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Version history

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February 2015	MCA 5.1.1
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SANCO/10180/20	Data points containing amendments or additions and brief description MCA 5.1.1 MCA 5.8.1 at applicants adopt a similar approach foshowing revisions and dersion history as outlined in 13 Chapter 4 How to revise an Assessment Report.



	Table of Contents	
		Page 🖓
CA 5	TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE &	
	SUBSTANCE	©."5 ×
CA 5.1	Studies on absorption, distribution, metabolism and excretion in mammals	Ž 5 🔊
CA 5.1.1	Absorption, distribution, metabolism and excretion by organized with the control of the control	,
CA 5.1.2	Absorption, distribution, metabolism and excretion by other routes	. OFO.
CA 5.2	Acute toxicity	<i>ڰ</i> ؞ۜ10 ٍ ﴿
CA 5.2.1	Oral	100
CA 5.2.2	Dermal S S S S S S S S S S S S S S S S S S S	. N
CA 5.2.3	Inhalation	<u>1</u> 1
CA 5.2.4	Skin irritation	1 1 46°
CA 5.2.5	Eye irritation	, \$\P'
CA 5.2.6	Skin sensitization	1
CA 5.2.7	Phototoxicity	ے 12
CA 5.3	Short-term toxicity.	15
CA 5.3.1	Oral 28-day study@	17
CA 5.3.2	Oral 90-day study	17
CA 5.3.3	Other routes 2	17
CA 5.4	Genotoxicity testing	17
CA 5.4.1	In vitro studies A S S S S S S S S S S S S S S S S S S	18
CA 5.4.2	In vivo studies on sometic cells	
CA 5.4.3	In vivo studies in gorm cells	19
CA 5.5	Long-term foxicity and carcinogenicity?	20
CA 5.6	Reproductive toxicity	21
CA 5.6.1	Generational studies	
CA 5.6.2	Developmental to Acity studies Neuroto Science Science Studies Neuroto Science Science Studies Neuroto Science Science Studies Neuroto Science Science Studies Neuroto Science Science Studies Neuroto	22
CA 5.7	Neuroto de la virial de la viri	22
CA 5.7.1°	Neurotoxicity studies in rodents O	
CA 5.7.2	Defayed polyneur opathy studies	23
CA 5.8	Other toxicological studies	23
CA 5.8.1	Toxicio studies of notabolites	23
CA 5.8.2	Supplementary studies on the active substance	77
CA 5.8.3	Endocrine disrupting properties	77
CA 5.9	Medical data Similar S	77
CA 5,9,1	Medical surveillance on manufactoring plant personnel and monitoring studies	78
CA 5.9.2	Data collected on Humans	78
CA 5.9.3	Medical data	78
	Epiderniological studies	78
CA 5.9.5	Diagnosis poisoning (determination of active substance, metabolites),	
	specific signs of poisoning, clinical tests	79
CA 5.96	Proposed treatment: first aid measures, antidotes, medical treatment	79
CA 5.9.7	Expected offects of poisoning	79
, W	⊗ x	



CA 5 TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

Mesosulfuron-methyl is a herbicidal active substance and was included into Africa I of Directive 91714 in 2003 (Directive 2003/84/EC, dated 25th of September 2003/Entry into Efrec 1st of January 2004.

This Supplemental Dossier contains only summaries of studies, which were not available at the time of the first Annex I inclusion of mesosulfuron-methyl and were, therefore, not evaluated puring the first EU review of this compound. All studies, which were already submitted by Bayer CropScience for the first Annex I inclusion, are contained in the Monograph, its Addenda and are included in the Baseline dessier provided by Bayer CropScience. These old studies are not supmarized in detail again. For all new studies detailed summaries are provided with this Supplemental Dossier As a new study a phototoxicity study invitro in BALB/c 3T3 cells is summarized in chapter CA \$6.2.7.

For the soil metabolites AE F147447, AE F160460 and BCS V14885 new genotoricity studies were conducted to support e-fate risk assessment.

CA 5.1 Studies on absorption, distribution, metabolism and excretion in mammals CA 5.1.1 Absorption, distribution, metabolism and excretion by oral coute

Report:	2; ;1997;M393715-01 &
Title:	Wat preliminar Coxicolonetics: Absorption, dis Poution and elimination - oral low dose (10
	mg/labody, Right), and oral high dear (1000 mg/kg body weight) Code: (2-pyrimidyl-
	C 140) AE 1430060
Report No:	906347
Document No:	ØM-198Ø15-01-1
Guidelines:	OFCD: 417; USEPA (=EPA): OPOTS 870.7485; ot specified
GLP/GE	

Report:	6; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ;
Title:	Rest preligenary to acoki wines: Metabolion - oral low dose (10 mg/kg body weight) and
	Oral high dose (0000 mg/kg box) weigh Code:(2-14C-pyrimidyl)-AE F130060
Report No:	C000054 ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
Document 144:	M-19741791-1 48 69 69
Guidelin:	LOU (=F,OC): 94 09/EC; OECD: 417; USEPA (=EPA): F§ 85-1; not specified
GLP/GEP:	eves 4 ~ 2 ~ 2

Report:	₩997;M-193718-01
Title:	Rat - Sorp on, distroution and elimination - single oral low dose (10 mg/kg body
	weight) Code. (phepyl-U-14C) AE F130060
Report No.	Ç © 6348 ♥
Document No:	M-193 8-01-1
	OECS: 417; USEPA (=EPA): OPPTS 870.7485; not specified
GLO GEP	y s



	${\color{red} {\psi}}^{\circ}$
Report:	k; ;1999;M-193724-01
Title:	Rat - Excretion via the bile - single oral low dose (10 mg/kg body weight) Code: (phoyl-U-14C) AE F130060
Report No:	C006349
Document No:	M-193724-01-1
Guidelines:	OECD: 417; USEPA (=EPA): OPPTS 8 @7485; not specified
GLP/GEP:	yes yes
	yes The second s
Report:	1. · · · · · · · · · · · · · · · · · · ·
Title:	Rat metabolism - single oral low by se (10 mg/k body weight) (194C-menyl)-AE
	Rat metabolism - single oral low ose (10 mg/kg ody woght) (Q14C-penyl)-AE F130060
Report No:	C008356 O O O V V V
Document No:	M-197419-01-1
Guidelines:	EU (=EEC): 94/79/EC DECD 417; OSEPA (=EPA) F § 850; not specified
GLP/GEP:	yes Q X X X X X X X
Report:	:1998;M-147473-Qt
Title:	(Phenyl-U-14C) AE 16330060 Rat a corption distribution are elimination. Stal high
11010.	dose (1000 kg/kg kody weight)
Report No:	A67074 V
Document No:	M-147%73-01
Guidelines:	EU (EEC); 94/79/LC; OECD: 417; USEPA (EEPA): F. 521; not specified
GLP/GEP:	
Report:	♥゚
Title:	Ratmetabolism single esa high dese (100 mg/g/body reight) Code: (U-14C-phenyl)-
Title:	£ F136060 0 40 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4
Report No:	0C008395 2 4 5 6 6
Document No.	0°C008595 M-197418-00-1
Guidelings	EV (=EAC): 94/27/EC; OECD 17; USEPA (EPA): F § 85-1; not specified
GLP/GEP:	Des 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
Report:	h; 1999; M-19373001
Title:	Råt - Ansorptick, distribution od elimpation - repeated oral dose (7 x 250 mg/kg body
Title.	Tweix Q Cotx Inher 24 U-14 (X AF J 130060
Report No:	C006350 \$ A C C
Documer No.	k. ₩-193760)-01-1 ♥
Guidelines:	
GLP/GEP:	yeg V
· • • • • • • • • • • • • • • • • • • •	OECD: 417: USEPACEPAC OPPTS 870.7485; not specified yes 1.
Report:	;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
Title:	Rat Metabolism - receated oral dose (7 x 250 mg/kg body weight) (U-14C-phenyl)-AE
Report W:	C00830
Document Noo	M-197420-01-1
Griveline &	ELX = EEC): 94/79/EC; OECD: 417; USEPA (=EPA): F § 85-1; not specified
GLP/GES.	ves
	1 1 2 2 3

The toxicological profile of mesosulfuron-methyl was already investigated and evaluated.



The absorption, distribution, metabolism and excretion, including plasma and blood pharmacokinetics of mesosulfuron-methyl (AE F130060) was investigated in the Wistar rat following a nominal single of gavage dose of 10 or 1 000 mg/kg body weight and following up to 7 daily oral doses of 250 mg/kg body weight. There were no significant differences in the excretion profile of the phenyl- or pyrimical- labeled mesosulfuron-methyl and a very low level of metabolism of the compound was found. In yow of these results [U-14C-phenyl]-mesosulfuron-methyl was used for the majority of the studies, though [2007-pyrimidyl]-mesosulfuron-methyl was also dosed as part of the determination of the metabolism.

In the rat, mesosulfuron-methyl appeared to be moderately to poorly absorbed and rapidly excreted following a single oral dose of 10 or 1 000 mg/kg body weight, with a mean of 95.1% of the dose present in the 0-24 hour excreta at both dose levels. Faecal exerction was predominant, whilst only 12.8% and 1.3% of the low and high dose, respectively were found in the urine. There was no significant sox difference in the route of excretion and no exhalation of radiolabelled carbon dioxide during the first 24 hours after administration of a single high dose. Repeated dosing at 250 mg/kg by/day for 7 days had no significant effect on the excretion profile. More than 93% of the totally administered adioactivity were found in rat faeces.

The pharmacokinetic parameters of mesosulfuron-methyl showed that the maximum concentration of radioactivity in whole blood (C_{max}) occurred at 4 and 2 hours after dosing for the 1 000 mg/kg bw dose group. A monophasic elimination occurred with half-lives between 8 and 12 hours depending on test conditions. An examination of the Miary excretion of mesosulfuron-methyl confirmed the generally moderate absorption of mesosulfuron methyl after low-dose oral exposure. Following a single oral dose of 10 mg/kg body weight, the total amount absorbed, as determined by the adioactivity present in urine (measured in a separate study) and biles was only between 2022 and 22.8% of the dose.

The concentration of mesositifuron methyl residues in the tissues of rate 72 hours after dosing was generally low with several of the tissues containing residue levels that were below the limit of quantification at both dose levels. Following a single oral dose of 10 mg/kg body weight, only traces of radioactivity were detected in the organs and tissues. In the males, only liver (mean 0.17 µg equivalents/g) and plasma (mean 0.30 µg equivalents/g) showed radioactivity. In the females, the mean values in organs and tissues were below the limit of quantification. At the high-dose level (1 000 mg/kg body weight) all tissue residue levels were below the gouvelents/g tissue.

The metabolism of mesosulfuron-methyl was determined in the rat following dosing at 10 or 1000 mg/kg body weight. The predominant excretion product was unchanged mesosulfuron-methyl (>68 % in studies with single application) excreted mainly in the Paeces. A main metabolic pathway was identified, *i.e.* breakdown of the sulfonylured bridg leading to AE F092944 and AE F140584 which cyclised to AE F147447 Minor metabolic reactions observed were *O*-demethylation at the pyrimidine moiety leading to AE F160459, and cleavage of the methanesulfonamidomethyl side chain leading to AE F151015 and AE 019541. Abreakdown of the sulfonylurea-bridge of AE F160459 led to AE F119094, which was further metabolised by *O*-demethylation to AE F118772. The formation of the benzoic acid metabolite AE F154851 due to hydrorysis of the methyl ester by esterases was detected as a minor metabolic reaction in rats.

The metabolic profile of mesosulfuron-methyl in the rat is shown in Figure 5.1.1-1.

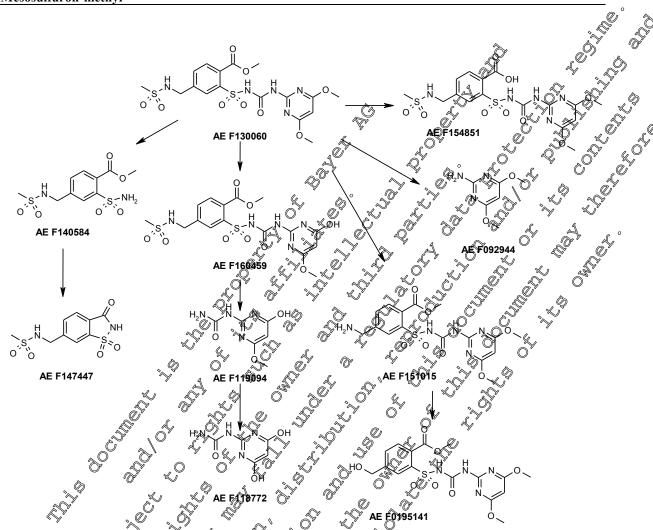


Figure 5.1.1-1: Metabolic profile of mesosulfuron methyl in rats

Report:	∃; 20¥3;M-470477-01
Title:	Pyrimid 1-2-140 mesosulfur off-methyl: Metabolic stability and profiling in
4,	hiver microsomes from rats and humans for Inter-Species Comparison
Repørt No:	Elisa-13-0029
Document No:	M-470497-01-J.
Guidelines	Regulation (EC) No. 1107/2009 (Europe) amended by the Commission
	Regulation (EU) No. 283/2013 (Europe) ES EPA OCSPP 870.SUPP; not specified
	US EPAOCSPF 870.SUPP; not specified
GLPGEP:	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-2-14C]-mesosulfuron (14C-mesosulfuror) 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 15 μM test item concentration was chosen in order to have enough sample material for possible identification of metabolities by chromatographic or spectroscopic methods. The sampling times were 0 and 1 hour after test start. The metabolic activity of the microsomes was demonstrated by determining 6β-hydrox testosterone that was formed from testosterone (some state of the form) testosterone (some s by testosterone 6β-hydroxylase. This biochemical reaction is well known for the CXP3A microsocial 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 HPI detection (HPLC-RAD).

Results

Results

The recovery of radioactivity was greasured in the microsome incubations and amounted to 107.1% (RLM) and 112.8% (HLM) for the 1 hour samples.

The results of the tests with 4C-mesosulfuron-methyl demonstrated that the privitry metabolism was only very slightly different between that's and humans.

While no metabolism was found in LM, as unresolved peak region (MOZ) was detected in the 1 h HPLCchromatogram of the HLM incultation that accounted to only \$1% of the total relative percentage (calculated from beak area values). Because of the approximately similar HPLC chromatograms of the incubations with rat and human microsomes (only a.i.), as further investigations were deemed necessary regarding peak region M-2. With regard to the Peak region M-2 with the incubations of human microsomes our expert stated that this was not a clearly recognized in "Peak" (= metabolite), but a peak region which was not isolated, or a direct Qution of a.i. from the column. Our experts decided that chances of success for a further isolation of this peak region which apyway was <10% would be minimal if at all.

Conclusion

Overall, the results suggest that phase I metabolism is not significantly involved in the biotransformation of mesosulfuron-methyl in rat and human liveronicrosomes.



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

Report:	i;	;2000;M-198123-01		, O	Ó
Title:	In vivo dermal absorption in	the rat using an oil susp	pension form@ation	(14C)-AE	\$ T
	F130060 Code: AE F130060	0 01 1K12 A7	4		,¥
Report No:	C009130	ĈA	.		W.
Document No(s):	M-198123-01-1	₩.	Ů,		
Guidelines:	EU (=EEC): 87/302 EEC;	OECD: Draft June 199	6; st specified	v S)
GLP/GEP:	yes	4©"		Q, S	`

Following 6-h dermal exposure to 14C-mesosulfuron-methyl in an oil flowable formulation to rate only limited systemic absorption was observed, i.e., nox. 14 & after 24 h using a 10 fold druted spray solution, and of max. 9% after 72 hours using the concentrated product

CA 5.2 Acute toxicity

Mesosulfuron-methyl exhibited a lox acute toxicity to mammals invespective of the route of exposure (oral, dermal administration, inhalation exposure). The repracting the part acut of the repracting the part acut of the part weight. The rat acute inhalation C₅₀ (4 hour) was > 193 mg/L air which was the highest achievable concentration and did not cause mortality.

No new studies were conducted since the birst submission.

Report:	4; 1996; N-1404(8-01 × C
Title:	Loe 13()60; Sub tance Occhnical (Code: Hoe 20060 DZC96 0001) - Testing for acute
	For all twicity in the male and for all y tar rate of the control
Report No:	A54612 4 A A A A A A A A A A A A A A A A A A
Document No(s):	MQ 4040\$01-1
Guidelines:	U (= EPA): 92/69 B. JMAIO; OEO: 401OUSEPA (=EPA): 81-1; Deviation not
	Specified & Survey &
GLP/GEP:	you or a

(2)	
Report:	3; 1996, M-140406-01
Title:	Hoe 130060, Substants, techocal; (Code: Hoe 130060 00 ZC96 0001) - Testing for acute
	der and toxicay in the male and female Wistar rat
Report No:	A 96613 O
Document No(s)	M-140496-01-4
Guidelines: O	EU EEC): 92/69 B.3; JMAF: ; OECD: 402; USEPA (=EPA): 81-2; Deviation not
	SIOCHIEU C
GLP/GF.	



CA 5.2.3 Inhalation

Report:		9;M-186735-02; An		
Title:	Testing for acute dust inhalation to	oxicity in male and	female Spræue D	Dawley rats 4-hour
	LC50 AE F130060 substance tech	nical Code: AE F13	30060 00 <u>4</u> 1 C95 00	001
Report No:	C003755	Ĉ	, V	
Document No(s):	M-186735-02-1	N.	W .	
Guidelines:	EU (=EEC): 67/548 B.2.; OECD	: 4036 USEPA (=E	P. §81-3;Devi	Oion na specifod
GLP/GEP:	yes	40' ~	Ý ~	

CA 5.2.4 Skin irritation

AE F130060 was not irritating to rabbit skin and only shightly firitating to rabbit eyes, but no classification is needed. No evidence of skin sensitisation was seen in a gamea pig maximisation test.

Report:	;1996;M-140524;0\(\frac{1}{2}\)
Title:	Hoe 130060; Substant, technocal; (Ode: How 1300, O 00 ZG 0 000). Testing for
	primary decimal irritation in the rabation of the primary decimal irritation of the primary decimal irri
Report No:	A56736 V (
Document No(s):	M-140324-01
Guidelines:	EU (EEC), 92/69 B4; JMSF: ; QECD: 4M; USOPA (EPA): 81-5; Deviation not specified
	specified V
GLP/GEP:	Bes Day of Land

CA 5.2.5

Report: \$; \$996;M\$140517601
Title: Hard 3006 Substance, teomical; (Code: Joe 13, 60 00 ZC96 0001) - Testing for
Mimary of entritation in the rabity
Report No: \\ \Delta 5672\text{2} \text{ \ \text{ \text{ \text{ \text{ \text{ \text{ \text{ \text{ \text{ \
Document No(s): M-140517-99-1
Guidelines: FS (=EFS): 92/5 B.5; WIAF: ; OECTO 405; USEPA (=EPA): 81-4; Deviation not
O Official C C C C C C C C C C C C C C C C C C C
GLP/GEP: yes O Y Y

Report:	6; ;1998;M-148033-01
Title:	Sensitizing properties in the Pirbright-White guinea pig in a maximization test AE
	F130000 substance, teQuical Code: AE F130060 00 1C95 0001
Report No:	A62065 Q
Document Vo(s):	M948033€01-1 ° 9
Guidel Ces:	EU (=DEC): B.6., 96/54/EEC; 67/548/EEC; JMAF: 1985; OECD: 406, 1981 / update
	1992; USEPA (=EPA): §81-6, Subdiv. F; Deviation not specified
GLA GEP	y g S



CA 5.2.7 **Phototoxicity**

According to the new data requirements (Commission Regulation (EU) No. 283/2015 of 1 March 20 Official Journal of the European Union, L 93/1, 3.4.2013), the conduct of a photofoxicity study is required under certain conditions.

