mg/kg bw	8000 ppm M: 1776.1 mg/ kg bw F: 1881.5 mg/ kg bw/day	> 8000 ppm No treatment-related adverse effects	Ö; ; 1988; M-123311-01-1
Subchronic oral toxicity (13-week feeding study) in the NMRI mouse	0, 320, 1600, 4000, 8000 ppm M: 53.5, 254, 648.5, 1297.3 mg/kg bw F: 54.1, 274.2, 698, 1384.8 mg/kg bw	8000 ppm M: 1297.3 mg/ kg bw F: 1384.8 mg/ kg bw	> 8000 ppm No treatment-related relevant adverse effects	,; ; 1989; M-123328-01-1
1-month (Range-finding-test) in Beagle dogs	0, 400, 2000, 10000 ppm M: 25.6, 129.9, 657.9 mg/kg bw F: 23.7, 121.2, 610.7 mg/kg bw/day	400 ppm M: 25.6 mg/kg bw F: 23.7 mg/kg bw/day	2000 ppm Discoloration of the kidneys and accentuated white pulp of the spleen	,; 1988; M-121473-01-1
3-month feeding study in Beagle dogs	0, 80, 400, 2000 ppm M: 6.5, 32.8, 175.4 mg/ kg bw F: 5.7, 28.8, 144.1 mg/ kg bw	2000 ppm M: 175.4 mg/ kg bw F: 144.1 mg/kg bw	> 2000 ppm No treatment-related relevant adverse effects	,; 1989; M-123323-01-1
52-week oral toxicity (feeding) study in the dog	0, 125, 500, 2000, 8000 ppm M: 3.8, 15.9, 72.3, 261.4 mg/kg bw F: 4.2, 17.2, 66.4, 271.9 mg/kg bw	8000 ppm M: 261.4 mg/ kg bw F: 271.9 mg/kg bw	> 8000 ppm No treatment-related relevant adverse effects	,; ; ; 1993; M-132042-01-1

M= male, F= female, RBC = red blood cell, Hb = hemoglobin, Hct = hematocrit

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CA 5.3.1 Oral 28-day study

Studies submitted and evaluated for the first inclusion of amidosulfuron on Annex I:

Report:	KCA 5.3.1/01 ; ; 1988; M-123241-01-1
Title:	Hoe 075032 - substance technical (Code: Hoe 075032 OH ZC97 0001) Subchronic oral toxicity - dose range finding - (28-day feeding study) in the Wistar rat
Report No.:	A40518
Document No.:	M-123241-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Report:	KCA 5.3.1/02 ,; ; 1988; M-123311-01-1
Title:	Hoe 075032 - substance technical (Code: Hoe 075032 OH ZC97 0001) Subchronic oral toxicity - dose range finding - (28-day feeding study) in the NMRI mouse
Report No.:	A40568
Document No.:	M-123311-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Report:	KCA 5.3.1/03 ; 1988; M-121473-01-1
Title:	Hoe 075032 - technical substance (Code: Hoe 075032 OH ZC97 0001) Testing for toxicity by repeated oral administration to Beagle dogs for 1 month (Range-finding-Test)
Report No.:	A39899
Document No.:	M-121473-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

CA 5.3.2 Oral 90-day study

Studies submitted and evaluated for the first inclusion of amidosulfuron on Annex I:

Report:	KCA 5.3.2/01 ; ; 1989; M-123312-01-1
Title:	Hoe 075032 - substance technical (Code: Hoe 075032 OH ZC97 0001) Subchronic oral toxicity (13-week feeding study) in the Wistar rat
Report No.:	A40569
Document No.:	M-123312-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Report:	KCA 5.3.2/02 ,; ; 1989; M-123328-01-1
Title:	Hoe 075032 - substance technical (Code: Hoe 075032 OH ZC97 0001) Subchronic oral toxicity (13-week feeding study) in the NMRI mouse
Report No.:	A40572
Document No.:	M-123328-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

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Report:	KCA 5.3.2/03 [redacted],; 1989; M-123323-01-1
Title:	Hoe 075032 - substance technical (Code: Hoe 075032 OH ZC94 0001) Testing for toxicity by repeated oral administration to Beagle dogs (3-month feeding study)
Report No.:	A40570
Document No.:	M-123323-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Report:	KCA 5.3.2/04 [redacted],; [redacted]; [redacted]; 1993; M-132042-01-1
Title:	52-week oral toxicity (feeding) study with Hoe 075032 substance technical Code: Hoe 075032 00 ZD99 0001 in the dog
Report No.:	A51061
Document No.:	M-132042-01-1
Guideline(s):	EU (=EEC): 87/302/EEC B p. 27; MAFF: Japan, NohSan No. 4200; OECD: 452 (1981); USEPA (=EPA): § 83-1 (1984)
Guideline deviation(s):	--
GLP/GEP:	**yes**

CA 5.3.3 Other routes

Studies submitted and evaluated for the first inclusion of amidosulfuron on Annex I:

Report:	KCA 5.3.3/01 [redacted],; [redacted]; 1992; M-136551-01-1
Title:	Hoe 075032; substance, technical Code: Hoe 075032 00 ZC95 0001 Testing for subchronic inhalation toxicity (21 applications within 29 days) in male and female Wistar rats
Report No.:	A47877
Document No.:	M-136551-01-1
Guideline(s):	OECD: 412 (1981); USEPA (=EPA): § 82-4
Guideline deviation(s):	
GLP/GEP:	**yes**

Report:	KCA 5.3.3/02 [redacted],; [redacted]; 1989; M-123306-01-1
Title:	Hoe 075032 - substance technical (Code: Hoe 075032 00 ZC97 0001) Cumulative dermal toxicity (6 treatments in 8 days) in the male and female Wistar rat
Report No.:	A40567
Document No.:	M-123306-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Report:	KCA 5.3.3/03 [redacted],; [redacted]; 1990; M-127700-01-1
Title:	Hoe 075032 - substance technical (Code: Hoe 075032 OH ZC97 0001) Subchronic dermal toxicity (21 treatments in 30 days) in the Wistar rat
Report No.:	A44849
Document No.:	M-127700-01-1
Guideline(s):	OECD: 410 (1981); USEPA (=EPA): § 82-2
Guideline deviation(s):	--
GLP/GEP:	**yes**

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CA 5.4 Genotoxicity testing

Amidosulfuron was tested in a sufficient range of *in vitro* and *in vivo* mutagenicity assays measuring different mutagenic endpoints like gene mutation in bacterial and mammalian cells, chromosomal aberration and unscheduled DNA synthesis *in vitro* as well as an *in vivo* micronucleus test in mice.

These studies did not give evidence of a mutagenic potential of amidosulfuron.

Classification:
No genotoxic effects were observed in the *in vivo* or *in vitro* studies with amidosulfuron, therefore no classification is required.

Table CA 5.4-1: Summary of genotoxicity tests with amidosulfuron

Study	Dose range	Results	Reference
	In vitro studies		
Reserve mutation assay (S. typhi-murium TA 98, TA 100, TA 1535, TA 1537 and TA 1538; E. coli WP2uvrA)	0, 4, 20, 100, 500,2500 and 5000 µ/plate (dissolved in DMSO)	Negative (+/- S-9 mix)	███,; 1988; M-120200-01-1
Chinese hamster V79 cell/HGPRT locus gene mutation assay	500, 1000, 1500 and 2000 µ/ml (dissolved in DMSO)	Negative (+/- S-9 mix)	███,; 1988; M-123019-01-1
Chromosomal aberration assay in cultured human lymphocytes	0, 0.1, 0.6 and 1.1 mg/ml (dissolved in DMSO)	Negative (+/- S-9 mix)	███,; ███; 1989; M-122350-01-1
Unscheduled DNA synthesis assay in mammalian cells (permanent human cell line A 549)	0, 1, 3, 10, 30, 100, 300, 1000 µ/ml (dissolved in DMSO)	Negative (+/- S-9 mix)	███,; 1988; M-120201-01-1
	In vivo studies		
Micronucleus test in NMRI mice	0, 1250, 2500, 5000 mg/kg bw/d (suspended in starch mucilage)	Negative	███,; 1988; M-120202-01-1

CA 5.4.1 In vitro studies

Studies submitted and evaluated for the first inclusion of amidosulfuron on Annex I:

Report:	KCA 5.4.1/01 ███,; 1988; M-120200-01-1
Title:	Hoe 075032 - substance, technical (Code: Hoe 075032 OH ZC98 0001) Study of the mutagenic potential in strains of Salmonella typhimurium (Ames Test) and Escherichia coli
Report No.:	A38573
Document No.:	M-120200-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

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Report:	KCA 5.4.1/02 ,; ; 1989; M-122350-01-1
Title:	Hoe 075032 - substance technical (Code: HOE 075032 OH ZC 94 0001) Chromosome aberration assay in human lymphocytes
Report No.:	A40034
Document No.:	M-122350-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Report:	KCA 5.4.1/03 ,; 1988; M-123019-01-1
Title:	Hoe 075032 - substance, technical (Code: Hoe 075032 OH ZC97 0001) Detection of gene mutations in somatic cells in culture HGPRT-test with V79 cells
Report No.:	A40409
Document No.:	M-123019-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

CA 5.4.2 In vivo studies in somatic cells

Studies submitted and evaluated for the first inclusion of amidosulfuron on Annex I:

Report:	KCA 5.4.2/01 ,; 1988; M-120202-01-1
Title:	Hoe 075032 - substance, technical (Code: Hoe 075032 OH ZC96 0001) Micronucleus test in male and female NMRI mice after oral administration
Report No.:	A38575
Document No.:	M-120202-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Report:	KCA 5.4.2/02 ,; 1988; M-120201-01-1
Title:	Evaluation of Hoe 075032 - substance, technical (Code: Hoe 075032 OH ZC97 0001) in the unscheduled DNA synthesis test in mammalian cells *in vitro*
Report No.:	A38574
Document No.:	M-120201-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

CA 5.4.3 In vivo studies in germ cells

Since the genotoxicity studies did not reveal evidence of a genotoxic potential and since in the reproduction studies no evidence of a germ cell-damaging potential was seen, the conduct of *in vivo* germ cell studies was not necessary.

CA 5.5 Long-term toxicity and carcinogenicity

The chronic toxicity and carcinogenicity of amidosulfuron has been investigated in rats and mice.
In a 2-year combined chronic toxicity and carcinogenicity study in rats, with dietary concentrations of 0, 400, 2000, 10000 and 20000 ppm, dose-related retardation of the body weight gain was noted in the males at ≥ 2000 ppm during the first half of the treatment period. At 2000 ppm this trend was completely reversible by the end of the study. In females increased water uptake at ≥ 2000 ppm was seen. At 20000 ppm, slight retardation of the body weight gain was noted during the second part of the

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treatment period, whereas at 400 and 2000 ppm females had slightly increased body weight gain in the first part of the treatment period which was regarded as a slight adaptive reaction.

For changes in hematological parameters at 20000 ppm which were only marginally different from those of the controls, no correlation could be found between these findings and the histopathological data. There was no evidence of an oncogenic potential of amidosulfuron.

The NOAEL was established at 2000 ppm in males and at 10000 ppm in females (equivalent to 97.8 and 614.2 mg/kg bw for males and females, respectively).

In a mouse oncogenicity study with dietary concentrations of 0, 400, 3500 and 7000 ppm, no treatment related effect up to the highest dose level tested was seen since changes of haematology and clinical chemistry parameters were not considered to be treatment-related since no dose relationship could be observed. There was no evidence of an oncogenic potential of amidosulfuron. A NOAEL was established at 7000 ppm (equivalent to 961 mg/kg bw/d for males and of 1260.2 mg/kg bw/d for females).

Based on both studies, it is concluded that amidosulfuron has no oncogenic potential.

Classification:
Since there is no evidence of a carcinogenic potential of amidosulfuron rats and mice, no classification is proposed.

Table CA 5.5-1: Summary of long-term toxicity and carcinogenicity in rat and mouse

STUDY/ SPECIES	DOSES TESTED	NOEL/ NOAEL	LOEL / EFFECTS	REFERENCE
Chronic toxicity and oncogencity study in Wistar rats via diet up to 111 weeks	0, 400, 2000, 10000, 20000 ppm M: 19.5, 97.8, 495.4, 1044.1 mg/kg bw/day F: 23.6, 118.7, 614.2, 1300.5 mg/kg bw/day	2000 ppm M: 97.8 mg/kg bw F: 614.2 mg/kg bw	Retardation of the body weight gain (m,f), increased water uptake (f), RBC decreased (f), increased MCV (f), decreased MCHC (m, f), decreased glucose (m,f), total protein (m) **No oncogenic potential**	,; ; ; 1992; M-138500-01-1
Oncogenicity study in mice for 78 to 91 weeks	0, 400, 3500, 7000 ppm M: 54, 474.6, 961 mg/kg bw/day F: 72.8, 671.7, 1260.2 mg/kg bw/day	7000 ppm M: 961 mg/kg bw F: 1260.2 mg/kg bw	7000 ppm No treatment-related adverse effects **No oncogenic potential**	,; ; ; 1992; M-138055-01-1

M= male, F= female, RBC = red blood cell, MCV = mean corpuscular volume, MCHC = mean corpuscular haemoglobin concentration, Hb = hemoglobin, Hct = hematocrit

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Studies submitted and evaluated for the first inclusion of amidosulfuron on Annex I:

Report:	KCA 5.5/01 ; ; ; 1992; M-138500-01-1
Title:	Hoe 075032 substance technical (Code: Hoe 075032 00 ZC96 0002) Combined chronic toxicity/oncogenicity (feeding) study in the rat
Report No.:	A49450
Document No.:	M-138500-01-1
Guideline(s):	MAFF: Japan, NohSan No. 4200; OECD: 451 (1981); USEPA (=EPA): § 83-5 (1984)
Guideline deviation(s):	--
GLP/GEP:	**yes**

Report:	KCA 5.5/02 ,; ; ; 1992; M-138055-01-1
Title:	Hoe 075032 substance technical (Code: Hoe 075032 00 ZC96 0002) Oncogenicity (feeding) study in mice
Report No.:	A48971
Document No.:	M-138055-01-1
Guideline(s):	MAFF: Japan, NohSan No. 4200; OECD: 451 (1981); USEPA (=EPA): § 83-2 (1984)
Guideline deviation(s):	--
GLP/GEP:	**yes**

CA 5.6 Reproductive toxicity

In a dose-range finding test for the two generation study of rats, slightly and statistically not significantly decreased mean body weights of male and female pups after birth were noted on day 0 post-partum at 10000 ppm. Furthermore, slightly decreased mean body weight gain during the lactation period and slightly decreased mean body weights of male and female pups were seen from day 1 to day 28 post-partum. None of the reproduction parameters were affected by the administration of the test substance.

Based on this study, the dose levels were chosen for the two generation study in rats to be 0, 400, 2000 and 10000 ppm.

In the two generation study on rats, at 10000 ppm, food consumption was occasionally decreased in the P and F1 generation females and F1 generation males, and at 2000 ppm in the F1 generation females during the prepairing and gestation periods. Body weights were slightly decreased at 2000 and 10000 ppm in the F1 and F2 pups. As in the dose-range finding test, none of the reproduction parameters were affected by the test substance.

Organ weight changes were noted in parents and pups of the 2000 ppm and the 10000 ppm dose level, but were not regarded as treatment-related. The NOAEL for both parental and reproductive effects was established at 400 ppm (equivalent to 22.5 mg/kg bw/day in males and to 29.7 mg/kg bw/day in females).

In conclusion, there was no evidence of a reproduction toxic potential of amidosulfuron.

Classification:
No treatment-related effects on reproduction parameters were observed in a rat multi-generation reproduction toxicity study which would trigger the classification for reproductive toxicity.

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Table CA 5.6-1: Summary of reproduction toxicity

STUDY DOSE LEVELS	NOEL parental mg/kg bw	NOEL develop-mental mg/kg bw	EFFECTS ppm	REF.
Preliminary study to the 2-generation reproduction study in the rat 0, 400, 2000, 10000 ppm	10000 ppm	Developmental: 2000 ppm Reproduction: 10000 ppm	Pups: Slightly decreased body weights at birth and slightly decreased body weight gain during the lactation period	,; ; ; 1991; M-130388-01-1
Two generation study in the rat 0, 400, 2000, 10000 ppm M: 22.5, 113.2, 568.0 mg/kg bw F: 29.7, 153.4, 732.3 mg/kg bw	400 ppm M: 22.5 mg/kg bw F: 29.7 mg/kg bw	Developmental:400 ppm M: 22.5 mg/kg bw F: 29.7 mg/kg bw Reproduction: 10000 ppm M: 568.0 mg/kg bw F: 732.3 mg/kg bw	Parents: Slightly reduced food consumption Pups: Slightly reduced mean body weight	,; ; ; 1992; M-135662-01-1

CA 5.6.1 Generational studies

Studies submitted and evaluated for the first inclusion of amidosulfuron on Annex I:

Report: KCA 5.6.1/01 S; ; ; 1991; M-130388-01-1
Title: Hoe 075032 substance technical (Code: Hoe 075032 00 ZC95 0001) Preliminary study to the two-generation reproduction study in the rat
Report No.: A46328
Document No.: M-130388-01-1
Guideline(s): --
Guideline deviation(s):
GLP/GEP: yes

Report: KCA 5.6.1/02 ,; ; ; 1992; M-135662-01-1
Title: Hoe 075032 substance technical (Code: Hoe 075032 00 ZC95 0001) Two-generation reproduction study in the rat
Report No.: A47236
Document No.: M-135662-01-1
Guideline(s): OECD: 416 (1981); USEPA (=EPA): § 83-4
Guideline deviation(s):
GLP/GEP: yes

CA 5.6.2 Developmental toxicity studies

Two developmental toxicity studies in rats with repeated oral administration of a dose of 1000 mg/kg bw from gestation day 7 to 16 in both studies in the sensitive phase of organogenesis did not impair the general health condition of the dams, interfere with the course of gravidity or delivery, or cause any disturbance of the intrauterine or postnatal development of the offspring. No teratogenic effect was observed. Therefore, the maternal and the fetal NOAEL in both studies was established at 1000 mg/kg bw/day.

A rabbit developmental toxicity study was conducted with administration of 1000 mg/kg bw on gestation days 6 to 18 in both studies. The dams delivered by caesarean section on day 29 of gravidity and the fetuses were kept for 24 hours in an incubator as a viability check and were then examined morphologically for developmental disturbances. The study showed that repeated oral administration

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of amidosulfuron at a dose level of 1000 mg/kg bw during the sensitive phase of organogenesis had no harmful effect on the general condition of the dams or on the intrauterine development of the conceptuses. There was also no effect on the viability of the fetuses during the first 24 hours in the incubator. The maternal and the fetal NOAEL was established at 1000 mg/kg bw/day.

Based on the studies in rats and rabbits, it can be concluded, that there is no evidence of a teratogenic potential of amidosulfuron.

Classification:
No treatment-related effects on fetal development were observed in the rat and rabbit developmental studies which would trigger the classification for developmental toxicity.

Table CA 5.6-2: Summary of developmental toxicity studies

STUDY DOSE LEVELS	NOEL maternal mg/kg bw/day	NOEL developmental mg/kg bw/day	DEVELOPMENTAL EFFECTS mg/kg bw/day	REF.
Testing for embryo-toxicity and effects on postnatal devel-opment in rats 0, 1000 mg/kg bw	1000 mg/kg bw	1000 mg/kg bw	No embryonic/fetal and postnatal toxicity	1991; M-130678-01-1
Postnatal develop-mental toxicity study in rats 0, 1000 mg/kg bw	1000 mg/kg bw	1000 mg/kg bw	No developmental toxic effects	,; 1988; M-123121-01-1
Teratogenicity in the Himalayan rabbit 0, 1000 mg/kg bw	1000 mg/kg bw	1000 mg/kg bw	No developmental toxic effects	,; 1988; M-123111-01-1

Studies submitted and evaluated for the first inclusion of amidosulfuron on Annex I:

Report: KCA 5.6.2/01 ,; 1988; M-123121-01-1
Title: Hoe 075032 - substance technical (Code: Hoe 075032 OH ZC97 0001) Testing for embryotoxicity in the Wistar rat after oral administration (Limit Test)
Report No.: A40449
Document No.: M-123121-01-1
Guideline(s): -
Guideline deviation(s): -
GLP/GEP: yes

Report: KCA 5.6.2/02 ; ; 1991; M-130678-01-1
Title: Hoe 075032 - substance technical (Code: Hoe 075032 00 ZC94 0001) Testing for embryotoxicity and effects on post-natal development in Wistar rats after oral administration (limit test)
Report No.: A46861
Document No.: M-130678-01-1
Guideline(s): OECD: 414; USEPA (=EPA): § 83-3
Guideline deviation(s): --
GLP/GEP: yes

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Report:	KCA 5.6.2/03 █; 1988; M-123111-01-1
Title:	Hoe 075032 - substance technical (Code: Hoe 075032 OH ZC97 0001) Testing for embryotoxicity in the Himalayan rabbit after oral administration (Limit Test)
Report No.:	A40445
Document No.:	M-123111-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Report:	KCA 5.6.2/04 █,; 2004; M-249539-01-1
Title:	Inhouse historical control data Wistar rat for study report no: 88.0318 (█, 1988) Hoe 075032 (Amidosulfuron) rat embryotoxicity study on finding: Individual scull bones - slight or non-ossification
Report No.:	C048111
Document No.:	M-249539-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Report:	KCA 5.6.2/05 █,; 2004; M-249538-01-1
Title:	Inhouse historical control data Himalayan rabbit for study report no: 89.0319 (█, 1988) Hoe 075032 (Amidosulfuron) rabbit embryotoxicity study on finding: at 13th thoracic vertebra - short or normally long, uni- or bilateral
Report No.:	C048110
Document No.:	M-249538-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

CA 5.7 Neurotoxicity studies

Amidosulfuron is a sulfonylurea herbicide which has no structural relationship to neurotoxic substances and furthermore, there was no evidence of a neurotoxic potential in the conducted apical studies. Consequently, no neurotoxicity studies were required, and no special studies have been conducted for this endpoint.

CA 5.7.1 Neurotoxicity studies in rodents

Since there was no evidence of a neurotoxic potential in the conducted apical studies, no neurotoxicity studies were required, and no specific studies have been conducted for this endpoint.

CA 5.7.2 Delayed polyneuropathy studies

Since amidosulfuron does not belong to the class of organophosphates or carbamates from which some candidates have the potential to cause polyneuropathy, and since in the other toxicology studies no evidence of any nerve effects was seen, it was not necessary to conduct such testing with amidosulfuron.

CA 5.8 Other toxicological studies

Two new genotoxicity studies on the soil metabolite AE F094206 were conducted (see below).

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CA 5.8.1 Toxicity studies of metabolites

AE F094206 was identified as a minor soil metabolite of Amidosulfuron with a predicted PEC_{gw} value of << 0.75 µg/L > 0.1 ug/L which triggers groundwater relevance assessment according to EU Council Directive 91/414/EEC (SANCO/221/2000 –rev. 10, 25 February, 2003). Two new studies; an in-vitro gene mutation assay and an in-vitro chromosome aberration assay, were conducted and are summarized below. An Ames test on AE F094206, [redacted],; 1992; M-138010-01-1, was submitted and evaluated for the first inclusion of amidosulfuron on Annex 1. All three in vitro genotoxicity studies indicate that AE F094206 does not have a mutagenic potential. An acute oral toxicity test in the Wistar rat on AE F094206, [redacted]; 1992; M-138216-01-1, was submitted and evaluated for the first inclusion of amidosulfuron on Annex 1, the LD50 value was found to be > 5000 mg/kg bw. in both sexes.

Amidosulfuron soil metabolite AE F094206: gene mutation assay in Chinese hamster V79 cells *in vitro* (V79/HPRT)

Report:	KCA 5.8.1/08 [redacted],; 2015; M-534290-01-1
Title:	Amidosulfuron soil metabolite AE F094206: Gene mutation assay in Chinese hamster V79 cells *in vitro* (V79 / HPRT)
Report No.:	1691501
Document No.:	M-534290-01-1
Guideline(s):	Ninth Addendum to OECD Guidelines for Testing of Chemicals, February 1998, adopted July 21, 1997, Guideline No. 476: Commission Regulation (EC) No. 440/2008 B17, dated May 30, 2008; United States Environmental Protection Agency Health Effects Test Guidelines, OPPTS 870.5300, *In Vitro* Mammalian Cell Gene Mutation Test, EPA 712-C-98-221, August 1998; Japanese Guidelines: Kanpoan No. 287 -- EPA Eisei No. 127 -- Ministry of Health & Welfare Heisei 09/10/31 Kikyoku No. 2 -- Ministry of International Trade & Industry; Ministry of Agriculture, Forestry and Fisheries of Japan. MAFF Notification No. 12 Nousan-8147, 24 November 2000
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive summary:
In this study the potential of Amidosulfuron soil metabolite AE F094206 to induce gene mutations at the HPRT locus in V79 cells of the Chinese hamster was investigated.

The study was performed in two independent experiments, using identical experimental procedures. In the first experiment the treatment period was 4 hours with and without metabolic activation. The second experiment was performed with a treatment time of 4 hours with and 24 hours without metabolic activation.

The maximum test item concentration of the pre-experiment and in the main experiments (1270 µg/mL) was equal to a molar concentration of about 10 mM.

No relevant and reproducible dose dependent increase of the mutation frequency was observed in the main experiments up to the maximum concentration with and without metabolic activation. The mutant frequency did not exceed the historical range of solvent controls. The threshold of three times the corresponding solvent control was exceeded in the second culture of the first experiment without metabolic activation at an intermediate concentration of 317.5 µg/mL. However, the threshold was not exceeded at any other, even higher concentration or in the parallel culture under identical conditions. Consequently, the isolated increase was judged as biologically irrelevant.

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Appropriate reference mutagens, used as positive controls, induced a distinct increase in mutant colonies and thus, showed the sensitivity of the test system and the activity of the metabolic activation system.

It can be concluded that under the experimental conditions the test item did not induce gene mutations at the HPRT locus in V79 cells. Therefore, Amidosulfuron soil metabolite AE F094206 is considered to be non-mutagenic in this HPRT assay.

I. Material and methods

A. Materials

1. Test material: **Amidosulfuron soil metabolite AE F094206**

Batch No.:	PW 210/213
Batch code:	AE F094206 00 1C99 0001
Purity:	99.5 % (w/w)
Appearance:	Light beige
Stability in Solvent:	Not indicated by the Sponsor
Storage:	In the refrigerator (+2 to +8 °C)
Expiry / Retest Date:	30 January 2021

2. Vehicle control:

Concurrent solvent controls (deionised water (local tap water, deionised at Harlan CCR)) were performed.

3. Positive Control Substances

Without metabolic activation

Name:	EMS; ethylmethane sulfonate
Purity:	≥ 99 %
Dissolved in:	Nutrient medium
Concentration:	0.15 mg/mL = 1.2 mM

With metabolic activation

Name:	DMBA; 7,12-dimethylbenz(a)anthracene
Purity:	≥ 95 %
Dissolved in:	DMSO; dimethylsulfoxide (final concentration in nutrient medium 0.5%)
Final concentration:	1.1 µg/mL = 4.3 µM (experiment I) 2.2 µg/mL = 8.6 µM (experiment II)

The dilutions of the stock solutions were prepared on the day of the experiment and used immediately.

The stability of both positive control substances in solution was proven by the mutagenic response in the expected range.

4. Activation

Due to the limited capacity for metabolic activation of potential mutagens in *in vitro* methods an exogenous metabolic activation system is necessary.

Phenobarbital/β-naphthoflavone induced rat liver S9 was used as metabolic activation system. The S9 was prepared and stored according to the currently valid version of the Harlan CCR SOP for rat liver S9 preparation. Each batch of S9 was routinely tested for its capability to activate the known mutagens benzo[a]pyrene and 2-aminoanthracene in the Ames test.

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An appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution to result in a final protein concentration of 0.75 mg/mL in the cultures. S9 mix contained MgCl2 (8 mM), KCl (33 mM), glucose-6-phosphate (5 mM) and NADP (4 mM) in sodium-ortho-phosphate-buffer (100 mM, pH 7.4).