The circumstances in which a phototoxicity study, according to the new data requirements, is required a "where the active substance absorbs electromagnetic radiation in the range 290-700 pm and is liable to reach the eyes or light-exposed areas of the skin, either by direct contact or through systemic distribution. x mol⁻¹ x cm⁻¹, no toxicity testing is required.

As the Ultraviolet / visible molar extinction absorption coefficient of the active substance exceeds the trigger of 10 L x mol⁻¹ x cm⁻¹, a cytotoxicity study has been performed in vitro asing BALB/c 3T3 cells.

Report:	64 (2014; M-476223-01 5 6
Title:	Mesosulfuron-methyl (AF F) 30060 technical: Colorovio ty as an in vitro with
	BALB/c31\$ c31 colls: Neutral Rod (NR) test doring simultaneous igradiation
	With artificial satisfied A
Report No:	1592100
Document No:	M-476222-01-1
Guidelines:	Commission regulation (EC) No. 440/2008 B 41, dated May 30, 2008;
	Commutee for Proprotary Medicinal Products (CPMP) Note for Guidance on
	Photosafety testing, EMEA, CPNP/SWP, 398/01, adopted 27 June 2002, into
20	operation in Dec 2002. DECD Guideline for Festing of Chemicals: Guideline:
	1732, 18 YILLO 313 INKO phologoxicil@lest (servised and approved by the National
- Q	Co-ordinators in May 2002 approved by Council April 2004);not specified
GLP/GEP\$	yes a second sec

Executive summar

The phototoxic potentiat of Mesosulfuron-methyl (AE F130060) technical, was tested in this assay using BALB/c 3T3 cells. The experiment was performed wice. The first experiment served as a range finding experiment (RFE), the second one was the main experiment (ME).

Cytotoxic effects did not occur after exposure of the test item to the cells, neither in the presence nor in the absence of irradiation with artificial sunlight in the RFE as well as in the ME. Therefore, ED50-values or a PIF could not be calculated. The could make MPE value was 0.048 and 0.000, respectively.

ults show that the lest item is not phototoxic.

Materials and meth Therefore, the res

Materials and methods

Mesosulfuron-methyl (AE F130060) technical

AE F130060-01-02 Origin Batch No.: EFME000144



Purity: 97.4% w/w (dose calculation was adjusted to purity)

Expiry Date:

Storage Conditions:

Stability in Solvent:

2. Vehicle:

3. Positive control:

4. Test system:

BALB/c 3T3 cells clone Cells:

Thawed stock cultures Master Cell Stock of the BALB/c 3 73 c3 cell line supplied by Cell cultures:

DMSO (further diluted in EBSS, final concentration of DMSO in EBSS was 1% (v/v)

Chlorpromazine dissolved in EBSS°

3T3 cells clone

tock cultures (Master Cell Stock of the BALB/c 3f3 c34 cell line supplies as done with about 1 £010° cells per ledium (DMEM) Seeding was done with about 1 20106 cells perdiask in 5 m of Dubbecco's Minimal essential Meditirn (DMEM), Supplemented with 1900 NCS. The cells were sub-cultured twice weekly The coll cultures were incubated at 37 ± in a 7.5 ± 0.5% carbon

dioxide atmosphere

B. Study design and nethods

The experiment was performed wice of he first experiment served as a range 1. Treatments:

Pinding experiment (RFE), the second one was the main experiment (ME).

Doses:

		. ~~~	(M	O	
٥,	Ø est i	tem	M.	+1-	Final concentrations in µg/mL
Š		Y *	V . (WV _`>	
*	Meso	sulfõ	řon 🔊	+/	24 1, 14 .81, 29.64, 118.55, 237.10, 474.19,
(Q",	, Š			948.00
Ĉ	Chlor	prom	azine ^		6.25, 12.5, 25, 37.5, 50, 75, 100, 200
	O ^y	29		+ 0	25, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 4.0
9/	Solve	n co	ntrol	% - %	EBSS containing 1 % (v/v) DMSO
Ş	1 1	•	Y &	y` .~Q`	

2. Experimental procedures

De Hönle Sol 500 solar simulator (filter H1 was used to keep the UVB

Grradiation as low as possible).

About 2 x 10⁴ cells per well were seeded in 100 µL culture medium (two

plates, one was exposed to artificial sunlight, one was kept in the dark).



Treatment:

24 hours after seeding the cultures were treated with the test item. The cultures were washed with EBSS. 8 dilutions of the solved test item were tested on two 96-well plates (100 μ L/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/cm² (4.95 J/cm²), the other plate was stored in the dark (each 50 min, about 25-26 °C). 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 about 22 - 23 hours at 37 \pm 1.5 °C and 7.5 \pm 0.5% CO2.

Cytotoxicity determination:

The medium was removed and 0.1 mb serum free medium ontaining 50 µg Neutral Red / mL added to each well. The plates were incubated for another 3 hours for the uptake of the vital die into the lysosomes of viable cells. There after, the medium was removed completely and the cells were washed with EBSS. For extraction of the eye 0.15 mL of a solution of 39% (vs.) decimised water, 50% (v/v) ethanol and 1% (v/v) accetioncid were added to each well.

After approx. 10 minutes at room temperature and a brief a plation, the plates were transferred to a microplate reader (Versamax), Molecular bevices) 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.

Number of measurements.

Mesosultirone, positive control. 6 times Solvent control. 12 times

Data Recording:

Arithmetic means ± standard deviation were alculated for every test group.

The ED50 values, the Photo Firitance Factor (PIF), as well as the Mean

Phototoxic Effect (MPE), were calculated using the software Phototox (Version 20) (discributed by ECD guideline). The ED50 values (effective dose where only 50% of the cells suroved) were determined by curve-fitting software.

Evaluation criteria: If PIF 2 or MPE 0.1: no phototoxic potential predicted.

If PIF > 2 and < for MPE > 0.1 and < 0.15 a probable phototoxic potential is predicted. If PIF > 5 or MPE > 0.15 a phototoxic potential predicted.

A. Results and discussion

The first experiment served as a range finding experiment (RFE), the second one was the main experiment (ME). The following concentrations of the test item solved in DMSO (further diluted in EBSS, final concentration of DMSO in EBSS was 1% (v/v); dose calculation was adjusted to purity) were tested in the presence and in the absence of irradiation in both experiments: 7.41, 14.81, 29.64, 59.28, 118.55, 237.10, 474.19, 948.06. As solvent control 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/cm² UVA, resulting in an irradiation dose of 4.95 J/cm² UVA. Another lest group of test item treated cells were kept in the dark for 50 minutes.

Cytotoxic effects did not occur after exposure of the test item to the cells, neither in the presence nor in the absence of irradiation with artificial sunlight in the RFE as well as in the ME. Therefore, ED50 values or a PIF could not be calculated. The resulting MPE value was 0.048 and 0.000, respectively.

Therefore, the results show that the test item is not phototoxic.

The results are summarized in table CA 5.2.7-1.

Table CA 5.2.7-1 - Summary of Results

	Substance	ED ₅₀ (+UV) [μg/mL]	ED ₅₀ (PUV) P	IE MPE	of irradiate	f solvent control ed versus non- ted plate
RFE	Test Item	-		- , , , 0.048	. 1 4 6 8	08.1
Kre	Positive control	0.28	3.11 \$ 47	02 0499	D 0 10	
ME	Test Item	- Q"	\$ - \$ \$	- 9.000		S .1 , &
ME	Positive control	6,16	8.94 8.55	.60 0.763	\$ 50°9	03,3

CA 5.3 Short-term toxicity

The short-term toxicity of mesosulfuron-methyl was assessed in an earlier EU review of the active substance, however these data are sommarized here for reference in graphype. Further infavailable in the Baseline Dossier provided by Bayer Crop Science and in the Monograph. substance, however these data are sommarized here for reference in grap type. Further information is



				W 4
TYPE OF STUDY	SPECIES / STRAIN	Dose levels (ppm)	NOAEL	Reference
Rat 90-d feeding study + 4 wk recovery	Wistar rat (Hoe:WISK)	0; 240; 1200; 6000; 12000	12000 ppm males: 908 mg/kg by females: 976 mg/kg	M-187497-61 KCA 5.3 201
Mouse 90-d feeding study	CD-1 mouse	0; 140; 1000; 7000	7000 ppm males: 123 kpg/kg bw females: 160 mg/kg bw	Doc C000016 M4194489 ₂ 01-1 RA 5,3002
Dog 28-d range finding	Beagle dog (Marshall)	0; 400; 2000; 100 % ; 20000	200% ppm 5 775 mg/kg/jv m/ws: 775 mg/kg/jv fogoles: 869.5 mg/l@bw	Do \$\infty\$ \text{A59274} \\ \text{M\$\text{4295}\$\$\text{\$\exitin{\ext{\$\text{\$\text{\$\text{\$\text{\$\text{\$\exitin{\ext{\$\exitin{\ext{\$\text{\$\exitit{\$\text{\$\text{\$\exititt{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\tex
Dog 90-d feeding study	Beagle dog	0; 2000; 7000; 7	2000 ppm Males: A mg/k w males: 034 mg/k bw	
Dog 12 month feeding study	Beagle dog	Ø, 400; 4080; 16668	1 (50) ppm) (50) males: Q1 mg/kg bw females, 646 mg/kg bw	,,000a 10. N° (009410 M-1985Q-01-1 KCA5 3/01

No adverse effects were seen at any dose level of up to the highest jested dose of 1000 mg/kg bw/day of mesosulfuron-methyl in rats mice and dogs. In all studies the NOAEL was the highest dose level given. This can be explained by the low toxicity of the test substance in combination with a non-linear absorption from the gastromestinal tractoile., up to 23 % after or allow doses of 10 mg/kg bw and only 2 % or slightly above at the limit dose of 1,000 mg/kg bw after or all exposure.

Slight changes of some bjochemical anothematological parameters which were seen in the subchronic study in rats did not show a clear dose-related pattern and were not consistently seen in both sexes and were therefore in the absence of any morphological consequence; regarded as findings due to variability.

No new short torm toxicity study has been performed, and there are no new scientific findings that influence the regulatory interpretation of the official evaluation of the active substance.

Report:	§; ;; ;2000;M-198511-01
Title;	Dog 12 month dietary voxicin Study Code: AE F130060 00 1C95 0001
Report No:	C069410 ~ 0 ~ 0
Document No(s)	M-19851 401-1 0 0
Guidelines: 🙏 4	EU (=0.000): 83/302/10.000, V B; JMAF: 4200; OECD: 452; USEPA (=EPA): 83-1;not
	"specified ""
GLP/GEP\$	yo & o
GLP/GEP.	



CA 5.3.1 Oral 28-day study

Report:	i;	;1997;M-142958-01		
Title:	AE F130060 - substance te	echnical; Code: AE F1300	60 00 1C94 © 001 -	28 day repeated
	dose toxicity study in dogs	(range finding study with	n dietary administrat	tion)
Report No:	A59274	ĈA	<i>. , , , , , , , , , ,</i>	
Document No(s):	M-142958-01-1	N. T.	W .	
Guidelines:	JMAF: (1985); OECD: 40	09; USEPA (=EPA): 82-	1;D viation not sp	cified &
GLP/GEP:	yes	40°		Q S 4

CA 5.3.2 Oral 90-day study

Report:	l; ;1999 M -187 49 7-01
Title:	Subchronic (90 days feeting) on toxicity study in rats Hoe 1300,60 sub Quice tonnical
	Code: Hoe 130060 00 CC96 0002
Report No:	C004205
Document No(s):	M-18/49/-01-1
Guidelines:	EU (=EEC): 8Q302/FEC Annex V Part B; JYAF: 1085; OF D: 438; USF A
	EU (=EEC): 8Q302/EEC Annex V Part B; JAAF: 035; OCCD: 448, USE A (=EPA): §87,1; Deviation not specified
GLP/GEP:	yes S S S

Report:	₩ \$999·M-194489-01 <>
Title:	Subchronic 400 days feeding oral pxicity Qudy in mice How 1300 substance technical
	(Side: Has 1300 to 00 ZC 96 0000
Report No:	\$\$\text{\$\ext{\$\text{\$\text{\$\text{\$\text{\$\text{\$\exitt{\$\exitt{\$\ext{\$\ext{\$\ext{\$\ext{\$\ext{\$\ext{\$\ext{\$\ext{\$\ext{\$\ext{\$\ext{\$\exitt{\$\exitt{\$\exitt{\$\ext{\$\ext{\$\ext{\$\ext{\$\ext{\$\ext{\$\ext{\$\exitt{\$\exitt{\$\ext{\$\exitt{\$\ext{\$\exitt{\$\exitt{\$\ext{\$\exitt{\$
Document No(s):	M ₇ 104489-10471 X
Guidelines:	EEC): 88@02/EEC Anney V Part S; JM OF: 1985, OECD: 408; USEPA
	EPAOF § 820; not secification of the second
GLP/GEP:	yes O O

Report:	у;	32000 Nr-1980 2-02; Amended: 2001-03-27
Title:	Dog Aral 90 by repened do	SEXoxicity study Metary administration) AE F130060 E F13660 00 fC95 0001
	substance to hnical Vode:	F F 136 80 00 1 C 95 0001
Report No:	Q09014\$ 6	
Document No.	M-198012-02, P	
Guidelines: 4	JM : 198 OEC 409	SEPA (=EPA): §82-1;not specified
GLP/GEP	Lys J J Q	~ <u>~</u>

CA 3.3.3 Other routes

Studies with other routes are not generally required and were not conducted.

CA 5.4 Senotoxicity testing

Testing for possible genotoxic properties of mesosulfuron-methyl technical in several *in vitro* and *in vivo* test systems on different indpoints gave negative results. The *in vitro* testing battery comprised investigations for gene mutation in bacterial and mammalian cells, examination of chromosomal aberration in Chinese Hamster cells and testing for unscheduled DNA-synthesis in primary rat hepatocytes. Furthermore, a mouse micronucleus assay on chromosomal aberration *in vivo* was performed.



experiments in two species, further testing, e. g. tests using germ cells was not triggered. The following table 5.4-1 presents a summary of genotoxicity testing conditions and results that were conditions the first European evaluation

Since all 5 tests we	Since all 5 tests were negative and no evidence for carcinogenic properties was seen in life-time					
experiments in two species, further testing, e. g. tests using germ cells was not triggered. The following						
table 5.4-1 presents a summary of genotoxicity testing conditions and results that were evaluated during						
he first European e	valuation	l .		A		
			Ď	Ž"		
Гаble CA 5.4-1 - F	Results of	genotoxicity tests	\			
Endpoint	Purity (%)	Test system	Consentration (dose) levels	Result .	Author & S	
Bacterial reverse mutation (Ames test)	95.6 %	Salmonella typhimurium TA 100, 1535, 1537, 98 E. coli WP2uvra	4 – 5000°µg/pl	Signative C	Author 9 1996; Doc. NA56749 M-149530-014 KØA 5.4.1(0)1	
Chromosomal aberration in vitro	94.6 %	Chinese Hamst W 79 lung fibrobla	250 - 300 μg/QL +/- 59 mix	o egativi o	1998 (7) Doc N° A6755 M-197927-04-1	
Mammalian cell gene mutation (HPRT test)	94.6 %	HPRT lows V78 Chin@e Ham or lung of fibrolasts	25 – ©00 μg/L +/ ₂ 9 mix	Chegatic C	1998© Doc A A67981 M-047480-01-1 I&A 5.4 703	
DNA damage and repair (UDS test)	94.6 %	Rat SD primary	1-2500 μg/mL \$\infty\$ \(\text{S} - 9 \) mis \(\text{V} \)	Negation 1	1998c Dog A° A67689 MC148054-01-1 DCA 5.4.1/04	
Chromosomal aberration (micronucleus test) in vivo	946	HscWin:NMRI mice	2000 000, 200 m2 kg bw	O S Negat@e	1998e Doc. N° A67143 M-147538-01-1 KCA 5.4.2/01	

In vitro studies

Report:	®; 3996;M€J4053Q±01
Title:	Het 13006, Substance, te Jinical; Code Hoe 130060 00 ZC96 0001) - Bacterial reverse
Q ₁	Antation Dest A A A
Report No:	\$\tilde{\text{A5676}}\$
Document No(s):	M-120530-61-1 Q
Guideline	M-190330 67-1 DJ (=EFO): 92 69 B 13 2/69 B 14; OECD: 471, 472; USEPA (=EPA): 798.5100,
	798.5265, not specified
GLR EP:	V yes A O V

Report:	;1998;M-147927-01
Title: O'	In vice man valian chromosome aberration test in V79 Chinese hamster lung cells AE
Title.	F150060 substance (lechnical Code: AE F130060 00 1C95 0001
Report No.	\$\tilde{Q}_{6}^{\tilde{7}} 555 \tilde{\tilde{Q}}^{\tilde{7}}
Document No.	M-147927-01-1
Guid Mines:	EU EEC): 92/69, L383A Annex B.10; OECD: 473, May 1983; USEPA (=EPA):
Document No.	78.5375, Fed.Reg.50, Subp. F;not specified
GLP/GO ³ :	yes



Report:	k; ;1998;M-147480-01		
Title:	AE F130060; substance, technical; Code: AE F13006	60 00 1C95 00 % - In vi	tro chino
	hamster lung V79 cell HPRT mutation test		
Report No:	A67081	, O	
Document No(s):	M-147480-01-1		
Guidelines:	EU (=EEC): 87/302, L133, 1987; OECD 76, 198-	4; USKPA (=EPA): 79	7.500, Part
	700 to end, 1986; not specified		
GLP/GEP:	yes	O N	
	(//)	M.	W

Report:	\$; ;1998;M-148054-01
Title:	Detection of DNA strand breaks in primary her diocytes of mahorats in itiro. U.S-teachin primary rat hepatocytes AE 6 3006 (Substance, technical Code: AE 6 3006 (Substance, technical Code: AE 6 3006 (Substance))
	primary rat hepatocytes AE 3006@ ubstacoe, technical Cove: AE 3006000 1CV
	0001
Report No:	A67689
Document No(s):	M-148054-01-1
Guidelines:	EU (=EEC): 88/30Q L 123 OEC 482, 1986; USEPA (PA) 98.55 y, 1985 not
	specified 2 0' 2' 2' 2' 2' 2' 2'
GLP/GEP:	yes Q a a a a a a a a a a a a a a a a a a

In vivo studies in somatic celts **CA 5.4.2**

Report:	*: 1998 N-1475 98-01 *E F F 130060; Obstance technical; Code: AE F 30060 0 1C25 0001 - Mouse microstucles; 2st **AC 143 **A
Title:	@E F130060; @stanc@techn; al; Co. AE F93006; 0 1C950001 - Mouse
	micro Rucleus Pest
Report No:	/ A6\(\)143 \(\frac{1}{2}\)
Document No(s)	NF-147508-01-10
Guidelines:	TEU (=YEC): 92/69, L383 A, Annex S12; OFCD: 47A, 1983; USEPA (=EPA):
CL D/CE	7985395, 30bparr 1, 1985390t spectred
GLF/GF/	
	### AC 143 ### 147538-01-1 PEU (=PEC): 92/69, L383 A. Annex 5.12; Of CD: 474, 1983; USEPA (=EPA): 798,5395, Subpart 1985 obt specified vivo studies in germ cells
Ď	
CA 5 4 3 ØIn	vis Q tudo in form all Q
CA 3.4.3	vipo studies in Serii Seris
Based on the toxici	ty and genotogicity profile, in vivo studies in germ cells were not required.
√ n	
\$ 1 m	
$igcup_{}$	



CA 5.5 Long-term toxicity and carcinogenicity

Type of study	Dose levels (ppm)	NOAEL (mg/kg bw/d)	REFERENCES
Wistar Rat combined		16000 ppm	,2000 (**)
Chronic	0, 160, 1600, 16000	Males: 865 mg/kg bod	M-198434-0141 KCA 5.5/01
		Fent res: 1056 mg/kg 0w/d	KCA 5.5/0 C 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
Oncogenicity	0, 160, 1600, 16000	Males: 764 mg/kg bw/	
		Females: 950mg/kg by/d	
M	0.00.00010000	800 ppg	2000b Doc. No.: C009460
Mouse oncogenicity	0, 80, 800 and 8000	Rates: 103 in k/kg bw/d Feyales: 100 mg/kg/bw/d	M\$98596-01-1

In a combined chronic toxicity and one ogenicity study in rats, continuous dietary treatment for 106 weeks with dose levels of up to 16 000 ppm, which were appropriately equivalent to the limit dose of 1000 mg/kg bw/day, did not produce any evidence of toxicity or of phoogenicity thring their natural lifespan. Also in mice, dietary treatment with up to 3000 ppm (car 1000 mg/kg bw/d) for 80 consecutive weeks, with the exception of slight reductions in body weight gain in females, and not show any evidence of an oncogenic activity in this species.

Report:	k; (2000)M-198434-01
Title:	Otechnical substance Code: AED130060000000000000000000000000000000000
	Otechnical substance Code: AEQ13006 00 165 0006,
Report No: 💉	C0 x 9379 x 3 6 7 6 2
Document No(s):	M 984 3 01-1
Guidelines:	U (= 5.C): 88/302/EG; JMAQ: 4200, OECO. 453; USEPA (=EPA): 83-5;not
	Specified Q Q Q Q Q A
GLP/GEP:	

Report: (32000;M-198596-01
Title: Mouse die by on Lenicia (18 months) study AE F130060 technical substance Code: AE
Title: Mouse dia by on Senicio (18 months) study AE F130060 technical substance Code: AE
Report No: C009460 > 2
Document No(s): M-498596-07-1
Guidelines: EU (=EFU): 88/302/EFO JMAF: 4200; OECD: 451; USEPA (=EPA): Series 83-2;not specific
specifies of the specif
CID/CED: O' & Type & X/
GLP/GEP: Vyes Vyes Vyes Vyes Vyes Vyes Vyes Vyes



CA 5.6 Reproductive toxicity

Study type	Species	Dose levels	NOAEL	Reference	
Multigeneration study	S.D. rats (Hsd:SD)	0, 160, 1600, 16 000 ppm	parents: 16000 ppm Offspring: 16000 ppm Malc©1175 mg/kg bw Females: 1388 mg/kg	2000 Dat N° C010081 D M-198366-01-1 KCA 5.6.1/10	
Developmental toxicity study	S.D. rats (Hsd:SD)	0, 100, 315, 1000 mg/kg bw/d		Doc. N. 2003932 M-187036-01	
Developmental toxicity study	Himalayan rabbits	0, 100, 315, 600 mg/kg bw/d	Maternal and foetal.	19%b Doc. 1%C000843 M-181336-021 KOA 5.62/04	

It was concluded that mesosulfuron-methyl did not have a reproduction or developmental coxicity potential. There was also no evidence of any teratogenic potential. No reproductive toxicity was seen, although due to the extremely high test substance intake during certain phases of dictary treatment the limit dose of 1 000 mg/kg bw/d was exceeded

New studies were not conducted.

CA 5.6.1 Generational studies

In the multigeneration study administration of mesosulfuron-methyl at dietary concentrations of up to and including 16 0000 ppm (Equivalent to merovimetal), 500 ppm (Equ including 16 000 ppm (equivalent to approximately 800 mg/kg bw/day up to 3000 mg/kg bw/day, depending on the food constamption in the different phase of the study did not cause any substancerelated adverse effects on reproduction, fertility mating behaviour or malformations in the offspring in a multigereration study in rats.

Report:	;2006;M-198304-01	
Title:	Harry e tukeng teoding-reproduction studies a rat two-generation reproduction toxicity	
	Quidy & F130960 sulp ance to hnical code: AE F130060 00 1C95 0001	
Report No:	C01056 2 2 2 2	
Document (s):	M-19830491-1 4+ 6 0	
Guideling:	MAF QOECD. 416, 981; USEPA (=EPA): §83-4, 1984; Deviation not specified	
GLP/GEP:	Yyes A Y Y Y	

Report: 2000;M-198366-01
Title: Rat typ-geney ion feeling-reproduction toxicity study with AE F130060 substance technical Code: AE@130060 00 1C95 0001
teclerical Code: AE@130060 00 1C95 0001
Report No. 7 GM 008]
Documer No(s) M-198666-01-1
Guidelines: JMAP: 1985; OECD: 416, 1981; USEPA (=EPA): OPPTS 870.3000, 1998; Deviation
Guidelines: JMAY: 1985; OECD: 416, 1981; USEPA (=EPA): OPPTS 870.3000, 1998; Deviation newspecified
GLP/GES: yes



CA 5.6.2 Developmental toxicity studies

Also the developmental toxicity studies in rats and rabbits, performed at the same laboratory like the reproduction toxicity study did not show substance-related adverse findings up to and including the limit dose level of 1000 mg/kg bw/d, which formed the NOAEL for dams and fetuses in both studies.

			. الم		\bigcirc \vee			~
Report:	V;		;M2147		4	*		
Title:	Range finding embryo	otoxicity stud	fter o	oral adm	instration	rin spragu	e dawley r	a r S
	substance, technical C	Code: AE F	0060 00) 1C9 5 √	001 💞	Q,	, O'Y	
Report No:	A67310	&	Ò °		4 N	, O >		
Document No(s):	M-147697-01-1	0′.	, O, "	N.	\$. <i>a</i>			W .
Guidelines:	EU (=EEC): 88/302/	EWA, 1987	JMAR	1985	OECIO	414, 12-M	[ay 1981;	SEPA .
	(=EPA): F 83-3, 1984	4;Deviațion	not spec	eified	1 1			
GLP/GEP:	yes		. W	. «)	

Report:	6; 1999; M-187036-01 S
Title:	Rat oral development toxici (teratogenici) study (EF129060 sapstance echnical
	Rat oral development toxici (teratogenicis) study (EF139060 satisfance) echnical Code: AE730060 (1C950001)
Report No:	C003932 ² (
Document No(s):	M-187@36-01-0
Guidelines:	EU (ZEEC) 88/302, JMA 1985 OECD; 414; USEPA EPA 883-3; not specified
GLP/GEP:	

Report:	;1998M-14 76 96-01
Title:	Range finding embryoto ocity study after sal administration in rabbits substance,
\ \text{\tin}\\ \text{\texi}\text{\tin}\text{\text{\text{\text{\text{\texi}\text{\text{\text{\texi}\text{\text{\texi}\text{\text{\texi}\text{\texi}\text{\texi}\tex{\texi}\text{\texi}\text{\texit{\texi}\text{\texi}\text{\texi}\ti	&hnic &Code: &E F & 6060 00 CC95 Q001
Report No:	7A67389
Document No(s):	M-447696-01-1
Guidelines.	by viation not specified
GLP/GEP:	Des N & S & S

1

Report:	;1998;M-139336-02; Amended: 2001-03-16
Title:	Rabbit ral developmental toxorty (temogenicity) study AE F130060 substance technical
4	Cos QAE PS 0060 AV 1C9 5 0001 2.
Report No	Q000843 \$\frac{1}{2} \frac{1}{2} \text{Q} \text{Q}
Documer No(s):	₩-181396-02-1 × × ×
Guidelines:	EU (4EEC): 38/302 JMAJ OohSan No. 4200 (1985) ; OECD: 414; USEPA
Y	(=FOA): F. S3-3;no specifical
GLP/GEP:	\ yes \C \ \ yes \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \

CA 5,7 Neurotoxica studies

No special dudies in a neurotoxic potential of mesosulfuron-methyl have been performed. The extensive toxicity data from all apical studies which are available do not provide evidence of any direct neurotoxic effects. Therefore, no additional testing was required and based on the available data, mesosulfuron-methyl does not present a neurotoxic hazard.



CA 5.7.1 **Neurotoxicity studies in rodents**

Based on the available information, mesosulfuron-methyl does not present a neurotoxic hazard. Therefore, neurotoxicity studies were not conducted.

CA 5.7.2 Delayed polyneuropathy studies

Mesosulfuron-methyl does not belong to the class of reganophosphates from which some have an OPIDP potential. Therefore, an OPIDP study was not necessary and not preformed.

Other toxicological studies **CA 5.8**

Other toxicological studies with the active in gredient was sufficiently demonstrated.

The equivalence of the test material used in the key toxicological studies was demonstrated in a position paper. Prease refer to the confidential part of the dossier. &

Genotoxicity studies were conducted with the metabolities AE F147447 CV14885.

AE F147447 F160460 and BCS-AE F147447

Report:	p; \$\tilde{Q}012; \tilde{Q}428741 \tilde{O}1\$
Title:	Salmonella tychimurjum reverse mutation assay with AE F147447
Report No:	
Document No:	M 5 42874€01-1
Guidelines:	ØECDNo. 47 Cadopted July 1, 1997
	ECNG. 440√2008 BÎ3∕14. dâted May 30. 2008
	EPA OPP \$8 870, \$100, EFA 712 -98-247, August 1998; not specified
	yes of or its or

Executive summar

This study the potential of AE F147447 to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the Salmonella typhimurium strains TAT535, TA 1527, TA 98, TA 100, and TA 102 was investigated.

The askey was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test from was tested at the following concentrations:

Pre-Experiment/Experiment I: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

Experiment II: 33; 100; 333; 1000; 2500; and 5000 µg/plate



The plates incubated with the test item showed normal background growth up to 5000 µg state with and without S9 mix in all strains used.

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 AE F147447 at any dose level, neither in the presence for 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 forder of biological relevance.

Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Therefore, AE F147447 is considered to be non-muragenic in this Salmonella typhimurium reverse mutation assay.

I. Material and methods.

A Materials

1 Test Material:

Description:
Lot/Batch:

Stability of test compound:

Solvent used:

AE F147447

SES 10681-2-3

98.9 w/w. Dose calculation adjusted to purity

not performed as part of this study.

/Germai

Germany; purity > 99 %).

2 Control materials

Negative: Solvent:

Miture medium

DMS@

Positive: nortactive fron (-89 mi

10 µg/plate for TA1535 and TA100

50 μg/plate for TA1537 and

10 μg/plate for TA98

methyl methane sulfonate:

 $3.0 \,\mu\text{L/plate}$ for TA 102

activation (S9 mix)

2-aminoanthracene, 2-AA:

2.5 µg/plate for TA 1535, TA 1537, TA 98, TA 100, and 10 µg/plate for TA

102

3 Matakalic activation

S9 derived from Wistar rats (Hsd Cpb: WU)



Phenobarbital/β-naphthoflav	one induced rat liver S9 were used as the metabolic activation system. The S9
is prepared from 8 - 12 week	ks old male Wistar rats (Hsd Cpb: WU; weight approx. 200 – 320 g,
	, The Netherlands) induced by intraperitone applications of 80
mg/kg b.w. phenobarbital (, Germany) and by peroral administrations of β
naphthoflavone (Germany) each on three
consecutive days. Each batch	h of S9 mix is routinely tested with 2-aminoanthracene as well as
benzo[a]pyrene. Cofactors a	re added to the S9 mix to reach the following concentrations in the S9 mix. 8
mM MgCl ₂ , 33 mM KCl, 5 t	mM Glucose-6-phosphate and 4 mM NAPP in 100 mM sodium-orthor
phosphate-buffer, pH 7.4.	
4 Test organisms:	Salmonella typhimurium, strains TA1\$35, T&1537, FA98, TA100 and
	TA 102 (from
	Regular checking of the properties of the strain regarding the membrane
	permeability, ampicilling and totracycline-resistance as well as
	spontaneous mutation rates are performed in the laboratory of
5 Test Concentrations:	
Pre-Experiment/Experiment	9. 3; 10, 33; 30; 33\$, 1000; 2500; and 5000 μg/plate
Experiment II:	33; 100; 333; 1900; 2500; and 3000 µg/plate
B Study Design and Metho	ids of the state o
Test performance	The study was conducted at
	Germany) from January 19th to February
14 th ,	
	ZOÍ2. TO O TO
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
4 5 11 1 ( ) ( ) ( )	

## 

To evaluate the topicity of the test item pre-experiment was performed with all strains used. Eight concentrations were tested for toxicity and mutation induction with each 3 plates. For each strain and dose level, including the control three plates were used.

Toxicity of the test item can be evident as a reduction of the number of spontaneous revertants or a clearing of the bacterial background lawn. In this assay  $100~\mu L$  test solution (solvent or reference mutagen solution (positive control)) 500  $\mu L$  89 mis S9 mix substitution buffer and 100  $\mu L$  bacterial suspension were mixed in a test tube and overlay agar (45 °C) was added to each tube. The mixture was poured on minimal agar plates and after solidification the plates were incubated upside down for at least 48 hours at 37 °C in the dark.

## 2 Pre-incubation assay:

In the pre-incubation assay  $100~\mu L$  test solution (solvent or reference mutagen solution (positive control)), 500  $\mu L$  Symix / Symix abstitution buffer and 100  $\mu L$  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 and after solidification the plates were incubated upside down for at least 48 hours at 37 °C in the dark.



### 3 Statistics:

According to OECD guideline 471, a statistical analysis of the data is not mandator

## 4 Acceptability of the assay:

The Salmonella typhimurium reverse mutation assay is considered acceptable if it meets the 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 our historical data the positive control substances should produce a significant increase in mutant colors a minimum of five analysable dose levels should be present with at least the howing no signs of toxic effects, evident as a reduction in the indication factor of 0.5.

  tion criteria:

  1 is considered to the indicator of the i

## 5 Evaluation criteria:

A test item is considered as a mutagen if a piologically revent picrease in the number of revertants exceeding the threshold of twice (strains TA 98, TA 100, and PA 100) or three (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 experiments.

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

## II Results and Discussion

## A Analytical determinations:

The stability of technical AE F147447 and the stability and homogeneity of technical AE F147447 in the solvent and analysis of achieved concentration were not performed as part of this study.

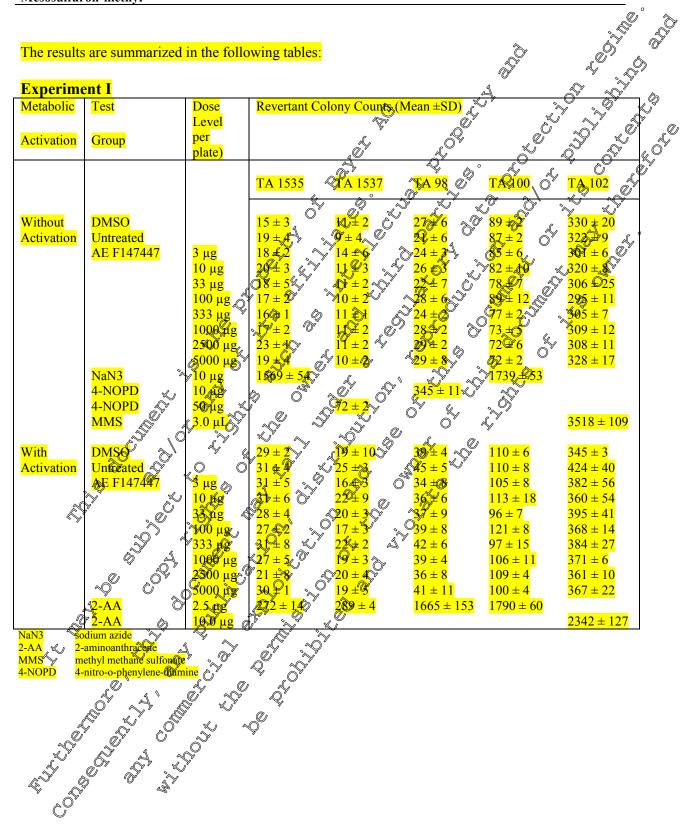
## B Preliminary cytotoxicity assay:

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without S9 mix in all strains used No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.3), occurred in the test groups with and without metabolic activation.

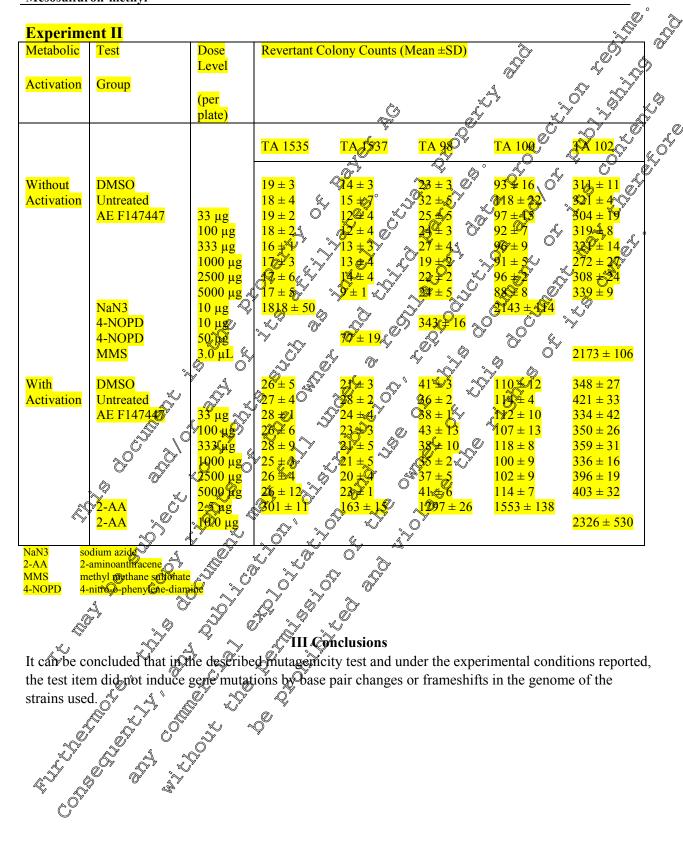
## C Mutation assays;

No substantial increase in reversant colony numbers of any of the five tester strains was observed following treatment with AE 147447 at any concentration level, neither in the presence nor absence of metabolic activation (\$9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Approprate reference mutagens were used as positive controls. They showed a distinct in-crease in induced revertant colonies.