The protein concentration of the S9 preparation was 35.0 mg/mL (Lot. No.: 290115) in the pre-experiment and in experiments I and II

5. Test System

Chinese Hamster V79 cells

The V79 cell line has been used successfully in *in vitro* experiments for many years. Especially the high proliferation rate (doubling time 12 - 16 h in stock cultures) and a good cloning efficiency of untreated cells (as a rule more than 50%) both necessary for the appropriate performance of the study, recommend the use of this cell line. The cells have a stable karyotype with a modal chromosome number of 22.

Culture conditions

Large stocks of the V79 cell line (supplied by Laboratory for Mutagenicity Testing; Technical University, Germany) are stored in liquid nitrogen in the cell bank of Harlan CCR allowing the repeated use of the same cell culture batch in many experiments. Before freezing, the level of spontaneous mutants was depressed by treatment with HAT-medium. Each batch is screened for mycoplasm contamination and checked for karyotype stability and spontaneous mutant frequency. Consequently, the parameters of the experiments remain similar because of the reproducible characteristics of the cells.

Thawed stock cultures are propagated at 37 °C in 75 cm2 plastic flasks. About 5×105 cells were seeded into each flask with 15 mL of MEM (minimal essential medium) containing Hank’s salts supplemented with 10% fetal bovine serum (FBS), neomycin (5 µg/mL) and amphotericin B (1%). The cells were sub-cultured twice weekly. The cell cultures were incubated at 37°C in a 1.5% carbon dioxide atmosphere (98.5% air).

Test Item Preparation

On the day of the experiment (immediately before treatment), the test item was suspended in deionised water. 2M sodium hydroxide was added until the substance was completely dissolved. If required, more deionised water was added until the intended concentration of the stock solution was reached. The final concentration of deionised water in the culture medium was 10% (v/v). The solvent was chosen to its solubility properties and its relative non-toxicity to the cell cultures.

The osmolarity and the pH-value were determined in culture medium of the solvent control and of the maximum concentration in the pre-experiment without metabolic activation.

Table CA 5.8.1-1: Osmolarity and pH values

	Solvent control	Test Item 1270 µg/mL
Osmolarity [mOsm]	286	318
pH value	7.37	7.55

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B. Study design and methods

1. Pre-Test on Toxicity

In a pre-test the concentration range for the mutagenicity experiments was determined. The general culture conditions and experimental conditions in this pre-test were the same as described for the mutagenicity experiment below. In this pre-test the colony forming ability of approximately 500 single cells (duplicate cultures per concentration level) after treatment with the test item was observed and compared to the controls. Toxicity of the test item is indicated by a reduction of the cloning efficiency (CE).

2. Dose selection

According to the current OECD Guideline for Cell Gene Mutation Tests at least four analysable concentrations should be used in two parallel cultures. For freely-soluble and non-cytotoxic test items the maximum concentration should be 5 mg/mL, 5 µL/mL or 10 mM, whichever is the lowest. For cytotoxic test items the maximum concentration should result in approximately 10 to 20% relative survival or cell density at sub-cultivation and the analysed concentrations should cover a range from the maximum to little or no cytotoxicity. Relatively insoluble test items should be tested up to the highest concentration that can be formulated in an appropriate solvent as solution or homogenous suspension. These test items should be tested up or beyond their limit of solubility. Precipitation should be evaluated at the beginning and at the end of treatment by the unaided eye.

The pre-experiment was performed in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation. Test item concentrations between 9.9 µg/mL and 1270 µg/mL (equal to a molar concentration of approximately 10 mM) were used. The highest concentration in the pre-experiment was chosen with regard to the purity (99.5% (w/w)) and the molecular weight (127.10 g/mol) of the test item.

Neither cytotoxic effects nor precipitation occurred up to the maximum concentration with and without metabolic activation following 4 and 24 hours treatment.

There was no relevant shift of osmolarity and pH values of the medium even at the maximum concentration of the test item.

The dose range of the main experiments went up to 1270 µg/mL or 10 mM as well. The individual concentrations were spaced by a factor of 2.0.

To overcome problems with possible deviations in toxicity the main experiments were started with more than four concentrations.

Table CA 5.8.1-2: Doses applied in the gene mutation assay with Amidosulfuron soil metabolite AE F094206 (concentrations given in bold letters were chosen for the mutation rate analysis)

		Experiment I					
4 hours	-	39.7	**79.4**	**158.8**	**317.5**	**635.0**	**1270.0**
4 hours	+	39.7	**79.4**	**158.8**	**317.5**	**635.0**	**1270.0**
		Experiment II					
24 hours	-	39.7	**79.4**	**158.8**	**317.5**	**635.0**	**1270.0**
4 hours	+	39.7	**79.4**	**158.8**	**317.5**	**635.0**	**1270.0**

C. Experimental Performance

Culture Medium

For seeding and treatment of the cell cultures the complete culture medium was MEM (minimal essential medium) containing Hank's salts, 10% FBS (except during 4 hour treatment), neomycin (5 µg/mL) and amphotericin B (1%). For the selection of mutant cells the complete medium was supplemented with 11 µg/mL 6-thioguanine. All cultures were incubated at 37 °C in a humidified atmosphere with 1.5% CO_2.

Seeding

Two to three days after sub-cultivation stock cultures were trypsinized at 37 °C for 5 minutes. Then the enzymatic digestion was stopped by adding complete culture medium with 10% FBS and a single cell suspension was prepared. The trypsin concentration for all sub-culturing steps was 0.2% in PBS. The PBS is composed as follows (per litre):

NaCl	8000 mg
KCl	200 mg
KH_2PO_4	200 mg
Na_2HPO_4	150 mg

Prior to the trypsin treatment the cells were rinsed with PBS buffer containing 200 mg/l EDTA (ethylene diamine tetraacetic acid). Approximately 1.5×106 (single culture) and 5×102 cells (in duplicate) were seeded in plastic culture flasks. The cells were grown for 24 hours prior to treatment.

Treatment

After 24 hours the medium was replaced with serum-free medium containing the test item, either without S9 mix or with 50 µl/mL S9 mix. Concurrent solvent and positive controls were treated in parallel. After 4 hours this medium was replaced with complete medium following two washing steps with "saline G". In the second experiment the cells were exposed to the test item for 24 hours in complete medium, supplemented with 10% FBS, in the absence of metabolic activation.

The "saline G" solution had the following constituents (per litre):

NaCl	8000 mg
KCl	400 mg
Glucose	1100 mg
$Na_2HPO_4 \times 2H_2O$	192 mg
KH_2PO_4	150 mg

Data Recording

The data generated were recorded in the raw data. The results are presented in tabular form, including experimental groups with the test item, solvent, and positive controls.

Acceptability of the Assay

The gene mutation assay is considered acceptable if it meets the following criteria:

a) the numbers of mutant colonies per 106 cells found in the solvent controls fall within the laboratory historical control data range.
b) the positive control substances should produce a significant increase in mutant colony frequencies.
c) the cloning efficiency II (absolute value) of the solvent controls must exceed 50%.

The data of this study comply with the criteria stated in the report.

Evaluation of Results
A test item is classified as positive if it induces either a concentration-related increase of the mutant frequency or a reproducible and positive response at one of the test points.

A test item producing neither a concentration-related increase of the mutant frequency nor a reproducible positive response at any of the test points is considered non-mutagenic in this system.

A positive response is described as follows:

A test item is classified as mutagenic if it reproducibly induces a mutation frequency that is three times above the spontaneous mutation frequency at least at one of the concentrations in the experiment.

The test item is classified as mutagenic if there is a reproducible concentration-related increase of the mutation frequency. Such evaluation may be considered also in the case that a threefold increase of the mutant frequency is not observed.

However, in a case by case evaluation this decision depends on the level of the corresponding solvent control data. If there is by chance a low spontaneous mutation rate within the laboratory´s historical control data range, a concentration-related increase of the mutations within this range has to be discussed. The variability of the mutation rates of solvent controls within all experiments of this study was also taken into consideration.

Statistical Analysis
A linear regression (least squares) was performed to assess a possible dose dependent increase of mutant frequencies. The number of mutant colonies obtained for the groups treated with the test item was compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05. However, both, biological and statistical significance were considered together.

II. Results and discussion

The amidosulfuron soil metabolite AE F094206 was assessed for its potential to induce gene mutations at the HPRT locus using V79 cells of the Chinese hamster.

The study was performed in two independent experiments, using identical experimental procedures. In the first experiment the treatment period was 4 hours with and without metabolic activation. The second experiment was performed with a treatment period of 4 h with and 24 h without metabolic activation. No precipitation of the test item was noted up to the maximum concentration with and without metabolic activation.

No relevant and reproducible toxic effects occurred up to the maximum concentration of 270.0 µg/mL with and without metabolic activation. In the second culture of experiment II with metabolic activation the cell density was below 50% at all evaluated concentrations. However, this effect was solely based on a relatively large cell count of the solvent control and does not indicate true cytotoxicity. The corresponding relative cloning efficiency I values did not show any cytotoxicity at all.

No relevant and reproducible increase in mutant colony numbers/10^6 cells was observed in the main experiments up to the maximum concentration. The mutant frequency did not exceed the historical range of solvent controls. The threshold of three times the corresponding solvent control was exceeded in the second culture of the first experiment without metabolic activation at an intermediate concentration of 317.5 µg/mL. However, the threshold was not exceeded at any other, even higher concentration or in the parallel culture under identical conditions. Consequently, the isolated increase described above was judged as biologically irrelevant.

A linear regression analysis (least squares) was performed to assess a possible dose dependent increase of mutant frequencies. No significant dose dependent trend of the mutation frequency indicated by a probability value of <0.05 was determined in any of the experimental groups. In both experiments of this study (with and without S9 mix) the range of the solvent controls was from 8.2 up to 39.2 mutants per 106 cells; the range of the groups treated with the test item was from 4.2 up to 44.8 mutants per 106 cells.

EMS (150 µg/mL) and DMBA (1.1 µg/mL in experiment I and 2.2 µg/mL in experiment II) were used as positive controls and showed a distinct increase in induced mutant colonies. The DMBA control of the first culture of the first experiment with metabolic activation fell short of the historical range of positive controls even though the induction factor was 4.1. The data are valid however, as the mean value of both parallel cultures (59.6 + 119.8 equal to a mean of 89.7) remained well within the historical range.

Thus, it can be concluded that the test item did not induce gene mutations at the HPRT locus in V79 cells. Therefore, amidosulfuron soil metabolite AE F094206 is considered to be non-mutagenic in this HPRT assay.

Amidosulfuron soil metabolite AE F094206: *In vitro* chromosome aberration test in Chinese hamster V79 cells

Report:	KCA 5.8.1/09 [redacted],; 2015; M-541548-01-1
Title:	Amidosulfuron soil metabolite AE F094206: *In vitro* chromosome aberration test in Chinese hamster V79 cells
Report No.:	1691502
Document No.:	M-541548-01-1
Guideline(s):	OECD Guidelines for Testing of Chemicals No. 473; US EPA (TSCA) OPPTS 870.5375; METI, MHLW, MAFF
Guideline deviation(s):	Deviation No. 1 Acceptability Criteria: The chromosome aberration assay will be considered acceptable if it meets the following criteria: Reason for the deviation (printed in bold letters): Typing error in the general study plan. This deviation has no detrimental impact on the outcome of the study
GLP/GEP:	**yes**

Executive summary

The test item Amidosulfuron soil metabolite AE F094206, suspended in deionised water, was assessed for its potential to induce structural chromosomal aberrations in V79 cells *in vitro* in two independent experiments. The following study design was used:

Table CA 5.8.1-3: Study design

	Without S9 mix		With S9 mix
	Exp. I	Exp. II	Exp. I & II
Exposure period	4 hrs	18 hrs	4 hrs
Recovery	14 hrs	—	14 hrs
Preparation interval	18 hrs	18 hrs	18 hrs

In each experimental group two parallel cultures were analysed. Per culture at least 150 metaphases were evaluated for structural chromosomal aberrations, except for the positive control in Experiment I, in the absence of S9 mix, where only 50 metaphases were scored.

The highest applied concentration in this study (1270.0 µg/mL of the test item, approx. 10 mM) was chosen with regard to the molecular weight of the test item and with respect to the current OECD Guideline 473.

Dose selection of the cytogenetic experiment was performed considering the toxicity data and the occurrence of test item precipitation in accordance with OECD Guideline 473.

In Experiment I and II in the absence and presence of S9 mix no cytotoxicity was observed up to the highest evaluated concentration.

In both independent experiments, no biologically relevant increase in the number of cells carrying structural chromosomal aberrations was observed after treatment with the test item.

In Experiment II without metabolic activation after 18 hours continuous treatment statistically significant increases in chromosomal aberrations were observed at 317.5 and 635.0 µg/mL (3.5 and 3.0 % aberrant cells, excluding gaps). Since the values are within the laboratory historical solvent control data (0.0 – 3.5 % aberrant cells, excluding gaps) the findings can be considered as biologically irrelevant. No relevant increase in the frequencies of polyploid metaphases was found after treatment with the test item as compared to the frequencies of the controls.

Appropriate mutagens were used as positive controls. They induced statistically significant increases in cells with structural chromosome aberrations.

In conclusion, it can be stated that under the experimental conditions reported, the test item did not induce structural chromosomal aberrations in V79 cells *in vitro*. Therefore, Amidosulfuron soil metabolite AE F094206 is considered to be non-clastogenic in this chromosome aberration test, when tested up to precipitating or the highest evaluable concentrations.

I. Material and methods

A Materials

1. Test item

Identification:	Amidosulfuron soil metabolite AE F094206
Batch:	PW210/213
Batch code:	AE F094206 00 1C99 0001
Purity:	99.5% (w/w)
Molecular Weight:	127.10 g/mol
Appearance:	White powder
Expiry Date:	30 January 2021
Storage Conditions:	In the refrigerator (+2 to +8 °C)
Stability in Solvent:	Not indicated by the sponsor

2. Control materials:

Solvent controls

Concurrent solvent controls (culture medium with 10.0 % deionised water (local tap water deionised at Harlan CCR)) were performed.

Positive controls

Without metabolic activation

Name:	EMS; ethylmethane sulfonate
Purity:	99 %
Dissolved in:	Nutrient medium

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Concentration: 1000.0 µg/mL (Exp. I)
500.0 µg/mL (Exp. II)

With metabolic activation
Name: CPA; cyclophosphamide
Purity: 97 - 103 %
Dissolved in: Saline (0.9 % NaCl [w/v])
Concentration: 1.4 µg/mL (Exp. I and II)

The dilutions of the stock solutions were prepared on the day of the experiment. The stability of the positive control substance in solution is unknown but a mutagenic response in the expected range is a sufficient biological evidence for chemical stability.

3. Metabolic activation S9 mix
Phenobarbital/β-naphthoflavone induced rat liver S9 was used as the metabolic activation system. The S9 was prepared and stored according to the currently valid version of the Harlan CCR SOP for rat liver S9 preparation. Each batch of S9 was routinely tested for its capability to activate the known mutagens benzo[a]pyrene and 2-aminoanthracene in the Ames test.
An appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution to result in a final protein concentration of 0.75 mg/mL in the cultures. S9 mix contained $MgCl_2$ (8 mM), KCl (33 mM), glucose-6-phosphate (5 mM) and NADP (4 mM) in sodium-ortho-phosphate-buffer (100 mM, pH 7.4).
The protein concentration of the S9 preparation used for this study was 35.0 mg/mL.

4. Test cells:
The V79 cell line has been used successfully for many years in *in vitro* experiments. The high proliferation rate (doubling time of V79 cells in stock cultures: approximately 13 hours, determined on December 17, 2010) and a reasonable plating efficiency of untreated cells (as a rule more than 70 %) both necessary for the appropriate performance of the study, support the use of this cell line. The cells have a stable karyotype with a modal chromosome number of 22 ± 1. Before freezing each batch is screened for mycoplasm contamination and checked for karyotype stability. Consequently, the parameters of the experiments remain similar because of the reproducible characteristics of the cells.

5. Dose selection:
Dose selection was performed according to the current OECD Guideline for chromosomal aberration studies. The highest test item concentration should be 10 mM, 2 mg/mL or 2 µL/mL, whichever is the lowest. At least three test item concentrations should be evaluated for cytogenetic damage.
With regard to the molecular weight of the test item, 1270.0 µg/mL (approx. 10 mM) was applied as top concentration for treatment of the cultures in the pre-test. Test item concentrations ranging from 5.0 to 1270.0 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity. In the pre-test for toxicity, precipitation of the test item was observed at the end of treatment at 317.5 µg/mL and above in the absence of S9 mix and at 158.8 µg/mL and above in the presence of S9 mix. Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I.
No cytotoxic effects were observed after 4 hours treatment in the absence and presence of S9 mix. Therefore, 1270.0.0 µg/mL was chosen as top treatment concentration for Experiment II.
The cytogenetic evaluation of concentrations in Experiment II (without S9 mix) higher than indicated in the following table was impossible due to precipitation on the slides.

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Table CA 5.8.1-4: Test concentrations:

Exp.	Prep. interval	Exposure period	Concentrations in µg/mL								
Without S9 mix											
I	18 hrs	4 hrs	5.0	9.9	19.8	39.7	**79.4**	**158.8**	**317.5**^{P}	635.0^{P}	1270.0^{P}
II	18 hrs	18 hrs	5.0	9.9	19.8	39.7	79.4	**158.8**	**317.5**	**635.0**	1270.0^{P}
With S9 mix											
I	18 hrs	4 hrs	5.0	9.9	19.8	**39.7**	**79.4**	**158.8**^{P}	317.5^{P}	635.0^{P}	1270.0^{P}
II	18 hrs	4 hrs			19.8	**39.7**	**79.4**	**158.8**^{P}	317.5^{P}	635.0^{P}	1270.0^{P}

Evaluated experimental points are shown in bold characters
^{P} Precipitation was observed at the end of treatment

B Study Design and Methods:

1. Study performance:
The study was conducted at ██████████ ███). The experimental start and completion dates of the study were April 9th 2015 and June 16th 2015, respectively.

2. Culture Medium and Conditions:
Thawed stock cultures were propagated at 37 °C in 80 cm^{2} plastic flasks. About 5 x 105 cells per flask were seeded in 15 mL of MEM (minimal essential medium) containing Hank's salts, glutamine and Hepes (25 mM). Additionally, the medium was supplemented with penicillin/streptomycin (100 U/mL/100 µg/mL) and 10 % (v/v) fetal bovine serum (FBS). The cells were sub-cultured twice a week.

3. Seeding of the Cultures:
Exponentially growing stock cultures more than 50 % confluent were rinsed with Ca-Mg-free salt solution containing 8000 mg/L NaCl, 200 mg/L KCl, 200 mg/L KH_2PO_4 and 150 mg/L Na_2HPO_4. Afterwards the cells were treated with trypsin-EDTA-solution at 37 °C for approx. 5 minutes. Then, by adding complete culture medium including 10 % (v/v) FBS the enzymatic treatment was stopped and a single cell suspension was prepared. The trypsin concentration for all sub-culturing steps was 0.25 % (w/v) in Ca-Mg-free salt solution. For experimental performance the cells were seeded into Quadriperm dishes containing microscopic slides. Into each chamber 1 x 104 – 6 x 104 cells were seeded with regard to the preparation time. All incubations were done at 37 °C in a humidified atmosphere with 1.5 % carbon dioxide (98.5 % air).

4. Preliminary cytotoxicity test:
A preliminary cytotoxicity test was performed to determine the concentrations to be used in the main experiment. Cytotoxicity is characterized by the percentages of mitotic suppression and/or relative increase in cell count. The experimental conditions in this pre-test phase were identical to those required and described below for the main experiment.
The pre-test was performed with 9 concentrations of the test item separated by no more than a factor of √10 and a solvent and positive control. All cell cultures were set up in duplicate. Exposure time was 4 hours (with and without S9 mix). The preparation interval was 18 hours after start of the exposure.

5. Cytogenetic Experiment

Pulse exposure:
The culture medium of exponentially growing cell cultures was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL culture medium was added. After 4 hours the cultures were washed twice with "Saline G" (pH 7.2) containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose•H_2O, 192 mg/L Na_2HPO_4•$2H_2O$ and 150 mg/L KH_2PO_4. The cells were then cultured in complete medium containing 10 % (v/v) FBS for the remaining culture time of 14 hours.

Continuous exposure (without S9 mix):
The culture medium of exponentially growing cell cultures was replaced with complete medium containing 10 % (v/v) FBS including the test item. The medium was not changed until preparation of the cells.

6. Preparation of metaphases
Cultures were treated with the metaphase-arresting substance colcemid (final concentration: 0.2 µg/mL) approximately two to three hours before the requested harvest time. The cells were treated on the slides in the chambers with hypotonic solution (0.4 % KCl) for 20 min at 37 °C. After incubation in the hypotonic solution the cells were fixed with a mixture of methanol and glacial acetic acid (3+1 parts, respectively). The slides were stained with Giemsa, mounted after drying and covered with a slid. All slides were labelled with a computer-generated random code to prevent scorer bias.

7. Evaluation

Cytotoxicity
Cytotoxicity was assessed by the determination of the relative increase in cell counts (RICC).

$$\text{RICC} = \frac{\text{(Increase in number of cells in treated cultures (final-starting))}}{\text{(Increase in number of cells incontrol cultures (final-starting))}} \times 100$$

Cytotoxicity [%] = 100 – RICC

In addition, coded slides were evaluated for mitotic index, 1000 cells per culture were scored and values were expressed as a percentage of the solvent controls.

Cytogenetic damage
At least 150 well-spread metaphases were scored per culture for structural aberrations, except for the positive control in Experiment I in the absence of S9 mix, where only 50 metaphases were scored. Only metaphases containing a number of centromeres equal to a number of 22 ± 2 were included in the analysis. Breaks, fragments, deletions, exchanges and chromosomal disintegrations are recorded as structural chromosomal aberrations. Gaps are recorded as well, but they are not included in the calculation of the aberration rates. If the analysis of the slides results in a large frequency of aberrant cells (at least 30 – 50 % cells with aberrations) the sample size can be reduced to 50 metaphases per culture. For borderline results the number of metaphases evaluated for cytogenetic damage may be increased to 300 per culture. Although the purpose of the test is to detect structural chromosomal aberrations, it is important to record polyploidy (tetraploid karyotype or more) and endoreduplication when these events are seen.

In addition, the number of polyploid cells in 500 metaphase cells per culture (% polyploid metaphases) was evaluated.

8. Data Recording
The data generated were recorded in the laboratory protocol. The results are presented in tabular form, including experimental groups with the test item, solvent, and positive controls.

9. Interpretation of Results

Providing that all of the acceptability criteria are fulfilled, a test item is considered to be clearly negative if, in all of the experimental conditions examined:

- None of the test item concentrations exhibits a statistically significant increase compared with the concurrent solvent control
- There is no concentration-related increase
- The results in all evaluated test item concentrations should be within the range of the laboratory historical solvent control data

The test item is then considered unable to induce chromosomal aberrations in this test system.

Providing that all of the acceptability criteria are fulfilled, a test item is considered to be clearly positive if, in any of the experimental conditions examined:

- At least one of the test item concentrations exhibits a statistically significant increase compared with the concurrent solvent control
- The increase is concentration-related in at least one experimental condition
- The results are outside the range of the laboratory historical solvent control data

When all of the criteria are met, the test item is then considered able to induce chromosomal aberrations in this test system.

There is no requirement for verification of a clear positive or negative response. In case the response is neither clearly negative nor clearly positive as described above and/or in order to assist in establishing the biological relevance of a result, the data should be evaluated by expert judgment and/or further investigations. Scoring additional cells (where appropriate) or performing a repeat experiment possibly using modified experimental conditions (e.g. narrow concentration spacing, other metabolic activation conditions, i.e. S9 concentration or S9 origin) could be useful.

However, results may remain questionable regardless of the number of times the experiment is repeated. If the data set will not allow a conclusion of positive or negative, the test item will therefore be concluded as equivocal.

10. Statistical analysis

Statistical significance was confirmed by using the Fisher's exact test (modified) ($p < 0.05$), using the validated R Script FisherMidP_V1.rnw for those values that indicate an increase in the number of cells with chromosomal aberrations compared to the concurrent solvent control.

II. Results and discussion

The test item Amidosulfuron soil metabolite AE F094206, suspended in deionised water, was assessed for its potential to induce chromosomal aberrations in V79 cells *in vitro* in the absence and presence of metabolic activation by S9 mix.

Two independent experiments were performed. In Experiment I the exposure period was 4 hours with and without S9 mix. In Experiment II the exposure periods were 4 hours with S9 mix and 18 hours without S9 mix. The chromosomes were prepared 18 hours after the start of treatment with the test item. In each experimental group two parallel cultures were analysed. At least 150 metaphases per culture were scored for structural chromosomal aberrations, except for the positive control in Experiment I, in the absence of S9 mix, where only 50 metaphases were scored due to strong clastogenic effects. To show cytotoxic effects the RICC and the MI were determined.

The highest treatment concentration in this study, 1270.0 µg/mL (approx. 10 mM) was chosen with regard to the molecular weight of the test item and with respect to the OECD Guideline for *in vitro* mammalian cytogenetic tests. Precipitation of the test item in the culture medium was observed at

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158.8 µg/mL and above in Experiment I and II in the presence of S9 mix. In the absence of S9 mix, precipitation occurred at 317.5 µg/mL and above in Experiment I and at 1270.0 µg/mL in Experiment II. No relevant influence on osmolarity or pH value was observed.

In Experiment I and II in the absence and presence of S9 mix no cytotoxicity was observed up to the highest evaluated concentration.

In both experiments, in the absence and presence of S9 mix, no biologically relevant increase in the number of cells carrying structural chromosome aberrations was observed (see Table CA 5.8.1-5). The aberration rates of the cells after treatment with the test item (1.3 – 3.5 % aberrant cells, excluding gaps) were close to the range of the solvent control values (1.0 – 3.0 % aberrant cells, excluding gaps) and within the range of the laboratory historical solvent control data.

In Experiment II without metabolic activation after 18 hours continuous treatment statistically significant increases in chromosomal aberrations were observed at 317.5 and 635.0 µg/mL (3.5 and 3.0 % aberrant cells, excluding gaps). Since the values are within the laboratory historical solvent control data (0.0 – 3.5 % aberrant cells, excluding gaps) the findings can be considered as biologically irrelevant.

In both experiments, no biologically relevant increase in the rate of polyploid metaphases was found after treatment with the test item (2.1 – 3.7 %) as compared to the rates of the solvent controls (2.4 – 3.4 %) (Table CA 5.8.1-5).

In both experiments, either EMS (1000 or 500 µg/mL) or CPA (1.4 µg/mL) were used as positive controls and showed distinct increases in cells with structural chromosome aberrations.

III. Conclusions

In conclusion, it can be stated that under the experimental conditions reported, the test item did not induce structural chromosomal aberrations in V79 cells *in vitro*.

Therefore, Amidosulfuron soil metabolite AE F094206 is considered to be non-clastogenic in this chromosome aberration test, when tested up to precipitating or the highest evaluable concentrations.

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Table CA 5.8.1-5: Summary of results

Exp.	Preparation interval	Test item concentration in µg/mL	Polyploid cells in %	Endomitotic cells in %	RICC in % of control	Mitotic indices in % of control	Aberrant cells in %		
							incl. gaps*	excl. gaps*	with exchanges
Exposure period 4 hrs without S9 mix									
I	18 hrs	Solvent control[1]	2.8	0.1	100.0	100.0	2.3	2.0	0.3
		Positive control[2#]	n.d.	n.d.	67.3	58.1	33.0	**30.0**[S]	21.0
		79.4	2.1	0.0	96.1	92.2	2.0	2.0	0.0
		158.8	2.3	0.2	99.1	94.8	1.7	1.0	0.0
		317.5[P]	2.3	0.0	97.1	89.6	1.0	1.0	0.0
Exposure period 18 hrs without S9 mix									
II	18 hrs	Solvent control[1]	2.4	0.1	100.0	100.0	1.3	1.0	0.0
		Positive control[3]	n.d.	n.d.	60.7	53.8	18.0	**17.7**[S]	5.0
		158.75	2.8	0.0	91.9	94.3	1.7	1.3	0.0
		317.50[##]	3.1	0.1	92.3	121.5	4.0	**3.5**[S]	0.2
		635.00	3.7	0.0	79.1	100.3	3.0	**3.0**[S]	0.0
Exposure period 4 hrs with S9 mix									
I	18 hrs	Solvent control[1]	3.4	0.9	100.0	100.0	4.3	3.0	0.0
		Positive control[4]	n.d.	n.d.	82.1	80.6	21.7	**20.7**[S]	4.3
		39.7	3.7	1.3	96.6	102.8	2.0	1.7	0.7
		79.4	2.7	0.5	93.7	119.8	2.7	2.3	0.0
		158.8[P]	2.8	0.7	99.9	114.9	2.3	2.0	0.0
II	18 hrs	Solvent control[1]	2.8	0.7	100.0	100	2.3	1.7	0.0
		Positive control[4]	n.d.	n.d.	90.3	63.3	19.3	**19.3**[S]	6.3
		39.69	2.7	0.9	110.8	94.3	2.7	2.3	0.0
		79.38	2.4	0.5	109.3	95.5	1.3	1.3	0.3
		158.75[P]	2.8	0.4	116.1	100.5	2.3	1.3	0.0

Summary of previously submitted toxicological data on metabolites

Amidosulfuron-ADHP (AE F094206, Hoe 094206) – Soil metabolite

An Ames test was found to be negative, indicating no genotoxicity potential in this assay. In an acute oral toxicity test in the rat, the LD50 value was found to be > 5000 mg/kg bw. in both sexes.