Report:	KCA 5.8.1 /02; 2012;M-433931-02; Amended: 2015-01-30
Title:	In vitro chromosome aberration test in Chinese hamster V79 cells with AE F147447
Report No:	1462102
Document No:	M-433931-02-1
Guidelines:	Ninth Addendum to the OECD Guidelines for Testing of Chemicals, February 1998 adopted July 21, 1997, Guideline No. 473 devitro Mammatan Chromosome Aberration Test; Commission Regulation (EC) No. 440/2008, B10: Mutagenicity - Idevitro Mammalian Chromosome Aberration Lest, dated May 30, 2008; United States Environmental Protection Agency Health Effects Test Guidelines, ODPTS 870.5375 on Vitro Mammalian Chromosome Aberration Test, EPA 712-C-98-233, August 1998 not specified
GLP/GEP:	yes y y y y y y

## Executive summary

The test item AE F147447, dissolved in conture medium was assessed for its potential to induce structural chromosome aberrations in V79 cells of the Chinese hanster in vitroin two independent experiments. The following study design was performed.

	U// h SX h	(//S) JI S)		
	Wothout 89 mi		With Somix	
***	Exp.	Exp. J.	Exp. Y	Esp. II
Exposure period 🗸	Chours \$	18 hours	4 hours	4 hours
Recovery	14 hours		1# hours	ĺ¥ hours ∕
Preparation interval	18 hours/	Le hours	18 hours	18 hours

In each experimental group two parallel cultures were set up 200 metaphases per culture were evaluated for structural chromosome aberrations.

The highest applied concentration (2936.0 µg/mL) approx. 10 mM) was chosen with regard to the molecular weight and the purity 98.9 % of the lest item and with respect to the current OECD Guideline 473. Test item concentrations between 11.5 and 2936.0 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity. Since the cultures falfilled the requirements for cytogenetic evaluation, this preliminary test was designated experiment. If since no cytotoxicity and test item precipitation was observed in the first experiment, concentrations between 11.5 and 2936.0 µg/mL in the absence of S9 mix and between 183.5 and 2936.0 µg/mL in the presence of S9 mix were chosen for the second experiment. No clastogenicity was observed at the concentrations evaluated either with or without metabolic activation. In Experiment I in the absence of S9 mix statistically significant increases were observed after treatment with 1468 and 336.0 µg/mL, however, the values are within the range of the historical solvent control data (0.0 4.0 % aberrant cells, excluding gaps) and therefore considered as being biologically irrefevant. No evalence of an increase in polyploid metaphases was found after treatment with the test item as compared to the frequencies of the control cultures.

Appropriate positive controls induced statistically significant increases in cells with structural chromosome aberrations.



Thus, it can be stated that under the experimental conditions reported, the test item did not induce structural chromosome aberrations in V79 cells (Chinese hamster cell line) in vitro. Cherefore, AE
F147447 is considered to be non-clastogenic in this chromosome aberration test up to the highest required concentration.

I. Material and methods

A Materials

1 Test Material:

Description:
Lot/Batch:
SES 10681-2-2
Purity:
Stability of test compound:
Solvent:

(chosen

AE F147447

98.92 w/w. Dose calculation adjusted to purity
not performed as part of this study
MEM culture recdium from
Cermany

2 Control materials:
Negative:

MEM Tissue Culture medium structural chromosome aberrations in V79 cells (Chinese hamster cell line) in vitro. Therefore, AE

1 Prssue Enlture medium Negative:

Positive, w/o

ENS; ethylmethane sulfonate, Ossolved in Nutrient medium, final Concentration: 1000.0 μg/mL @experiment I) 600.0 μg/mL

c activation:

PA; coclophosphamide, dissolved in Saline (0.9 % [w/v]), nal Concentration 1.4 µg/mL

The dilutions of the stock solutions were prepared on the day of the experiment. The stability of the positive control substance (EMS and CPA, respectively) in solution is unknown but a mutagenic responsion the expected range was sufficient biological evidence of chemical stability.

### S9 from male Wistar rats (Hsd Cpb: WU) 3 Metabolic activation \$9 mix:

The S9 was prepared from 4 - 12 weeks of male Wistar rats (Hsd Cpb:

The Netherlands) weight approx. 220 - 320 g induced by intraperitoneal applications of 80 mg/kg b, W. phenobarbital ( Germany) and by orally administrations of 80 mg/kg b.y β-naphthoflavone ( Germany) each, on three consecutive days Each batch of S9 mix was routinely tested with 2-aminoanthracene as well as benzo(2) benzo(2) benzo(2) 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 V79 cell line has been used successfully for many years in in vitro experiments with a high proliferation and a reasonable plating efficiency.



The cells have a stable karyotype with a modal chromosome number of  $22 \pm 1$ .

## **5 Test concentrations:**

Preparation	Exposure	Concentration			∂ _A	***	%		
interval	period	in μg/mL				Ű,			
		Without S9 m	ix	L		Ö.	.,0	- \$9'	
18 hours	4 hours	11.5 22.9	45.9	91.80	183.5	36 7.0 <b>734.0</b>	1468.0	<b>29</b> 36.0 0	
							\$ ~		_@`
18 hours	18 hours	11.5 22.9	45.9	91.8	183.5	₹367 <b>.0© 734.0</b>	14689	2936.0	
			<u> </u>	Į Š			\$		
		With S9 mix	0	« »				<i>y 4</i>	ĺ
18 hours	4 hours	11.5 22.9	45.9	<b>90</b> 7.8	Ø83.5 ⊀	₽367.0 <b>₽734.0</b>	1468.0	29360	a.°
				* ^					O Y
18 hours	4 hours				18375	<b>36</b> 7.0 <b>734.0</b>	1468.0	<u> 2</u> 936.0	ď
		_ (	24 7					» O ~	

Evaluated experimental points are showing bold characters

## B Study Design and Methods

1 Study performance:

The study was conducted at

respectivelv

Germany). The experimental start and completion Ontes of the stody were January 25th 2012 and Major 26th 2012,

## 2 Culture Medium and Conditions:

For seeding and treatment of the cell cultures the colture medium was MEM (minimal essential medium) containing Hank's salts, glutamine and Hepes (25/mM), Additionally, the medium was supplemented with penicillin Streptomycin (000 U/ML/ 100 µg/mL) and 10 % (40) feta bovine serum (FBS). All cultures were incubated at 37 % in a numidified atmospher with 1.5 % CO2 (98.5 % air).

## 3 Seeding of the Cultures:

Exponentially frowing stock cultures more than 50% confident were rinsed with Ca-Mg-free salt solution containing 8000 mg/L Na(S), 200 mg/L Kcl, 200 mg/L KH2PO4 and 150 mg/L Na2HPO4. Afterwards the cells were created with pypsin-DDTA foliution at 37% 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-Mgfree salt solution. The cell's were seeded into Quadriperm dishes, which contained microscopic slides. Into each chamber 1 x 10 4 6 x 10 4 cell were solded with regard to the preparation time.

## 4 Treatment after the hour exposure period:

The culture medium of exponentially growing cell cultures was replaced with serum-free medium containing the test frem. For the treatment with metabolic activation 50 µL S9 mix per mL culture medium were added.

After A 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 • H₂O, 192 mg/L Na₂HPO4 • 2 H₂O and 150 mg/L KH₂PO₄. The cells were



then cultured in complete medium containing 10 % (v/v) FBS for the remaining culture time of 14 hours. All cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % CO₂ (9.55 % air).

Colcemid was added to the culture medium (0.2 µg/mL) 15.5 hours after the start of the treatment. The cells were treated, 2.5 hours later, on the slides in the chambers with hypothesic solution (0.4 % Cell) for methanol and  $\frac{1}{2}$  and  $\frac{1}$ methanol and glacial acetic acid (3:1 parts, respectively). After preparation the cells were stained with Giemsa and labelled with a computer-generated random code to prevent scorer bias

### **6 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 becounting 10 defined fields per coded slide. The cell number of the treatment groups is given in percentage compared to the respective solvent control.

## 7 Analysis of Metaphase Cells

Evaluation of the cultures was performed according to the OECD grideling using NIKON microscopes with 100x objectives. Breaks fragments, deletions exchanges, and chromosome disingegrations were recorded as structural chromosome aberrations Gaps were also recorded but not included in the calculation of the aberration rates. 100 well spread metaphases per culture were evaluated for cytogenetic damage on coded slides.

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

## 8 Acceptability of the Test:

The chromosome abernation test is considered acceptable, if it meets the following criteria:

- The number of structural aberrations found in the solvent controls falls within the range of the laboratory historical confrol 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 historical control data.

## 9 Evaluation of Results:

A test item is classified as non-classogenis in the number of induced structural chromosome aberrations in all evaluated dose groups in the range of the aboratory historical control data and no significant increase of the Rumber of structural Romonosome aberrations is observed.

A test item icclassified as castogeoic if the number of induced structural chromosome aberrations is not in the range of the Yaboratory historical control data and either a concentration-related or a significant increase of the Rumber of structural chromosome aberrations is observed.

Both prological 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.



In this study together with chromosome aberration also polyploids and endoreduplications were considered. A test item can be classified as an eugenic if the number of induced numerical aberrations is not in the range of the laboratory historical control data.

## II. Results and discussion

Two independent experiments were performed. In Experiment I the exposure period was 4 hours with and without S9 mix. In Experiment II the exposure period was 4 hours with 59 mix and 18 hours without S9 mix. The chromosomes were prepared 18 hours (Exp. 11) after stant of treatment with the test item. In each experimental group two parallel cultures were set up. 100 metaphases per valuated for structural chromosome aberrations.

No relevant influence on osmolarity was observed in Experiment II the exposure period was 4 hours with 59 mix and 18 hours with 39 mix and 18 hours with 30 mix and 30 mix

No relevant influence on osmolarity was observed. In Experiment I as well as in Experiment II, the pH of the stock solutions (2.94 mg/mL without S9 mix) and 9.09 mg/mL with S9 mix) was adjusted prior to the preparation of the dilution series by using small amounts of 2N NaOH.

No cytotoxicity, indicated by reduced medices of reduced cell numbers could be observed in both experiments up to the highest applied 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. The aberration rates of the cells after treatment with the test item (0.5—2.5 % aberrant cells, excluding gaps) were within the range of the solvent control values (0.0—2.5 % aberrant cells, excluding gaps) and within the range of the laboratory historical solvent control data.

Two statistically significant increases were observed in Experiment I after treatment with 1468.0 and 2936.0 μg/mL. Since these increases of 2.0% aberrant cells, excluding gaps were within the laboratory historical solvent control data range (0.0 – 4.0% aberrant cells, excluding gaps), this has to be regarded as being biologically irrelevant.

No evidence of an increase in polyploid metaphases was found after treatment with the test item as compared to the frequencies of the control cultures.

In both experiments, either EMS (1000.0 or 600.0  $\mu$ FmL) or CPA(1.4  $\mu$ g/mL) were used as positive controls which showed district increases in cells with structural phromosome aberrations.

The results are summarized in the following table:



Exp.	Preparation interval	Test item concentration in µg/mL	Cell numbers in % of control	Mitotic indices in % of control	incl. gaps*	Aberrant cells  thy% excly gaps*	with exchanges
		-	Exposure p	oeriod 4 hrs with	out S9 mix	0.0 8	0.0 0.5 0.0 0.0 0.5
I	18 hrs	Solvent control ¹	100.0	10000		<mark>0.0</mark>	0.0
		Positive control ²	n.d.	<b>₹19.8</b>	21.0	20.5°	145
		734.0	98.1	115.8	1.50°	2 1.5 5	0.5
		1468.0	90.2	J11.1	)	o sos	2 0.5
		2936.0	96.9	115.0	2.0 °	7 1.5 7 2.0 ^s	0.0
			Exposure p	eriod 18 krrs with	2.0 1.5 1.5 2.5 2.5 2.5 2.5 2.5	Q 1.	
II	18 hrs	Solvent control ¹	100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%, 100.0%	100 % 100 % 81.5 85.9 107.5	2.5 ×	72.5 10.5 10.5	/ 4 <mark>0.5</mark>
		Positive control ³	S nat.	8 <u>1.5</u>	% 120°	10.5°	2.5
		734.0	91.6 %	85.9 107/5	0 <mark>1.5</mark>		0.0
		1468.0	7 <mark>790</mark>	10%5	, <del>0</del>	0.5	0.0
		29369 O	<b>3</b> 0.3	904.4 ~	<b>2.5</b>	0.5	0.0
		Positive control 734.0	<b>Exposure</b>	P04.4	h S9 mix		
I	18 hrs	Solvent control ¹	<b>100.0</b>	¥100.0	<b>3.0</b> /	2.0	0.0
	Ô	Positive control*	n.d.y	59 <b>9</b>	44.0 ©	42.0 ^S	13.0
	\$ ⁰	734.0	<b>2009</b>	<b>3</b> 88.0	\$ <b>3.0</b>	2.0	1.0
	~~~	1468.0 Q	102.3	599 588.0 104.2	<b>2</b> 0.5	0.5	0.5
4		2936.0	102.3 102.8 102.8 200.0	\$88.0 \$104.2 \$100.4	0.5	2.0	0.0
II	18 hrs	Solvent control	\$ 000.0 ×	\$\frac{100}{5}2	4.0	3.5	1.5
	Ö		4 " O'	Oʻ 😞	<mark>24.5</mark>	24.0 ^S	<mark>6.0</mark>
		1468.0 2936.0 447 showled no say	9 <mark>4.6</mark> . O	93.0	2.5	2.5	0.0
		1468.0	96 8 °	100.4	2.5	2.5	0.0
			(U' 📉 🔉	&j			



Report:	∃; ;2012;M-433935-01
Title:	Gene mutation assay in Chinese hamster V79 cells in vitro (V79 / HRRT) - AE F147047
Report No:	1462103
Document No:	M-433935-01-1
Guidelines:	Ninth Addendum to the OECD Guidelines for Testing of Chemicals, Section No. 46: In vitro Mammalian Cell Gene Mutation Test adopted July 24, 1997; Commission Regulation (EC) No. 440/2008, B17, date May 30, 2008 United States Environmental
	Cell Gene Mutation Test, EPA 712-698-221, August 1998;not specified
GLP/GEP:	yes of the second of the secon

Executive summary:

The study was performed to investigate the potential of AE F 47447 to indice genemutations at the HPRT locus in V79 cells of the Chinese hamster.

The assay was performed in two independent experiments, using two parallel cultures each. The first main experiment was performed with and will out liver microsoma activation and a treatment period of 4 hours. The second experiment was performed with a treatment time of 4 hours with and 24 hours without metabolic activation.

No substantial and reproducible dose dependent increase of the mutation frequence exceeding the historical range of solvent controls was observed in both main experiments.

Appropriate reference mutagens, used as positive controls, induced a distinct increase in mutant colonies and thus, showed the sensitivity of the lost system and the activity of the metabolic activation system. In conclusion it can be stated that under the experimental conditions reported the test item did not induce gene mutations at the HPR Tocus in V79 cells. Therefore, AF @147447 is considered to be nonmutagenic in this HPRT ssay.

I Material and methods

A Materials:

1 Test Material

SE**S** 106862

Lot/Batch: Purity:

% w/w, Dose calculation adjusted to purity

Sability of test compound

not performed as part of this study.

Solvent used∻

2 Control materials:

Tissue Culture medium

Ethyl methanesulphonate (EMS) dissolved in nutrient medium.

Final concentration: 0.15 mg/mL (1.2 mM).

Postrive: with activation (+S9 mix):

DMBA; 7,12-dimethylbenz(a)anthracene dissolved in DMSO.

Final concentration 1.1 μ g/mL (= 4.3 μ M).



3 Metabolic activation S9 mix: S9 from male Wistar rats

Phenobarbital/B-Naphthoflavone induced rat liver S9 was used as the metabolic activation system. Phe S9 was prepared from 8 - 12 weeks old male Wistar rats (Hsd Cpb: WU, weight approx. 220 – 320 g/s , The Netherlands) induced by i.p. applications of 80 mg/kg b.w. phenobarbital and by peroral administrations of β-naphthoflavone each, on three consecutive days. The livers were prepared 24 fours after the last treatment. The S9 fractions were produced by diffution of the liver homogenate with a KClo solution (1+3) followed by centrifugation at 9000 g. Cofactors were added to the S9 supernatable to reach following concentrations in the S9 mix were: 8 mM MgCl₂, 33 mM KCl, 5 mM glucose-6-phosphate and 4 mMNADP in 100 mM sodium-ortho-phosphate-butter, pH 7.4

4 Test cells:

The V79 cell line has been used successfully in vitto experiments for many years. Especially the high, proliferation rate (doubling time 12 -16 h is stock cultures) and a good cloning officiency of untreated cells (as a rule more than 50 %) recommend the use of this cell line. The cells have a stable karyotype with a model abromosome number of \$22. with a modal chromosome number of \$2

5 Test concentrations:

		<i>i</i> ~-	~* ~~			<u> </u>	<u>(2)</u>
Exposure	S9	Concentr	adions in us	g/m I Ø	10,		· S
period	mix	لئے '	4				
	*	Experime	ent/Q		, O' (
4 hours	- 0	156.3	3 12.5 0	625	4250 C	2500 √	3000
4 hours		1 56.3 √	312.5	625.0	P1250 @	2500	3000
		Experime	ent _e II 🏻 🔈				7
24 hours	- &	156പ്പ	3 2.5 ₆ 0	625,0	1250	×2500×	3000
4 hours	+0"	156.3	312.5	62 \$ Ø	¥250 S	2500	3000

Concentrations given in bold letters were chosen for the mutation rate analysis

was conducted at

1 Study performance:

The study

Completion Germany). The experimental start and completion dates of the study were January 26th 2012 and March 29th 2012 respectively.

2 Culture medium and conditions:

For seeding and treatment of the cell cultures the complete culture medium was MEM (minimal essential medium) containing Hank salts, neomycin (5 µg/mL) and amphotericin B (1 %). For the selection of mutant cets the complete medium was supplemented with 11 μg/mL 6-thioguanine. All cultures were 35°C in a humi@fied atmosphere with 1.5 % CO₂ (98.5 % air).



3 Seeding of the cultures:

Two to three days after sub-cultivation stock cultures were trypsinized at 37 °C for Sminutes. There he 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 PSS. EDTA (ethylene diamine tetraacetic acid). Approximately 1.3 x 10⁶ (single culture) and 6 x 10² cells (in duplicate) were seeded in plastic culture flasks. The cells were group for the college group for the college group for the cells were gro

4 Treatment:

After 24 hours the medium was replaced with sering-free medium containing the test from, 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 ph was adjusted to 7.2 The colonies used to determine the closing efficiency (survival) were fixed and stained approx. 7 days after treatment. Three or four days after treatment 3.5x 1 cells per experimental point were subcultivated in 175 cm² flasks containing 30 medium. Following the expression time of 7 dates five 80 cm² cell culture flasks were seeded with about 3 – 5 x 0 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 humical fied atmosphere with 1.5 % CO2 for about 8 days. The colonies were stained with 10 % methylene blue in \$61 % KOH solution.

The stained colonies with more than So cells were counted. In doubt the colony size was checked with a preparation pricroscope.

6 Acceptability of the Assavo

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

- the numbers of mutant commission of mutant commissi laboratory historical control data (see Annex).
- the positive control substances should produce a significant increase in mutant colony frequencies.
- the loning efficiency Il absolute values of the solvent controls should exceed 50 %.

7 Evaluation of Results:

A test item is classified as positive if it induces wither a concentration-related increase of the mutant frequency or a producible 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 of any of the test points is considered non-mutagenic in this system.

A positive response is described as follows:

A test item is lassified as mutagenic if it reproducibly induces a mutation frequency that is three times above the spontantous nutation frequency at least at one of the concentrations in the experiment. The test tem 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. 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 into consideration.

8 Statistical Analysis:

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

II Results and discussion

No relevant toxic effects occurred up to the maximum concentration of 3000 yeg/ml equal to approximately 10 mM.

No relevant and reproducible increase of matant colony numbers 106 cells was observed in the main experiments up to the maximum concentration. The majant frequency remained well within the historical range of solvent controls. An increase of the rouction in the second experiment without metabolic activation at 2500 µg/mL (custure II) and 2000 µg/mL (both cultures), and in the second culture of the second experiment with metabolic activation at \$12.5, \$250, \$200, and 3000 ag/mL were judged as biologically irrelevant Quetuations sirve they were based on a rather low mutation frequency of the solvent controls (4.9.4.4, and 3.6). The absolute values of the mutation requestry even remained within the actual range of the solvent controls of this study (3.6 to 20% colories per 0.6 cells).

A linear regression analysis (least squares) was performed to assess a possible dose dependent increase of mutant frequency. A agnificant dose dependent trend of the mutation frequency indicated by a probability value of \$0.05 was determined in the second experiment at both cultures without metabolic activation. However, the trend was judged as biologically irrelevant since the mutation frequency did not exceed the historical solvent control range discussed dove.

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

The results of experiments I and I are summarized in the following table:

₩	_~>/	>	A 100 m							
	Z rélative 1	relative r	elative	myrant		<mark>relative</mark>	<mark>relative</mark>	relative	<mark>mutant</mark>	
	<mark>89</mark> clon o ğ	cell, c	clowing a	wionies	<mark>induct</mark>	cloning	<mark>cell</mark>	cloning	colonies	<mark>inducti</mark>
ration 🔏	1		?, d(,)		<mark>ion</mark>					<mark>on</mark>
μ/mL n	nix efficience			10 ⁶	<mark>factor</mark>	efficiency	density	efficiency efficiency of the second of the s	10 ⁶	<mark>factor</mark>
	Cy <mark>i</mark> ~~	I N	eficiency 10	<mark>cells</mark>		<mark>I</mark>		<mark>II</mark>	<mark>cells</mark>	
	%	<mark>%</mark> 9	<mark>%</mark>			<mark>%</mark>	<mark>%</mark>	<mark>%</mark>		
Column 1 2	2 3 3 . V	4 5	5	<mark>6</mark>	<mark>7</mark>	8	<mark>9</mark>	<mark>10</mark>	<mark>11</mark>	12
Experiment 14 h			<mark>culture I</mark>					<mark>culture II</mark>		
treatment										
Solvent -	100.0	100.0	100.0	<mark>9.6</mark>	1.0	100.0	100.0	100.0	<mark>9.2</mark>	1.0



MICSUST	ılturon-	metnyi										
control												
with												
DMSO Positive	150.0		88.7	122.8	85.8	81.6	8.5	80.1	82.1	62.8	1054	
control	100.0	_	00.7	122.0	00.0	01.0	(Ö	82.1 A	, <mark>02.0</mark>		
(EMS)								₹ 		Č		
Test item		<u>-</u>	95.2		ture was no	t continue	ed*			()	continue	() X
Γest item		<u>-</u>	92.3	110.9	106.3	14.1	<mark>√12.5</mark>	94.2	1000	1 1 1 1 2	3.4	0.4 @
Γest item		-	88.7	111.5	99.6	7.5	0.8	86.5	3107.5	83.8 C	9.3	1.0
Γest item		-	13.9	102.6	92.3	33.5	₩	93.7	99.6 V	919.5	8.7/°	1 2.3
Γest item Γest item			87.9 92.5	108.7 124.1	99.7	9.7 7 16.3	1.0 @		97.©° 2 <mark>8</mark> 4.5	91.3	7.6 (20.7)	0.80
Solvent	2000.0	+	100.0	100.0	89.7 100.0	٥. /	21.0	100 n C	100.0 T	1060	7.0°	2.0
control			100.0	100.0		, <mark>1</mark> 4.9		100.0 C				
with				4) á			
<mark>OMSO</mark>				654.3 654.3		*					**************************************	
Positive	1.1	+	45.5	63/3 6	91.7	³ 481.	32.3	44.0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	8 <mark>8</mark>	9 <mark>3.9</mark>	₹ <mark>465.0</mark>	<mark>56.0</mark>
control DMBA)			*				4	~ ~				
Test item		+	105.4	© cul	tore was no	t continue	torio de la company de la com	98.7 98.7	& cult	was not	t continue	ed*
Γest item		+ 4	99.2 <	90.3	93.6	9.4		95.5	917.4 \$		13.2	1.6
	60.7.0		♠ \		Mac d					7		1.0
<mark>Γest item</mark>	625.0		107.9 8	<mark>⊕</mark> 02.9	96.4	12.9	0.9	94.30°	<mark>1405.6</mark> U	94. 2	14.7	1.8
Γest item	1250,0	+	103.9	1013 05	2.8	5.1 \$	0.3	2 <mark>102.9</mark>	<mark>106.6</mark>	94. 3	11.5	<mark>1.4</mark>
Γest item		+ ~	96.5 «	92.3	7 950	29.9	10.7	2 ⁷ / ₉	110.1	94.	11.4	1.4
<mark>Γest item</mark>	3000.0	& S	Ø33	A 9	83 7 N	105	0.7	101.9	90.4	6 92.	<mark>7.9</mark>	1.0
		¥				- 65 ×				6		
Experime reatment	ent 145 <mark>24</mark>	h		Q,	100.0		I)		<u>(</u>	culture II		
Solvent	<mark>/</mark>	<u>-</u> «	100.0	100.6	100.0	3.9	1.0	100.0	100.0	100	<mark>4.4</mark>	1.0
control (()	O,		100.00 Q					<mark>.0</mark>		
with	Ő											
OMSO Positive	1500		950	844	≈ <mark>87.6</mark>	535.	108.1	102.0	92.9	<mark>62.</mark>	615.2	138.5
control (3 <mark>07.0</mark>	5 5	100.1	102.0	94.)	9	013.2	136.3
EMS)		ř Ş		7 								
Test item	<u> </u>	- 0	102.8		ture was no		e <mark>d*</mark>	<mark>99.7</mark>	cult	ture was not		ed*
<mark>Γest item</mark>	912.5	-	<mark>99.5</mark>	<mark>96.7</mark>	100.0	<mark>5.6</mark>	<mark>1.1</mark>	101.2	112.2	<mark>97.</mark>	<mark>8.5</mark>	1.9
										2		



1,100000	iiiui vii-)
Test item	625.0	•	103.8	<mark>96.5</mark>	<mark>96.7</mark>	8.7	1.8	<mark>99.7</mark>	112.8	81.	8.6	(1), (
Test item	1250.0	-	101.2	90.7	104.3	12.1	2.5	102.4	93.3	100 .1	10.0	2.2	
Test item	2500.0	-	101.6	<mark>98.9</mark>	<mark>86.0</mark>	11.1	2.2	<mark>≱</mark> 100.1	104.8	74. 8 Č	13.8 200	3.1	
Test item	3000.0	_	101.7	<mark>71.0</mark>	84.0	19.4	3.65×	100.5	903.6	7 4.	1950	4.4	J. Ó
Experime treatment						\$\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	, D		1000				
Solvent control with DMSO	l	+	100.0	100.0	100.0	20.4 20.4 20.4 20.4 20.4 20.4 20.4 20.4	4.0 4.0 4.0 4.0 4.0 4.0 4.0 4.0	2100.0 P	10000	100 .0	3.6 A	1.0 2	
Positive control (DMBA)	1.1	+	<mark>49.6</mark>	56.6 \$\infty\$	89.4 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	988. 8		58/6 95.4		950 6	11850 2	327.0	
Test item	<mark>156.3</mark>	+	<mark>96.1</mark>	cultu	re was hot o	ontinue	<mark>d*</mark> 🎓 🖔	95.8	culture	was no	t continue	<mark>d*</mark>	
Test item	312.5	+	99.1		<mark>940</mark>	9.7 2	0,5	92.7 «		§3. ₹ 6	13.9	3.8	
Test item		<mark>+</mark>	92.3	98.1		13.7	<mark>0.₹</mark> ∑	96.8	124.0 ×	<mark>95.</mark> 9	<mark>3.3</mark>	<mark>0.9</mark>	
Test item		20 +0 20	9 33	106.0	90.4 ~	14.7	9 <mark>0.7</mark> 3		97.9°	96. 2	13.6	3.8	
Test item	2500 o	<mark>+</mark>	95.3	10 <u>1</u> .\$	91,4	3 75.7	X	93.5 «) /	<mark>96.7</mark>	<mark>95.</mark> 6	<mark>16.9</mark>	<mark>4.7</mark>	
Test item		+	9 <mark>92.8</mark>	93.4 ()	93.3 ***	15 <mark>9</mark>		94.9 2	97.5	91. 2	<mark>14.0</mark>	3.9	
* culture	was not	continue	ed sonce a	min mum q	only four	analysal	ole conce	ntrations is	required				
				~, 0		~~~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~						-

Technical AE F147447 did not demonstrate inutagenic potential in this in vitro HPRT cell mutation assay, under the experimental conditions described.



AE F160460

Report:	6;	;2012;M-428745	-01 g	7	\$ & \(\tilde{\chi} \)
Title:	Salmonella typhimuriun	n reverse mutation assay	with AE F160460		
Report No:	1462301	۵	Į, Š	, O'	
Document No:	M-428745-01-1			, W	
Guidelines:	OECD No. 471, adopte		Q)"
	EC No. 440/2008 B13/1		Z,		
	EPA OPPTS 870.5100.	, EPA 712- 6 ,98-247, Au	guSt 1998;not sp	crified "	
GLP/GEP:	yes		y 03 4		

Executive summary:

This study was performed to investigate the potential of AE @16046@ to induce gene mutations according to the plate incorporation test (experiment H and the pre-incubation test (experiment II) using the Salmonella typhimurium strains TA 1535 TA 1537, TA 98, TA 100, and TA 102.

The assay was performed in two independent experiments both without ther microsomal activation. Each concentration, including the controls, was tested in triple ate. The test frem was tested at the following concentrations:

Pre-Experiment/Experiment I: 3; 10; 33; 100; 333; 1000; 2500; and 5000 ug/plate

© 33; Φ00; 333; 100 € 2500; and 5000 μg/g rate Experiment II:

The plates incubated with the test item showed formal background growth up to 5000 µg/plate with and without metabolic activation in both independent 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 nambers of any of the five tester strains was observed following treatment with AEF 160460 at any dosedevel, wither in the presence nor absence of metabolic activation (\$9 mix). There was also no tendence of higher mutation rates with increasing concentrations in the range below the generally orknowledged border of biological relevance.

Appropriate reference mutagens as positive controls showed a distinct increase of induced revertant colonies.

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test stem did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used?

genome of the strains used. Therefore, AE F160460 as considered to be non-mutagenic in this Salmonella typhimurium reverse mutation assay.

I. Material and methods

If white powder SES 11562-12-4

96.7 % w/w, Dose calculation adjusted to purity

tability of test compound: not performed as part of this study.

Solvent used: **DMSO**



					<i>_</i>
2 Control materials:					
Negative:		Culture medium		ð	
Solvent:		DMSO			
Positive:	non- activation	ı (-S9 mix):		A .	
		sodium azide: 10 ug	/plate for TA1535	and TA100	or TA 1537
		4-nitro-o-phenylene	Miamine, 4-NOPD	o: 50 ug/plate f	or TA 1537
		and 10 ug/plate for	ГА98		
		sodium azide: 10 µg 4-nitro-o-phenylene and 10 µg/plate for methyl methane sulf 59 mix): 2-aminoanthracene, TA 98, TA 100 and	Conate. MAS: 3.0 a	uL/plate for T	102
	activation (+ S	59 mix) :			
		2-aminoanthracene?	2-AA 2.5 2.5 2.5 play	for TA 1535	% TA 1507.
		TA 98, TA 100 Jand	10 ug/plate for T	X 102	ý "V"
3 Metabolic activatio		S9 mix derived from	nale Wistar rats		
Phenobarbital/β-naphth	noflavone induc	edorat liyer \$9 were us	sed as the metabol	c activation sy	stem The S9
is prepared from 8 – 12					
		The Netherlands) in			
mg/kg b.w. phenobarbi	ital (any and by oral		
naphthoflavone (, Germa	ny)Æach batcl	h of S9 mix
is routinely tested with	2-aminoanthon	cene as wellas benzo	a]pyrene. Coractor	sare added to	the S9 mix
to reach the following of	concentrations i	n the S9 mix: 8 mM N	12C12, 334mM KC	1.5 mM Gluce	ose-6-
phosphate and 4mM N	JADP_in_700 m/	sodium-ortho-phosi	Pate-byffer, pH 7	.4. D uring the (experiment
the S9 mix was stored	m an ice bath				-
		Sodium-ortho-phosics (1980)		V	
4 Test organisms:	D" ~ «				