Amidosulfuron-desmethyl (AE F101630, Hoe 101630) – Rat and Plant metabolite

An Ames test was found to be negative, indicating no genotoxicity potential in this assay. In an acute oral toxicity test in the female rat, the LD50 value was found to be > 5000 mg/kg bw.

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Amidosulfuron-ADMP (AE F092944, Hoe 092944) – Metabolite found in the water sediment

An Ames test was found to be negative, indicating no genotoxicity potential in this assay. In an acute oral toxicity test in the rat, the LD50 value was found to be between 2000 and 5000 mg/kg bw.

AE F128870 – Soil and Plant metabolite

An expert statement is provided on the justification for not conducting an acute toxicity study. In addition, rationale is given as to why data generated on the parent and on metabolites that are structurally similar, can be used to bridge to AE F128870, to predict the acute toxicity and genotoxicity potential of this metabolite. AE F128870 is predicted to have a low acute toxicity potential and not to be genotoxic.

<u>*Studies submitted and evaluated for the first inclusion of amidosulfuron on Annex I:*</u>

Report:	KCA 5.8.1/01 ; 1992; M-137603-01-1
Title:	Hoe 101630 - substance technical (Code: Hoe 101630 00 ZC93 0001) Mutagenic potential in strains of Salmonella typhimurium (Ames test) and Escherichia coli
Report No.:	A48491
Document No.:	M-137603-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Report:	KCA 5.8.1/02 ,; ; 1992; M-137602-01-1
Title:	Hoe 101630, substance, technical (Code: Hoe 101630 00 ZC93 0001) Testing for acute oral toxicity in the male and female Wistar rat
Report No.:	A48490
Document No.:	M-137602-01-1
Guideline(s):	OECD: 401 (1981); USEPA (=EPA): § 81-1 (1984)
Guideline deviation(s):	
GLP/GEP:	**yes**

Report:	KCA 5.8.1/03 ; 1992; M-138010-01-1
Title:	2-Amino-4,6-dihydroxypyrimidin (Code: Hoe 094206 00 ZC99 0003) Study of the mutagenic potential in strains of Salmonella typhimurium (Ames test) and Escherichia coli
Report No.:	A48922
Document No.:	M-138010-01-1
Guideline(s):	
Guideline deviation(s):	--
GLP/GEP:	**yes**

Report:	KCA 5.8.1/04 ,; 1992; M-138216-01-1
Title:	Hoe 094206, substance, technical (Code: Hoe 094206 00 ZC99 0003) Testing for acute oral toxicity in the male and female Wistar rat
Report No.:	A49744
Document No.:	M-138216-01-1
Guideline(s):	OECD: 401 (1987); USEPA (=EPA): § 81-1
Guideline deviation(s):	--
GLP/GEP:	**yes**

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Report: KCA 5.8.1/05 [redacted],; 1992; M-137963-01-1
Title: Hoe 092944 - substance, technical (Code: Hoe 092944 00 ZD99 0001) Study of the mutagenic potential in strains of Salmonella typhimurium (Ames Test) and Escherichia coli
Report No.: A48871
Document No.: M-137963-01-1
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **yes**

Report: KCA 5.8.1/06 [redacted]U; 1995; M-138232-02-1
Title: Hoe 092944; substance technical (Code: Hoe 092944 00 ZD99 0001) Testing for acute oral toxicity in the male and female Wistar rat
Report No.: A49161
Document No.: M-138232-02-1
Guideline(s): OECD: 401 (1987); USEPA (=EPA): § 81-1 (1984)
Guideline deviation(s): --
GLP/GEP: **yes**

Report: KCA 5.8.1/07 [redacted]; 2002; M-229593-01-1
Title: Statement on acute toxicity of AE F128870, a soil and plant metabolite of amidosulfuron (AE F075032) (position paper)
Report No.: C031417
Document No.: M-229593-01-1
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **no**

"AIR process" - new studies submitted

The following studies are not part of the baseline dossier. They have been submitted to former RMS (Austria) to support the post Annex I process of Amidosulfuron. They were evaluated by Austria and are part of the DAR Addendum (Feb. 2011 – Addendum to monograph prepared in the context of post Annex I procedure (new Annex II data)).

BCS-CO41839 (metabolite Amidosulfuron-guanidine) was identified as a major soil metabolite of Amidosulfuron with a predicted PEC_{gw} value of > 0.75 µg/L << 10 ug/L which triggers groundwater relevance assessment according to EU Council Directive 91/414/EEC (SANCO/221/2000 –rev. 10, 25 February, 2003). Hereafter are presented the toxicological data generated on this metabolite to demonstrate its non-relevance. This includes 3 *in vitro* genotoxicity tests, an acute oral toxicity study and a 28-day oral toxicity study in the rat.

These studies demonstrate that BCS-CO41839 (metabolite Amidosulfuron-guanidine) is not genotoxic and not toxic after an acute or subacute oral administration.

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Report:	KCA 5.8.1/10 [redacted] V; 2010; M-370258-01-1
Title:	BCS-CO41839 (metabolite amidosulfuron-guanidine) - Acute toxicity in the rat after oral administration
Report No.:	AT05921
Document No.:	M-370258-01-1
Guideline(s):	Regulation (EC) No 1907/2006 (Reach); EEC Directive 440/2008 Part B - Method B.1. tris; OECD 423 (2001); EPA Health Effects Test Guidelines (OPPTS 870.1100); EPA 712-C-98-190 (1998)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Material and methods:

Test Material: BCS-CO41839 (Amidosulfuron-guanidine)

- Lot/Batch: BCOO 5900-29-4
- Purity: 98.6%
- Stability of test compound: Stable for the duration of the dosing phase
- Vehicle: Polyethylene glycol 400

Test animals:

- Species: Rat (female)
- Strain: HsdCpb:Wu Wistar
- Age: 8 to 12 weeks approximately
- Weight at dosing: 163 to 200 g
- Source: [redacted]

Diet: Standard diet, [redacted] Maus/Ratte Haltung, [redacted], ad libitum

Water: Tap water, ad libitum

Housing: Animals were housed in groups within treatment groups, in polycarbonate cages on low dust wood granulate bedding ([redacted], Germany

The test substance was tested using a stepwise procedure, each step using three female rats. The starting dose was selected from one of four fixed levels, 5, 50 300 and 2000 mg/kg bw. In the first instance, three animals were dosed at 300 mg/kg bw, in the absence of mortality, a further three animals were dosed at this dose level. As there was no mortality at 300 mg/kg, an additional three animals were dosed at 2000 mg/kg bw; as no mortality occurred, a further three animals were dosed at 2000 mg/kg bw.

The animals were randomly assigned to their groups. Following a fasting period of approximately 16 – 24 hours, each group received a single dose of 300 or 2000 mg/kg of BCS-CO41839 by gavage. The test substance was administered in polyethylene glycol 400 at a volume of 10 mL/kg bw. Clinical signs and mortality rates were determined several times on the day of administration and subsequently at least once daily for an observation period of at least 14 days. Body weights were recorded on days 1, 8 and 15. On day 15, all animals were sacrificed by carbon dioxide, dissected and examined for gross pathological changes.

Findings:

No deaths and no clinical signs were observed during the observation period, neither at 300 nor at 2000 mg/kg bw. Body weights of the rats were not affected. No gross abnormalities at necropsy were found.

Conclusion:

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The acute oral LD_{50} value of Amidosulfuron-Guanidine in the Wistar female rat was greater than 2000 mg/kg bw. No classification and labelling is triggered, neither according to DSD nor to CLP.

Report:	KCA 5.8.1/11 [redacted]; 2010; M-385894-01-1
Title:	BCS-CO41839 (metabolite amidosulfuron-guanidine) - 28-day toxicity study in the rat by dietary administration
Report No.:	SA 09173
Document No.:	M-385894-01-1
Guideline(s):	O.E.C.D. guideline 407 (July, 1995); E.E.C. Directive 96/54/EC, Method B.7 (July, 1996)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Material and methods:

Test Material: BCS-CO41839 (metabolite Amidosulfuron-guanidine)
- Lot/Batch: BCOO 5900-29-4
- Purity: 98.6%
- Stability of test compound: Stable in rodent diet for a period covering the study duration
- Vehicle: None

Test animals:
- Species: Rat
- Strain: Wistar Rj:WI (IOPS HAN)
- Age: 6 to 7 weeks approximately
- Weight at dosing: 241 to 281 g for the males, 175 to 205 g for the females
- Source: [redacted] France

Diet: Certified rodent powdered and irradiated diet [redacted] from [redacted] [redacted], France), ad libitum

Water: Tap water, ad libitum

Housing: Animals were caged individually in suspended stainless steel wire mesh cages

The purpose of this study was to assess subacute toxicity of Amidosulfuron-guanidine when administered to rats in their diets for a period of 28 days.

<u>Animal assignment and treatment:</u>
There were 5 animals of each sex per dose group. Animals were assigned to dose groups randomly by body weight. Amidosulfuron-guanidine was administered in the diet for 28 days to Wistar rats at the following doses – 0, 400, 2000 and 10000 ppm (equating to 0, 31, 152 and 778 mg/kg bw/d in males and 0, 34, 174 and 867 mg/kg bw/day in females). The dose levels were based on the evaluation of the results of 28 days and 13 weeks rat study with the parent compound Amidosulfuron, where the only observed effect was reduced body weight gain seen in males at the highest dose level tested (10000 ppm). A negative control group received plain diet. Animal housing and husbandry were in accordance with the regulations of the Guide for the Care and Use of Laboratory Animals (Public Health Service, National Institute of Health, NIH publication N°86-23, revised 1985) and "Le Guide du Journal Officiel des Communautés Européennes L358, 18 Décembre 1986, N°86/609/CEE du 24 Novembre 1986".

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Table CA 5.8.1-6: Diet concentration and daily dose levels

Test group	Concentration in diet (ppm)	Dose per animal (study averages)		Animals assigned	
		Male (mg/kg bw/day)	Female (mg/kg bw/day)	Male	Female
1	0	0	0	5	5
2	400	31	34	5	5
3	2000	152	174	5	5
4	10000	778	867	5	5

Diet preparation, analysis and administration:
Amidosulfuron-guanidine was ground to a fine powder and incorporated into the diet by dry mixing to provide the required concentrations. There was one preparation per week for each concentration. The stability was demonstrated during the course of the study at concentrations of 400 and 10000 ppm for a time which covered the period of usage and storage for the study. Homogeneity at the lowest and highest dietary concentrations and concentration checks at all dose levels were within the range 85-90% of nominal concentrations.

Clinical observations:
The animals were observed twice daily for moribundity and mortality (once daily on weekends or public holidays). Animals were observed for clinical signs at least once daily, in addition, detailed physical examinations were performed at least weekly during the treatment period. The nature, onset, severity, reversibility, and duration of any clinical signs were recorded.

Food consumption and body weight:
Food consumption was recorded weekly; the weekly mean achieved dosage intake for each week and for weeks 1 to 4 was calculated for each sex. Body weights were recorded at least once during the acclimatization phase, on the first day of test substance administration, then at weekly intervals throughout the treatment periods. Diet-fasted animals were weighed before necropsy (terminal body weight) at Day 30 or 31.

Opthalmoscopic observation:
An ophthalmoscopic examination was performed on all animals during the acclimatization phase and on control and high dose group animals during week 4. After instillation of an atropinic agent (Mydriaticum, Merck Sharp and Dohme) each eye was examined by means of an indirect ophthalmoscope.

Neurotoxicity assessment:
All animals were subjected to a neurotoxicity assessment (exploratory locomotor activity, open field observations, sensory reactivity and grip strength) during week 4.

Haematology and Clinical Chemistry:
On study days 30 or 31, blood samples were taken from all animals in all groups by puncture of the retro-orbital venous plexus. Animals were diet-fasted overnight prior to blood sampling and anesthetized by inhalation of Isoflurane. Blood was collected on EDTA for hematology, on and clot activator for serum clinical chemistry and on sodium citrate for coagulation parameters.

The following hematology parameters were assayed using an Advia 120 (, France): red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocyte count, white blood cell count and differential count evaluation and platelet count. A blood smear of Wright's stain was examined when results of Advia 120 determinations were abnormal.

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A blood smear was prepared and stained using May-Grunwald-Giemsa method. It was examined when the results of Advia 120 determinations were abnormal.

Prothrombin time and activated partial thromboplastin time were assayed on an ACL Elite Pro (, France).

The following clinical chemistry parameters were assayed on serum using an Advia 1650 (, France): total bilirubin, glucose, urea, creatinine, chloride, sodium, potassium, calcium, inorganic phosphorus, total cholesterol, triglycerides, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma-glutamyltransferase activities, total protein and albumin concentrations. Globulin concentrations and albumin/globulin ratio values were calculated. Any significant change in the general appearance of the plasma and the serum was recorded.

Urine analysis:
On study day 27, overnight urine samples were collected from all animals. Feed and water were not accessible during urine collection. Any significant change in the general appearance of the urine was recorded. Urinary volume was measured, pH was assayed using a Clinitek 500 and Multistix dipsticks (, France). Urinary refractive index was measured using a RFM320 refractometer (, France). Glucose, bilirubin, ketone bodies, occult blood, protein and urobilinogen were assayed using a Clinitek 500 and Multistix dipsticks (, France). Microscopic examination of the urinary sediment was performed after centrifugation of the urine. The presence of red blood cells, white blood cells, epithelial cells, bacteria, casts and crystals was graded.

Sacrifice and pathology:
On study days 30 or 31, a complete necropsy was performed on all animals. Animals were deeply anesthetized by Isoflurane inhalation, then exsanguinated before necropsy. All animals were diet-fasted prior to scheduled sacrifice. The necropsy included the examination of all major organs, tissues and body cavities. Macroscopic abnormalities were recorded, sampled and examined microscopically. Adrenal gland, brain, epididymis, heart, kidney, liver, ovary, pituitary gland, prostate gland, spleen, testis, thyroid gland (with parathyroid gland) and uterus (including cervix) were weighed fresh at scheduled sacrifice only. Paired organs were weighed together.

The following organs or tissues were sampled: adrenal gland, aorta, articular surface (femoro-tibial), bone (sternum), bone marrow (sternum), brain, epididymis, oesophagus, exorbital (lachrymal) gland, eye and optic nerve, Harderian gland, heart, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidney, larynx/pharynx, liver, lung, lymph nodes (submaxillary, mesenteric), mammary gland, nasal cavities, ovary, pancreas, pituitary gland, prostate gland, sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, submaxillary (salivary) gland, testis, thymus, thyroid gland (with parathyroid), tongue, trachea, urinary bladder, uterus (with cervix), vagina. A bone marrow smear was prepared from femur, stained with May-Grünwald Giemsa, but not examined as no treatment related changes were observed in haematology or bone marrow histology. Tissues samples were fixed by immersion in neutral buffered 10% formalin with the exception of the eye, optic nerve, Harderian gland, epididymis and testis that were fixed in Davidson's fixative. Histopathological examinations were performed on all tissues specified above (except exorbital lachrymal gland, larynx/pharynx and nasal cavities), on all animals in the control group and the highest dose group. Kidney, liver, lung, thyroid gland were examined in the intermediate dose groups. Significant macroscopic findings of all animals were examined microscopically.

Findings:
Clinical signs and mortality:
There was no mortality in the study. There were no treatment-related clinical signs in any group.

Body weight and body weight gain:
No relevant changes in mean body weight or mean body weight gain were observed at any dose level in either sex.

Food consumption:
There was no impact on food consumption in either sex at any dose level tested.

Ophthalmoscopy:
No ocular abnormalities were induced by treatment.

Haematology and Clinical Chemistry:
Hematological and clinical chemistry examination showed no treatment-related findings. The only statistically significant difference observed was absolute lymphocyte count at 2000 ppm in females only; this was considered to be incidental since it was not seen at higher dose level.

Urine analysis:
Urinalysis revealed no treatment-related findings.

Functional observation battery:
No treatment-related neurotoxicity findings were observed.

Sacrifice and pathology:
There were no changes in terminal body weights or organ weights at any dose in either sex.
There were no treatment-related macroscopic or microscopic changes at any dose in either sex.

Conclusion:
The No Observed Effect Level (NOEL) of Amidosulfuron-guanidine was 10000 ppm, equivalent to 778 and 867 mg/kg bw/d in males and females, respectively.

Report:	KCA 5.8.1/12 ,; 2009; M-358599-01-1
Title:	Salmonella typhimurium reverse mutation assay with BCS-CO41839 (metabolite amidosulfuron-guanidine)
Report No.:	1271703
Document No.:	M-358599-01-1
Guideline(s):	Ninth Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 471: Bacterial Reverse Mutation Test, adopted July 21, 1997; Commission Regulation (EC) No. 440/2008 B13/14, dated May 30, 2008; EPA Health Effects Test Guidelines, OPPTS 870.5100, Bacterial Reverse Mutation Test EPA 712-C-98-247, August, 1998
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Material and methods:

Test Material: BCS-CO41839 (metabolite Amidosulfuron-guanidine)
Lot/Batch: RDL 603-16-20
Purity: 98.3%
Stability of test compound: Stable for the duration of the study

Control Materials:
Negative: Culture medium
Solvent: Dimethyl sulfoxide (DMSO), (MERCK, Darmstadt, Germany)

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Positive:

- Non activation (-S9): Sodium azide, NaN_3 ([redacted], Germany) for TA 1535 and TA 100 at 10 µg/plate, 4-nitro-o-phenylene-diamine, 4-NOPD ([redacted], Germany) for TA 98 at 10 µg/plate and for TA 1537 at 50 µg/plate , methyl methane sulfonate, MMS ([redacted], Hohenbrunn, Germany) for TA 102 at 3 µg/plate
- Activation (+S9): 2-Aminoanthracene, 2-AA ([redacted], Germany) for TA 1535, TA 1537, TA 98, and TA 100 at 2.5 µg/plate and for TA 102 at 10 µg/plate
- Activation: The S9 fraction was isolated from the livers of Phenobarbital/β-Naphthoflavone induced rats

Test organism: Histidine-dependent auxotrophic mutants of Salmonella typhimurium TA 1535, TA 100, TA 1537, TA 98 & TA 102

Source: Strains of S. typhimurium were obtained from Trinova [redacted], Germany

Test concentrations:

Experiment I (plate incorporation): For all strains with or without S9 mix: 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate

Experiment II (pre-incubation test): For all strains with or without S9 mix: 33, 100, 333, 1000, 2500 and 5000 µg/plate

To evaluate the toxicity of the test item a pre-experiment was performed with all strains used. Eight concentrations were tested for toxicity and mutation induction with each 3 plates. The experimental conditions in this pre-experiment were the same as described for the experiment I below (plate incorporation test).

Toxicity of the test item can be evident as a reduction in the number of spontaneous revertants or a clearing of the bacterial background lawn.

The pre-experiment is reported as main experiment I, since the following criteria are met:
Evaluable plates (>0 colonies) at five concentrations or more in all strains used.

In the pre-experiment the concentration range of the test item was 3 – 5000 µg/plate. The pre-experiment is reported as experiment I. Since no toxic effects were observed 5000 µg/plate was chosen as maximal concentration.

The concentration range included two logarithmic decades. The following concentrations were tested in experiment II: 33; 100; 333; 1000; 2500; and 5000 µg/plate

For each strain and dose level, including the controls three plates were used.
The following materials were mixed in a test tube and poured onto the selective agar plates:

- 100 µL Test solution at each dose level (solvent or reference mutagen solution (positive control)),
- 500 µL S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
- 100 µL Bacteria suspension (cf. test system, pre-culture of the strains),
- 2000 µL Overlay agar

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In the pre-incubation assay 100 µL test solution (solvent or reference mutagen solution (positive control)), 500 µL S9 mix / S9 mix substitution buffer and 100 µL bacterial suspension were mixed in a test tube and incubated at 37 °C for 60 minutes. After pre-incubation 2.0 mL overlay agar (45 °C) was added to each tube. The mixture was poured on minimal agar plates.

After solidification the plates were incubated upside down for at least 48 hours at 37 °C in the dark.

The Salmonella typhimurium reverse mutation assay is considered acceptable if it meets the following criteria:

- regular background growth in the negative and solvent control
- the spontaneous reversion rates in the negative and solvent control are in the range of the Harlan Laboratory historical data
- the positive control substances should produce a significant increase in mutant colony frequencies

A test item is considered as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice (strains TA 98, TA 100, and TA 102) or three times (strains TA 1535 and TA 1537) the colony count of the corresponding solvent control is observed.

A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration.

An increase exceeding the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.

A dose dependent increase in the number of revertant colonies below the threshold is regarded as an indication of a mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remain within the historical range of negative and solvent controls such an increase is not considered biologically relevant.

Findings:
No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with BCS-CO41839 (metabolite Amidosulfuron-guanidine) at any concentration level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

The concurrent positive controls demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

Results are presented in the following tables:

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Table CA 5.8.1-7: Summary of Results Pre-Experiment and Experiment I (without activation)

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	TA 102
Without Activation	**DMSO**		14 ± 5	14 ± 5	32 ± 7	123 ± 9	475 ± 97
	Untreated		15 ± 2	14 ± 5	32 ± 5	157 ± 19	455 ± 14
	BCS-CO41839	**3 µg**	15 ± 2	13 ± 3	33 ± 8	139 ± 9	478 ± 20
		10 µg	17 ± 1	16 ± 3	33 ± 3	151 ± 15	471 ± 23
		33 µg	17 ± 2	11 ± 1	36 ± 7	131 ± 7	467 ± 33
		100 µg	18 ± 3	11 ± 2	31 ± 4	138 ± 12	425 ± 44
		333 µg	16 ± 1	14 ± 3	31 ± 4	147 ± 11	492 ± 16
		1000 µg	14 ± 1	16 ± 5	34 ± 6	140 ± 3	496 ± 34
		2500 µg	14 ± 1	13 ± 3	34 ± 3	138 ± 8	481 ± 10
		5000 µg	16 ± 2	12 ± 2	29 ± 4	151 ± 6	462 ± 14
	NaN_3	**10 µg**	1898 ± 67			1868 ± 42	
	4-NOPD	**10 µg**			337 ± 27		
	4-NOPD	**50 µg**		78 ± 6			
	MMS	**3.0 µL**					3432 ± 963

Table CA 5.8.1-8: Summary of Results Pre-Experimental and Experiment I (with activation)

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	TA 102
With Activation	**DMSO**		19 ± 3	19 ± 3	52 ± 7	188 ± 10	616 ± 24
	Untreated		21 ± 9	15 ± 2	52 ± 3	192 ± 14	655 ± 59
	BCS-CO41839	**3 µg**	19 ± 3	20 ± 4	46 ± 9	194 ± 20	620 ± 28
		10 µg	20 ± 3	20 ± 5	40 ± 7	167 ± 7	626 ± 29
		33 µg	19 ± 0	18 ± 5	43 ± 2	161 ± 10	622 ± 35
		100 µg	21 ± 2	18 ± 3	37 ± 7	166 ± 31	610 ± 32
		333 µg	18 ± 2	20 ± 3	48 ± 1	161 ± 18	617 ± 39
		1000 µg	17 ± 2	17 ± 5	47 ± 10	165 ± 12	605 ± 74
		2500 µg	14 ± 2	18 ± 1	43 ± 4	164 ± 8	610 ± 28
		5000 µg	18 ± 3	18 ± 4	45 ± 4	160 ± 3	607 ± 18
	2-AA	**2.5 µg**	355 ± 6	342 ± 39	1921 ± 203	2569 ± 79	
	2-AA	**10.0 µg**					2122 ± 142

Key to Positive Controls	
NaN_3	sodium azide
2-AA	2-aminoanthracene
MMS	methyl methane sulfonate
4-NOPD	4-nitro-o-phenylene-diamine

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Table CA 5.8.1-9: Summary of Results Experiment II (without activation)

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	TA 102
Without Activation	DMSO		17 ± 3	9 ± 1	32 ± 5	135 ± 5	417 ± 22
	Untreated		9 ± 1	12 ± 3	28 ± 4	158 ± 9	424 ± 10
	BCS-CO41839	33 µg	15 ± 6	9 ± 1	22 ± 1	122 ± 18	360 ± 22
		100 µg	17 ± 4	7 ± 2	29 ± 3	134 ± 11	380 ± 5
		333 µg	18 ± 3	10 ± 3	30 ± 3	131 ± 5	394 ± 24
		1000 µg	17 ± 3	10 ± 2	30 ± 3	104 ± 14	366 ± 41
		2500 µg	12 ± 3	9 ± 1	29 ± 1	125 ± 10	354 ± 34
		5000 µg	16 ± 4	10 ± 3	27 ± 9	123 ± 23	356 ± 2
	NaN_3	10 µg	1786 ± 79			1884 ± 24	
	4-NOPD	10 µg			500 ± 19		
	4-NOPD	50 µg		133 ± 58			
	MMS	3.0 µL					1690 ± 230

Table CA 5.8.1-10: Summary of Results Experiment II (with activation)

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	TA 102
With Activation	DMSO		16 ± 1	13 ± 4	38 ± 4	147 ± 2	550 ± 20
	Untreated		15 ± 3	12 ± 6	41 ± 6	175 ± 13	526 ± 64
	BCS-CO41839	33 µg	17 ± 5	15 ± 3	47 ± 7	135 ± 16	481 ± 16
		100 µg	12 ± 4	14 ± 4	39 ± 11	139 ± 20	537 ± 27
		333 µg	17 ± 4	14 ± 1	37 ± 6	134 ± 2	562 ± 52
		1000 µg	16 ± 3	10 ± 4	40 ± 3	124 ± 2	521 ± 40
		2500 µg	20 ± 4	11 ± 2	43 ± 2	139 ± 6	525 ± 38
		5000 µg	16 ± 4	11 ± 1	39 ± 4	141 ± 7	547 ± 38
	2-AA	2.5 µg	274 ± 28	157 ± 19	1410 ± 148	1787 ± 386	
	2-AA	10.0 µg					2406 ± 66

Key to Positive Controls	
NaN_3	sodium azide
2-AA	2-aminoanthracene
MMS	methyl methane sulfonate
4-NOPD	4-nitro-o-phenylene-diamine

Conclusion:

It is concluded that Amidosulfuron-guanidine shows no evidence of mutagenic activity in this *in vitro* bacterial system.

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Report:	KCA 5.8.1/13 [redacted],; 2009; M-357870-01-1
Title:	BCS-CO41839 (metabolite amidosulfuron-guanidine) - *In vitro* chromosome aberration test in Chinese hamster V79 cells
Report No.:	1271705
Document No.:	M-357870-01-1
Guideline(s):	Ninth Addendum to the OECD Guidelines for Testing of Chemicals, February 1998, adopted July 21, 1997, Guideline No. 473 *In vitro* Mammalian Chromosome Aberration Test; Commission Regulation (EC) No. 440/2008, B10: Mutagenicity - *In vitro* Mammalian Chromosome Aberration Test, dated May 30, 2008; United States Environmental Protection Agency Health Effects Test Guidelines, OPPTS 870.5375, *In vitro* Mammalian Chromosome Aberration Test, EPA 712-C-98-223, August 1998
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Material and methods:

Test Material:	BCS-CO41839 (metabolite Amidosulfuron-guanidine)
Lot/Batch:	RDL 603-16-20
Purity:	98.3%
Stability of test compound:	Stable for the duration of the study
Solvent:	DMSO; Dimethyl sulfoxide ([redacted], Germany, lot no. K39250731847 / K40013731920)
Control Materials:	
Negative:	Culture medium
Positive:	EMS; Ethylmethane sulfonate ([redacted], Belgium, lot no. A0246840) without S9 mix at 600 and 1000 µg/ml CPA; Cyclophosphamide ([redacted], Germany, lot no. 097K1311) with S9 mix at 1.4 µg/ml
Cell line:	Chinese hamster V79 lung cells
Source:	Cells obtained from [redacted], Germany
Test concentrations:	Experiment I (pre-test): 10.4, 20.8, 41.6, 83.1, 166.3, 332.5, 665.0, 1330.0 and 2660 µg/ml. Experiment II: 41.6, 83.1, 166.3, 332.5, 665.0, 1330.0 and 2660 µg/ml

The highest concentration used in the cytogenetic experiments was chosen considering the current OECD Guideline for *in vitro* mammalian cytogenetic tests requesting for the top concentration clear toxicity with reduced cell numbers or mitotic indices below 50 % of control, which is the lowest concentration, and/or the occurrence of precipitation. In case of non-toxicity the maximum concentration should be 5 mg/mL, 5 µL/mL or 10 mM, whichever is the lowest, if formulation in an appropriate solvent is possible.

With respect to the molecular weight of the test item 2660 µg/mL of BCS-CO41839 (metabolite Amidosulfuron-guanidine) was applied as top concentration for treatment of the cultures in the pre-test. Test item concentrations between 10.4 and 2660 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity. No precipitation of the test item was observed. Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I.

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Since no cytotoxicity and test item precipitation was observed in the first experiment, concentrations between 41.6 and 2660 µg/mL in the absence and presence of S9 mix were chosen for the second experiment.

Table CA 5.8.1-11: Doses applied in the Chromosome aberration test with BCS-CO41839

Preparation	Exposure	Exp.	Concentration								
interval	period		in µg/mL								
			Without S9 mix								
18 hrs	4 hrs	I	10.4	20.8	41.6	83.1	166.3	332.5	665.0	1330.0	2660.0
18 hrs	18 hrs	II			41.6	83.1	166.3	332.5	665.0	1330.0	2660.0
			With S9 mix								
18 hrs	4 hrs	I	10.4	20.8	41.6	83.1	166.3	332.5	665.0	1330.0	2660.0
18 hrs	4 hrs	II			41.6	83.1	166.3	332.5	665.0	1330.0	2660.0

Exponentially growing stock cultures more than 50 % confluent were treated with trypsin-EDTA-solution at 37 °C for approx. 5 minutes. Then the enzymatic treatment was stopped by adding complete culture medium and a single cell suspension was prepared. The trypsin concentration for all sub-culturing steps was 0.5 % (w/v) in Ca-Mg-free salt solution (, Germany).

Prior to the trypsin treatment the cells were rinsed with Ca-Mg-free salt solution, which was composed as follows (per litre):

NaCl	8000 mg
KCl	200 mg
KH_2PO_4	200 mg
$Na_2HPO_4 \cdot 7H_2O$	2160 mg

The cells were seeded into Quadriperm dishes (, Germany) that contained microscopic slides (at least 2 chambers per dish and test group). In each chamber 1 x 104 - 6 x 104 cells were seeded with regard to the preparation time. The medium was MEM with 10 % FCS (complete medium), 1 % 100x Penicillin/ Streptomycin and 1 % Amphotericin B.

Exposure period 4 hours

The culture medium of exponentially growing cell cultures was replaced with serum-free medium with 1 % 100x Penicillin/Streptomycin-solution and 1 % Amphotericin B-solution containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL medium were used. Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed twice with "Saline G" and then the cells were cultured in complete medium for the remaining culture time.

The "Saline G" solution was composed as follows (per litre):

NaCl	8000 mg
KCl	400 mg
$Glucose \cdot H_2O$	1100 mg
$Na_2HPO_4 \cdot 2\ H_2O$	192 mg
KH_2PO_4	150 mg

pH was adjusted to 7.2.

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Exposure period 18 hours
The culture medium of exponentially growing cell cultures was replaced with complete medium (with 10 % FCS, 1 % 100x Penicillin/-Streptomycin-solution, 1 % Amphotericin B-solution) containing different concentrations of the test item without S9 mix. The medium was not changed until preparation of the cells.

All cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % CO_2 (98.5 % air).

Preparation of the Cultures
Colcemid was added (0.2 µg/mL culture medium) to the cultures 15.5 hours after the start of the treatment. The cells on the slides were treated 2.5 hours later, in the chambers with hypotonic solution (0.4 % KCl) for 20 min at 37 °C. After incubation in the hypotonic solution the cells were fixed with a mixture of methanol and glacial acetic acid (3:1 parts, respectively). Per experiment two slides per group were prepared. After preparation the cells were stained with Giemsa ([redacted] [redacted] Germany).

Evaluation of Cell Numbers
The evaluation of cytotoxicity indicated by reduced cell numbers was made after the preparation of the cultures on spread slides. The cell numbers were determined microscopically by counting 10 defined fields per coded slide. The cell number of the treatment groups is given in percentage compared to the respective solvent control.

Analysis of Metaphase Cells
Evaluation of the cultures was performed (according to standard protocol of the "Arbeitsgruppe der Industrie, Cytogenetik") using NIKON microscopes with 100x oil immersion objectives. Breaks, fragments, deletions, exchanges, and chromosome disintegrations were recorded as structural chromosome aberrations. Gaps were recorded as well but not included in the calculation of the aberration rates. At least 100 well spread metaphases per culture were evaluated for cytogenetic damage on coded slides, except for the positive control in Experiment I without metabolic activation, where only 50 metaphases were evaluated.
Only metaphases with characteristic chromosome numbers of 22 ± 1 were included in the analysis. To describe a cytotoxic effect the mitotic index (% cells in mitosis) was determined.

Evaluation criteria
A test item is classified as non-clastogenic if:
- the number of induced structural chromosome aberrations in all evaluated dose groups is in the range of the laboratory's historical control data range

and/or
- no significant increase of the number of structural chromosome aberrations is observed.

A test item is classified as clastogenic if:
- the number of induced structural chromosome aberrations is not in the range of the laboratory's historical control data range

and
- either a concentration-related or a significant increase of the number of structural chromosome aberrations is observed.

Statistical significance was confirmed by means of the Fisher's exact test ($p < 0.05$). However, both biological and statistical significance should be considered together. If the criteria mentioned above for the test item are not clearly met, the classification with regard to the historical data and the biological relevance is discussed and/or a confirmatory experiment is performed.

Although the inclusion of the structural chromosome aberrations is the purpose of this study, it is important to include the polyploids and endoreduplications. The following criterion is valid:

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A test item can be classified as aneugenic if:

- the number of induced numerical aberrations is not in the range of the laboratory's historical control data range.

Acceptability criteria:
The chromosome aberration test performed was considered acceptable, if the following criteria were met:

- The number of structural aberrations found in the solvent controls falls within the range of the testing laboratory's historical control data
- The positive control substances produce significant increases in the number of cells with structural chromosome aberrations, which are within the range of the laboratory's historical control data

Findings:
No precipitation of the test item in the culture medium was observed. No relevant increase in the osmolarity or pH value was observed (Exp. I: solvent control: 373 mOsm, pH 7.3 versus 377 mOsm and pH 7.3 at 2660.0 µg/mL; Exp. II: solvent control: 391 mOsm, pH 7.4 versus 383 mOsm and pH 7.4 at 2660.0 µg/mL).

Neither reduced mitotic indices nor clearly reduced cell numbers could be observed up to the highest evaluated concentrations of the test item.

In both experiments, in the absence and presence of S9 mix, no biologically relevant increase in the number of cells carrying structural chromosome aberrations was observed. The aberration rates of the cells after treatment with the test item (0.0 - 3.5 % aberrant cells, excluding gaps) were close to the range of the solvent control values (1.0 - 2.5 % aberrant cells, excluding gaps) and within the range of the laboratory's historical control data (0.0 - 4.0 % aberrant cells, excluding gaps). Only the aberration rate after treatment with 1330.0 µg/mL (3.5 % aberrant cells excluding gaps) slightly exceeded the laboratory's historical solvent control range. As this value was not statistically significantly increased it is considered biologically not relevant.

No evidence of an increase in polyploid metaphases was noticed after treatment with the test item as compared to the controls.

In both experiments, either EMS (600 or 1000 µg/mL) or CPA (1.4 µg/mL) were used as positive controls and showed distinct increases in the number of cells with structural chromosome aberrations.

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Table CA 5.8.1-12: Summary of results of the chromosomal aberration study with BCS-CO41839 (without activation)

Exp.	Preparation interval	Test item concentration in µg/mL	Cell numbers in % of control	Mitotic indices in % of control	Aberrant cells in % incl. gaps*	Aberrant cells in % excl. gaps*	Aberrant cells in % with exchanges
Exposure period 4 hrs without S9 mix							
I	**18 hrs**	**Solvent control[1]**	100.0	100.0	2.5	2.5	2.0
		Positive control[2#]	n.t.	61.3	42.0	42.0^{S}	30.0
		665.0[##]	106.3	98.1	3.9	3.0	1.0
		1330.0	90.7	96.2	0.5	0.5	0.0
		2660.0[##]	104.9	100.4	3.8	3.5	0.3
Exposure period 18 hrs without S9 mix							
II	**18 hrs**	**Solvent control[1]**	100.0	100.0	1.5	1.5	0.0
		Positive control[3#]	n.t.	60.0	37.0	37.0^{S}	17.0
		665.0	87.3	125.2	2.5	2.5	0.0
		1330.0	74.3	108.6	3.5	3.5	0.0
		2660.0	73.7	111.0	1.5	1.5	0.0

* Inclusive cells carrying exchanges
[#] Evaluation of 50 metaphases per culture
[##] Evaluation of 200 metaphases per culture
n.t. Not tested
[S] Aberration frequency statistically significant higher than corresponding control values
[1] DMSO 0.5 % (v/v)
[2] EMS 1000.0 µg/mL
[3] EMS 600.0 µg/mL

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Table CA 5.8.1-13: Summary of Results of the chromosomal aberration study with BCS-CO41839 (with activation)

Exp.	Preparation interval	Test item concentration in µg/mL	Cell numbers in % of control	Mitotic indices in % of control	Aberrant cells in % incl. gaps*		
					incl. gaps*	excl. gaps*	with exchanges
Exposure period 4 hrs with S9 mix							
I	**18 hrs**	**Solvent control[1]**	100.0	100.0	1.0	1.0	0.0
		Positive control[4]	n.t.	69.2	9.5	9.5[S]	3.5
		665.0	102.7	89.4	2.5	1.0	0.0
		1330.0	74.1	91.2	2.5	2.5	1.0
		2660.0	102.0	83.5	0.0	0.0	0.0
II	**18 hrs**	**Solvent control[1]**	100	100.0	1.5	1.5	0.5
		Positive control[4]	n.t.	93.2	11.5	10.5[S]	4.5
		665.0	103.0	140.8	1.5	1.0	0.0
		1330.0	107.1	142.3	1.0	1.0	0.5
		2660.0	96.4	123.4	0.5	0.5	0.0

* Inclusive cells carrying exchanges
n.t. Not tested
S Aberration frequency statistically significant higher than corresponding control values
1 DMSO 0.5 % (v/v)
4 CPA 1.4 µg/mL

Conclusion:
Under the experimental conditions reported, the test item BCS-CO41839 (metabolite Amidosulfuron-guanidine), did not induce structural chromosome aberrations in V79 cells (Chinese hamster cell line), when tested up to the highest required concentration.

Report: KCA 5.8.1/14 [redacted],; 2010; M-363077-01-1
Title: BCS-CO41839 (metabolite amidosulfuron-guanidine) - Gene mutation assay in Chinese hamster V79 cells *in vitro* (V79 / HPRT)
Report No.: 1271704
Document No.: M-363077-01-1
Guideline(s): OECD 476; EC 440/2008 B.17 (2008); OPPTS 870.5300; US EPA 712-C-98-221 (1998)
Guideline deviation(s): not specified
GLP/GEP: **yes**

Material and methods:

Test Material: BCS-CO41839 (metabolite Amidosulfuron-guanidine)
Lot/Batch: RDL 603-16-20
Purity: 98.3%
Stability of test compound: Stable for the duration of the study

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Control Materials:

Negative:	Culture medium
Solvent:	Dimethyl sulfoxide (DMSO), supplier , Germany, purity 99.9%, lot No. K40013731920 / K39250731847
Positive:	
- Non activation (-S9):	Ethylmethane sulfonate (EMS), supplier Belgium, purity ≥ 98%, lot No. A0259466, dissolved in culture medium. Final concentration 0.15 mg/mL = 1.2 mM (experiment I), 0.075 mg/ mL = 0.6 mM (experiment II)
- Activation (+S9):	7, 12-dimethylbenz(a)anthracene (DMBA), supplier , Germany, purity 95%, lot No. 096K 1881, dissolved in DMSO. Final concentration 1.1 µg/mL = 4.3 µM
Metabolic activation:	The S9 fraction was isolated from the livers of Phenobarbital/ß-Naphthoflavone induced male Wistar rats. (protein content 35.6 mg/mL in the pre-experiment, 32.3 mg/mL in experiment I and 31.7 mg/mL in experiment II) and was kept frozen at -80°C. Each batch of S9 mix is routinely tested with 2-aminoanthracene as well as benzo(a)pyrene.
Cell line:	Chinese hamster V79 lung cells
Source:	Cell line supplied by , Germany
Culture condition:	Incubation performed at 37°C in a humidified atmosphere with about 4.5% CO_2.
Test concentrations:	BCS-CO41839 was used at concentrations ranging from 21.7 to 2780 µg/mL in the pre-experiment. Maximum concentration was equal to approximately 10 mM. In the main experiments the concentrations used were 173.8, 347.5, 695, 1390 and 2780 µg/mL

The selection of V79 forward mutations is based on the resistance of induced mutants to the purine analogue 6-thioguanine (6-TG). This resistance is a result of a mutation at the X-chromosome-linked HPRT locus rendering the cells unable to use 6-TG for DNA synthesis. Therefore, cell colonies formed in the presence of 6-TG are considered to represent mutants at the HPRT gene.

Dose Selection

According to the recommendations of the guidelines, several concentrations (usually at least four) of the test item should be used. These should yield a concentration-related toxic effect. The highest concentration should produce a low level of survival and the survival in the lowest concentration should approximate the solvent control. Relatively insoluble substances should be tested up to their limit of solubility under culture conditions. For freely-soluble nontoxic substances the maximum concentration should be 5 mg/mL or 10 mM. If the maximal concentration is based on cytotoxicity the cloning efficiency should be reduced to less than 50 % and/or culture growth at subcultivation should be at least 20 % of the corresponding solvent control.

In the range finding pre-experiment the intended concentration range of the pre-experiment was 21.7 to 2780 µg/mL to evaluate toxicity in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation. The maximum concentration was equal to approximately 10 mM.

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Since neither precipitation nor cytotoxic effects occurred under those conditions, the maximum concentration of the main experiments was again 2780 µg/mL equal to about 10 mM in the first and second experiment. The individual concentrations were spaced by a factor of 2.
There was no relevant shift of osmolarity and pH values of the medium even in the stock solution of the test item.

Table CA 5.8.1-14: Doses applied of BCS-CO41839

	concentrations in µg/mL					
	Experiment I					
without S9 mix*	86.9	173.8	347.5	695	1390	2780
with S9 mix*	86.9	173.8	347.5	695	1390	2780
	Experiment II					
without S9 mix**	86.9	173.8	347.5	695	1390	2780
with S9 mix*	86.9	173.8	347.5	695	1390	2780

* 4 hours treatment ** 24 hours treatment

The cultures at the lowest concentration were not continued, since a minimum of only four analysable concentrations is required by the guidelines.

Seeding
Two days old (experiment II) or three days old (experiment I) exponentially growing stock cultures (more than 50 % confluent) were trypsinized at 37 °C for 5 minutes. Then the enzymatic digestion was stopped by adding complete culture medium and a single cell suspension was prepared. The trypsin concentration for all subculturing steps was 0.2 % in Ca-Mg-free salt solution (Trypsin: USA).

The Ca-Mg-free salt solution had the following constituents (per litre):
NaCl 8000 mg
KCl 400 mg
Glucose 1000 mg
$NaHCO_3$ 350 mg

Prior to the trypsin treatment the cells were rinsed with Ca-Mg-free salt solution containing 200 mg/l EDTA (ethylene diamine tetraacetic acid).

The cell suspension was seeded into plastic culture flasks (, Germany). Approximately 1.5×10^6 (single culture) and 5×10^2 cells (in duplicate) were seeded in MEM with 10 % FCS (complete medium) for the determination of mutation rate and toxicity, respectively.

Table CA 5.8.1-15: Treatment

Treatment time		
	Without S9 mix	**With S9 mix**
Experiment I	4 hours	4 hours
Experiment II	24 hours	4 hours

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24 hours after seeding the medium of each culture in parallel was replaced with serum-free medium containing the test item, either with S9 mix (50 µL/mL) or without S9 mix.

After 4 hours (first experiment with and without S9 mix, second experiment with S9 mix) this medium was replaced with complete medium (MEM) following two washing steps with "saline G". In the second experiment without metabolic activation (24 hours treatment time) the medium containing the test item was MEM with 10 % FCS.
Concurrent solvent and positive controls were treated in parallel.

The "saline G" solution is composed as follows (per litre):

NaCl	8000 mg
KCl	400 mg
Glucose	1100 mg
$Na_2HPO_4 \times 7H_2O$	290 mg
KH_2PO_4	150 mg

The pH was adjusted to 7.2

The colonies used to determine the cloning efficiency (survival) were fixed and stained 7 days (experiment I) and 6 days (experiment II) after treatment as described below.

Three days after treatment 1.5×106 cells per experimental point were subcultivated in 175 cm² flasks containing 30 mL medium.

Following the expression time of approximately 6 days five 80 cm² cell culture flasks were seeded with about 3 - 5×105 cells each in medium containing 6-TG (11 µg/mL). Two additional 25 cm² flasks were seeded with approx. 500 cells each in non-selective medium to determine the viability. The cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % CO_2.

After 7 – 10 days the colonies were stained with 10 % methylene blue in 0.01 % KOH solution (████ ████, Germany).

Colonies with more than 50 cells were counted manually. In doubt the colony size was checked with a preparation microscope (Nikon, 40407 Düsseldorf, Germany).

Acceptance criteria:
The gene mutation assay is considered acceptable if it meets the following criteria:

- the numbers of mutant colonies per 106 cells found in the solvent controls falls within the laboratory historical control data from 2006 - 2008 (see Annex).
- the positive control substances should produce a significant increase in mutant colony frequencies (number of mutant colonies per 106 cells at least three times the number of mutant colonies/106 of the corresponding solvent control).
- the cloning efficiency II (absolute value) of the solvent controls should exceed 50 %

Evaluation criteria:
A test item is classified as positive if it induces either a concentration-related increase of the mutant frequency or a reproducible and positive response at one of the test points.

A test item producing neither a concentration-related increase of the mutant frequency nor a reproducible positive response at any of the test points is considered non-mutagenic in this system.

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A positive response is described as follows:
A test item is classified as mutagenic if it reproducibly induces a mutation frequency that is three times above the spontaneous mutation frequency at least at one of the concentrations in the experiment.

The test item is classified as mutagenic if there is a reproducible concentration-related increase of the mutation frequency. Such evaluation may be considered also in the case that a threefold increase of the mutant frequency is not observed.

However, in a case by case evaluation this decision depends on the level of the corresponding solvent control data. If there is by chance a low spontaneous mutation rate in the range normally found (0.6 – 31.7 mutants per 10^6 cells) a concentration-related increase of the mutations within this range has to be discussed. The variability of the mutation rates of solvent controls within all experiments of this study was also taken into consideration.

Statistical analysis:
A linear regression (least squares) was performed to assess a possible dose dependent increase of mutant frequencies using SYSTAT®11 ([redacted] USA) statistics software. The number of mutant colonies obtained for the groups treated with the test item were compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05. However, both, biological and statistical significance were considered together.

Table CA 5.8.1-16: Statistical results

Experimental group	**p-value**
experiment I, culture I without S9 mix	0.188
experiment I, culture II without S9 mix	0.690
experiment I, culture I with S9 mix	0.333
experiment I, culture II with S9 mix	0.653
experiment II, culture I without S9 mix	0.473
experiment II, culture II without S9 mix	0.069
experiment II, culture I with S9 mix	0.008*
experiment II, culture II with S9 mix	0.135

* inverse trend without biological relevance

Findings:
Precipitation of the test item was solely observed at the maximal concentration in experiment I without metabolic activation.

No relevant cytotoxic effects indicated by a relative cloning efficiency below 50% of the solvent control occurred in both main experiments with and without metabolic activation.

No relevant and reproducible increase in mutant colony numbers/10^6 cells was observed in the main experiments up to the maximal concentration. The induction factor of three times the corresponding solvent control was not reached or exceeded in any of the experimental parts. The mutation frequency exceeded the historical range of solvent controls in culture I of experiment II without metabolic activation. However this effect was not reproduced in the parallel culture under identical experimental conditions and was therefore, judged as biologically irrelevant fluctuation.

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A linear regression analysis (least squares) was performed to assess a possible dose dependent increase of mutant frequencies using SYSTAT® statistics software. A significant dose dependent trend of the mutation frequency indicated by a probability value of <0.05 was solely detected in the first culture of the second experiment with metabolic activation. This trend however, was judged as irrelevant since it actually was reciprocal, going down versus increasing concentrations.

In both experiments of this study (with and without S9 mix) the range of the solvent controls was from 9.6 up to 17.4 mutants per 10^6 cells; the range of the groups treated with the test item was from 4.8 up to 36.6 mutants per 10^6 cells.

EMS (150 µg/mL in experiment I and 75 µg/mL in experiment II) and DMBA (1.1 µg/mL) were used as positive controls and showed a distinct increase in induced mutant colonies.

Table CA 5.8.1-17: Summary of results

	conc. µg per mL	S9 mix	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/ 10^6 cells	induction factor	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/ 10^6 cells	induction factor
Column	1	2	3	4	5	6	7	8	9	10
Experiment I / 4 h treatment			culture I				culture II			
Solvent control with DMSO		-	100.0	100.0	9.6	1.0	100.0	100.0	11.9	1.0
Positive control with EMS	150.0	-	77.3	86.8	165.8	17.2	83.7	99.6	115.7	9.7
Test item	86.9	-	80.8	culture was not continued#			99.3	culture was not continued#		
Test item	173.8	-	89.7	92.8	25.7	2.7	96.7	92.2	23.3	2.0
Test item	347.5	-	107.8	103.9	11.1	1.2	99.5	97.6	7.0	0.6
Test item	695.0	-	107.0	100.9	12.2	1.3	92.0	91.3	20.1	1.7
Test item	1390.0	-	101.0	100.0	13.1	1.4	93.2	98.3	5.5	0.5
Test item	2780.0 (p)	-	98.5	91.0	31.0	3.2	99.3	101.9	13.3	1.1
Solvent control with DMSO		+	100.0	100.0	13.8	1.0	100.0	100.0	12.7	1.0
Positive control with DMBA	1.1	+	34.8	49.8	1325.5	96.0	40.1	62.0	1272.2	100.3
Test item	86.9	+	102.4	culture was not continued#			98.3	culture was not continued#		
Test item	173.8	+	94.5	97.8	7.9	0.6	101.7	80.1	9.3	0.7
Test item	347.5	+	96.3	100.9	7.7	0.6	82.7	89.9	22.5	1.8
Test item	695.0	+	97.1	102.9	11.7	0.8	98.7	107.7	18.5	1.5
Test item	1390.0	+	93.8	99.7	23.6	1.7	101.6	105.7	4.8	0.4
Test item	2780.0	+	96.6	85.7	14.8	1.1	98.7	107.6	12.8	1.0
Experiment II / 24 h treatment			culture I				culture II			
Solvent control with DMSO		-	100.0	100.0	13.8	1.0	100.0	100.0	14.9	1.0
Positive control with EMS	75.0	-	83.0	57.8	447.9	32.4	86.8	81.3	201.2	13.5
Test item	86.9	-	93.9	culture was not continued#			96.9	culture was not continued#		
Test item	173.8	-	89.5	103.9	36.6	2.7	97.2	88.3	28.8	1.9
Test item	347.5	-	97.6	100.9	19.1	1.4	96.9	86.4	20.1	1.3
Test item	695.0	-	86.6	93.9	36.0	2.6	98.0	91.6	19.4	1.3
Test item	1390.0	-	80.6	95.0	22.6	1.6	97.1	94.7	7.2	0.5
Test item	2780.0	-	83.3	83.8	13.6	1.0	98.0	100.6	5.4	0.4
Experiment II / 4 h treatment										
Solvent control with DMSO		+	100.0	100.0	16.5	1.0	100.0	100.0	17.4	1.0
Positive control with DMBA	1.1	+	74.3	82.1	643.1	39.0	78.5	83.0	510.0	29.2
Test item	86.9	+	98.9	culture was not continued#			105.2	culture was not continued#		
Test item	173.8	+	96.8	97.7	18.1	1.1	101.2	88.1	16.3	0.9
Test item	347.5	+	98.2	82.4	18.3	1.1	100.5	81.0	22.8	1.3
Test item	695.0	+	96.1	100.4	11.9	0.7	102.6	81.3	14.2	0.8
Test item	1390.0	+	93.5	147.2	13.3	0.8	88.6	65.0	17.3	1.0
Test item	2780.0	+	82.6	151.0	5.3	0.3	95.3	85.8	10.6	0.6

induction factor = mutant colonies per 10^6 cells divided by mutant colonies per 10^6 cells of the corresponding solvent control
# culture was not continued since a minimum of only four analysable concentrations is required
p = precipitation

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Conclusion:
In conclusion it can be stated that under the experimental conditions reported the test item did not induce gene mutations at the HPRT locus in V79 cells.

Therefore, BCS-CO41839 (metabolite Amidosulfuron-guanidine) is considered to be non-mutagenic in this HPRT assay.

Amidosulfuron-Desmethyl-Chloropyrimidine was identified as a major soil metabolite of Amidosulfuron with a predicted PECgw value of << 0.75 µg/L > 0.1 ug/L which triggers groundwater relevance assessment according to EU Council Directive 91/414/EEC (SANCO/221/2000 –rev. 10, 25 February, 2003). The submitted toxicological studies include three *in vitro* and two *in vivo* genotoxicity tests. It should be noted that in all the genotoxicity studies conducted, BCS-CO78570 (the sodium salt of BCS-CO41838), was used. Following an initial positive result in the in-vitro Chromosome Aberration test, which subsequently proved to be negative in repeat testing, two *in vivo* genotoxicity tests were conducted; a Mouse Micronucleus test and a UDS assay, to conclusively demonstrate the non-genotoxic potential of BCS-CO41838. The in-vitro Chromosome Aberration test is recognized as having a high false positive rate. Additionally, a rationale for the lack of a need to conduct an Acute Oral Toxicity study with this metabolite is provided.

The studies provided demonstrate that Amidosulfuron-Desmethyl-Chloropyrimidine is not genotoxic and most probably of low acute toxicity. According to SANCO/221/2000 –rev. 10, 25 February, 2003, the acute toxicity testing for this metabolite is not triggered anyhow.