Salmonella typhimurio	m, strains TAIS	35, TA1537 TA98 T	A 199 and TA 102	obtained from	1
7/25	Ge:	rmany). Régular checl	king of the propert	ies of the strain	
the membrane permeat	ity, appicillic	and tetracycline-rest	stance as well as s	pontaneous mi	
are performed in the la	boratory of				
			å "		
5 Test Concentration)		
Pre-Experiment/Experi	iment P.	3; (0); 33; (00); 330;	1000; 2500; and 5	000 μg/plate	
Experiment II:		33 ; 100, 333; 1000;	2500; and 5000 μg	g/plate	
			,	. 1	
B Study Design and	Methods: 🦠				
1 Test performance:		The study was cond	ucted at		
		A second	/Germany)	from January	19 th to
		March 2 nd 2012.	3 /	,	
the membrane permeatare performed in the falls Test Concentration Pre-Experiment Experiment II: B Study Design and Market Present Performance:	Î"				



2 Assay procedure:

Preliminary cytotoxicity/mutation assay:

To evaluate the toxicity of the test item a pre-experiment was performed with all strains used. Pight concentrations were tested for toxicity and mutation induction with each 3 plates. Toxicity of the test items background lawn. In this assay 100 μ L test solution (solvent or reference mutagen solution (positive) control)), 500 μ L S9 mix / S9 mix substitution buffer and 100 μ L test solution (positive) test tube and overlay agar (45 °C) was added to each tube. The mixture was poured on mormal agar plates and after solidification the plates were incubated topside down for at least 48 hours at 30°C in the day.

Pre-incubation assay:

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 bacternal suspension were mixed in a test tobe and incubated at 37 °C for 60 minutes. After pre-iocubation 2.0 on Love May agair (45 °C) was added to each tube. The mixture was poured on minimal agar plates and after solidification the plates were incubated upside down for at least 48 hours at 37 % in the dark.

3 Statistics:

3 Statistics:
According to OECD 471 a statistical analysis is not mandators.

4 Acceptability of the assay:

The Salmonella typinimum 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 our kiistorical data 🔑
- the positive control swostances should produce a significant increase in mutant colony frequencies
- a minimum of five analysable dose levels should be present with at least three dose levels showing no signs of toxic effects, evident as a reduction in the number of revertants below the indication factor of 0.5

5 Evaluation criteria:

A test item is considered as a nutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice (strains TA 98, TA 100, and TA 102) or thrice (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. In increase exceeding the threshold at only one concentration is judged as biologically relevant if romoduced in an independent second experiment.

A dose dependent increase in the number of revertant colonies below the threshold is regarded as an indication of 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 increasors not considered biologically relevant.



II. Results and Discussion

1 Analytical determinations:

The stability of AE F160460 and the stability and homogeneity of AE F160460 in the solvent and analysis of achieved concentration were not determined as part of this study.

2 Preliminary cytotoxicity assay:

The plates incubated with the test item showed normal background growth up-to 5000 μg/plate with and without S9 mix in all strains used. No toxic effects, and dent as a reflection of the resolution of the

without S9 mix in all strains used. No toxic effects, wident as a reduction of the member of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation.

3 Mutation assays:

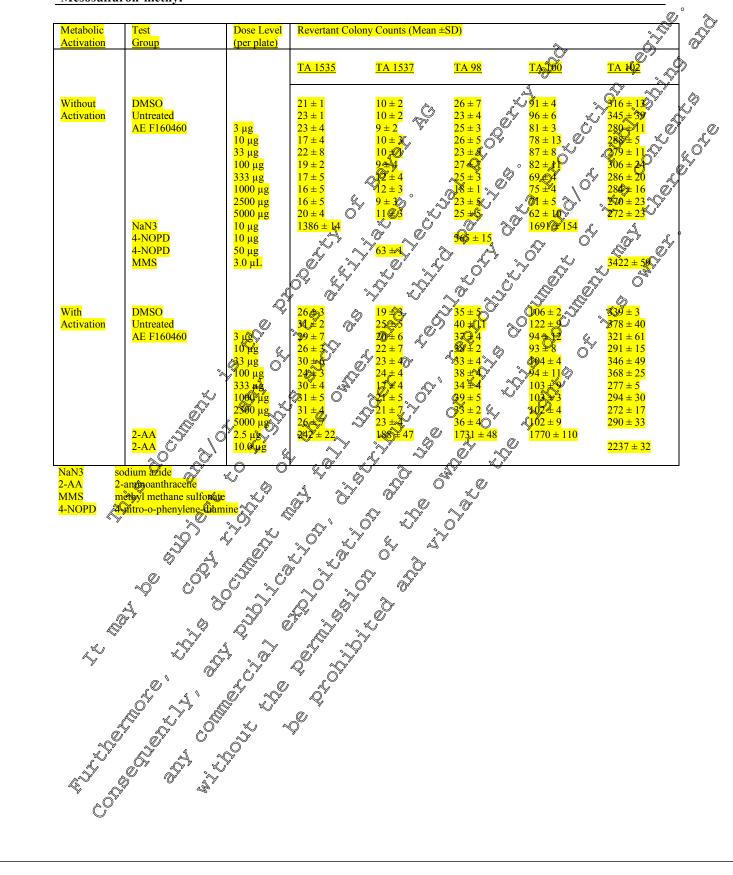
No substantial increase in revertant colony numbers of any of the rive tester strums was observed following treatment with AE F160460 arony dose level neither in the presence nor absence of metabolic activation (S9 mix). There was also not enderby of higher multiation rates with increasing Concentrations in

activation (S9 mix). There was also not endeave of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

Appropriate reference mutagens were used as positive controls and showed a distinguine increase of induced revertant colonies.

The results are summarized in the following tables:







Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colo	ony Counts (Mean =	÷SD)		TA 102
			<u>TA 1535</u>	<u>TA 1537</u>	<u>TA 98</u>	<u>TA</u>	TA 1/02
Without Activation	DMSO Untreated AE F160460	33 μg 100 μg 333 μg 1000 μg	$ \begin{array}{c} 18 \pm 3 \\ 14 \pm 2 \\ 17 \pm 3 \\ 16 \pm 2 \\ 18 \pm 7 \\ 16 \pm 3 \end{array} $	16 ± 6 23 ± 8 17 ± 4 15 ± 2 18	25 ± 5 21 ± 4 25 ± 4 25 ± 7 18 ± 2 31	104 ± 6 124 ± 14 104 ± 15 108 ± 11 109 ± 28 87 ± 9	
	NaN3 4-NOPD 4-NOPD MMS	2500 μg 5000 μg 10 μg 10 μg 50 μg 3.0 μL	12 ± 4 15 ± 2 1889 ± 33	デュ 13±3 13±3 14±20	29 ± 8 29 ± 5 372 € 24	96.913 101¥15 20077±83	399 ± 17 399 ± 17 394 ± 14 368 ± 23 340 ± 9 3580 ± 505
With Activation	DMSO Untreated AE F160460	33 µg 100 µg 33	21 ± 2 25 ± 4 20 0 4 18 ± 4 26 ± 3 22 ± 6 19 ± 4	23 ± 8 17 ± 4 15 ± 2 18 2 19 ± 6 27 ± 2 13 ± 3 20 ± 1 26 ± 3 18 ± 2 19 ± 4	$ \begin{array}{c} 32 \pm 6 \\ 22 \pm 5 \\ 32 \pm 5 \end{array} $ $ \begin{array}{c} 33 \pm 3 \\ 34 \pm 1 \\ 34 \pm 2 \\ 38 \pm 2 \\ 38 \pm 3 \end{array} $	13 + 12 13 + 7 13 + 7 143 + 15 143 + 10 122 - 7 130 + 10 224 + 21	483 ± 70 570 ± 81 464 ± 58 439 ± 32 449 ± 63 515 ± 35 516 ± 74
	2-AA 2-AA sodium azita	2.5 µg 10 Q µg	22 ± 6 19 ± 4 24 ± 8 326 ± 27	19 ± 4 246 ± 22	38 ± 52 1936 ± 52	J 1929 341	$\frac{523 \pm 47}{2967 \pm 656}$
NaN3 2-AA MMS 4-NOPD	4-nitro o-pheny one	fonate diamine		26 ± 3 18 ± 2 19 ± 4 24 5 ± 22 0 10 clusions		Y"	
Ŕ			III. C	omelusions			

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported AE E160460 and not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Report:	6; 2012; M-433910-02; Amended: 2015-01-30
Title	In vitro chroprosom aberration test in Chinese hamster V79 cells with AE F160460
Report No:	1462302
Document No: ©	M-4339 0-02-10
Guidelines:	Nintle Addendism to the OECD Guidelines for Testing of Chemicals, February 1998,
Guidelines:	adopted July 21, 1997, Guideline No. 473 In vitro Mammalian Chromosome Aberration
	Test; Commission Regulation (EC) No. 440/2008, B10: Mutagenicity - In vitro
	Mampalian Chromosome Aberration Test, dated May 30, 2008; United States
	Environmental Protection Agency Health Effects Test Guidelines, OPPTS 870.5375, In
A SA	specified
GLP/ĜĔP:	yes



Executive summary:

	t item AE F1604605, di			_	Al 39	
chromo	some aberrations in V7	9 cells of the	Chinese hamster	in vitro in two inde	ep e ńdent exp	eriments. The
followi	ng study design was pe	rformed:		A	ĺ.	
				Ö Ü		
		Withou	ut S9 mix	With S9	mix 💍	
		Exp. I	Exp. II	Exp	Exp II	
	Exposure period	4 hrs	18 hrs .	hrs	4 hrs	
	Recovery	14 hrs	4 . 0	0 140 rs	hrs	
	Preparation interval	18 hrs	hrs hrs	∂l8 hrs	5 18 hrs	
		Q,				

In each experimental group two parallel cultures were set up 100 metaphases per culture were evaluated for structural chromosome aberrations, except for the positive control in Experiment II without \$9 mix, where only 50 metaphases were evaluated. The highest applied concentration (24000 µg/mL) was chosen with regard to the solubility properties of the jest item and with respect to the current OECD Guideline 473. Dose selection was performed considering the toxicity data and the occurrence of precipitation. In the absence and presence of \$9 mix to cytotoxicits was observed up to the highest applied concentration. No clastogenicity was poserved at the concentrations evaluated either with or without metabolic activation. No evidence of the increase in polyploid metaphases was noticed after treatment with the test item as compared to the control cultures.

Appropriate positive controls induce ostatistically significant increases in cells with structural chromosome berrations.

It can be stated that under the experimental conditions reported, the test item did not induce structural chromosome aberrations in V D cells (Chinese hanger cell line) in vitro. Therefore, AE F160460 is considered to be not clastosenic in this chomosome aberration test, when tested up to the highest applied concentration.

I Material and methods

A Material:

Description:

Office the powder of the highest applied to the highest

Lot/Batch: SES 11562-12-4

26.7% w/w, Dose calculation adjusted to purity

not performed as part of this study.

DMSO

Šegative: MEM Tissue Culture medium

Positive - Without metabolic activation:



> EMS; ethylmethane sulfonate, dissolved in Nutrient medium. Final Concentration: 1000.0 µg/mL (Experiment I) 600.0 µg/ml (Experiment II)

Positive - With metabolic activation:

CPA; cyclophosphamide, dissolved in Saline (0.9 Final Concentration: 4 µg/mL

The dilutions of the stock solutions were prepared on the day of the experiment. The stability positive control substances (EMS and CPA, respectively) in solution is unknown but a moragenic response in the expected range was sufficient biological evidence of chemical stability.

3 Metabolic activation S9 mix:	S9 mix fro	m male Wist	ar râts	4		O"	-\$\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot
The S9 was prepared from 8 - 12 wee	ks old male	Vistar rats (
The Netherlands) w	eight approx	. 22 % 320 g					
80 mg/kg b.w. phenobarbital (, Ge	rmary) a	and by a	orally adj	Phistration	s of 80
mg/kg b.w. β-naphthoflavone (//N				ěrmaříy) ead	ch, on
three consecutive days. Each batch of	Somix was	routiprely test	ed with	2-amun	oantloace	ne as well a	as
benzo(a)pyrene. For the experiments	an appropria	te quantity of	f S9 supe	eri ca tant	was thav	oed and mix	xed
with S9 cofactor solution to result in	a final proteil	V concentration	on of 0.₹	\$ mg/m	L'in thos	cultures. S9	mix
contained MgCl ₂ (8 mM), KCl (33 ml	M), glucose-6	o-phosphate	5 mM) a	ind NAI	DP (4 m)	A) in sodiur	n-
ortho-phosphate-buffer 100 mM, pH	Ø.4).			& ,			

5 Test cells: The V79 cell line has been used successfully for many years on in vitro experiments. The high proliferation rate 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.

interval	P *****	u 29				<i>,</i>	Concentra				
		Q	<i>©</i> ′				Without S	9 mix			
18 hours		(), o'		~ ~ 4	37.5	75.0	150.0	300.0	600.0	1200.0	2400.0 ^P
18 hours	18 hours		9.4	18.80	37.5	75.0	150.0	300.0	600.0	1200.0	2400.0
	5° ~4°			Ą,			With S9	mix			
18 hours	45 brours	I K	9.4	18.8	37.5	75.0	150.0	300.0	600.0	1200.0	2400.0 ^P
18 hoors	24 hours	, B					150.0	300.0	600.0	1200.0	2400.0

Evaluated experimental points are shown in bold characters

Precipitation occurred at the end of treatment



B Study Design and Methods:

1 Study performance: The study was conducted at

/Germany). The experimental start completion dates of the study were January 25th 2012, respectively.

2 Culture Medium and Conditions:

and

For seeding and treatment of the cell cultures the culture medium was MEM (minimal essential medium) containing Hank's salts, glutamine and Hepes (25 m). Additionally, the medium was sopplemented with penicillin/streptomycin (100 U/mL/ 100 μg/mL) and 10 % (v/v) fetal boxine serum (FBS). All cultures were incubated at 37 °C in a humidified throughere with 1.5 % CO2 (98.5 % air).

3 Seeding of the Cultures:

Exponentially growing stock cultures more than 30 % onfluent were insed with CoMg free salt solution containing 8000 mg/L NaCl, 200 mg/L KCl 200 mg/L KH₂PO₄ and 150 mg/L Na₂HPO₆ Afterwards the cells were treated with trypsin-EDTA-solution at \$7°C for approx. 5 minutes. Then, by adding complete culture medium including 10 % () FBS the enzymanic treatment were stopped and a single cell suspension was prepared. The pypsing concentration for all sub-culturing steps was 0.5 % (w/v) in Ca-Mgfree salt solution. The cells were seeded into Quadriperm dishes, which Contained microscopic slides. Into each chamber $1 \times 10^4 - 6 \times 10^4$ cells were seeded with regard to the preparation time.

4 Treatment after the 4-hour Exposure period

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 uL S9 mix per mL culture medium were added. After 4 bours the cultures were washed twice with "Saline G" (pH 7.2) containing 8000 mg/L NaCl, 400 mg/L KaCl, 1100 mg/L glucose • H₂00, 192 mg/L Na₂HPO₄ • 2 H₂O and 150 mg/L KH₂PO₄. The cells were then cultured in complete medium containing 10 % (v/v) FBS for the remaining culture time of 14 hours. All cultures were incubated at 37°C in a humidified atmosphere with 1.5 % CO2 (98.5 % air).

5 Slides preparation:

Colcemid was added to the culture medium ((2) µg/mL) 15.5 hours after the start of the treatment. The cells were treated, 2.5 hours later, on the slides in the chambers with hypotonic solution (0.4 % KCl) for 20 min at 37°C. After incuration to the hypotonic solution the cells were fixed with a mixture of methanol and glacial acooc acid (3:1 prots, respectively). After preparation the cells were stained with Giemsa and labelled with a computer-generated random code to prevent scorer bias.

6 Naluation of Cell Numbers:

The eyalization 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.

7 Analysis of Metaphase Cells: Evaluation of the cultures was performed according to the OFCD guideline using NIKON incress of with 100x objectives. Breaks, fragments, deletions, exchanges, and chromesome disintegrations were recorded as structural chromosome aberrations. Gaps were recorded as well but not included in the calculation of the aberration rates. 100 well spread metaphases per culture were evaluated for cytogenetic damage on coded slides.

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.

8 Acceptability of the Test:

The chromosome aberration test is considered acceptable, if it meets the following enteria:

- The number of structural abertations found in the selvent controls falls within the range of the laboratory 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 historical control data.

9 Evaluation of Results:

A test item is classified as non-lastogenic if:

- the number of induced structural chromosome aberrations in all evaluated dose groups is in the range of the laboratory historical control data.
- no significant increase of the number of structural chromosome aborrations is observed.

A test item is classified as clastogenic if

- number of induced structural chromosome aberrations is not in the range of the laboratory historical control data and
- either a concentration-related or a significant increase of the number of structural chromosome aberrations is observed

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.

In this study also polyploids and endoreduplications were considered together with chromosome aberration. A test item cap be classified as an eugenic if the number of induced numerical aberrations is not in the range of the laboratory historical control data

II. Results and discussion

Two inderendent experiments were performed. In Experiment I the exposure period was 4 hours with and without \$9 mix in Experiment II the exposure period was 4 hours with \$9 mix and 18 hours without \$9 mix. The chromosomes were prepared 18 hours after start of treatment with the test item.

Inceach experimental group two parallel cultures were set up. 100 metaphases per culture were evaluated for structural chromosome aberrations, except for the positive control in Experiment II without S9 mix, where only 50 metaphases were evaluated. The highest treatment concentration in this study, 2400.0



μg/mL was chosen with regard to the solubility properties of the test item and with respect to the OΕΦ Guideline for in vitro mammalian cytogenetic tests.

Visible precipitation of the test item in the culture medium was observed in Experiment I at 2400 ug/m in the absence and presence of S9 mix at the end of treatment. In the absence and presence of S9 mix procytotoxicity was observed up to the highest applied concentration.

In the absence and presence of S9 mix, no biologically relevant increase in the number of cells carrying structural chromosome aberrations was observed. The abstration rates of the cells after freatment with the test item (0.0 - 3.5 % aberrant cells, excluding gaps) were within the range of the solvent control values (2.0 -4.0 % aberrant cells, excluding gaps) and within the range of the laboratory historical solvent control data. No evidence of an increase in polyploid metaphases was noticed after treatment with the test item as compared to the control cultures. In both experiments, either ENTS (600:0 or 1000.0 jp/mL) or CPA (1.4 μg/mL) were used as positive controls which showed distinct increases in cells with structural chromosome aberrations.

The results are summarized in the following table:

Exp.	Preparation	Test item	vell numbers	Mototic in Coces		Abecant cells	ý
	interval	concentration in unique	in (%) of control	in % of pontrol *	incl.:«gaps*	©in %	with
		in µg/mL		e .	y mci.zgaps	N i Co	exchanges
		· 🛋	Exp ® ure p	erod 4 hrs with	out S9 mix		
I	18 hrs	Positive control 600.0	Expoure p	~ ************************************	$\mathbb{O}^{2.5}$	2.0	0.0
	Ô	Positive control ²	n,d.	70°	10.5	9.5 ⁸	5.0
			O 4 <mark>997.5</mark>	700.0 70.0 90.9 95.3	§ 2.0	1.5	0.0
		1200.0	4 99.8 ×	95.3 O	2.0	2.0	0.0
		2 400.0	<u></u>	y , y	2.5	2.0	0.5
	*			eriod 18 hrs wit	out S9 mix		
II	18 hrs	Solvent control ¹	1000		3.5	3.5	0.5
	18 hrs	Softent control Positive Control 600.0	1,0%0 On.d.	73.2	42.0	39.0 ⁸	16.0
		60.0 D	2 104.10	107.7	1.0	0.0	0.0
		1200.		107.7	3.5	3.5	0.5
4		2400,0	109.5	<mark>95.6</mark>	3.5	3.5	0.5
·	Whrs A		Exposure	period 4 hrs wi	th S9 mix		
I	18 hrs	Solvent control	1800	100.0	<mark>4.0</mark>	2.5	0.5
		Postave control ⁴	n.d.	74.1	9.0	9.0 ⁸	5.5
		6000	80.0	107.0	1.5	1.5	0.5
		2 2 2 0 0 . 0	71.7	110.5	3.0	3.0	1.0
~	\$ Pa	2400.0 ^P	<mark>87.7</mark>	104.2	2.0	2.0	0.5
II II	18 hrs	Solvent control ¹	100.0	100.0	<mark>4.5</mark>	<mark>4.0</mark>	0.5



					6n © 7
Positive control ⁴	n.d.	<mark>76.1</mark>	18.5	18.5 ^S	6.19 F
600.0	81.0	85.7	3.0	3.0	Ø <mark>0.5</mark>
1200.0	<mark>77.2</mark>	93.8	2.5	2.5	0.5
2400.0	100.4	114.0	3.5	3.0	
		\(\)	// /	7	· · · · · · · · · · · · · · · · · · ·

III. Conclusions

In conclusion, it can be stated that under the experimental conditions reported, the test item AE F1.00460 did not induce structural chromosomal aberrations in 79 cells of the Chinese handler in 71 tro, when tested up to the highest applied concentration.

Report:	; 2012; N-433923-02; Amended 2015-01-19
Title:	Gene mutation assay in Chipaese ham ver V79 cells in vitro (V79 / HPKT) - AE F16000
Report No:	1462303
Document No:	M-433923-02-10