Report:	KCA 5.8.1/16 ., 2009; M-359561-01-1
Title:	Salmonella typhimurium reverse mutation assay with BCS-CO78570 (metabolite amidosulfuron-desmethyl-chloropyrimidine)
Report No.:	1285201
Document No.:	M-359561-01-1
Guideline(s):	Ninth Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 471: Bacterial Reverse Mutation Test, adopted July 21, 1997; Commission Regulation (EC) No. 440/2008 B13/14, dated May 30, 2008; EPA Health Effects Test Guidelines, OPPTS 870.5100, Bacterial Reverse Mutation Test EPA 712-C-98-247, August, 1998
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Material and methods:

Test Material:	BCS-CO78570 (metabolite Amidosulfuron-desmethyl-chloropyrimidine)
Lot/Batch:	BCOO 5766-3-3
Purity:	88.7%, test substance used contained 5.4% of the respective disodium salt and 5.9% water
Stability of test compound:	Stable for the duration of the study
Control Materials:	
Negative:	Culture medium
Solvent:	Dimethyl sulfoxide, DMSO (, Germany)
Positive:	
- Non activation (-S9):	Sodium azide, NaN_3 (, Germany) for TA 1535 and TA 100 at 10 µg/plate, 4-nitro-ophenylene-diamine, 4-NOPD (, Germany) for TA 98 at 10 µg/plate and for TA 1537 at 50 µg/plate, methyl methane sulfonate, MMS (, , Germany) for TA 102 at 3 µg/plate.

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- Activation (+S9): 2-Aminoanthracene, 2-AA ([redacted], Germany) for TA 1535, TA 1537, TA 98, and TA 100 at 2.5 µg/plate and for TA 102 at 10 µg/plate

- Activation: The S9 fraction was isolated from the livers of Phenobarbital/β-Naphthoflavone induced rats

Test organism: Histidine-dependent auxotrophic mutants of Salmonella typhimurium:TA 1535, TA 100, TA 1537, TA 98 & TA 102

Source: Strains of S. typhimurium were obtained from [redacted] Germany

Test concentrations:

Experiment I: For all strains with or without S9 mix: 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate

Experiment II: For all strains with or without S9 mix: 33,100, 333, 1000, 2500 and 5000 µg/plate

Pre-Experiment for Toxicity

To evaluate the toxicity of the test item a pre-experiment was performed with all strains used. Eight concentrations were tested for toxicity and mutation induction with each 3 plates. The experimental conditions in this pre-experiment were the same as described for the experiment I below (plate incorporation test).

Toxicity of the test item can be evident as a reduction in the number of spontaneous revertants or a clearing of the bacterial background lawn.

The pre-experiment is reported as main experiment I, since the following criteria are met:

- Evaluable plates (>0 colonies) at five concentrations or more in all strains used.

Dose Selection

In the pre-experiment the concentration range of the test item was 3 – 5000 µg/plate. The pre-experiment is reported as experiment I. Since no toxic effects were observed 5000 µg/plate was chosen as maximal concentration.

The concentration range included two logarithmic decades. The following concentrations were tested in experiment II: 33; 100; 333; 1000; 2500; and 5000 µg/plate

Experimental Performance

For each strain and dose level, including the controls three plates were used.

The following materials were mixed in a test tube and poured onto the selective agar plates:

- 100 µl test solution at each dose level (solvent or reference mutagen solution (positive control)),
- 500 µl S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
- 100 µl Bacteria suspension (cf. test system, pre-culture of the strains),
- 2000 µL Overlay agar

In the pre-incubation assay 100 µL test solution (solvent or reference mutagen solution (positive control)), 500 µL S9 mix / S9 mix substitution buffer and 100 µL bacterial suspension were mixed in a test tube and incubated at 37 °C for 60 minutes. After pre-incubation 2.0 mL overlay agar (45 °C) was added to each tube. The mixture was poured on minimal agar plates.

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After solidification the plates were incubated upside down for at least 48 hours at 37 °C in the dark.

Acceptance criteria:
The Salmonella typhimurium reverse mutation assay is considered acceptable if it meets the following criteria:

- regular background growth in the negative and solvent control
- the spontaneous reversion rates in the negative and solvent control are in the range of the Harlan Laboratory historical data
- the positive control substances should produce a significant increase in mutant colony frequencies

Assessment criteria:
A test item is considered as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice (strains TA 98, TA 100, and TA 102) or three times (strains TA 1535 and TA 1537) the colony count of the corresponding solvent control is observed.

A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration.

An increase exceeding the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.

A dose dependent increase in the number of revertant colonies below the threshold is regarded as an indication of a mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remain within the historical range of negative and solvent controls such an increase is not considered biologically relevant.

Findings:
The plates incubated with the test item normal background growth in all strains up to 5000 µg/plate with and without S9 mix in both experiments.

No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with BCS-CO78570 (metabolite Amidosulfuron- desmethyl-chloropyrimidine) at any concentration level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

The concurrent positive controls demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

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Table CA 5.8.1-18: Summary of Results Pre-Experimental and Experiment 1 (without activation)

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	TA 102
Without Activation	DMSO		11 ± 5	11 ± 3	22 ± 3	104 ± 8	325 ± 9
	Untreated		16 ± 8	10 ± 2	26 ± 1	132 ± 7	317 ± 21
	BCS-CO78570 (Metabolite Amidosulfuron-desmethyl-chloropyrimidine)	3 µg	12 ± 4	11 ± 3	30 ± 10	110 ± 13	301 ± 6
		10 µg	17 ± 3	9 ± 1	24 ± 4	116 ± 12	329 ± 3
		33 µg	16 ± 2	11 ± 4	20 ± 6	119 ± 19	327 ± 24
		100 µg	13 ± 3	11 ± 3	25 ± 1	135 ± 16	344 ± 25
		333 µg	11 ± 1	11 ± 3	30 ± 12	110 ± 27	326 ± 8
		1000 µg	11 ± 1	11 ± 4	20 ± 4	115 ± 10	315 ± 23
		2500 µg	13 ± 4	14 ± 3	21 ± 5	117 ± 13	363 ± 30
		5000 µg	14 ± 5	8 ± 1	24 ± 8	111 ± 8	344 ± 24
	NaN_3	10 µg	1413 ± 10			1754 ± 165	
	4-NOPD	10 µg			304 ± 3		
	4-NOPD	50 µg		313 ± 61			
	MMS	3.0 µL					2364 ± 146

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Table CA 5.8.1-19: Summary of Results Pre-Experimental and Experiment 1 (with activation)

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	TA 102
With Activation	DMSO		18 ± 2	14 ± 2	35 ± 7	129 ± 5	367 ± 34
	Untreated		13 ± 4	15 ± 2	29 ± 1	138 ± 5	447 ± 38
	BCS-CO78570 (Metabolite Amidosulfuron-desmethyl-chloropyrimidine)	3 µg	18 ± 2	16 ± 4	26 ± 6	123 ± 8	394 ± 33
		10 µg	18 ± 3	11 ± 1	42 ± 2	133 ± 15	406 ± 27
		33 µg	18 ± 7	12 ± 4	29 ± 3	118 ± 3	376 ± 68
		100 µg	16 ± 4	13 ± 3	30 ± 3	128 ± 13	379 ± 81
		333 µg	17 ± 3	15 ± 1	27 ± 6	128 ± 8	371 ± 58
		1000 µg	17 ± 9	18 ± 8	30 ± 1	128 ± 7	379 ± 88
		2500 µg	18 ± 5	11 ± 3	39 ± 15	119 ± 6	420 ± 80
		5000 µg	17 ± 5	14 ± 2	31 ± 6	141 ± 9	436 ± 14
	2-AA	2.5 µg	252 ± 6	214 ± 11	1428 ± 167	1952 ± 128	
	2-AA	10.0 µg					1393 ± 106

Key to Positive Controls	
NaN_3	sodium azide
2-AA	2-aminoanthracene
MMS	methyl methane sulfonate
4-NOPD	4-nitro-o-phenylene-diamine

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Table CA 5.8.1-20: Summary of Results Experiment II (without and with activation)

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	TA 102
Without Activation	DMSO		15 ± 2	13 ± 1	28 ± 2	102 ± 14	367 ± 5
	Untreated		12 ± 2	11 ± 3	27 ± 5	110 ± 5	354 ± 26
	BCS-CO78570 (Metabolite Amidosulfuron-desmethyl-chloropyrimidine)	33 µg	13 ± 1	14 ± 4	28 ± 2	99 ± 13	336 ± 19
		100 µg	15 ± 3	15 ± 2	26 ± 2	100 ± 10	387 ± 17
		333 µg	15 ± 2	12 ± 3	29 ± 2	104 ± 11	378 ± 12
		1000 µg	14 ± 3	13 ± 2	22 ± 5	95 ± 21	327 ± 26
		2500 µg	13 ± 1	12 ± 4	20 ± 1	114 ± 11	356 ± 17
		5000 µg	11 ± 4	12 ± 5	19 ± 8	99 ± 9	362 ± 25
	NaN_3	10 µg	1739 ± 57			1998 ± 32	
	4-NOPD	10 µg			286 ± 16		
	4-NOPD	50 µg		83 ± 6			
	MMS	3.0 µL					1942 ± 125
With Activation	DMSO		18 ± 5	16 ± 3	33 ± 10	109 ± 3	516 ± 23
	Untreated		17 ± 4	16 ± 1	41 ± 9	130 ± 12	531 ± 47
	BCS-CO78570 (Metabolite Amidosulfuron-desmethyl-chloropyrimidine)	33 µg	19 ± 6	17 ± 3	37 ± 2	119 ± 22	491 ± 37
		100 µg	19 ± 2	15 ± 3	40 ± 8	116 ± 16	491 ± 27
		333 µg	17 ± 3	15 ± 1	34 ± 3	117 ± 5	511 ± 37
		1000 µg	19 ± 3	14 ± 5	36 ± 2	120 ± 14	456 ± 38
		2500 µg	14 ± 4	13 ± 3	42 ± 2	117 ± 4	489 ± 15
		5000 µg	12 ± 2	17 ± 2	29 ± 1	122 ± 11	494 ± 24
	2-AA	2.5 µg	310 ± 14	239 ± 10	1739 ± 116	1997 ± 138	
	2-AA	10.0 µg					2168 ± 38

Key to Positive Controls	
NaN_3	sodium azide
2-AA	2-aminoanthracene
MMS	methyl methane sulfonate
4-NOPD	4-nitro-o-phenylene-diamine

Conclusion:
It is concluded that BCS-CO78570 (metabolite Amidosulfuron-desmethyl-chloropyrimidine) showed no evidence of mutagenic activity in this *in vitro* bacterial system.

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Report:	KCA 5.8.1/17 ,; 2010; M-370255-01-1
Title:	BCS-CO78570 (metabolite amidosulfuron-desmethyl-chloropyrimidine) - Chromosome aberration test in Chinese hamster V79 cells
Report No.:	1285203
Document No.:	M-370255-01-1
Guideline(s):	Ninth Addendum to the OECD Guidelines for Testing of Chemicals, February 1998, adopted July 21, 1997, Guideline No. 473 *In vitro* Mammalian Chromosome Aberration Test; Commission Regulation (EC) No. 440/2008, B10: Mutagenicity - *In vitro* Mammalian Chromosome Aberration Test, dated May 30, 2008; United States Environmental Protection Agency Health Effects Test Guidelines, OPPTS 870.5375, *In vitro* Mammalian Chromosome Aberration Test, EPA 712-C-98-223, August 1998
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Material and methods:

Test Material: BCS-CO78570 (metabolite Amidosulfuron-desmethyl-chloropyrimidine)

- Lot/Batch: BCOO 5766-3-3
- Purity: 88.7%, substance used contained 5.4% of the respective disodium salt and 5.9% water
- Stability of test compound: Stable for the duration of the study
- Solvent: Deionised water

Control Materials:

- Negative: Culture medium
- Positive: EMS; Ethylmethane sulfonate (, Belgium, lot no. A0246840) without S9 mix at 1000 µg/ml. for Experiments IA and IB, at 600 µg/ml for Experiment II.
  CPA; Cyclophosphamide (, Germany, lot no. 097K1311) with S9 mix at 1.4 µg/ml

Cell line: Chinese hamster V79 lung cells

- *Source:* Cells obtained from , Germany

Test concentrations:

Experiment IA: 17.9, 35.9, 71.9, 143.8, 287.5, 575.0, 1150.0, 2300.0 and 4600.0 µg/ml

Experiment IB: 287.5, 575.0, 1150.0, 1725.0, 2300.0, 3450.0 and 4600.0 µg/ml

Experiment II (without S9 mix): 35.9, 71.9, 143.8, 287.5, 575.0, 1150.0, 2300.0 and 4600.0 µg/ml

Experiment 11 (with S9 mix): 143.8, 287.5, 575.0, 1150.0, 2300.0 and 4600.0 µg/ml

Determination of cytotoxicity:

The highest concentration used in the cytogenetic experiments was chosen considering the current OECD Guideline for *in vitro* mammalian cytogenetic tests requesting for the top concentration clear toxicity with reduced cell numbers or mitotic indices below 50 % of control, whichever is the lowest concentration, and/or the occurrence of precipitation. In case of non-toxicity the maximum concentration should be 5 mg/mL, 5 µL/mL or 10 mM, whichever is the lowest, if formulation in an appropriate solvent is possible.

With respect to the solubility of the test item, 4600.0 µg/mL of BCS-CO78570 (metabolite Amidosulfuron-desmethyl-chloropyrimidine) was applied as top concentration for treatment of the

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cultures in the pre-test. Test item concentrations between 17.9 and 4600.0 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity. No precipitation of the test item was observed. Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment IA.

A confirmatory Experiment IB with the same top test item concentration was performed in the absence and presence of S9 mix to verify the positive results obtained in Experiment IA, which could not be confirmed.

Dose selection of Experiment II was influenced by the results obtained in Experiment IB. No clear cytotoxicity was observed up to the highest applied concentration. Therefore, 4600.0 µg/mL was chosen as top treatment concentration for Experiment II.

Table CA 5.8.1-21: Doses applied in the Chromosome aberration test with BCS-CO78570

Preparation interval	Exposure period	Exp.	Concentration in µg/mL								
			Without S9 mix								
18 hrs	4 hrs	IA	17.9	35.9	71.9	143.8	287.5	**575.0**	**1150.0**	**2300.0**	**4600.0**
18 hrs	4 hrs	IB			287.5	575.0	1150.0	**1725.0**	**2300.0**	**3450.0**	**4600.0**
18 hrs	18 hrs	II		35.9	71.9	143.8	287.5	**575.0**	**1150.0**	**2300.0**	**4600.0**
			With S9 mix								
18 hrs	4 hrs	IA	17.9	35.9	71.9	143.8	287.5	**575.0**	**1150.0**	**2300.0**	**4600.0**
18 hrs	4 hrs	IB			287.5	575.0	1150.0	**1725.0**	**2300.0**	**3450.0**	**4600.0**
18 hrs	4 hrs	II				143.8	287.5	**575.0**	**1150.0**	**2300.0**	**4600.0**

Evaluated experimental points are shown in bold characters

Seeding of the Cultures

Exponentially growing stock cultures more than 50 % confluent were treated with trypsin-EDTA-solution at 37 °C for approx. 5 minutes. Then the enzymatic treatment was stopped by adding complete culture medium and a single cell suspension was prepared. The trypsin concentration for all sub-culturing steps was 0.5 % (w/v) in Ca-Mg-free salt solution ([redacted], Germany).

Prior to the trypsin treatment the cells were rinsed with Ca-Mg-free salt solution, which was composed as follows (per litre):

NaCl	8000 mg
KCl	200 mg
KH_2PO_4	200 mg
$Na_2HPO_4 \cdot 7\ H_2O$	2160 mg

The cells were seeded into Quadriperm dishes ([redacted], Germany) that contained microscopic slides (at least 2 chambers per dish and test group). In each chamber 1 x 10^4 - 6 x 10^4 cells were seeded with regard to the preparation time. The medium was MEM with 10 % FCS (complete medium), 1 % 100x Penicillin/ Streptomycin and 1 % Amphotericin B.

Exposure period 4 hours

The culture medium of exponentially growing cell cultures was replaced with serum-free medium with 1 % 100x Penicillin/-Streptomycin-solution and 1 % Amphotericin B-solution containing the test item.

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For the treatment with metabolic activation 50 µL S9 mix per mL medium were used. Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed twice with "Saline G" and then the cells were cultured in complete medium for the remaining culture time.

The "Saline G" solution was composed as follows (per liter):

NaCl	8000 mg
KCl	400 mg
Glucose•H_2O	1100 mg
Na_2HPO_4•$2H_2O$	192 mg
KH_2PO_4	150 mg

pH was adjusted to 7.2.

Exposure period 18 hours
The culture medium of exponentially growing cell cultures was replaced with complete medium (with 10 % FCS, 1 % 100x Penicillin/-Streptomycin-solution, 1 % Amphotericin B-solution) containing different concentrations of the test item without S9 mix. The medium was not changed until preparation of the cells.

All cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % CO_2 (98.5 % air).

Preparation of the Cultures
Colcemid was added (0.2 µg/mL culture medium) to the cultures 15.5 hours after the start of the treatment. The cells on the slides were treated 2.5 hours later, in the chambers with hypotonic solution (0.4 % KCl) for 20 min at 37 °C. After incubation in the hypotonic solution the cells were fixed with a mixture of methanol and glacial acetic acid (3:1 parts, respectively). Per experiment two slides per group were prepared. After preparation the cells were stained with Giemsa (Germany).

Evaluation of Cell Numbers
The evaluation of cytotoxicity indicated by reduced cell numbers was made after the preparation of the cultures on spread slides. The cell numbers were determined microscopically by counting 10 defined fields per coded slide. The cell number of the treatment groups is given in percentage compared to the respective solvent control.

Analysis of Metaphase Cells
Evaluation of the cultures was performed (according to standard protocol of the "Arbeitsgruppe der Industrie, Cytogenetik) using NIKON microscopes with 100x oil immersion objectives. Breaks, fragments, deletions, exchanges, and chromosome disintegrations were recorded as structural chromosome aberrations. Gaps were recorded as well but not included in the calculation of the aberration rates. At least 100 well spread metaphases per culture were evaluated for cytogenetic damage on coded slides, except for the positive control without metabolic activation, where only 50 metaphases were evaluated.

Only metaphases with characteristic chromosome numbers of 22 ± 1 were included in the analysis. To describe a cytotoxic effect the mitotic index (% cells in mitosis) was determined.

Evaluation criteria:
A test item is classified as non-clastogenic if:

- the number of induced structural chromosome aberrations in all evaluated dose groups is in the range of the laboratory's historical control data range.

and/or

- no significant increase of the number of structural chromosome aberrations is observed.

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A test item is classified as clastogenic if:

- the number of induced structural chromosome aberrations is not in the range of the testing laboratory's historical control data range.

and

- either a concentration-related or a significant increase of the number of structural chromosome aberrations is observed.

Statistical significance was confirmed by means of the Fisher's exact test ($p < 0.05$). However, both biological and statistical significance should be considered together. If the criteria mentioned above for the test item are not clearly met, the classification with regard to the historical data and the biological relevance is discussed and/or a confirmatory experiment is performed.

Although the inclusion of the structural chromosome aberrations is the purpose of this study, it is important to include the polyploids and endoreduplications. The following criterion is valid:

A test item can be classified as aneugenic if:

- the number of induced numerical aberrations is not in the range of the laboratory's historical control data range.

Acceptability criteria:
The chromosome aberration test performed was considered acceptable, if the following criteria were met:

- The number of structural aberrations found in the solvent controls falls within the range of the laboratory's historical control data.
- The positive control substances produce significant increases in the number of cells with structural chromosome aberrations, which are within the range of the laboratory's historical control data.

Findings:
Neither precipitation of the test item in the culture medium nor relevant increase in the osmolarity or pH value was observed (Exp. IA: solvent control: 291 mOsm, pH 7.4 versus 307 mOsm and pH 7.3 at 4600.0 µg/mL; Exp. IB: solvent control: 292 mOsm, pH 7.4 versus 311 mOsm and pH 7.3 at 4600.0 µg/mL; Exp. II: solvent control: 298 mOsm, pH 7.3 versus 304 mOsm and pH 7.3 at 4600.0 µg/mL).

In Experiment IA in the absence of S9 mix the cell numbers were reduced to 65.5 % of control at the highest evaluated concentration (4600.0 µg/mL). In Experiment IB in the presence of S9 mix the cell numbers were reduced to 64.9 % of control at the highest evaluated concentration (4600.0 µg/mL). In Experiment II in the absence of S9 mix the mitotic index was reduced to 58.8 % of control and the cell numbers were reduced to 55.3 % of control at the highest evaluated concentration (4600.0 µg/mL). In all other experimental parts no clear cytotoxicity was observed up to the highest applied concentration of 4600.0 µg/mL.

In Experiment IA statistically significant increases in the number of aberrant cells excluding gaps (3.0, 6.0 and 4.0 %, respectively) were observed after treatment with 2300.0 and 4600.0 µg/mL (without S9 mix) and 4600.0 µg/mL (with S9 mix). The values obtained at the highest evaluated concentrations (6.0 and 4.0 % aberrant cells excluding gaps) exceeded the laboratory's historical solvent control data range (0.0 –3.5 % aberrant cells excluding gaps). In addition, in the absence and presence of S9 mix all values increased in a dose-related manner. To clarify these results a confirmatory experiment with the same conditions was performed. In this Experiment IB in the absence and presence of S9 mix no clastogenicity was observed up to the highest applied concentration of 4600.0 µg/mL. Therefore, the positive results obtained in Experiment IA could not be confirmed. In Experiment II in the absence and presence of S9 mix no statistically significant increase in the number of aberrant cells were observed up to the highest evaluated concentration. However, the values obtained in the presence of

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S9 mix after treatment with 1150.0 and 2300.0 µg/mL (5.3 and 4.3 % aberrant cells excluding gaps, respectively) slightly exceeded the laboratory´s historical solvent control data range (0.0 – 3.5 % aberrant cells excluding gaps). As these values were neither statistically significant nor increased in a dose-dependent manner this effect is not defined as biologically relevant.

No biologically relevant increase in the rate of polyploid metaphases was found after treatment with the test item (2.1 - 4.5 %) as compared to the rates of the solvent controls (2.2 - 4.0 %).

A small number of endomitotic cells was observed only in Experiment IB without S9 mix. This finding is regarded to be without biologically relevance, since they were not reproducible in Experiments IA and II.

Either EMS (1000.0 and 600.0 µg/mL) or CPA (1.4 µg/mL) were used as positive controls and showed distinct increases in the number of cells with structural chromosome aberrations.

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Table CA 5.8.1-22: Summary of results of the chromosomal aberration study with BCS-CO78570 (without activation)

Exp.	Preparation interval	Test item concentration in µg/mL	Polyploid cells in %	Endomitotic cells in %	Cell numbers in % of control	Mitotic indices in % of control	Aberrant cells in % incl. gaps*	Aberrant cells in % excl. gaps*	Aberrant cells in % with exchanges
		Exposure period 4 hrs without S9 mix							
IA	**18 hrs**	**Solvent control[1]**	2.6	0.0	100.0	100.0	0.5	0.5	0.0
		Positive control[2#]	3.0	0.0	n.t.	91.7	46.0	46.0[S]	33.0
		1150.0	3.5	0.0	71.7	114.2	2.0	1.5	0.5
		2300.0	4.3	0.0	72.1	109.2	3.5	3.0[S]	0.0
		4600.0[##]	3.2	0.0	65.5	86.1	6.3	6.0[S]	1.8
IB	**18 hrs**	**Solvent control[1]**	3.9	0.1	100.0	100.0	1.5	1.0	0.5
		Positive control[2]	3.9	0.0	n.t.	79.0	27.5	27.0[S]	20.0
		2300.0	3.5	0.0	79.6	89.8	2.0	1.5	0.5
		3450.0	4.1	0.4	112.8	112.1	1.0	0.5	0.0
		4600.0	3.3	0.0	91.3	100.6	1.0	0.5	0.0
		Exposure period 18 hrs without S9 mix							
II	**18 hrs**	**Solvent control[1]**	2.2	0.0	100.0	100.0	1.0	1.0	0.0
		Positive control[3]	1.5	0.0	n.t.	57.8	22.0	21.0[S]	8.0
		1150.0	2.6	0.0	68.1	118.0	0.5	0.5	0.0
		2300.0	2.1	0.0	62.8	61.6	2.5	1.5	0.0
		4600.0	2.6	0.0	55.3	58.8	4.0	3.5	0.0

* Inclusive cells carrying exchanges
[#] Evaluation of 50 metaphases per culture
[##] Evaluation of 200 metaphases per culture
n.t. Not tested
[S] Aberration frequency statistically significant higher than corresponding control values
[1] Deionised water 10.0 % (v/v)
[2] EMS 1000.0 µg/mL
[3] EMS 600.0 µg/mL

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Table CA 5.8.1-23: Summary of results of the chromosomal aberration study with BCS-CO78570 (with activation)

Exp.	Preparation interval	Test item concentration in µg/mL	Polyploid cells in %	Endomitotic cells in %	Cell numbers in % of control	Mitotic indices in % of control	Aberrant cells in % incl. gaps*	Aberrant cells in % excl. gaps*	Aberrant cells in % with exchanges
		Exposure period 4 hrs with S9 mix							
IA	**18 hrs**	**Solvent control[1]**	4.7	0.0	100.0	100.0	1.0	0.5	0.0
		Positive control[2]	4.8	0.0	n.t.	95.9	12.0	11.5[S]	4.0
		1150.0	4.5	0.0	88.5	114.8	2.5	1.5	1.0
		2300.0	3.5	0.0	93.7	126.1	2.0	2.0	0.5
		4600.0	4.0	0.0	82.4	108.8	4.5	4.0[S]	2.0
IB	**18 hrs**	**Solvent control[1]**	2.8	0.0	100.0	100.0	2.0	1.5	0.5
		Positive control[2]	3.5	0.0	n.t.	63.7	12.0	11.0[S]	2.0
		2300.0	3.2	0.0	80.7	101.4	1.5	1.0	0.0
		3450.0	3.0	0.0	87.5	104.6	2.5	1.5	1.0
		4600.0	2.7	0.0	64.9	101.4	2.0	2.0	1.0
II	**18 hrs**	**Solvent control[1]**	4.0	0.0	100.0	100.0	2.5	2.5	0.0
		Positive control[2]	3.7	0.0	n.t.	66.1	20.5	18.5[S]	5.5
		1150.0	2.6	0.0	100.2	102.3	6.0	5.3	1.5
		2300.0[##]	3.6	0.0	93.6	105.2	4.5	4.3	1.0
		4600.0[##]	3.1	0.0	91.9	121.2	4.0	2.5	0.5

* Inclusive cells carrying exchanges
[#] Evaluation of 50 metaphases per culture
[##] Evaluation of 200 metaphases per culture
n.t. Not tested
[S] Aberration frequency statistically significant higher than corresponding control values
[1] Deionised water 10.0 % (v/v)
[2] CPA 1.4 µg/mL

Conclusion:
The results of the *in vitro* test for structural chromosome aberrations in V79 cells (Chinese hamster cell line) gave equivocal and inconclusive results. Although it was generally assumed that Amidosulfuron-desmethyl-chloropyrimidine does not have the potential to induce chromosome aberrations *in vitro*, two *in vivo* studies; a micronucleus test and UDS test were conducted in order to confirm or negate the equivocal findings in the *in vitro* test.
The *in vivo* micronucleus assay and in vivo UDS assay with BCS-CO78570 (Amidosulfuron-desmethyl-chloropyrimidine) were both found to be negative. Thus, BCS-CO78570 (Amidosulfuron-desmethyl-chloropyrimidine) is considered to be non-mutagenic and negates the above findings in the *in vitro* test.

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Report:	KCA 5.8.1/18 L; 2010; M-363087-01-1
Title:	BCS-CO78570 (metabolite amidosulfuron-desmethyl-chloropyrimidine) - Gene mutation assay in Chinese hamster V79 cells *in vitro* (V79 / HPRT)
Report No.:	1285202
Document No.:	M-363087-01-1
Guideline(s):	OECD 476; EC 440/2008 B.17 (2008); OPPTS 870.5300; US EPA 712-C-98-221 (1998)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Test Material: BCS-CO78570 (metabolite Amidosulfuron-desmethyl-chloropyrimidine)

Lot/Batch: BCOO 5766-3-3

Purity: 88.7%, test substance used contained 5.4% of the disodium salt and 5.9% water

Stability of test compound: Stable for the duration of the study

Control Materials:

Negative: Culture medium

Solvent: Deionised water

Positive:

- Non activation (-S9): Ethylmethane sulfonate (EMS), supplier Belgium, purity ≥ 98%, lot No. A0259466, dissolved in culture medium. Final concentration 0.15 mg/mL = 1.2 mM (experiment I), 0.075 mg/ mL = 0.6 mM (experiment II)

- Activation (+S9): 7, 12-dimethylbenz(a)anthracene (DMBA), supplier Germany, purity 95%, lot No. 096K 1881, dissolved in dimethyl sulfoxide (DMSO), supplier , Germany. Final concentration 1.1 µg/mL = 4.3 µM

Metabolic activation: The S9 fraction was isolated from the livers of Phenobarbital/ß-Naphthoflavone induced male Wistar rats. (protein content 32.3 mg/mL in the pre-experiment and in experiment I and 32.7 mg/mL in experiment II) and was kept frozen at -80°C. Each batch of S9 mix is routinely tested with 2-aminoanthracene as well as benzo(a)pyrene

Cell line: Chinese hamster V79 lung cells

Source: Cell line supplied by , Germany

Culture conditions: Incubation performed at 37°C in a humidified atmosphere with about 4.5% CO_2.