Guidelines:	Ninth Addendum to the OECLO Guidednes for resting of Chemicals, Section 4, No. 476: In vitro Managorian Cell Gene Mutation Test adopted July 201997; Commission Regulation (EC), No. 440,0008, B17, dated May 30, 2008; United States Environmental Protection Agency Health Effects Test Quidelines, OPPTS 870,5300, In Vitro Mammalian Cell Gene Mutation Cest, EPO 712-C-98-221, August 1998; and specified
GLP/GEP:	yes of a of a

Executive summar

The study was performed to investigate the potential of AE F 60460 to induce gene mutations at the HPRT locus in 79 cells of the Chinese hamster.

The assay was performed in two independent experiments, using two parallel cultures each. The first main experiment was performed without liver microsomal activation and a treatment period of 4 hours. The second experiment was performed with a treatment time of 4 hours with and of 24 hours without metabolic activation.

The highest concentration of the pre-experiment (2400 μ g/mL) was limited by the solubility properties of the test item in DMSO The concentration range of the major experiments was limited by cytotoxic effects and the solubility of the test item.

No substantial and reproducible lose dependent increase of the mutation frequency was observed in both main experiments.

Appropriate reference mutagens, used as positive controls, induced a distinct increase in mutant colonies and thus, showed the sensitivity of the test system and the activity of the metabolic activation system. In conclusion it can be stated that under the experimental conditions reported the test item did not induce gene mutations at the HPRF locus in V79 cells. Therefore, AE F160460 is considered to be non-mutagenic in this HPRT assay.



A Materials

1 Test Material:

Description: Lot/Batch:

Purity:

Stability of test compound:

Solvent used:

2 Control materials:

Negative:

Positive: non- activation (-S9 mix)

I. Material and methods

AE F160460

Off white powder SES 11562-12-4

96.7 % w/w, Dose calculation adjusted to purity not performed as part of this study. dimethylsulfoxide (DMSO).

Tissue Culture medium

nix)

Chyl methane sulphonare (EMS) dissolved in nutrient medium.

Final concentration 0.15 mg/mL (12 mM)

Positive: with activation (+\$9 mix)

Final concentration 0.15 mg/mL (12 mM)

mixto

pMBA; 7,12-dimethyloenz(a) anthracene dissolved in DMSO.

Final concentration 1.1 µg/n/L (4.3 µM)

3 Metabolic activation; S9 mix from male Wistar rats Phenobarbital/β-Naphthoflavone induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from & 12 weeks old male Wistar rats (Hsa Cpb; WU, weight approx. 220 – 320 g,

The Netherlands finduced by intraperitorical applications of 80 mg/kg b.w. phenobarbital and by peroral administrations of β-naphthoffervone each, on three consecutive days.

An appropriate quantity of Sy supernatant was that wed and mixed with 89 cofactor solution to result in a final protein concentration of 0.75 mg/m in the cultures. Cofactors were added to the S9 supernatant to reach following concentration on the \$9 mix were: 7 mM MgCl₂ 33 mM KCl, 5 mM glucose-6phosphate and 4 mMNADK in 100 mM sodium on tho-phosphate buffer, pH 7.4. During the experiment, the S9 mix was stored in an ice both.

4 Test cells:

The V79 cell line has been used specessially in vitroexperiments 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 30 %) both necessary for the appropriate performance of the study, recommend the use Lustave a stable kar of this cell line. The cells have a stable karyotype with a modal chromosome number of 22.



Document MCA: Section 5 Toxicological and metabolism studies Mesosulfuron-methyl

5 Test compound concentrations used (µg/mL):

Exposure	S9				ntrations	19	ő
period	mix			in μ	g/mJ		
				Exper	iment I	Q,	
4 hours	-	75	150	300 🔊	600	(° 1200	2400
4 hours	+	75	150	3000	600 🦠	1200	2400
				Experi	ment M		
24 hours	-	150	300		#2 00 \$	2400	\$3600 ×
4 hours	+	300	600	, 1200° ×	©1800°	2400 P	3600 P

Concentrations given in bold letters were chosen for the mutation rate anal

B Study Design and Methods:

1 Test performance:

The study was conducted at

Germany). The experimental start and completion detes of the study were bebruard 2nd 2012 and March 27th 2012, respectively. March 27th 2012, respectively.

2 Culture medium and conditions:

2 Culture medium and conditions: Some seeding and conditions of the cell cultures the complete culture dedium was MEM (minimal essential medium) containing Hank's salts, 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 semidified atmosphere with \$5 % \$62 (98.5% air).

3 Seeding of the cultures:

Two to three days after sub-cultivation stock cultures were the psinized at 37 °C for 5 minutes. Then the enzymatic disestion was stopped by adding complete culture medium with 10 % FBS and a single cell suspension was prepared. The trypsin coocentration for all sub-culturing steps was 0.2 % in PBS. Prior to the trypsin treatment the cells were rinsed with Ca-Mg-free salt solution containing 200 mg/l EDTA (ethylene diagram tetraacetic acid) Approximately 1.5 x 106 (single culture) and 5 x 102 cells (in duplicate) were seeded in plastic culture flasks. The cells were grown for 24 hours prior to treatment.

4 Cell treatment:

After 24 hours the medium was replaced with serum-free medium containing the test item, either without S9 mix @with 59 µl/mb 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 pH was adjusted to 7.2 Three or four 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 7 days five 80 cm² cell culture flasks

P Precipitation



were seeded with about 3 - 5×105 cells each in medium containing 6-TG. Two additional 25 cm² flasts were seeded with approx. 500 cells each in non selective medium to the containing forms of the containing forms. were seeded with approx. 500 cells each in non-selective medium to determine the vability.

The cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % CO₂ for about 8 days. The

5 Evaluation of cell numbers:The stained colonies with more than 50 cells were counted. In doubt the colony size was checked with a preparation microscope. **6 Acceptability of the Assay:**The gene mutation assay is considered acceptable if it meets the following criteria:

- the numbers of mutant colonies per 10⁶. Color following criteria:

- the numbers of mutant colonies per 10⁶ cells found in the solvent.

 the position
 - the positive control substances should produce a significant increase in mutant colony frequencies.
 - the cloning efficiency II (absolute value) of the solvent controls should exceed 500%

7 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 af one of the test points.

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

A positive response is described as follows:

A test item is classified as fautageonic if i Creproducibly 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 mance a low spontaneous invitation 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 cates of solvent controls within all experiments of this study was also taken into consideration.

8 Statistical Analysis:

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

AII. Results and discussion

The assay was performed in two independent experiments, using two parallel cultures each. The first main experiment was performed with and without liver microsomal activation and a treatment period of 4 hours. The following concentrations were tested: 150, 300, 600, 1200 and 2400 µg/mL.



The second experiment was performed with a treatment time of 4 hours with and 24 hours without metabolic activation. The following concentrations were tested: 300, 600, 1200, 2400 and 3600 µg/mL without S9 and 600, 1200, 1800, 2400 and 3600 µg/mL with S9. No relevant and eproducible increases in mutant colony numbers/10⁶ cells was observed in the main experiments up to the maximum concentration.

The threshold was reached or exceeded at 2400 µg/mL in the second culture of the first experiment without metabolic activation. This increase however, was reither reproduced in the parallel culture under identical conditions nor in the second experiment without metabolic activation and consequently, judged as biologically irrelevant. A similar increase occurred in both cultures of the first experiment with metabolic activation at 2400 μg/mL. The historical range of solvent controls was exceeded in vulture one but not in culture II. Again, this increase was not reproduced in the second experiment at 2400 μg/mL or at any other, even higher concentration and therefore Judged as irrelevant. In a linear regression analysis of (least squares) a significant dose dependent trend of the mutation frequency indicated by a probability value of <0.05 was determined in both cultures of the first experiment with metabolic activations. This trend was judged as irrelevant since the values of the mutation frequency remained either within the historical range of solvent controls or the increase was not reproduced as discussed above. EMS (150 μg/mL) and DMBA (151 μg/m)L) were used as positive controls and showed a distinct increase in induced mutant colonies.

The results are summarized in the following table

	- Q's'	- AO		~~~	. V	<u> </u>	· · ·			
AE F160460			S ~				~\\			
HPRT , 1462303		\(\sigma'\) (1)	~ <i>V</i>		. ~					
Conc.	S9 Relative	Relatio 1	Relative cloning	Matent Slopie	Inductio n factor	Rélative clonir	Relativ	Relative cloning	Mutant colonie	Induction factor
μg/mb	mi efficienc	e call density	cloning efficienc	s/ 10 ⁶	en racion Os	efforienc	density	efficienc	s/ 10 ⁶	II lactor
	x · yı · · ·	f	y II	cetty		N I		y II	cells	
	\$ <mark>%</mark> \$	<mark>%</mark> ?	<mark>%</mark> 0" *	· <mark>J</mark> Y &	, 3	<mark>%</mark>	<mark>%</mark>	<mark>%</mark>		
Column 1 2			<mark>6</mark>	' <mark>7</mark>	<mark>8</mark> 🍣	<mark>9</mark>	<mark>10</mark>	<mark>11</mark>	<mark>12</mark>	13
Experiment I / 4 b	Cultur				8 8	Culture II				
treatment 4			0" 0)						
Solvent	- 2400 0 4	01000 als	100.0	19.8	1.0	100.0	100.0	100.0	<mark>9.2</mark>	1.0
control	- A 72.7 0			Ó,						
with Solution DMSO				7						
								_		
Positice 150.0 (CEMS)	- A \\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	78%	80÷Q,*	<mark>94.1</mark>	<mark>4.8</mark>	<mark>69.8</mark>	<mark>97.8</mark>	<mark>94.2</mark>	<mark>112.4</mark>	12.2
control ,										
(EMS)	F O S	F								
Test item 75.0	- 4 96.1	Culture wa	s not contir	nued#			Culture v	vas not conti	nued [#]	
Test item 1500	87.9		<mark>87.8</mark>	<mark>6.1</mark>	0.3	<mark>90.3</mark>	<mark>100.9</mark>	100.5	<mark>21.7</mark>	2.3
Test item 300.0	- ² 85.2	<mark>78.3</mark>	<mark>79.7</mark>	10.7	0.5	<mark>82.7</mark>	<mark>104.8</mark>	<mark>115.6</mark>	<mark>11.8</mark>	1.3
Test item 600.0	<mark>- 84.4</mark>	<mark>76.1</mark>	<mark>82.0</mark>	10.3	<mark>0.5</mark>	<mark>78.8</mark>	102.3	103.7	<mark>12.3</mark>	<mark>1.3</mark>



Wesosunur on-metnyi													
Test item	1200. 0		-	82.3	<mark>79.2</mark>	83.3	2.4	0.1	<mark>70.1</mark>	101.6	132.5	11.9	
Test item	2400. 0		-	0.0	<mark>48.1</mark>	<mark>71.6</mark>	12.6	0.6	0.0	2	0 <mark>96.2</mark>	31.3	3,4
Solvent control	l		+	<mark>100.0</mark>	100.0	<mark>100.0</mark>	8.3	1.0 ©	100.0	1460.0	100.0	8.5	71.0 × 1
with DMSO							کہ	Ü,	Ő Ź	\$	100.0		7.0 × 7.0 × 7.25
Positice	1.1		+	<mark>70.4</mark>	112.2	84.4	403.2	48.7	6 0 /.3	7100 7 G	258.0 v O	394%	726
control (DMBA)							403.20°)P % 1	9108.7 V			
Test item	75.0		+	97.0	Culture v	vas not conti	inued		89 <u>%1</u>	Culture	was not conti		
Test item	150.0		+	<mark>96.7</mark>	104.3	85Q4 (1 2 3 3 6 6	Z <mark>1.9</mark>	88.6	1000	923	7.9°	<u>√.4</u>
Test item	300.0		+	<mark>94.1</mark>	104.7	0.85.7 (C)	14.0	1.7	875 81.5	97.7		9.5	1.7
Test item Test item	600.0 1200.		+	98.3 84.1	110.8Q"	68.1 87.6	17.5 010.3	2.1 71.2	81.5 71.1	109.2	8 9	№ .6	1.8
Test item	0 2400.		+	62.4	87.00	. ~	50°B	₩	36.6	105.3	86.9 86.9	22.6	<mark>4.1</mark>
	0				4				₹		86.9 3		
Experime treatment				Culture I	2				Sulture II))		
Solvent				100.9	100.0	100.0 ^	11.4	1.0		100.0	100.0	23.1	1.0
<mark>control</mark>		8											
with DMSO			0			4 8			7 V V V V V V V V V V V V V V V V V V V				
	\$50.0		- %	9 2.3		84.8	231-87	20Q*	9 ¥.4	<mark>79.3</mark>	109.6	236.0	10.2
control (EMS)			39		Š,								
Test item	<mark>150.0</mark>	On.	- - (2 9.5	Culture	vas not conti	inued >		<mark>96.3</mark>	Culture v	was not conti	inued#	
Test item	300.0	P	<u>.</u> Ĉ	102.	132.8	189,5	\.\.\.\.\.\.\.\.\.\.\.\.\.\.\.\.\.\.\.	0.6	106.5	<mark>97.8</mark>	110.7	13.8	0.6
Test item	69 0	Ц		99.7	300.0	80.4 ©	19.4	<mark>1.7</mark>	108.2	<mark>105.4</mark>	116.9	10.5	0.5
Test item	\$200. 0		- \$	124.1 Q	131.4	92.3	1 5 9	<mark>1.4</mark>	<mark>94.4</mark>	100.3	<mark>115.6</mark>	8.2	0.4
Test item	2400. 0	((// n	<u> </u>	l al.	*, <mark>}}39.5</mark>	\$08.2 \$	9.4	0.8	<mark>40.4</mark>	<mark>96.3</mark>	111.0	<mark>4.6</mark>	0.2
Test item	3600			120	155.6	98.7 2	17.1	1.5	97.1	103.3	100.1	<mark>7.5</mark>	0.3
Experiment treatment	Spy II / 4				*	ř					l		
Softent			\$\frac{1}{\pi_{+}}	200.0	100.0	100.0	14.3	1.0	100.0	100.0	100.0	9.9	1.0
control with	O ,												



					•			•				<u></u>	%
DMSO													Ţ
Positice	1.1		+	<mark>84.7</mark>	102.9	<mark>71.8</mark>	512.1	<mark>35.9</mark>	<mark>86.3</mark>	<mark>101.7</mark>	\$ 5	1 (<i>D</i>))	<i>y</i> I
control											D ^y	495.6	
(EMS)		<u> </u>								_4			ð
Test item	300.0		+	<mark>93.0</mark>	Culture v	was not cont	inued [#]	Ö	<mark>91.8</mark>	101.4	was not conti	inwed" 👋 👋	i
Test item	<mark>600.0</mark>		+	<mark>93.5</mark>	102.4	<mark>90.2</mark>	<mark>13.7</mark>	1.0 E	92.0) <mark>94.5</mark>	72.5	9.6	2
Test item	<mark>1200.</mark>		+	<mark>93.5</mark>	115.4	91.3	18.7	<mark>(21.3</mark>	93.6	<mark>88.6</mark>	77 .) ^y
	<mark>o</mark>							7		Ġ°	5 4		
Test item	<mark>1800.</mark>		+	<mark>94.2</mark>	121.4	83.6	<mark>5.1</mark>	0.4	61.0 %	92.7	86.9	15,10	
	<mark>o</mark>						\bigcirc			*			
Test item	<mark>2400.</mark>	P	+	91.0	127.0	86.2	14.9	1.0	<mark>969</mark>	1.6	8 5.7	21.8 A 2.2 ·	
	0								, × A		, 0		
Test item	<mark>3600.</mark>	P	+	<mark>99.2</mark>	121.3	<mark>79.6</mark>	14.6	\$\frac{1.0}{1.0} \times\$	91.4	99.6	97.7	2 <mark>1,6.7</mark> 2.7	
	0								11/02				
# culture v	was not co	ontin	ued si	nce a minir	num of 🔊	four analysa	ble concen	trations is re	quired 0			N. S.	
P Precipit	ation					, L)	O A			~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		**	
						7	40	<u> </u>	₩.		X	4	

III Conclusions ...

In conclusion it can be stated that under the experimental conditions reported the test item did not induce gene mutations at the PPRT locus in V79 cells. Therefore AE F100460 s considered to be non-T assay. mutagenic in this HORT

BCS-CV14885

Report:	9 ;
Title:	Salmonella tophimurum reverse mutation assay with BCS-CV14885
Report No:	1490201
Document No: Q	448998-01-1-10
Guidelines:	Ninth Addendim to QECD Guidelines for Testing of Chemicals, Section 4, No. 471:
4	Baccrial Reverse Mutation Test, adopted July 21, 1997; Commission Regulation (EC) No.
	440/2008 13/14 dated Vay 30, 2008; EPA Health Effects Test Guidelines, OPPTS
	\$70.5100, Bacterial Recerse Moration Test; EPA 712-C-98-247, August, 1998; none
GLP SEP:	yes A O O O O

Executive summary:

This study was performed to investigate the potential of BCS-CV14885 to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the Salmonella typhingunum strains TA 15350 TA 1537, TA 98, TA 100, and TA 102.

The assay was performed in two independent experiments both with and without liver microsomal activation Each concentration, including the controls, was tested in triplicate. The test item was tested at the following concentrations:

Pre-Experiment/Experiment I: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate



33; 100; 333; 1000; 2500; and 5000 µg/plate Experiment II: The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments. No toxic effects, evident as a reduction in the number of revertants (below the indication factor) occurred in the test groups with and without metabolic activation. No substantial increase in revertant colony numbers of any of the five tested strains was observed following treatment with BCS-CV14885 at any dose lever neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher putation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive coursels and showed a distinct increase of indiffeed revertant colonies. The test item did not induce gene mutations by base pair changes of frameshifts in the goome of the strains used. Therefore, BCS-CV14885 is spinsidered to be non-routage reverse mutation assay. A. Materials 1 Test material: Description: Lot/Batch: dose calculation will be adjusted to purity Purity: Stability of test compound Solvent used: non activation (-19 mix): 2 Control materials Negative: Sølvent: Positive: «sodium@azide:Φ0 μg/plate for TA1535 and TA100 4-nitro-o-phonylene diamine, 4-NOPD: 50 μg/plate for TA1537 and 10 pp plate for TA98 methyl methane sulfonate, MMS: 3.0 µL/plate for TA 102 2-aminoanthracene, 2-AA: 2.5 μg/plate for TA 1535, TA 1537, (A) 98 (FA) 100, and 10 µg/plate for TA 102

3 Metabolic activation: S9 mix derived from male Wistar rats

Phenobarbital/ β -naphthoffavone induce that liver S9 were used as the metabolic activation system. The S9 is prepared from 8 - 12 weeks old male Wistar rats (Hsd Cpb: WU; weight approx. 220 - 320 g,

The Netherlands) induced by intraperitoneal applications of 80

mg/kg b.w Shenobarbital (, Germany) and by peroral administrations of β-naphtho/Davone (, Germany). Each batch of S9

mix is outinely tested with 2-aminoanthracene as well as benzo[a]pyrene. Cofactors are added to the S9 mix to reach the following concentrations in the S9 mix: 8 mM MgCl₂, 33 mM KCl, 5 mM Glucose-6-



phosphate and 4mM NADP_in 100 mM sodium-ortho-phosphate-buffer, pH 7.4._During the experiment the S9 mix was stored in an ice bath.

4 Test organisms:

Salmonella typhimurium, strains TA1535, TA1537, TA98, TA100 and TA 107 obtained from Germany). Regular checking of the properties of the strains organding the membrane permeability, ampicillin- and tetracycline sistance as well as spontaneous mutation rates are performed in the laboratory of Harlan CCR.

5 Test Concentrations:

Pre-Experiment/Experiment I: 3; 10; 33, 100; 333; 1000; 2500; and 5000 µg/plate 33; 100; 333, 1000; 2500; and 5000 µg/plate 3

B Study Design and Methods:

1 Test performance:

The soudy was consucted at

August 1st , 2012

2 Assay procedure:

Preliminary cytotoxic (y/mutation **ssay:

To evaluate the toxicity of the test from a pre-experiment was performed with all strains used. Eight concentrations were tested for toxicity and mutation induction with each 3 plates. 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 lasince the following criteria are met: evaluable plates (>0 colonies) at five concentrations or more in all strains used.