Test concentrations: BCS-CO78570 was used at concentrations ranging from 36.3 to 4640 µg/mL in the pre-experiment. Maximum concentration was equal to approximately 10 mM. In the main experiments the concentrations used were 290, 580, 1160, 2320 and 4640 µg/mL

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The selection of V79 forward mutations is based on the resistance of induced mutants to the purine analogue 6-thioguanine (6-TG). This resistance is a result of a mutation at the X-chromosome-linked HPRT locus rendering the cells unable to use 6-TG for DNA synthesis. Therefore, cell colonies formed in the presence of 6-TG are considered to represent mutants at the HPRT gene.

Dose Selection
According to the recommendations of the guidelines, several concentrations (usually at least four) of the test item should be used. These should yield a concentration-related toxic effect. The highest concentration should produce a low level of survival and the survival in the lowest concentration should approximate the solvent control. Relatively insoluble substances should be tested up to their limit of solubility under culture conditions. For freely-soluble nontoxic substances the maximum concentration should be 5 mg/mL or 10 mM. If the maximal concentration is based on cytotoxicity the cloning efficiency should be reduced to less than 50 % and/or culture growth at subcultivation should be at least 20 % of the corresponding solvent control.

In the range finding pre-experiment the intended concentration range of the pre-experiment was 36.3 to 4640 µg/mL to evaluate toxicity in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation. The maximum concentration was equal to approximately 10 mM.

Since neither precipitation nor cytotoxic effects occurred under those conditions, the maximum concentration of the main experiments was again 4640 µg/mL equal to about 10 mM in the first and second experiment. The individual concentrations were generally spaced by a factor of 2. A closer step was used between the highest two concentrations to cover possible cytotoxic effects more closely.

There was no relevant shift of osmolarity and pH values of the medium even in the stock solution of the test item.

Table CA 5.8.1-24: Applied dose of BCS-CO78570

	concentrations in µg/mL					
	Experiment I					
without S9 mix*	145	290	580	1160	2320	4640
with S9 mix*	145	290	580	1160	2320	4640
	Experiment II					
without S9 mix**	145	290	580	1160	2320	4640
with S9 mix*	145	290	580	1160	2320	4640

* 4 hours treatment ** 24 hours treatment

The cultures at the lowest concentration were not continued, since a minimum of only four analysable concentrations is required by the guidelines.

Seeding
Two days old (experiment II) or three days old (experiment I) exponentially growing stock cultures (more than 50 % confluent) were trypsinized at 37 °C for 5 minutes. Then the enzymatic digestion was stopped by adding complete culture medium and a single cell suspension was prepared. The trypsin concentration for all subculturing steps was 0.2 % in Ca-Mg-free salt solution (Trypsin: [redacted] USA).

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The Ca-Mg-free salt solution had the following constituents (per litre):

NaCl	8000 mg
KCl	400 mg
Glucose	1000 mg
$NaHCO_3$	350 mg

Prior to the trypsin treatment the cells were rinsed with Ca-Mg-free salt solution containing 200 mg/l EDTA (ethylene diamine tetraacetic acid).

The cell suspension was seeded into plastic culture flasks ([redacted], Germany). Approximately 1.5×10^6 (single culture) and 5×10^2 cells (in duplicate) were seeded in MEM with 10 % FCS (complete medium) for the determination of mutation rate and toxicity, respectively.

Table CA 5.8.1-25: Treatment

Treatment time		
	Without S9 mix	**With S9 mix**
Experiment I	4 hours	4 hours
Experiment II	24 hours	4 hours

24 hours after seeding the medium of each culture in parallel was replaced with serum-free medium containing the test item, either with S9 mix (50 µL/mL) or without S9 mix.

After 4 hours (first experiment with and without S9 mix, second experiment with S9 mix) this medium was replaced with complete medium (MEM) following two washing steps with "saline G". In the second experiment without metabolic activation (24 hours treatment time) the medium containing the test item was MEM with 10 % FCS.

Concurrent solvent and positive controls were treated in parallel.

The "saline G" solution is composed as follows (per litre):

NaCl	8000 mg
KCl	400 mg
Glucose	1100 mg
$Na_2HPO_4\times7H_2O$	290 mg
KH_2PO_4	150 mg

The pH was adjusted to 7.2

The colonies used to determine the cloning efficiency (survival) were fixed and stained 7 days (experiment I) and 6 days (experiment II) after treatment as described below.

Three days after treatment 1.5×106 cells per experimental point were subcultivated in 175 cm² flasks containing 30 mL medium.

Following the expression time of approximately 6 days five 80 cm² cell culture flasks were seeded with about 3 - 5×105 cells each in medium containing 6-TG (11 µg/mL). Two additional 25 cm² flasks were seeded with approx. 500 cells each in non-selective medium to determine the viability. The cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % CO_2.

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After 7 – 10 days the colonies were stained with 10 % methylene blue in 0.01 % KOH solution (█ ████████████████ Germany).

Colonies with more than 50 cells were counted manually. In doubt the colony size was checked with a preparation microscope (Nikon, 40407 Düsseldorf, Germany).

Acceptance criteria
The gene mutation assay is considered acceptable if it meets the following criteria:

- the numbers of mutant colonies per 10^6 cells found in the solvent controls falls within the laboratory historical control data from 2006 - 2008 (see Annex).
- the positive control substances should produce a significant increase in mutant colony frequencies (number of mutant colonies per 10^6 cells at least three times the number of mutant colonies/10^6 of the corresponding solvent control).
- the cloning efficiency II (absolute value) of the solvent controls should exceed 50 %.

Evaluation criteria:
A test item is classified as positive if it induces either a concentration-related increase of the mutant frequency or a reproducible and positive response at one of the test points.

A test item producing neither a concentration-related increase of the mutant frequency nor a reproducible positive response at any of the test points is considered non-mutagenic in this system.

A positive response is described as follows:
A test item is classified as mutagenic if it reproducibly induces a mutation frequency that is three times above the spontaneous mutation frequency at least at one of the concentrations in the experiment.

The test item is classified as mutagenic if there is a reproducible concentration-related increase of the mutation frequency. Such evaluation may be considered also in the case that a threefold increase of the mutant frequency is not observed.

However, in a case by case evaluation this decision depends on the level of the corresponding solvent control data. If there is by chance a low spontaneous mutation rate in the range normally found (0.6 – 31.7 mutants per 106 cells) a concentration-related increase of the mutations within this range has to be discussed. The variability of the mutation rates of solvent controls within all experiments of this study was also taken into consideration.

Statistical analysis:
A linear regression (least squares) was performed to assess a possible dose dependent increase of mutant frequencies using SYSTAT®11 (████████████████████████████████████ ████████████████, USA) statistics software. The number of mutant colonies obtained for the groups treated with the test item were compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05. However, both, biological and statistical significance were considered together.

Table CA 5.8.1-26: Statistical results

experimental group	p-value
experiment I, culture I without S9 mix	0.881
experiment I, culture II without S9 mix	0.007
experiment I, culture I with S9 mix	0.389
experiment I, culture II with S9 mix	0.755
experiment II, culture I without S9 mix	0.177
experiment II, culture II without S9 mix	0.067
experiment II, culture I with S9 mix	0.404
experiment II, culture II with S9 mix	0.832

Findings:
Relevant cytotoxic effects occurred at the maximum concentration of 4640 µg/mL in both cultures of experiment I without metabolic activation. No relevant cytotoxic effects were noted in the second experiment without metabolic activation even though the cells were treated with the test item for 24 hours. However, 10% serum has to be added to the medium during 24 hours treatment so, the lower cytotoxicity may indicate protein binding effects of the test item.

No relevant and reproducible increase in mutant colony numbers/106 cells was observed in the main experiments up to the maximum concentration. The mutant frequencies generally remained well within the historical range of solvent controls. The induction factor of three times the corresponding solvent control was exceeded at 1160 µg/mL and above in the second culture of experiment I without metabolic activation. However, the number of mutant colonies per 106 cells did not exceeded the range of historical solvent control data except at the maximum concentration which however induced severe cytotoxic effects, there was no dose relationship in mutant colonies between 1160 µg/ml and 2320 µg/mL and the increase was not reproduced in the parallel culture performed under identical conditions or in the second experiment without metabolic activation. The increased induction factors were judged to be based upon the rather low solvent control of just 4.8 mutant colonies/106 cells.

A linear regression analysis (least squares) was performed to assess a possible dose dependent increase of mutant frequency using SYSTAT® statistics software. A single significant dose dependent trend of the mutation frequency indicated by a probability value of <0.05 was determined in culture II of the first experiment without metabolic activation. However, the trend was judged as biologically irrelevant as discussed above.

In both experiments of this study (with and without S9 mix) the range of the solvent controls was from 4.8 up to 22.6 mutants per 106 cells; the range of the groups treated with the test item was from 4.8 up to 38.0 mutants per 106 cells.

EMS (150 µg/mL in experiment I and 75 µg/mL in experiment II) and DMBA (1.1 µg/mL) were used as positive controls and showed a distinct increase in induced mutant colonies.

Table CA 5.8.1-27: Summary of results

	conc. µg per mL	S9 mix	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/ 10^6 cells	induction factor	relative cloning efficiency I %	relative cloning efficiency II %	mutant colonies/ 10^6 cells	induction factor
Column	1	2	3	4	5	6	7	8	9	10
Experiment I / 4 h treatment			culture I				culture II			
Solvent control with water		-	100.0	100.0	22.6	1.0	100.0	100.0	4.8	1.0
Positive control with EMS	150.0	-	94.6	110.5	122.8	5.4	93.3	84.8	73.8	15.4
Test item	145.0	-	88.3	culture was not continued#			113.6	culture was not continued#		
Test item	290.0	-	88.6	106.1	8.8	0.4	102.4	83.9	4.8	1.0
Test item	580.0	-	81.4	110.9	20.0	0.9	102.4	58.4	8.7	1.8
Test item	1160.0	-	79.2	121.7	13.6	0.6	99.8	64.7	22.2	4.6
Test item	2320.0	-	61.9	120.9	14.1	0.6	41.1	57.1	16.6	3.5
Test item	4640.0	-	2.1	118.6	16.7	0.7	0.0	56.2	38.0	7.9
Solvent control with water			100.0	100.0	12.7	1.0	100.0	100.0	16.5	1.0
Positive control with DMBA	1.1	+	56.4	76.5	742.4	58.3	55.9	70.1	1046.9	63.6
Test item	145.0	+	100.6	culture was not continued#			107.8	culture was not continued#		
Test item	290.0	+	92.9	85.7	19.0	1.5	107.6	82.6	13.6	0.8
Test item	580.0	+	101.7	57.6	31.2	2.5	104.5	81.5	28.3	1.7
Test item	1160.0	+	101.0	86.2	18.9	1.5	102.4	106.2	10.9	0.7
Test item	2320.0	+	99.5	78.1	14.5	1.1	98.1	112.6	10.5	0.6
Test item	4640.0	+	79.6	103.1	12.2	1.0	79.9	99.3	16.8	1.0
Experiment II / 24 h treatment			culture I				culture II			
Solvent control with water		-	100.0	100.0	12.9	1.0	100.0	100.0	13.1	1.0
Positive control with EMS	150.0	-	97.6	44.6	362.5	28.2	83.1	60.6	203.7	15.5
Test item	145.0	-	113.2	culture was not continued#			87.9	culture was not continued#		
Test item	290.0	-	109.0	97.9	11.2	0.9	91.6	125.4	11.3	0.9
Test item	580.0	-	110.0	86.7	15.1	1.2	98.2	123.4	12.8	1.0
Test item	1160.0	-	117.0	87.0	22.9	1.8	102.3	104.2	10.8	0.8
Test item	2320.0	-	97.1	91.1	22.6	1.8	73.7	101.0	5.3	0.4
Test item	4640.0	-	86.8	90.0	17.6	1.4	70.8	87.8	29.6	2.3
Experiment II / 4 h treatment										
Solvent control with water		+	100.0	100.0	13.6	1.0	100.0	100.0	8.1	1.0
Positive control with DMBA	1.1	+	35.0	69.1	687.8	50.6	30.9	53.2	952.8	118.1
Test item	145.0	+	101.8	culture was not continued#			93.0	culture was not continued#		
Test item	290.0	+	100.8	91.9	14.1	1.0	81.5	99.9	19.3	2.4
Test item	580.0	+	102.1	102.7	11.2	0.8	91.3	104.3	12.5	1.5
Test item	1160.0	+	117.9	92.0	6.0	0.4	87.6	95.1	14.1	1.7
Test item	2320.0	+	104.9	88.6	16.0	1.2	97.8	73.0	20.2	2.5
Test item	4640.0	+	94.9	85.0	16.1	1.2	76.4	87.1	12.6	1.6

induction factor = mutant colonies per 10^6 cells divided by mutant colonies per 10^6 cells of the corresponding solvent control

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

Conclusion:

In conclusion it can be stated that under the experimental conditions reported the test item did not induce gene mutations at the HPRT locus in V79 cells.

Therefore, BCS-CO78570 (metabolite Amidosulfuron-desmethyl-chloropyrimidine) is considered to be non-mutagenic in this HPRT assay.

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Report:	KCA 5.8.1/19 ; 2010; M-366697-01-1
Title:	Micronucleus assay in bone marrow cells of the mouse with BCS-CO78570 (Amidosulfuron-Desmethyl-Chloropyrimidine)
Report No.:	1298100
Document No.:	M-366697-01-1
Guideline(s):	OECD Section 4, No 474 (July 21, 1997) EC 440/2008 dated May 05, 2008 EPA OPPTS 870.5395 (August 1998)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Material and methods:

Test Material:	BCS-CO78570 (metabolite Amidosulfuron-desmethyl-chloropyrimidine)
Lot/Batch:	BCOO 5766-3-3
Purity:	88.7%, substance used contained 5.4% of the respective disodium salt and 5,9% water
Stability of test compound:	Stable for the duration of the study
Solvent:	30% DMSO / 70% PEG 400, supplier , Germany
Control Materials:	
Negative:	-
Positive:	Cyclophosphamide (CPA), supplier , Germany
Test animals:	
Species:	Mouse
Strain:	NMRI
Age:	8 to 12 weeks approximately
Weight:	Males; 37.4 g (SD ±2.6 g), Females; 28.4g (SD ±1.4 g)
Source:	, The Netherlands
Number of animals per dose:	Pre-Experiment: 2 animals/sex/group
Micronucleus assay:	6 animals/sex/group
Dose levels:	Pre-Experiment: 200 and 2000 mg/kg bw Micronucleus assay: 0, 500, 1000 and 2000 mg/kg bw

Pre-Experiment on Toxicity

A preliminary study on acute toxicity was performed with two animals per sex under identical conditions as in the mutagenicity study concerning: animal strain, vehicle, route, frequency, and volume of administration.

The animals were treated orally with the test item and examined for acute toxic symptoms at intervals of approximately 1 h, 2-4 h, 6 h, 24 h, 30 h, and 48 h after administration of the test item.

Dose Selection

It is generally recommended to use the maximum tolerated dose or the highest dose that can be formulated and administered reproducibly or 2000 mg/kg as the upper limit for non-toxic test items.

The maximum tolerated dose level is determined to be the dose that causes toxic reactions without having major effects on survival within 48 hours.

The administered volume was 20 ml/kg bw.

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Three adequately spaced dose levels spaced by a factor of 2 were administered, and samples were collected at the central sampling interval 24 h after treatment. For the highest dose level an additional sample was taken at 48 h after treatment.

Test Groups
Six males and six females were assigned to each test group for the main study.

At the beginning of the treatment the animals (including the controls) were weighed and the individual volume to be administered was adjusted to the animal's body weight. The animals received the test item, the vehicle or the positive control substance once. Twelve animals, six males and six females, were treated per dose group and sampling time. The animals of all dose groups (excepted the positive control) were examined for acute toxic symptoms at intervals of approximately 1 h, 2 - 4 h, 6 h, 24 h and 48 h after administration of the test item.

Sampling of the bone marrow was done 24 and 48 hours after treatment, respectively.

Preparation of the Animals
The animals were sacrificed using CO_2 followed by bleeding. The femora were removed, the epiphyses were cut off and the marrow was flushed out with foetal calf serum using a syringe. The cell suspension was centrifuged at 1500 rpm (390 x g) for 10 minutes and the supernatant was discarded. A small drop of the re-suspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald ([redacted], Germany)/Giemsa ([redacted], Great Britain). Cover slips were mounted with EUKITT ([redacted], Germany). At least one slide was made from each bone marrow sample.

Analysis of Cells
Evaluation of the slides was performed using NIKON microscopes with 100x oil immersion objectives. Per animal 2000 polychromatic erythrocytes (PCE) were analysed for micronuclei. To describe a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 2000 erythrocytes. The analysis was performed with coded slides.

All animals per test group were evaluated as described.

Acceptance Criteria:
The study was considered valid as the following criteria are met:

- at least 5 animals per group and sex can be evaluated.
- PCE to erythrocyte ratio should not be less than 20 % of the negative control.
- the positive control shows a statistically significant and biological relevant increase of micronucleated PCEs compared to the vehicle control.

Evaluation Criteria:
A test item is classified as mutagenic if it induces either a dose-related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group. Statistical methods (nonparametric Mann-Whitney test) will be used as an aid in evaluating the results. However, the primary point of consideration is the biological relevance of the results.

A test item that fails to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes is considered non-mutagenic in this system.

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Historical Controls

Table CA 5.8.1-28: Historical controls 2003 - 2008

	Vehicle Controls			Positive Controls (CPA)		
	Males	**Females**	**Total**	**Males**	**Females**	**Total**
Mean* ± SD	0.094 ± 0.039	0.080 ± 0.037	0.088 ± 0.030	2.309 ± 0.697	1.898 ± 0.633	2.120 ± 0.614
Range**	0.01 - 0.20	0.0 - 0.19	0.01 - 0.18	0.70 -4.52	0.58 - 3.68	0.77 - 3.685
No. of Experiments	289	269	291	288	268	290

*: mean value (percent micronucleated cells)
**: range of the mean group values (percent micronucleated cells)

Findings:
As estimated by a pre-experiment 2000 mg BCS-CO78570 (Amidosulfuron-desmethyl-chloropyrimidine) per kg bw (the maximum guideline-recommended dose) was suitable dose.

The mean number of polychromatic erythrocytes was not decreased after treatment with the test item as compared to the mean value of PCEs of the vehicle control indicating that BCS-CO78570 (Amidosulfuron-desmethyl-chloropyrimidine) did not have any cytotoxic properties in the bone marrow.

In comparison to the corresponding vehicle controls there was no biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item. All results for the tested item regarding % PCEs with micronuclei were within the historical control data (range from 0.01% to 0.18%). The statistically significant increase in PCE with micronuclei (%) at 2000 mg/kg (24 hours treatment) is based on one female (Animal Nr 45) which had 6 micronucleated cells per 2000 PCEs. At 2000 mg/kg all other animals had 1 to 4 micronucleated cells per 2000 PCEs, which was completely in the range of the concurrent vehicle control. Therefore it is assumed that this finding has no biological significance.

40 mg/kg bw cyclophosphamide administered orally was used as positive control which showed a statistically significant increase of induced micronucleus frequency.

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Table CA 5.8.1-29: Summary of Micronucleus Test results

test group	dose mg/kg bw	sampling time (h)	PCEs with micronuclei (%)	range	PCE per 2000 erythocytes
vehicle	0	24	0.092	0 - 4	1206
test item	500	24	0.104	0 - 3	1200
	1000	24	0.096	0 - 4	1174
	2000	24	0.154	1 - 6	1174
positive control	40	24	3.229	14 -102	1093
test item	2000	48	0.108	1 - 4	1203

Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of the non-parametric Mann-Whitney test.

Table CA 5.8.1-30: Statistical results

Vehicle control versus test group	Significance	p
500 mg BCS-CO78570 (Amidosulfuron-desmethyl-chloropyrimidine)/kg bw; 24 h	-	0.3687
1000 mg BCS-CO78570 (Amidosulfuron-desmethyl-chloropyrimidine)/kg bw; 24 h	-	0.5000
2000 mg BCS-CO78570 (Amidosulfuron-desmethyl-chloropyrimidine)/kg bw; 24 h	+	0.0291
40 mg CPA/kg bw; 24 h	+	< 0.0001
2000 mg BCS-CO78570 (Amidosulfuron-desmethyl-chloropyrimidine)/kg bw; 48 h	-	0.3099

\+ = significant;
\- = not significant

Conclusion:
There was no indication of any clastogenic effect of BCS-CO78570 (Amidosulfuron-desmethyl-chloropyrimidine) in this *in vivo* micronucleus test.

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Report:	KCA 5.8.1/20[redacted]; 2010; M-387609-01-1
Title:	*In vivo* unscheduled DNA synthesis in rat hepatocytes with BCS-CO78570 (amidosulfuron-desmethyl-chloropyrimidine)
Report No.:	1298200
Document No.:	M-387609-01-1
Guideline(s):	OECD 486, adopted July 21, 1997 EEC Directive 200/32, B.39 May 19, 2000 EPA OPPTS 870.5550, EPA 712-C-98-230, August 1998
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Material and methods:

Test Material:	BCS-CO78570 (metabolite Amidosulfuron-desmethyl-chloropyrimidine
Lot/Batch:	BCOO 5766-3-3
Purity:	88.7%, substance used contained 5.4% of the respectivedisodium salt and 5.9% water
Stability of test compound:	Stable for the duration of the study
Solvent:	30% DMSO (dimethylsulfoxide) / 70% PEG (polyethylene glycol) 400, supplier [redacted], Germany
Control Materials:	
Negative:	-
Positive:	<u>4 hours preparation interval</u>: DMH; DMH; N,N´ dimethylhydrazinedihydrochloride, supplier Sigma-Aldrich, Deisenhofen, Germany, dissolved in 0.9% NaCl solution, dose used <u>16 hour preparation interval</u>: 2-AAF; 2-acetylaminofluorene, supplier [redacted], Germany, dissolved in DMSO / PEG 400 (1 + 9), dose used 100 mg/kg b.w
Test animals:	
Species:	Rat
Strain:	Wistar
Age:	6 to 12 weeks approximately
Weight:	Males: 177.8 g (SD ± 8.6 g)
Source:	[redacted], The Netherlands
Number of animals per dose:	Pre-Experiment: 2 animals/sex/group
Main-Experiment:	4 males/group
Dose levels:	Pre-Experiment: 2000 mg/kg bw Main-Experiment: 0, 1000 and 2000 mg/kg bw

Pre-Experiment on Toxicity

A preliminary study on acute toxicity was performed with two males and two females administered a single dose of 2000 mg/kg bw of BCS-CO78570 formulated in 30% DMSO / 70% PEG 400, under identical conditions as in the mutagenicity study concerning: animal strain; vehicle; route, frequency, and volume of administration.

The animals were treated orally (gavage) and examined for acute toxic symptoms at intervals of 1 h, 2-4 h, 6 h, and 24 h after administration of the test item.

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Main-Experiment
For genotoxicity investigations it is generally recommended to use the maximum tolerated dose or the highest dose that can be formulated and administered reproducibly.

The administered volume was 20 ml/kg bw.

The maximum tolerated dose is determined to be the dose that causes toxic reactions (e.g. reduced spontaneous activity, eyelid closure, apathy, etc.) without having major effects on survival within 24 hours. If no toxic reactions are observed the highest dose recommended by the OECD guideline to be used should be 2000 mg/kg b.w. The low dose will be 50 % of the high dose.

Test groups
Four male rats are assigned to each test group.

Before the beginning of the treatment the animals were weighed and the individual volume to be administered was adjusted to the body weight of the animals. The animals received the test item once and were examined for acute toxic symptoms at intervals of approx. 1 h, 2h and 4h for the 4 hours treatment, and 1h and 16 h for the 16 hours treatment after administration of the test item. Four animals (males) were treated per dose group.

Isolation of the Primary Hepatocytes
After anaesthetising the rats with 46% Ketamin (, Germany), 23% Xylazin (Rompun 2%, , Germany) and 31% Midazolan (, Germany) (approx. 2 mL/kg body weight) the liver was perfused through the vena portae with Hanks' balanced salt solution (, Germany) supplemented with collagenase (0.05 % (w/v), , Germany) adjusted to pH 7.4 and maintained at.

The isolated hepatocytes were washed twice with HBSS. The crude cell suspension was filtered through a stainless steel mesh to yield a single cell suspension. The quality of the performed perfusion was determined by the trypan blue dye exclusion method for cell viability. In addition, the number of the cells was determined.

Culture Conditions
The washed hepatocytes were centrifuged and transferred into Williams medium E (Germany) supplemented with:

Hepes	2.38 mg/ml	L-Glutamine	0.29	mg/ml
Penicillin	100 units/ml	Insulin	0.50	µg/ml
Streptomycin	0.10 mg/ml	Fetal calf serum (FCS)	100	µl/ml

This complete medium was adjusted to pH 7.6.

At least three cultures were established from each animal. Aliquots of 2.5 ml with freshly isolated hepatocytes in complete culture medium (2.0 × 105 viable cells/ml) were added to 35 mm six-well dishes (, Germany) containing one 25 mm round plastic coverslip (, Germany) per well coated with gelatine.

After an attachment period of approximately 1.5 h in a 95 % air/ 5 % CO_2 humidified incubator at 37° C the culture medium was discarded. Then, the cell layer was rinsed once with PBS to remove non-adherent cells. Subsequently, 3HTdR (5 µCi/ml, specific activity 20 Ci/mmol;

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████████████, Germany) in 2.0 ml culture medium (WME, 1 % (v/v) FCS) was added to the cultures. After a labelling time of 4 h the cells were washed twice with WME supplemented with 1 % (v/v) FCS and 0.25 mM unlabelled thymidine. Cultures were incubated overnight using the same medium. To prepare for autoradiography the medium was replaced by a hypotonic solution of 1 % (w/v) sodium citrate for 10 minutes to swell the nuclei for better grain detection. The cells on the coverslips were then fixed by three changes of methanol:acetic acid (3+1 v/v) for 20 minutes each, rinsed with 96 % (v/v) ethanol, and air-dried.

Autoradiographic Processing
The cover slips were mounted the side carrying the cells up on glass slides and coated with KODAK NTB (████████████, Germany) photographic emulsion in the dark. The coated slides were stored in light-proof boxes in the presence of a drying agent for 14 days (excepted the reserves slides for 7 days) at 4° C. The photographic emulsion was then developed with Ilford Phenisol (████████████Switzerland) at room temperature, fixed in Rapid Fixer (████████████, Switzerland) and stained with hematoxylin/eosin.

Quantification of UDS
Evaluation was performed microscopically on coded slides using NIKON microscopes with oil immersion objectives. The cells for scoring were randomly selected according to a fixed scheme. The number of silver grains in the nuclear area was counted automatically using the Sorcerer UDS device version 2.0 DT3152 (Perceptive Instruments). In addition, the number of grains of the most heavily labelled nuclear-sized cytoplasm area adjacent to the nucleus was counted. At least two slides per animal and 50 cells per slide were evaluated. Heavily radio-labelled cells undergoing replicative DNA synthesis were excluded from counting.

At least three animals per group were evaluated as described above.

Evaluation criteria
Nuclear and net grain counts are estimated together. Increased net grains should be based on enhanced nuclear grain counts rather than on decreased cytoplasmic grain counts.

A test item is classified as positive if the mean number of net grains is higher than five per nucleus at one of the test points.

A group average between 0 and 5 net grains is considered as a marginal response. A dose-related increase in nuclear and net grains and/or a substantial shift of the percentage distribution of the nuclear grain counts to higher values provide additional information to confirm a positive response with less than 5 net grains.

Statistical significance may give further evidence for a positive evaluation. Statistical significance can be evaluated by means of the non-parametric Mann-Whitney test.
A test item producing net grains not greater than 0 at anyone of the test points is considered non-effective in this system.

Statistical analysis
A statistical evaluation of the results was not necessary to perform as the number of net grain counts of the groups treated with the test item were in the range of the corresponding controls.

Findings:
The viability of the hepatocytes was not substantially affected by the *in vivo* treatment with the test item at any of the treatment periods or dose groups. The interindividual variations obtained for the numbers and the viabilities of the isolated hepatocytes are in the range of the performing Laboratories historical control data.

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No dose level of the test item revealed UDS induction in the hepatocytes of the treated animals as compared to the current vehicle controls. Neither the nuclear grains nor the resulting net grains were distinctly enhanced due to the *in vivo* treatment of the animals with the test item for 4 hours or 16 hours, respectively. Therefore, the net grain values obtained after treatment with the test item were consistently negative.

In addition, no substantial shift to higher values was obtained in the percentage of cells in repair.
Appropriate reference mutagens (DMH, 80 mg/kg b.w. and 2-AAF, 100 mg/kg b.w.) were used as positive controls. *In vivo* treatment with DMH or 2-AAF revealed distinct increases in the number of nuclear and net grain counts.

Table CA 5.8.1-31: Results of UDS test with 4 hours preparation interval

Test Group	Ani-mal No.	Mean Nuclear Grain Count		Mean Cytoplasmic Grain Count		Mean Net Grain Counts		Mean Nuclear Grains of Cells in Repair		% Cells in Repair
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Vehicle Control (30% DMSO/70% PEG 400)	1	10.39	4.18	15.07	5.75	-4.68	5.41	8.00	2.83	2
	2	9.56	7.58	11.16	9.23	-1.60	5.94	9.14	5.67	7
	3	10.39	6.17	12.00	5.48	-1.61	5.69	7.54	2.79	13
	4	11.27	4.76	13.59	5.22	-2.32	5.75	7.50	2.84	12
1000 mg/kg bw BCS. CO78570 (Amidosulfuron-desmethyl-chloro-pyrimidine)	5	8.78	4.97	14.19	6.37	-5.41	5.59	7.33	1.53	3
	6	10.81	5.51	16.19	6.85	-5.38	5.89	6.67	2.08	3
	7	11.84	6.66	17.07	7.88	-5.23	6.86	7.20	2.49	10
	8	9.51	5.14	10.84	5.16	-1.33	5.37	6.64	2.13	14
2000 mg/kg b.w. BCS. CO78570 (Amidosulfuron-desmethyl-chloropyrimidine	9	10.38	6.05	14.75	7.60	-4.37	6.32	6.75	2.71	8
	10	11.19	5.68	12.39	6.14	-1.20	5.47	9.00	3.77	12
	11	9.66	4.92	12.42	7.86	-2.76	7.50	7.17	2.33	12
	12	8.68	5.45	11.37	5.231	-2.69	5.17	8.00	5.14	6
Positive control (DMH 80 mg/kg b.w.)	13	41.38	13.86	13.97	5.93	27.41	11.69	27.65	11.50	99
	14	55.41	22.10	16.21	7.08	39.2	19.34	39.20	19.34	100
	15	52.36	24.37	15.65	8.16	36.71	20.05	37.40	19.66	98
	16	68.36	29.61	18.95	9.38	49.41	26.77	49.94	26.37	99

S.D. = Standard deviation. The Standard deviation shown for each animal is the deviation the 100 analyzed cells. The deviation shown for the mean of each group is the standard deviation between the results obtained for each test group consisting of three animals.

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Table CA 5.8.1-32: Results of UDS test with 16 hours preparation interval

Test Group	Animal No.	Mean Nuclear Grain Count		Mean Cytoplasmic Grain Count		Mean Net Grain Counts		Mean Nuclear Grains of cells in Repair		% Cells in Repair
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Vehicle Control (30% DMSO/70% PEG 400)	17	6.74	3.86	12.65	5.76	-5.91	5.87	6.67	2.08	3
	18	4.81	3.74	9.40	5.42	-4.59	4.50	5.00	0.00	1
	19	3.48	2.49	5.98	3.23	-2.50	3.52	9.00	0.00	2
	20	6.66	4.86	11.62	6.17	-4.96	5.47	7.80	1.30	5
1000 mg/kg b.w. BCS. CO78570 (Amidosulfuron-desmethyl-chloropyrimidine)	21	7.54	4.82	13.02	6.37	-5.48	5.12	5.00	0.00	1
	22	11.33	10.37	16.47	12.20	-5.14	7.54	11.00	9.54	3
	23	10.02	7.31	14.15	10.23	-4.13	8.34	9.14	3.98	7
	24	12.10	6.54	19.71	10.34	-7.61	9.19	7.17	2.64	6
2000 mg/kg b.w. BCS. CO78570 (Amidosulfuron-desmethyl-chloropyrimidine	25	8.80	4.84	13.08	6.74	-4.28	6.20	5.60	0.89	5
	26	7.89	4.08	13.28	5.46	-5.49	5.46	9.00	1.41	2
	27	7.71	5.52	11.65	7.79	-3.94	5.52	6.75	1.26	4
	28	8.38	7.81	12.40	9.13	-4.02	5.60	8.00	2.65	3
Positive control (2-AAF 100 mg/kg b.w.	29	24.90	11.85	14.37	7.29	10.53	7.55	13.05	6.28	79
	30	28.28	12.59	17.49	8.35	10.79	10.99	15.22	8.86	74
	31	22.09	12.16	12.06	6.10	10.03	9.57	14.55	7.83	69
	32	34.30	19.59	21.50	12.21	12.80	12.53	17.44	10.88	75

S.D. = Standard deviation. The Standard deviation shown for each animal is the deviation the 100 analyzed cells. The deviation shown for the mean of each group is the standard deviation between the results obtained for each test group consisting of three animals.

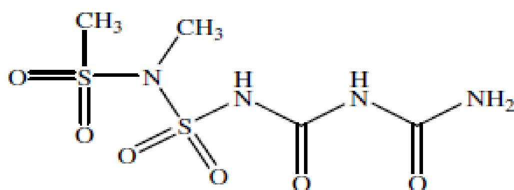
Conclusion:
In conclusion, it can be stated that under the experimental conditions reported, i.e. oral administration up to the Maximal Tolerated Dose of 2000 mg/kg, BCS-CO78570 (metabolite Amidosulfuron-desmethyl-chloropyrimidine) did not induce DNA-damage leading to increased repair synthesis in the hepatocytes of the treated rats.

BCS-CQ51287 (metabolite Amidosulfuron-biuret) was identified as a minor soil metabolite of Amidosulfuron with a predicted PEC_{gw} value of < 0.75 µg/L > 0.1 ug/L which triggers groundwater relevance assessment according to EU Council Directive 91/414/EEC (SANCO/221/2000 –rev. 10, 25 February, 2003). The studies conducted include 3 *in vitro* genotoxicity tests and the rationale for the lack of a need to conduct an Acute Oral Toxicity study with this metabolite.

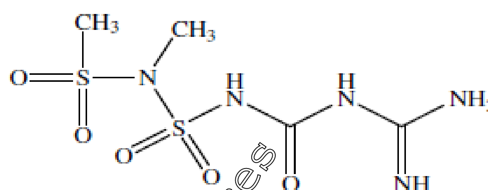
These 3 *in vitro* genotoxicity studies demonstrated that BCS-CQ51287 (metabolite Amidosulfuron-biuret) is not genotoxic. Amidosulfuron-biuret is probably of low acute toxicity, but according to SANCO/221/2000 –rev. 10, 25 February, 2003, the acute toxicity testing for this metabolite is not triggered anyhow.

Report:	KCA 5.8.1/21 [redacted],; 2010; M-395869-01-1
Title:	Bridging statement comparing the toxicity profile of BCS-CQ51287 (amidosulfuron-biuret) with BCS-CO41839 (amidosulfuron-guanidine
Report No.:	M-395869-01-1
Document No.:	M-395869-01-1
Guideline(s):	not specified
Guideline deviation(s):	not specified
GLP/GEP:	**no**

Executive summary:
This position paper compared the structural and toxicity profiles of BCS-CQ51287 (metabolite Amidosulfuron-biuret) with BCS-CO41839 (metabolite Amidosulfuron-guanidine).



BCS-CQ51287



BCS-CO41839

Both compounds are structurally very similar apart from the substitution of a carbonyl group with an imine group, have the same toxicity profile when a DEREK/LHASA search is conducted (with no specific alerts) and are non-mutagenic in *in-vitro* genotoxicity testing.

In addition, a Rat Acute Oral LD_{50} test has already been conducted on BCS-CO41839 (metabolite Amidosulfuron-guanidine). The conclusion from this study was as follows:

The acute oral LD_{50} value of BCS-CO41839 (metabolite Amidosulfuron-guanidine) in the Wistar female rat was greater than 2000 mg/kg bw. Due to the structural similarity of Amidosulfuron-guanidine and Amidosulfuron-biuret, it is concluded that Amidosulfuron-biuret is of low acute toxicity as well.

Report:	KCA 5.8.1/22 ,; 2010; M-387616-01-1
Title:	Salmonella typhimurium reverse mutation assay with BCS-CQ51287 (metabolite amidosulfuron-biuret)
Report No.:	1336800
Document No.:	M-387616-01-1
Guideline(s):	OECD Section 4, No. 471 adopted July 21, 1997 EC No 440/2008 B13/14, dated May 30, 2008 EPA OPPTS 870.5100, EPA 712-C-98-247, August 1998
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Material and methods:

Test Material: BCS-CQ51287 (metabolite Amidosulfuron-biuret)

- Lot/Batch: BCOO 6067-2-7
- Purity: 97.5%
- Stability of test compound: Stable for the duration of the study

Control Materials:

- Negative: Concurrent untreated and solvent controls
- Solvent: Dimethyl sulfoxide, DMSO (, Germany)

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Positive:

- Non activation (-S9): Sodium azide, NaN_3 (, Germany) for TA 1535 and TA 100 at 10 µg/plate, 4-nitro-o-phenylene-diamine, 4-NOPD (, Germany) for TA 98 at 10 µg/plate and for TA 1537 at 50 µg/plate , methyl methane sulfonate, MMS (, Germany) for TA 102 at 3 µg/plate.

- Activation (+S9): 2-Aminoanthracene, 2-AA (, Germany) for TA 1535, TA 1537, TA 98, and TA 100 at 2.5 µg/plate and for TA 102 at 10 µg/plate

Activation: The S9 fraction was isolated from the livers of Phenobarbital/β-Naphthoflavone induced rats

Test organism: Histidine-dependent auxotrophic mutants of Salmonella typhimurium:TA 1535, TA 100, TA 1537, TA 98 & TA 102

Source: Strains of S. typhimurium were obtained from , Germany

Test concentrations:

Pre-experiment/Experiment I: For all strains with or without S9 mix: 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate

Experiment II: For all strains with or without S9 mix: 33, 100, 333, 1000, 2500 and 5000 µg/plate

Pre-Experiment for Toxicity

To evaluate the toxicity of the test item a pre-experiment was performed with all strains used. Eight concentrations were tested for toxicity and mutation induction with each 3 plates. The experimental conditions in this pre-experiment were the same as described for the experiment I below (plate incorporation test).

Toxicity of the test item can be evident as a reduction in the number of spontaneous revertants or a clearing of the bacterial background lawn.

The pre-experiment is reported as experiment I, since the following criteria are met:
Evaluable plates (>0 colonies) at five concentrations or more in all strains used.

Dose Selection

In the pre-experiment the concentration range of the test item was 3 – 5000 µg/plate. The pre-experiment is reported as experiment I. Since no toxic effects were observed 5000 µg/plate was chosen as maximal concentration.

The concentration range included two logarithmic decades. The following concentrations were tested in experiment II: 33; 100; 333; 1000; 2500; and 5000 µg/plate

Experimental Performance

For each strain and dose level, including the controls three plates were used.

The following materials were mixed in a test tube and poured onto the selective agar plates:

- 100 µl test solution at each dose level (solvent or reference mutagen solution (positive control)),
- 500 µl S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
- 100 µl Bacteria suspension (cf. test system, pre-culture of the strains),
- 2000 µL Overlay agar

In the pre-incubation assay 100 µL test solution (solvent or reference mutagen solution (positive control)), 500 µL S9 mix / S9 mix substitution buffer and 100 µL bacterial suspension were mixed in a test tube and incubated at 37 °C for 60 minutes. After pre-incubation 2.0 mL overlay agar (45 °C) was added to each tube. The mixture was poured on minimal agar plates.

After solidification the plates were incubated upside down for at least 48 hours at 37 °C in the dark

Acceptance criteria:
The Salmonella typhimurium reverse mutation assay is considered acceptable if it meets the following criteria:

- regular background growth in the negative and solvent control
- the spontaneous reversion rates in the negative and solvent control are in the range of the Harlan Laboratory historical data
- the positive control substances should produce a significant increase in mutant colony frequencies

Assessment criteria:
A test item is considered as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice (strains TA 98, TA 100, and TA 102) or three times (strains TA 1535 and TA 1537) the colony count of the corresponding solvent control is observed.

A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration.

An increase exceeding the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.

A dose dependent increase in the number of revertant colonies below the threshold is regarded as an indication of a mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remain within the historical range of negative and solvent controls such an increase is not considered biologically relevant.

Findings:
No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with BCS-CQ51287 (metabolite Amidosulfuron-biuret) at any concentration level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

The concurrent positive controls demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

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Table CA 5.8.1-33: Summary of Results Pre-Experimental and Experiment I (without activation)

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	TA 102
Without Activation	DMSO		14 ± 2	8 ± 1	28 ± 3	128 ± 23	366 ± 27
	Untreated		14 ± 5	9 ± 3	27 ± 0	129 ± 4	379 ± 23
	BCS-CQ51287	3 µg	13 ± 6	10 ± 2	25 ± 4	144 ± 11	366 ± 16
		10 µg	13 ± 1	10 ± 2	26 ± 2	138 ± 2	368 ± 3
		33 µg	13 ± 1	9 ± 4	24 ± 4	140 ± 14	391 ± 20
		100 µg	14 ± 4	10 ± 3	27 ± 2	136 ± 11	418 ± 27
		333 µg	14 ± 3	9 ± 0	21 ± 2	137 ± 13	365 ± 19
		1000 µg	15 ± 4	9 ± 2	22 ± 3	123 ± 21	387 ± 27
		2500 µg	13 ± 5	11 ± 2	28 ± 2	133 ± 12	404 ± 7
		5000 µg	14 ± 2	11 ± 1	26 ± 4	140 ± 14	390 ± 35
	NaN_3	10 µg	1596 ± 47			1898 ± 38	
	4-NOPD	10 µg			328 ± 38		
	4-NOPD	50 µg		68 ± 4			
	MMS	3.0 µL					3291 ± 266

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Table CA 5.8.1-34: Summary of Results Pre-Experimental and Experiment I (with activation)

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	TA 102
With Activation	DMSO		14 ± 2	17 ± 6	35 ± 9	167 ± 18	593 ± 16
	Untreated		22 ± 3	17 ± 2	43 ± 12	158 ± 3	548 ± 73
	BCS-CQ51287	3 µg	17 ± 6	16 ± 4	33 ± 8	150 ± 7	568 ± 11
		10 µg	15 ± 5	14 ± 1	34 ± 7	153 ± 19	623 ± 2
		33 µg	19 ± 5	14 ± 2	36 ± 6	166 ± 22	657 ± 9
		100 µg	18 ± 3	15 ± 0	39 ± 4	149 ± 11	609 ± 35
		333 µg	19 ± 5	15 ± 3	31 ± 6	150 ± 9	525 ± 14
		1000 µg	15 ± 3	17 ± 5	27 ± 4	147 ± 22	604 ± 10
		2500 µg	21 ± 2	17 ± 4	32 ± 6	168 ± 13	606 ± 9
		5000 µg	20 ± 1	17 ± 4	28 ± 2	153 ± 3	603 ± 12
	2-AA	2.5 µg	375 ± 23	512 ± 36	3137 ± 362	3668 ± 135	
	2-AA	10.0 µg					2060 ± 217

Key to Positive Controls	
NaN_3	sodium azide
2-AA	2-aminoanthracene
MMS	methyl methane sulfonate
4-NOPD	4-nitro-o-phenylene-diamine

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Table CA 5.8.1-35: Summary of Results Experiment II (without activation)

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	TA 102
Without Activation	**DMSO**		16 ± 1	11 ± 2	23 ± 6	126 ± 23	402 ± 12
	Untreated		17 ± 3	11 ± 2	26 ± 3	170 ± 34	393 ± 29
	BCS-CQ51287	**33 µg**	14 ± 4	14 ± 2	21 ± 6	102 ± 7	355 ± 17
		100 µg	15 ± 2	16 ± 4	23 ± 4	120 ± 5	363 ± 20
		333 µg	13 ± 1	9 ± 4	27 ± 6	122 ± 3	361 ± 3
		1000 µg	18 ± 5	9 ± 1	23 ± 1	107 ± 11	359 ± 17
		2500 µg	17 ± 2	11 ± 2	20 ± 5	110 ± 3	394 ± 11
		5000 µg	9 ± 2	10 ± 2	24 ± 2	128 ± 2	382 ± 35
	NaN_3	**10 µg**	1876 ± 148			1902 ± 79	
	4-NOPD	**10 µg**			370 ± 8		
	4-NOPD	**50 µg**		92 ± 2			
	MMS	**3.0 µL**					1095 ± 214

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Table CA 5.8.1-36: Summary of Results Experiment II (with activation)

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	**TA 1537**	**TA 98**	**TA 100**	**TA 102**
With Activation	**DMSO**		22 ± 7	14 ± 1	30 ± 10	163 ± 19	519 ± 31
	Untreated		19 ± 5	18 ± 1	33 ± 5	164 ± 11	562 ± 43
	BCS-CQ51287	**33 µg**	17 ± 4	16 ± 6	26 ± 3	153 ± 6	524 ± 10
		100 µg	27 ± 2	14 ± 2	31 ± 6	138 ± 34	515 ± 33
		333 µg	20 ± 11	15 ± 2	28 ± 7	144 ± 12	472 ± 39
		1000 µg	22 ± 7	17 ± 3	31 ± 5	145 ± 7	531 ± 14
		2500 µg	24 ± 4	18 ± 2	29 ± 8	152 ± 14	482 ± 12
		5000 µg	19 ± 2	17 ± 3	37 ± 4	150 ± 10	517 ± 33
	2-AA	**2.5 µg**	416 ± 25	537 ± 35	1933 ± 228	3208 ± 148	
	2-AA	**10.0 µg**					2348 ± 168

Key to Positive Controls	
NaN_3	**sodium azide**
2-AA	**2-aminoanthracene**
MMS	**methyl methane sulfonate**
4-NOPD	**4-nitro-o-phenylene-diamine**

Conclusion:

It is concluded that BCS-CQ51287 (metabolite Amidosulfuron-biuret) showed no evidence of mutagenic activity in this *in vitro* bacterial system.

Report: KCA 5.8.1/23[redacted]B; 2010; M-389584-01-1

Title: BCS-CQ51287 (metabolite amidosulfuron-biuret) - *In vitro* chromosome aberration test with chinese hamster V79 cells

Report No.: 1337000

Document No.: M-389584-01-1

Guideline(s): OECD 473 (1997)
EC No. 440/2008, B10 dated May 30, 2008
EPA OPPTS 870.5374 (August 1998)

Guideline deviation(s): The historical data represented in Annex III refer to experiments performed in 2008 to 2009 (instead of January to December 2008 as stated in the study plan).
Reason for the deviation: updating
This deviation has no detrimental impact on the outcome of the study.

GLP/GEP: **yes**

Material and methods:

Test Material: BCS-CQ51287 (metabolite Amidosulfuron-biuret)
- Lot/Batch: BCOO 6067-2-7
- Purity: 97.5%
- Stability of test compound: Stable for the duration of the study
- Solvent: Culture medium (minimal essential medium: MEM)

Control Materials:
- Negative: Concurrent culture medium controls were performed
- Positive:
  - Non activation (-S9): EMS; Ethylmethane sulfonate ([redacted], Belgium, lot no. A0276402) at 1000 µg/ml (Experiment I) and 600 µg/ml (Experiment II)
  - Activation (+S9): CPA; Cyclophosphamide ([redacted], Germany, lot no. 097K1311) at 1.4 µg/ml

Cell line: Chinese hamster V79 lung cells
- *Source:* Cells obtained from [redacted] Germany

Test concentrations: Experiment I (pre-test): 10.7, 21.5, 43.0, 85.9, 171.9, 343.8, 687.5, 1375.0 and 2750 µg/ml.
Experiment II; Without S9 mix: 10.7, 21.5, 43.0, 85.9, 171.9, 343.8, 687.5, 1375.0 and 2750 µg /ml.
Experiment II; With S9 mix: 171.9, 343.8, 687.5, 1375.0 and 2750 µg /ml.

Determination of cytotoxicity:
The highest concentration used in the cytogenetic experiments was chosen considering the current OECD Guideline for *in vitro* mammalian cytogenetic tests requesting for the top concentration clear toxicity with reduced cell numbers or mitotic indices below 50 % of control, whichever is the lowest concentration, and/or the occurrence of precipitation. In case of non-toxicity the maximum concentration should be 5 mg/mL, 5 µL/mL or 10 mM, whichever is the lowest, if formulation in an appropriate solvent is possible.

With respect to the molecular weight (274.3 g/mol) of the test item 2750 µg/mL of BCS-CQ51287 (metabolite Amidosulfuron-biuret) (approx. 10 Mm) was applied as the highest concentration for treatment of the cultures in the pre-test. Test item concentrations between 10.7 and 2750 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity. No precipitation of the test item was observed. Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I.

Since no cytotoxicity and test item precipitation was observed in the first experiment up to the highest applied concentration, 2750 µg/mL was chosen as the highest concentration for Experiment II.

Table CA 5.8.1-37: Doses applied in the Chromosome aberration test with BCS-CQ51287

Preparation interval	Exposure period	Exp.	Concentration in µg/mL								
			Without S9 mix								
18 hrs	**4 hrs**	**I**	10.7	21.5	43.0	85.9	171.9	343.8	**687.5**	**1375.0**	**2750.0**
18 hrs	**18 hrs**	**II**	10.7	21.5	43.0	85.9	171.9	343.8	**687.5**	**1375.0**	**2750.0**
			With S9 mix								
18 hrs	**4 hrs**	**I**	10.7	21.5	43.0	85.9	171.9	343.8	**687.5**	**1375.0**	**2750.0**
18 hrs	**4 hrs**	**II**					171.9	343.8	**687.5**	**1375.0**	**2750.0**

Evaluated experimental points are shown in bold characters

Seeding of the Cultures

Exponentially growing stock cultures more than 50 % confluent were rinsed with Ca-Mg-free salt solution containing 8000 mg/L NaCl, 200 mg/L KCl, 200 mg/L KH_2PO_4 and 150 mg/L Na_2HPO_4. Afterwards the cells were treated with trypsin-EDTA-solution at 37 °C for approx. 5 minutes. Then, by adding complete culture medium including 10 % (v/v) FBS the enzymatic treatment were stopped and a single cell suspension was prepared. The trypsin concentration for all sub-culturing steps was 0.5 % (w/v) in Ca-Mg-free salt solution. The cells were seeded into Quadriperm dishes, which contained microscopic slides. Into each chamber 1×10^4 – 6×10^4 cells were seeded with regard to the preparation time.

Exposure period 4 hours

The culture medium of exponentially growing cell cultures was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL culture medium were added. Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed twice with "Saline G" and then the cells were cultured in complete medium containing 10 % (v/v) FBS for the remaining culture time of 14 hours.

The "Saline G" solution was composed as follows (per litre):

NaCl	8000 mg
KCl	400 mg
Glucose • H_2O	1100 mg
Na_2HPO_4 • 2 H_2O	192 mg
KH_2PO_4	150 mg

pH was adjusted to 7.2.

Exposure period 18 hours

The culture medium of exponentially growing cell cultures was replaced with complete medium containing 10 % (v/v) FBS, including the test item without S9 mix. The medium was not changed until preparation of the cells. Concurrent solvent and positive controls were performed.

All cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % CO_2 (98.5 % air).

Preparation of the Cultures

Colcemid was added (0.2 µg/mL culture medium) to the cultures 15.5 hours after the start of the treatment. The cells on the slides were treated 2.5 hours later, in the chambers with hypotonic solution (0.4 % KCl) for 20 min at 37 °C. After incubation in the hypotonic solution the cells were fixed with a

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mixture of methanol and glacial acetic acid (3:1 parts, respectively). Per experiment two slides per group were prepared. After preparation the cells were stained with Giemsa ([redacted] [redacted], Germany) and labelled with a computer-generated random code to prevent scorer bias.

Evaluation of Cell Numbers
The evaluation of cytotoxicity indicated by reduced cell numbers was made after the preparation of the cultures on spread slides. The cell numbers were determined microscopically by counting 10 defined fields per coded slide. The cell number of the treatment groups is given in percentage compared to the respective solvent control.

Analysis of Metaphase Cells
Evaluation of the cultures was performed (according to standard protocol of the "Arbeitsgruppe der Industrie, Cytogenetik") using NIKON microscopes with 100x oil immersion objectives. Breaks, fragments, deletions, exchanges, and chromosome disintegrations were recorded as structural chromosome aberrations. Gaps were recorded as well but not included in the calculation of the aberration rates. At least 100 well spread metaphases per culture were evaluated for cytogenetic damage on coded slides, except for the positive control without metabolic activation.

Only metaphases with characteristic chromosome numbers of 22 ± 1 were included in the analysis. To describe a cytotoxic effect the mitotic index (% cells in mitosis) was determined. In addition, the number of polyploid cells in 500 metaphases per culture was determined (% polyploid metaphases; in the case of this aneuploid cell line polyploid means a near tetraploid karyotype).

Evaluation criteria:
A test item is classified as non-clastogenic if:
- the number of induced structural chromosome aberrations in all evaluated dose groups is in the range of the laboratory's historical control data range.

and/or
- no significant increase of the number of structural chromosome aberrations is observed.

A test item is classified as clastogenic if:
- the number of induced structural chromosome aberrations is not in the range of the testing laboratory's historical control data range.

and
- either a concentration-related or a significant increase of the number of structural chromosome aberrations is observed.

Statistical significance was confirmed by means of the Fisher's exact test ($p < 0.05$). However, both biological and statistical significance should be considered together. If the criteria mentioned above for the test item are not clearly met, the classification with regard to the historical data and the biological relevance is discussed and/or a confirmatory experiment is performed.

Although the inclusion of the structural chromosome aberrations is the purpose of this study, it is important to include the polyploids and endoreduplications. The following criterion is valid:

A test item can be classified as aneugenic if:
- the number of induced numerical aberrations is not in the range of the laboratory's historical control data range.

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Acceptability criteria:
The chromosome aberration test performed was considered acceptable, if the following criteria were met:

- The number of structural aberrations found in the solvent controls falls within the range of the laboratory's historical control data.
- The positive control substances produce significant increases in the number of cells with structural chromosome aberrations, which are within the range of the laboratory's historical control data.

Findings:
Neither precipitation of the test item in the culture medium nor relevant increase in the osmolarity was observed (Exp. I: solvent control: 316 mOsm versus 334 mOsm and at 2750.0 µg/mL; Exp. II: solvent control: 322 mOsm versus 337 mOsm at 2750.0 µg/mL). The pH value of the stock solution was adjusted by addition of sodium hydroxide to 7.1 in Experiment I and to 7.2 in Experiment II. Before adjustment the pH value of the stock solution was 6.5 in Experiment I and 6.7 in Experiment II.

No toxic effects indicated by reduced mitotic indices or reduced cell numbers were observed after treatment with the test item.

In both experiments, in the absence and presence of S9 mix, no biologically relevant increase in the number of cells carrying structural chromosome aberrations was observed. The aberration rates of the cells after treatment with the test item (1.0 - 3.0 % aberrant cells, excluding gaps) were close to the range of the solvent control values (1.0 - 2.5 % aberrant cells, excluding gaps) and within the total range of the laboratory's historical control data (0.0 - 4.0 % aberrant cells, excluding gaps).

No reproducibly and biologically relevant increase in the rate of polyploid metaphases was found after treatment with the test item (2.1 - 4.7 %) as compared to the rates of the solvent controls (2.6 - 3.2 %).

Either EMS (1000.0 and 600.0 µg/mL) or CPA (1.4 µg/mL) were used as positive controls and showed distinct increases in the number of cells with structural chromosome aberrations.

In conclusion, it can be stated that under the experimental conditions reported, the test item BCS-CQ51287 (metabolite Amidosulfuron-biuret) did not induce structural chromosome aberrations in *V79* cells (Chinese hamster cell line), when tested up to the highest required test item concentration.

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Table CA 5.8.1-38: Summary of Results of the chromosomal aberration study with BCS-CQ51287 (without activation)

Exp.	Preparation interval	Test item conc. in µg/mL	Polyploid cells in %	Cell numbers in % of control	Mitotic indices in % of control	Aberrant cells in % incl. gaps*	Aberrant cells in % excl. gaps*	Aberrant cells in % with exchanges
					Exposure period 4 hrs without S9 mix			
I	**18 hrs**	**Solvent control[1]**	2.9	100.0	100.0	2.0	1.0	0.0
		Positive control[2]	2.4	n.t.	91.8	20.5	20.0[S]	10.5
		687.5	4.0	84.3	117.5	3.0	3.0	0.5
		1375.0	2.7	100.9	110.0	2.0	1.5	0.0
		2750.0	4.1	95.8	106.3	2.5	2.5	0.5
					Exposure period 18 hrs without S9 mix			
II	**18 hrs**	**Solvent control[1]**	3.2	100.0	100.0	1.5	1.5	0.5
		Positive control[3]	2.6	n.t.	73.0	19.5	19.0[S]	7.5
		687.5	2.5	89.2	88.0	1.0	1.0	0.0
		1375.0	3.4	69.8	97.9	2.0	2.0	0.0
		2750.0	3.4	87.9	93.1	2.0	2.0	1.5

* Inclusive cells carrying exchanges
n.t. Not tested
S Aberration frequency statistically significant higher than corresponding control values
1 Medium (MEM, minimal essential medium)
2 EMS 1000.0 µg/mL
3 EMS 600.0 µg/mL
4 CPA 1.4 µg/mL

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Table CA 5.8.1-39: Summary of Results of the chromosomal aberration study with BCS-CQ51287 (with activation)

Exp.	Preparation interval	Test item Conc. in µg/mL	Polyploid cells in %	Cell numbers in % of control	Mitotic indices in % of control	Aberrant cells in % incl. gaps*		
						incl. gaps*	excl. gaps*	with exchanges
			Exposure period 4 hrs with S9 mix					
I	**18 hrs**	**Solvent control[1]**	3.1	100.0	100.0	3.0	1.5	0.5
		Positive control[4]	2.5	n.t.	62.0	11.5	11.0[S]	3.0
		687.5	2.8	88.5	97.5	3.5	3.0	1.0
		1375.0	2.1	92.1	115.9	3.0	2.5	0.5
		2750.0	3.2	92.6	112.8	1.5	1.5	0.0
II	**18 hrs**	**Solvent control[1]**	2.6	100.0	100.0	2.5	2.5	0.0
		Positive control[4]	2.7	n.t.	82.9	12.0	11.5[S]	2.0
		687.5	3.2	85.5	98.6	2.5	2.5	1.0
		1375.0	4.7	88.6	126.3	3.0	2.0	0.0
		2750.0	2.2	100.1	91.7	1.5	1.5	0.5

* Inclusive cells carrying exchanges
n.t. Not tested
S Aberration frequency statistically significant higher than corresponding control values
1 Medium (MEM, minimal essential medium)
2 EMS 1000.0 µg/mL
3 EMS 600.0 µg/mL
4 CPA 1.4 µg/mL

Conclusion:

Under the experimental conditions reported, the test item BCS-CQ51287 (metabolite Amidosulfuron-biuret), did not induce structural chromosome aberrations in V79 cells (Chinese hamster cell line), when tested up to the highest required concentration.

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Report:	KCA 5.8.1/24 [redacted] T; 2010; M-393906-01-1
Title:	BCS-CQ51287 (metabolite amidosulfuron-biuret) - Gene mutation assay in Chinese hamster V79 cells *in vitro* (V79 / HPRT)
Report No.:	1336900
Document No.:	M-393906-01-1
Guideline(s):	OECD 476 (1998); EC 440/2008 B.17 (2008); US EPA OPPTS 870.5300; EPA 712-C-98-221
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Test Material: BCS-CQ51287 (metabolite Amidosulfuron-biuret)
- Lot/Batch: BCOO 6067-2-7
- Purity: 97.5%
- Stability of test compound: Stable for the duration of the study

Control Materials:
- Negative: Concurrent solvent control (culture medium)
- Solvent: Culture medium MEM (minimal essential medium) supplemented with 10% fetal bovine serum (FBS) and 1% neomycin
- Positive:
  - Non activation (-S9): Ethylmethane sulfonate (EMS), supplier [redacted], Belgium, purity ≥ 98%, lot No. A0276402, dissolved in culture medium. Final concentration 0.15 mg/mL = 1.2 mM
  - Activation (+S9): 7, 12-dimethylbenz(a)anthracene (DMBA), supplier [redacted], Germany, purity 95%, lot No. 096K 1881, dissolved in dimethylsulfoxide (DMSO), supplier [redacted] Germany. Final concentration 1.1 µg/mL = 4.3 µM
  - Metabolic activation: The S9 fraction was isolated from the livers of Phenobarbital/ß-Naphthoflavone induced male Wistar rats. (protein content 35.0 mg/mL in the pre-experiment and Experiment 1, 33.7 mg/mL in Experiment II) and was kept frozen at -80°C. Each batch of S9 mix is routinely tested with 2-aminoanthracene as well as benzo(a)pyrene

Cell line: Chinese hamster V79 lung cells
- *Source:* Cell line supplied by [redacted], Germany

Culture condition: Incubation performed at 37°C in a humidified atmosphere with about 1.5% CO_2 (98.5% air).

Test concentrations: BCS-CQ51287 was used at concentrations that ranged from 21.9 and 2800 µg/mL in the pre-experiment and at concentrations of 87.5, 175.0, 350.0, 700.0, 1400.0 and 2800 µg/mL in both the main experiments (Experiments I and II). Maximum concentration was equal to approximately 10 mM

The selection of V79 forward mutations is based on the resistance of induced mutants to the purine analogue 6-thioguanine (6-TG). This resistance is a result of a mutation at the X-chromosome-linked

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HPRT locus rendering the cells unable to use 6-TG for DNA synthesis. Therefore, cell colonies formed in the presence of 6-TG are considered to represent mutants at the HPRT gene.

Dose Selection
According to the recommendations of the guidelines, several concentrations (usually at least four) of the test item should be used. These should yield a concentration-related toxic effect. The highest concentration should produce a low level of survival and the survival in the lowest concentration should approximate the solvent control. Relatively insoluble substances should be tested up to their limit of solubility under culture conditions. For freely-soluble nontoxic substances the maximum concentration should be 5 mg/mL or 10 mM. If the maximal concentration is based on cytotoxicity then it should result in approximately 10 – 20% relative survival (cloning efficiency) or the relative total growth at subcultivation.

In the range finding pre-experiment the concentration range was between 21.9 and 2800 µg/mL to evaluate toxicity in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation. The maximum concentration was equal to approximately 10 mM. No relevant toxic effect occurred up to the maximum concentration with and without metabolic activation following 4 and 24 hours treatment.

The test medium was checked for precipitation or phase separation at the end of each treatment period (4 or 24 hours) prior to removal to the test item. Neither precipitation nor phase separation occurred was observed up to the maximum concentration of 2800.0 µg/mL.

The stock solution was neutralised with 2 N NaOH. There was no relevant shift of osmolarity of the medium even at the maximum concentration of the test item.

Based on the results of the pre-experiment, the individual concentrations of the main experiments were selected. The maximum concentration was again, 2800.0 µg/mL. A series of concentrations spaced by a factor of 2 was placed into the lower range.

Table CA 5.8.1-40: Doses applied of BCS-CQ51287

exposure period	S9 mix	concentrations in µg/mL					
		Experiment I					
4 hours	-	87.5	**175.0**	**350.0**	**700.0**	**1400.0**	**2800.0**
4 hours	+	87.5	**175.0**	**350.0**	**700.0**	**1400.0**	**2800.0**
		Experiment II					
24 hours	-	87.5	**175.0**	**350.0**	**700.0**	**1400.0**	**2800.0**
4 hours	+	87.5	**175.0**	**350.0**	**700.0**	**1400.0**	**2800.0**

The concentrations printed in bold were chosen for the mutation rate analysis.

Seeding
Two days (experiment I) or three days (experiment II) after sub-cultivation stock cultures were trypsinized at 37 °C for 5 minutes. Then the enzymatic digestion was stopped by adding complete culture medium and a single cell suspension was prepared. The trypsin concentration for all sub-culturing steps was 0.2 % in Ca-Mg-free salt solution.

The Ca-Mg-free salt solution had the following constituents (per litre):

NaCl	8000 mg
KCl	200 mg
KH_2PO_4	200 mg
Na_2HPO_4	150 mg

Prior to the trypsin treatment the cells were rinsed with Ca-Mg-free salt solution containing 200 mg/l EDTA (ethylene diamine tetraacetic acid).

The cell suspension was seeded into plastic culture flasks ([redacted], Germany). Approximately 1.5×10^6 (single culture) and 5×10^2 cells (in duplicate) were seeded in MEM with 10 % FBS (complete medium) for the determination of mutation rate and toxicity, respectively.

Treatment

After 24 h the medium was replaced with serum-free medium containing the test item, either without S9 mix or with 50 µl/mL S9 mix. Concurrent solvent and positive controls were treated in parallel. After 4 h this medium was replaced with complete medium following two washing steps with "saline G". In the second experiment the cells were exposed to the test item for 24 h in complete medium, supplemented with 10 % FBS, in the absence of metabolic activation.

The "saline G" solution had the following constituents (per litre):

NaCl	8000 mg
KCl	400 mg
Glucose	1100 mg
$Na_2HPO_4\times2H_2O$	192 mg
KH_2PO_4	150 mg

The pH was adjusted to 7.2.

The colonies used to determine the cloning efficiency (survival) were fixed and stained approx. 7 days after treatment as described below.

Three days (experiment II) or four days (experiment I) after treatment 1.5×10^6 cells per experimental point were sub-cultivated in 175 cm² flasks containing 30 mL medium. Following the expression time of 7 days five 80 cm² cell culture flasks were seeded with about 3 - 5×10^5 cells each in medium containing 6-TG. Two additional 25 cm² flasks were seeded with approx. 500 cells each in non-selective medium to determine the viability.

The cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % CO_2 for about 8 days. The colonies were stained with 10 % methylene blue in 0.01 % KOH solution.

The stained colonies with more than 50 cells were counted. In doubt the colony size was checked with a preparation microscope (Nikon, 40407 Düsseldorf, Germany).

Acceptance criteria

The gene mutation assay is considered acceptable if it meets the following criteria:

- the numbers of mutant colonies per 10^6 cells found in the solvent controls falls within the laboratory historical control data from 2006 - 2008 (see Annex).
- the positive control substances should produce a significant increase in mutant colony frequencies (number of mutant colonies per 10^6 cells at least three times the number of mutant colonies/10^6 of the corresponding solvent control).
- the cloning efficiency II (absolute value) of the solvent controls should exceed 50 %.

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Evaluation criteria:
A test item is classified as positive if it induces either a concentration-related increase of the mutant frequency or a reproducible and positive response at one of the test points.

A test item producing neither a concentration-related increase of the mutant frequency nor a reproducible positive response at any of the test points is considered non-mutagenic in this system.

A positive response is described as follows:
A test item is classified as mutagenic if it reproducibly induces a mutation frequency that is three times above the spontaneous mutation frequency at least at one of the concentrations in the experiment.

The test item is classified as mutagenic if there is a reproducible concentration-related increase of the mutation frequency. Such evaluation may be considered also in the case that a threefold increase of the mutant frequency is not observed.

However, in a case by case evaluation this decision depends on the level of the corresponding solvent control data. If there is by chance a low spontaneous mutation rate in the range normally found (0.6 – 31.7 mutants per 10^6 cells) a concentration-related increase of the mutations within this range has to be discussed. The variability of the mutation rates of solvent controls within all experiments of this study was also taken into consideration.

Statistical analysis:
A linear regression (least squares) was performed to assess a possible dose dependent increase of mutant frequencies using SYSTAT®11 (, USA) statistics software. The number of mutant colonies obtained for the groups treated with the test item were compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05. However, both, biological and statistical significance were considered together.

Table CA 5.8.1-41: Statistical results

experimental group	p-value
experiment I, culture I without S9 mix	0.666
experiment I, culture II without S9 mix	0.121
experiment I, culture I with S9 mix	0.036
experiment I, culture II with S9 mix	0.491
experiment II, culture I without S9 mix	0.772
experiment II, culture II without S9 mix	0.498
experiment II, culture I with S9 mix	0.039*
experiment II, culture II with S9 mix	0.547

* inverse trend without biological relevance

Findings:
No precipitation of the test item was observed up to the maximum concentration with and without metabolic activation.

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No relevant cytotoxic effects indicated by a relative cloning efficiency I below 50% occurred in any of the experimental parts.

No relevant and reproducible increase in mutant colony numbers/10^6 cells was observed in the main experiments up to the maximum concentration. All mutant frequencies remained well within the historical range of solvent controls. The induction factor exceeded the threshold of three times the corresponding solvent control in the second culture of the first experiment without metabolic activation at 2800.0 µg/mL. This effect however, was judged to be based upon the rather low solvent controls of 2.8 mutant colonies/10^6 cells. The absolute value of the mutation frequency was only 10.3 colonies per 10^6 cells.

A linear regression analysis (least squares) was performed to assess a possible dose dependent increase of mutant frequency using SYSTAT®11 statistics software. A single significant dose dependent trend of the mutation frequency indicated by a probability value of <0.05 was determined in the first culture of the first experiment with metabolic activation. However, the trend was judged as biologically irrelevant since the mutation frequency did not exceed the threshold of two times the corresponding solvent control. Another significant trend occurred in the first culture of the second experiment with metabolic activation. This trend however, was judged as irrelevant since it was reciprocal, going down versus increasing concentrations.

In both experiments of this study (with and without metabolic activation) the range of the solvent controls was from 2.8 up to 28.2 mutant colonies per 10^6 cells; the range of the groups treated with the test item was from 1.4 up to 40.3 mutant colonies per 10^6 cells.

EMS (150 µg/mL) and DMBA (1.1 µg/mL) were used as positive controls and showed a distinct increase in induced mutant colonies.

Table CA 5.8.1-42: Summary of results

	Conc. µg per mL	S9 mix	Relative Cloning Efficiency I	Relative Cloning Efficiency II	Mutant Colonies 10^6 cells	Induction Factor	Relative Cloning Efficiency I	Relative Cloning Efficiency II	Mutant Colonies 10^6 cells	Induction Factor
			%	%			%	%		
Column	1	2	3	4	5	6	7	8	9	10
Experiment I/4h treatment			**Culture I**				**Culture II**			
Solvent control with medium		-	100.0	100.0	15.3	1.0	100.0	100.0	2.8	1.0
Positive control with EMS	150.0	-	96.0	93.8	149.8	9.8	105.7	76.8	36.0	12.8
Test item	87.5	-	106.9	Culture was not continued#			103.7	Culture was not continued#		
Test item	175.0	-	106.1	91.9	40.3	2.6	102.7	73.0	8.0	2.8
Test item	350.0	-	101.1	86.9	27.8	1.8	92.5	88.8	7.2	2.5
Test item	700.0	-	96.9	99.9	15.1	1.0	110.5	96.7	4.7	1.7
Test item	1400.0	-	103.3	80.9	19.6	1.3	101.5	97.8	6.9	2.4
Test item	2800.0	-	107.3	121.5	21.4	1.4	99.7	103.9	10.3	3.7
Solvent control with medium		+	100.0	100.0	26.0	1.0	100.0	100.0	8.6	1.0
Positive control with DMBA	1.1	+	97.1	99.0	919.0	35.4	90.0	76.5	327.2	38.1
Test item	87.5	+	115.5	Culture was not continued#			102.2	Culture was not continued#		
Test item	175.0	+	104.3	97.9	18.5	0.7	103.0	102.4	14.2	1.6
Test item	350.0	+	105.3	86.9	18.9	0.7	105.0	92.8	5.8	0.7
Test item	700.0	+	103.7	104.6	20.1	0.8	107.1	80.6	1.4	0.2
Test item	1400.0	+	106.1	106.6	26.4	1.0	104.3	103.6	9.4	1.1
Test item	2800.0	+	104.1	92.2	35.8	1.4	101.4	68.0	4.6	0.5
Experiment II/24h treatment			**Culture I**				**Culture II**			
Solvent control with medium		-	100.0	100.0	27.1	1.0	100.0	100.0	24.6	1.0
Positive control with EMS	150.0	-	95.2	121.1	415.2	15.3	95.4	97.1	369.7	15.1
Test item	87.5	-	100.7	Culture was not continued#			95.8	Culture was not continued#		
Test item	175.0	-	106.1	129.2	14.0	0.5	92.7	78.6	36.0	1.5
Test item	350.0	-	108.3	125.3	31.6	1.2	90.0	90.4	15.1	0.6
Test item	700.0	-	111.9	123.9	17.3	0.6	90.8	90.6	20.6	0.8
Test item	1400.0	-	101.3	126.1	28.3	1.0	92.6	80.7	24.0	1.0
Test item	2800.0	-	94.8	120.4	24.9	0.9	88.2	101.3	19.0	0.8
Experiment II/4h treatment			**Culture I**				**Culture II**			

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Solvent control with medium		+	100.0	100.0	28.2	1.0	100.0	100.0	12.7	1.0
Positive control with DMBA	1.1	+	50.1	92.1	859.4	30.5	66.2	92.0	968.4	76.3
Test item	87.5	+	110.7	Culture was not continued#			116.1	Culture was not continued#		
Test item	175.0	+	100.1	110.0	22.4	0.8	121.6	106.2	13.5	1.1
Test item	350.0	+	112.2	111.1	25.8	0.9	130.4	102.2	18.4	1.5
Test item	700.0	+	105.5	108.7	30.2	1.1	127.3	99.6	23.6	1.9
Test item	1400.0	+	107.8	101.5	23.4	0.8	116.0	100.6	16.1	1.3
Test item	2800.0	+	101.0	102.2	11.3	0.4	113.0	102.3	18.4	1.5

Conclusion:
In conclusion it can be stated that under the experimental conditions reported BCS-CQ51287 did not induce gene mutations at the HPRT locus in V79 cells.

CA 5.8.2 Supplementary studies on the active substance

As a supplementary study on the active substance an acute toxicity study with intraperitoneal administration was performed which provided an LD50 of 1000 - 2000 mg/kg bw (Hoe 075032 - substance technical (Code: Hoe 075032 OH ZC96 0001) Testing for acute intraperitoneal toxicity in the male and female Wistar rat A39777, M-121351-01-1).

Studies submitted and evaluated for the first inclusion of amidosulfuron on Annex I:

Report: KCA 5.8.2/01 [redacted],; [redacted]; 1987; M-121351-01-1
Title: Hoe 075032 - substance technical (Code: Hoe 075032 OH ZC96 0001) Testing for acute intraperitoneal toxicity in the male and female Wistar rat
Report No.: A39777
Document No.: M-121351-01-1
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: no

Impurity AE F103452 (in Amidosulfuron technical)

A supplementary Ames test on the technical material of amidosulfuron containing the impurity AEF103452 was conducted in 2007 and is summarized below. The results of this study indicate that AE F103452 does not have a genotoxic potential.

Report: KCA 5.8.2/02 [redacted]R; 2007; M-295635-01-1
Title: Amidosulfuron (tested as amidosulfuron technical) - Salmonella/microsome test plate incorporation and reincubation method
Report No.: AT04288
Document No.: M-295635-01-1
Guideline(s): OECD 471; Directive 2000/32/EEC, Method B.13./14.(2000); OPPTS N°870.5100 (August 1998)
Guideline deviation(s): none
GLP/GEP: yes

Material and Methods:

Test Material: Amidosulfuron technical (containing impurity AEF 103452)
Lot/Batch: 2007-009013
Purity: 98.4%
Stability of test compound: Stable for the duration of the study

Control Materials:

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Negative:	Culture medium
Solvent:	Dimethyl sulfoxide (DMSO)
Positive:	
-Non activation (-S9):	Sodium azide, NaN_3 (, Germany) for TA 1535, Nitrofurantoin (NF,) for TA 100, 4-Nitro-1,2-phenylene diamine (4-NPDA,) for TA 98 and TA 1537, Mitomycin C (MMC,) for TA 102 in plate incorporation trials, Cumene hydroperoxide (Cumene,) for TA 102 in pre-incubation trials
-Activation (+s9):	2-Amoneanthracene, 2-AA ( ) for TA 1535, TA 1537, TA 98, TA 100 and TA 102

Activation: The S9 fraction was isolated from the livers of Arochlor 1254 induced rats

Test organism:	Histidine-dependent auxotrophic mutants of Salmonella typhimurium: TA 1535, TA 100, TA 1537, TA 98 and TA 102
Source:	Strains of S. typhimurium were obtained from
Test concentrations:	
Tests:	For all strains with or without S9 mix: 16, 50, 158, 500, 1581 and 5000 µg/plate
Pre-incubation trials:	For all strains with or without S9 mix: 100, 200, 400, 600, 800, 1000, 1200 and 3200 µg/tube

For the mutant count, three plates were used, both with and without S9 mix, for each strain and dose. An equal number of plates, filled with the solvent minus the test substance, comprised the negative control. Each positive control also contained three plates per strain. The amount of solvent for the test substance and for the controls was 0.1 ml/plate. The doses for the first trial were routinely determined on the basis of a standard protocol: if not limited by solubility 5000 µg or 5 µl per plate were used as the highest dose. At least five additional doses were routinely used. If less than three doses were used for assessment, at least two repeats were performed. The results of the first experiment were then considered as a pre-test for toxicity. However, in case of a positive response or if at least three doses could be used for assessment, the first trial was included in the assessment. If the second test confirmed the results of the first, no additional repeat were performed. Doses of repeats were chosen on the basis of the results obtained in the first experiment. The independent repeat was performed as preincubation in a water bath at 37°C for 20 minutes. At the end of the preincubation period 2 ml of molten soft agar was added to the tubes, the content mixed and plated.

The Salmonella typhimurium reverse mutation assay is considered acceptable if it meets the following criteria:
- regular background growth in the negative and solvent control
- the spontaneous reversion rates in the negative and solvent control are in the range of the Harlan Laboratory historical data
- the positive control substances should produce a significant increase in mutant colony frequencies

A test item is considered as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice (strains TA 98, TA 100, and TA 102) or three times (strains TA 1535 and TA 1537) the colony count of the corresponding solvent control is observed.

A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration.

An increase exceeding the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.

A dose dependent increase in the number of revertant colonies below the threshold is regarded as an indication of a mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remain within the historical range of negative and solvent controls such an increase is not considered biologically relevant.

Findings:
There was no indication of a bacteriotoxic effect at doses of up to and including 400 µg per plate. The total bacteria counts consistently produced results comparable to the negative controls, or differed only insignificantly. No inhibition of growth was noted as well. Higher doses had a strain-specific bacteriotoxic effect. Therefore they could only be used for assessment purposes up to and including 1600 µg per plate.

None of the five strains concerned showed in the plate incorporation test a dose-related and biologically relevant increase in mutant counts over those of the negative controls. This applied both to the tests with and without S9 mix and was confirmed by the results of the pre-incubation trials.

Results are presented in the following tables:

Table 5.8.2-1: Summary of mean values without S9 mix

Summary of Mean Values Without S9 Mix					
Table and Group	TA 1535	TA 100	Strain TA 1537	TA 98	TA 102
1-5 µg/Plate					
0	11	89	9	25	214
16	12	86	7	22	178
50	11	90	9	20	177
158	7	107	6	24	199
500	6	85	5	24	149
1581	5	88	6	20	62
5000	-	16	3	4	-
Na-azide	445				
NF		224			
4-NPDA			92	131	
MMC					664
6-11 µg/Plate					
0	8	126	9	35	290 169
100	12	111	11	31	279 147
200	11	113	7	26	280 141
400	10	128	8	27	339 180
600					132
800	9	116	8	28	198 103
1000					100
1200					87
1600	8	105	5	26	173
3200	5	71	3	10	14
Na-azide	464				

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NF		462			
4-NPDA			115	185	
Cumene					685 413

Table 5.8.2-2: Summary of mean Values with S9 mix

Summary of Mean Values With S9 Mix					
Table and Group	TA 1535	TA 100	Strain TA 1537	TA 98	TA 102
1-5 µg/Plate					
0	10	131	10	29	189
16	7	119	10	36	201
50	8	117	14	32	203
158	7	118	12	28	220
500	11	146	14	30	209
1581	7	136	8	25	112
5000	-	2	2	5	-
2-AA	88	1219	130	927	456
6-11 µg/Plate					
0	11	148	8	37	230 233
100	10	162	7	52	299 217
200	9	160	8	37	320 228
400	11	152	7	36	367 237
600					249
800	11	141	7	36	323 209
1000					222
1200					195
1600	12	149	8	34	267
3200	6	32	5	21	93
2-AA	143	1626	278	1594	841 803

Conclusion:

The Salmonella/microsome test, employing doses of up to 5000 µg per plate, showed that amidosulfuron produced bacteriotoxic effects, starting at 500 µg per plate. Therefore, 3200 µg per plate and above could not be used for assessment.

Evaluation of individual dose groups, with respect to relevant assessment parameters (dose effect, reproducibility) revealed no biologically relevant variations from the respective negative controls.

In spite of the low doses used, positive controls increased the mutant counts to well over those of the negative controls, and thus demonstrated the system's high sensitivity.

Despite this sensitivity, no indications of mutagenic effects of amidosulfuron could be found at assessable doses of up to 1600 µg per plate in any of the Salmonella typhimurium strains used.

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Due to these results, amidosulfuron (containing impurity AEF103452) is regarded to be non-mutagenic.

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CA 5.8.3 Endocrine disrupting properties

There is no evidence from the existing database that amidosulfuron has an effect on the endocrine system. No primary endocrine disrupting effects were observed *in vivo* and it is considered unlikely that any mechanistic study would add any relevant information. Amidosulfuron also does not fulfil the interim criteria for endocrine disruptors stated in the Regulation (EC) No. 1107.

CA 5.9 Medical data

A summary of medical data known for amidosulfuron by [redacted] is given in the following chapters.

CA 5.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

CA 5.9.1 contains **confidential** business data. Hence, the summary of these data can be found in document JCA.

CA 5.9.2 Data collected on humans

No cases of overexposures or intoxications with Amidosulfuron have been reported in literature.

CA 5.9.3 Direct observations

No cases of overexposures or intoxications with Amidosulfuron have come to the attention of Bayer CropScience.

CA 5.9.4 Epidemiological studies

No epidemiological studies have been published.

CA 5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

There are no reports on poisoning in humans.
Though it is a sulfonyl urea compound, amidosulfuron does not influence glucose metabolism.

CA 5.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

First Aid:

- Remove patient from exposure/terminate exposure
- Thorough skin decontamination with copious amounts of water and soap, if available with polyethylenglykol 300 followed by water.

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Note: Most formulations with this active ingredient can be decontaminated with water (and soap), so for formulations polyethyleneglykol 300 is not required.

- Flushing of the eyes with lukewarm water for 15 minutes

- Induction of vomiting does not seem to be required in regard of the low toxicity.

Treatment:

- Gastric lavage does not seem to be required in regard of the low toxicity.

- The application of activated charcoal and sodium sulphate (or other carthartic) might be considered in significant ingestions.

- As there is no antidote, treatment has to be symptomatic and supportive.

CA 5.9.7 Expected effects of poisoning

No persisting effects of poisoning are expected.

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