3 Statistics:

According to OEOD 471 a statistical analysis is not mandatory

4 Acceptability of the assay:

The Salmanella typhimurium reverse munition assay is considered acceptable if it meets the following criteria:

- regular back found growth in the hegative and solvent control
- the spontaneous reversion rates in the regative and solvent control are in the range of our historical data
- the positive control substances should produce a significant increase in mutant colony frequencies
- a manimum of five analysable dose levels should be present with at least three dose levels showing no signs of toxic effects, evident as a reduction in the number of revertants below the indication factor of 0.5.



5 Evaluation 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 thrice (strains TA 1535 and

A dose dependent increase is considered biologically relevant of the threshold at an one concentration. An increase exceeding the threshold at an one concentration.

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. In wever whenever the colony counts remain within the historical range of the colony counts remain within t II Results and Diverse.

1 Analytical determinations:

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The stel." I'.

The stability of BCS-CV14885 and the stability and homogeneity analysis of achieved concentration were not determined as part of this

2 Preliminary cytotoxicity assay: &

The plates incubated with the test item showed normal background growth up to 5000 ug/plate with and without S9 mix in all strains used No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the est groups with and without factabolic activation.

3 Mutation assay

3 Mutation assay The test item BES-CV44885 was assessed for its potential to induce generalizations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using Salmonella typhimurium strains TA 1535, TA 1537, TA 98 TA 100, and TA 102

The assay was performed in two independent experiments both will and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test item was tested at the following concentrations:

Pre-Experiment/Experiment J. 3; 10, 33; 100, 333, 1000, 2500; and 5000 μg/plate 33. 100; 33; 1000; 2500, and 5000 μg/plate Experiment II.

The plates incubated with the tespitem showed formal background growth up to 5000 µg/plate with and without netabolic activation in both independent experiments.

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

No substantial recrease in revertant colony numbers of any of the five tester strains was observed following treatment with BCS-CV44885 at any dose level, neither in the presence nor absence of metabolic activation (S90 ix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens as positive controls showed a distinct increase of induced revertant cotonies.

The results are summarized in the following tables:



Mesosulfu	ron-methyl							
Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colo	ony Counts (Mean	±SD)			
Without Activation	DMSO Untreated BCS-CV14885	3 µg 10 µg 33 µg 100 µg 333 µg	$ \begin{array}{c} $	11 ± 4 10 ± 3 12 ± 3 11 ± 4 11 ± 4 12 ± 2 11 ± 4 12 ± 2	29 ± 2 29 ± 2 26 ± 8 23 ± 7 29 ± 2 21 42 26 ± 3	TADO 167 ± 10 165 ± 8 174 ± 11 159 ± 12 168 ± 16 150 2 4	TA 162	
	NaN3 4-NOPD 4-NOPD MMS	1000 µg 2500 µg 5000 µg 10 µg 10 µg 50 µg 3.0 µL	20 ± 6 14 ± 2 12 ± 5 2006 ± 192 17 ± 3 22 ± 7	10 ± 2 10 ± 2 9 ± 3 77 ± 45	20±7 20±7 18±6 502±31	167 ± 10 165 ± 8 174 ± 11 159 ± 12 169 ± 5 168 ± 16 159 ± 4 163 ± 20 057 ± 5 170 ± 10 2068 → 137 166 → 19 168 ± 17 169 ± 10 175 ± 10 193 ± 2 169 ± 10 169 ± 10 160 ± 10	302 ± 35 409 ± 8 437 + 28 398 ± 21 305 ± 17 410 ± 25 426 ± 21 446 ± 5 307 ± 29 3693 ± 764 3693 ± 764 448 ± 34 458 ± 22 436 ± 31 480 ± 23	
With Activation	\ <u>\</u>	3 μgς 10 μg 33 μg	17 # 7	18 ± 6 19, ± 4 18, ± 5 16 ± 4 15 ± 2 20, ± 3 16 ± 8 24, 5 24, 5 27	25 ± 5 36 ± 6 32 ± 6 32 ± 7 43 ± 7 43 ± 7 42 ± 4 38 ± 2 28 ± 8 21 338 ± 147	1075 ± 10 193 ± 7 166 ± 7 158 ± 17 266 ± 19 164 ± 16 161 ± 14 162 ± 15 169 ± 26 1982 ± 114	\$\\ \text{38} \div \div 27\$ \$\\ \text{388} \div \div 67\$ \$\\ \text{520} \div 126\$ \$\\ \text{448} \div 34\$ \$\\ \text{458} \div 22\$ \$\\ \text{436} \div 31\$ \$\\ \text{480} \div 23\$ \$\\ \text{456} \div 74\$ \$\\ \text{462} \div 24\$ \$\\ \text{451} \div 109\$ \$\\ \text{1758} \div 337\$	
	2-AA 2			20 ¥ 3 (9 ± 5) 16 ± 3 216 ± 27 (17 ± 17 ± 17 ± 17 ± 17 ± 17 ± 17 ± 17 ±				



Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colo	ny Counts (Mean ±	=SD)	<u> </u>	
			<u>TA 1535</u>	TA 1537	<u>TA 98</u>	TA DOO	IA NVZ
Without Activation	DMSO Untreated BCS-CV14885	33 μg 100 μg 333 μg 1000 μg	17 ± 4 16 ± 5 16 ± 4 17 ± 4 15 ± 1 17 ± 5	19 ± 5 21 ± 6 17 ± 4 19 ± 4 15 ± 7	27 ± 4 28 ± 0 29 ± 7 25 ± 2 22 ± 3 25 € 2	1734 ± 9 179 ± 4 144 ± 15 146 ± 14 149 ± 14	344 ± 24 377 ± 18 378 ± 24 378 ± 20 365 ± 19 364 ± 21 380 ± 23
	NaN3 4-NOPD 4-NOPD MMS	2500 μg 5000 μg 10 μg 10 μg 50 μg 3.0 μL	13 ± 5 13 ± 5 1603 ± 27	20±2 20±2 (4) ± 12	26±3 7±8 618471	148 #3 143 #8 161 ¥ 10 0583 ± 28	364 ± 21 380 ≠ 23 1485 ≠ 236 1485 ≠ 236
With Activation	DMSO Untreated BCS-CV14885	33 µg 100 µg 33	21 ± 5 18 ± 4 18 ± 4 20 ± 4 23 ± 2	24 ± 6 32 ± 2 22 ± 2 24 ± 3 21 ± 7	38 ± 4 38 ± 12 37 ± 7 36 ± 11 38 ⊋ 3 89 ± 5	1/9 ± 4 144 ± 15 146 ± 14 149 ± 1 148 ± 6 142 ± 8 161 ± 10 0583 ± 28 140 ± 12 206 ± 24 044 ± 7 149 ± 1 160 ± 16 152 ± 9 261 ± 12 157 ± 10 158 ± 10	$ \begin{array}{c} 1488 \pm 236 \\ 432 \pm 55 \\ 520 \pm 20 \\ 418 \pm 45 \\ 390 \pm 14 \\ 386 \pm 16 \\ 390 \pm 49 \\ 416 \pm 66 \end{array} $
	2-AA 2-AA	2500 μg 5000 μg 2.5 μg 10 μg	18 ± 2 18 ± 2 265 ± 22	$ \begin{array}{c} 20 \pm 3 \\ 27 \pm 0 \\ 13 \\ 4 \end{array} $	35 ± 8 33 ± Q 1455 ± 222	157 ± 12 157 ± 10 1469 ± 140	$416 \pm 66 \\ 359 \pm 19$ 1432 ± 150
NaN3 2-AA MMS 4-NOPD	Damingarff Cmethyl met	hracene hane sell-tonate nenylene-diamin	e do a	21 ± 7 20 ± 3 27 ≠ 0 15 ± 21 15 ± 21		K," Q	

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item dict not include gene mutations by base pair changes or frameshifts in the genome of the strains used.

Report:	b; ;2913;M-449003-02; Amended: 2015-01-30
Title:	Vovitro Arromos@me aberration test in Chinese hamster V79 cells with BCS-CV14885
Report No:	¥490 <u>2</u> 02*
Document No:	M-449003-02-1
Guidelines:	Nighth Addendum to the QCD Guidelines for Testing of Chemicals, February 1998,
Guidelines:	adonted July 21 997 Avideline No. 473 In vitro Mammalian Chromosome Aberration
	Test Commission Regulation (EC) No. 440/2008 B10: Mutagenicity - In vitro Mammalian
	Chromosome Abertation 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;none
GLP GEP:	yęs 🗸 🗡
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Executive summary:

The test item BCS-CV14885, dissolved in culture medium (minimal essential medium), was assessed for



its potential to induce structural chromosome aberrations in V79 cells of the Chinese hamster in vitro in vitroun three independent experiments. The following study design was performed:

	Without S9 1		With S9 mix Exp. II
	Exp. IA	Exp. II	Éxp. IB Exp. II
Exposure period	4 hrs	18 hrs	4 hrs Q 4 hrs Q
Recovery	14 hrs	- 4 6	14 hrs 14 hrs
Preparation interval	18 hrs	18 hrs 💝	08 hrs 6 18 hrs

In each experimental group two parallel entures were set up. At Meast 600 metaphases per culture were evaluated for structural chromosome aberrations, except for the positive control in experiment IA in the absence of S9 mix, where only 50 meraphases were evaluated.

The highest applied concentration (4022: 9 µg/mb, approx. 10 mM) was chosen with regard to the molecular weight and the purity (97.8 %) of the test item and with respect to the current SECD Guideline 473.

Dose selection for the cytogenetic experiments was performed considering the toxicity data. In the absence and presence of \$9 mix concentration no contoxicity was observed up to the highest applied concentration.

No clastogenicity was observed at the concentrations evaluated. However, a single statistically significant increase was observed in Experiment 14A in the abservee of S9 mix after treatment with 2011.0 $\mu g/mL$ (4.0 % aberrant cells axcluding gaps). The value is in the aboratory historical solvent control data range (0.0 – 4.0 % aberrant cells, excluding gaps) and therefore biologically irrelevant.

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

Appropriate positive control mutagens innuced statistically significant increases in cells with structural chromosome aberrations

Under the experimental conditions reported the test item did not induce structural chromosome aberrations in V79 cells (Chinese ramster cell line) in otro. Therefore, BCS-CV14885 is considered to be non-classogenic in this chromosome aberration test in the absence and presence of metabolic activation, when tested up to the highest required concentration.

I. Material and methods

Stability of test compound:

🖒 Solvent used:

BCS-CV14885

white powder

BCS-CV14885-01-01

97.8 % w/w, dose calculation will be adjusted to purity

not performed as part of this study

culture medium, (MEM)



2 Control materials:

Negative:

Positive - Without metabolic activation:

EMS; ethylmethane solfonate, dissolved in Nutriem medium.
Final Concentration: 1000.0 μg/mL (Experiment) 600.0 μg/mL (Experiment) 1000.0 μg/mL (Experiment) 10000.0 μg/mL (Experiment) 1000.0 μg/mL (Experiment) 1000.0 μg/mL (Ex Experiment II) (Experiment) 600 μg/ml (Exper

Positive - With metabolic activation:

The dilutions of the stock solutions were prepared on the day of the experiment. The stability of the positive control substances (EMS and CPA respectively) in solution is unknown but in the expected range was sufficient biological evidence of chemical stability

3 Metabolic activation S9 mix: Somix from male Wistan rats

The S9 was prepared from 8 - 12 weeks old male Wiston rats (The Netherlands) weight approx 220 - 320 g induced by intraperiton at applications of Germany) and by orally administrations of 80 80 mg/kg b.w. phenobarbital? mg/kg b.w. β-naphthoflayone (. Germany) each. on three consecutive days. Each batch of S9 mix was foutinely tested with 2-aminoanthracene as well as benzo(a)pyrene. For the experiments an appropriate quantity of \$9 supernatant was thawed and mixed with S9 cofactor solution to result in a final protein concentration of \$75 mem L in the cultures. S9 mix contained MgC (8 mM), KCL (33 mM), glucose-6 phosphate (5 mM) and NADP (4 mM) in sodiumortho-phosphate-buffer (100 mM, pH 7.4)

4 Test cells:

The V79 cell line has been used successfully for many years in in vitro experiments. The high proliferation rate and a reasonable plating efficiency of Cuntreated 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 nun

5 Test concentrations:

Preparation interval	@period	Exp.	¥		Ş	(Concentrat in µg/m				
18 hrs			Withou	§9 mix							
18 hrs	4 hrs	IA T	15.7	31.4	62.8	125.7	251.4	502.8	1005.5	2011.0	4022.0
18 hrs	1840*	, Ti	15.7	31.4	62.8	125.7	251.4	502.8	1005.5	2011.0	4022.0
	4		With S9	mix							
18 hrs	4 hrs	IA*	15.7	31.4	62.8	125.7	251.4	502.8	1005.5	2011.0	4022.0



Prepar inter	1	Exp.		oncentrati in μg/mI	^	
18 ł	ars 4 hrs	IB	125.7	251.4	502.8 005.5	2011.0 4022.0
18 ł	ars 4 hrs	II	(A)	251.4	502. 1005.5	2071.0 4022.0

Evaluated experimental points are shown in bold characters

* Was repeated due to invalid solvent control data

B. Study design and methods

1 Study performance:

The study was conducted a

Germany). The experimental start •

and

Sentember 4 2012 Sespensively

2 Culture Medium and Conditions: s

For seeding and treatment of the cell cultures the culture medium was MEM (minimal essential medium) containing Hank's salts, glutomine and Hepos (25 mM). Additionally, the medium was supplemented with penicillin/streptomycin (100 U/mL/100 μ g/mL/and 10% (v/x) fetal boving serum (GBS). All cultures were incubated at 37 °C in a happidified atmosphere with 1.5% CQ (98.5 % air)

3 Seeding of the Altures

Exponentially growing spock cultures more than 50 % conflict were rinsed with Ca-Mg-free salt solution containing 8000 mg/L NaCk 200 mg/L KCk 200 m

4 4-hour exposure period:

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 μ L S9 mix per mL culture medium were 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 glueose • H/O, 192 mg/L Na₂HPO₄ • 2 H₂O and 150 mg/L KH₂PO₄. The cells were the cultured in complete medium containing 10 % (v/v) FBS for the remaining culture time of 14 hours.

5 18 bour exposure period

The fulture 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 in the absence and presence of metabolic activation.



All cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % CO₂ (98.5 % air).

5 Slides preparation:

Colcemid was added to the culture medium (0.2 µg/mL) 15.5 hours after the start of the treatment. The cells were treated, 2.5 hours later, on the slides in the chamber with hypotonic solution (0.4% KCl) for 20 min at 37 °C. After incubation in the hypotonic solution the cells were fixed with a mosture of methanol and glacial acetic acid (3:1 parts, respectively) after preparation the cells were stained with Giemsa and labelled with a computer-generated random code to prevent scorer bias.

6 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 incroscopically 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.

7 Analysis of Metaphase Cells:

Evaluation of the cultures was performed according to the OECD guideline using MKON microscopes with 100x 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 100 well spread metaphases per culture were evaluated for cytogenetic damage on coded slides, except for the positive control without S9 inix in experiment IA where only 50 metaphases were scaled.

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

8 Acceptability of the Test:

The chromosome aberration test is considered acceptable, if it meets the following criteria:

- The number of structural aberrations found in the solvent controls falls within the range of the laboratory instorical 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 historical control data.

9 Evaluation of Results:

A test item is classified as non-clastogenical

- the number of induced structural thromosome aberrations in all evaluated dose groups is in the range of the laboratory distorical control data and
- no significant increase of the number of structural chromosome aberrations is observed.

A test item is classified in classed enic in

- The number of induced structural chromosome aberrations is not in the range of the laboratory historical control data and
 - either a concentration-related or a significant increase of the number of structural chromosome observed.

Statistical significance was confirmed by means of the Fisher's exact test (7) (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 occursion of the structural chromosome aberrations is the purpose of this study, it is important to include the polypoids and endoreduplications. The following criterion is valid:

A test item can be classified as an eugenic if the number of induced numerical oberrations is not in the range of the laboratory historical control data.

II. Results and discussion

The test item BCS-CV14885, dissolved in culture medium (minimal essential medium), was assessed for its potential to induce structural chromosome aberrations in V79 cells of the Chinese harvier in vitro in the absence and presence of metabolic activation by S9 max.

Three independent experiments were performed. In Experiment 1A the exposure period was 4 hours without S9 mix. In Experiment 1B the exposure period was 4 hours with S9 mix. In Experiment 18 the exposure period was 4 hours with S9 mix and 18 hours without S9 mix. The chomosomes were prepared 18 hours (Exp. IA, IB & II) after the start of treatment with the jest item.

In each experimental group two parallel cultures were set up At least 100 spetaphases per culture were evaluated for structural chromosome aberrations, except for the positive control without \$9 mix in Experiment IA, where only 50 metaphases were scored.

The highest treatment concentration in this study, 40220 µg/mL was chosen with regard to the molecular weight and the purity (97.8 %) of the test item and with respect to the OEOD Guideline for *in vitro* mammalian cytogenetic tests.

Neither test item precipitation not relevant influence of osmothrity or pH value was observed. In the absence and presence of 59 mix no cytotoxicity was observed up to the highest applied concentration.

In all experimental parts no clastogenicity was observed at the concentrations evaluated. However, one single statistically significant increase was observed in Experiment A in the absence of S9 mix after treatment with 2011. In page 4.0 % aberrant cells, excluding gaps). The value is within the laboratory historical solvent control data range (0.044.0 % aberrant cells, excluding gaps) and therefore biologically irrelevant.

No evidence of an increase in polyploid metaphase was noticed ofter treatment with the test item as compared to the control cultures.

Either EMS (600 or 10000 μg/μμ) or CPA (2.0 μg/mP) were used as positive controls which showed distinct increases in cells with structural chromosome aberrations.

The results are summarized in the following table

		.*		9			
$Exp_{.}$	Preparation ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	Test item ~	Cell numbers	Mitotic indices		Aberrant cells	
Exp.	Preparation interval	concentration	ℤ″ <mark>in %</mark> ʹ⅀ʹ	in %		in %	
		⊘ <mark>m μg/pa∏</mark>	of control	of control	incl. gaps*	excl. gaps*	with
		_ √ (<u>exchanges</u>
	~ A		· Q		4.00	•	_
			Exposure po	<mark>eriod 4 hrs witho</mark>	ut 89 mix		
TA @	10 hr C	ilvent control!	100.0	100.0	2.0	1.5	0.0
IA «		MACHIE COMPANI	100.0	100.0	<u>2.0</u>	1.3	<mark>0.0</mark>
IA (18 hg So	ositive control ^{2#}	<mark>n.d.</mark>	<mark>92.0</mark>	<mark>47.0</mark>	46.0 ⁸	30.0
		2 10 905 5	91.9	117.9	2.0	2.0	1.0
		ar.	7 1.7		<u> </u>		
	ř	2011.0##	104.5	<mark>96.0</mark>	4.0	4.0 ^S	1.3
		4022.0	92.2	117.9	5.0	3.5	0.5



Meso	sulluron-meti	nyı					
			Exposure p	eriod 18 hrs with	out S9 mix		40.0
II	18 hrs	Solvent control ¹	100.0	100.0	1.5	1.5	7.5 7.5 9.0 0.0 0.0
		Positive control ³	<mark>n.d.</mark>	88.3	10.0	10.0 ^S	7.5 90 0.0
		1005.5	99.9	95 ©	1.5	1.5	
		2011.0	75.3	108.3	Q	0.5	
		4022.0	104.6	2 <mark>113.7</mark>	\$ <mark>0.0</mark>	0.0	Q' <mark>Y</mark>
			Exposure	oeriod 4 hrs wit			0.0
IE	18 hrs	Solvent control ¹	100.0	7100.0	4.0	SOS C	1 ₹0 ^y
		Positive control ⁴	n <u>A</u>	785°	22.5	21.0 ^S	6.5 0.5 0.5
		1005.5	77.7	106.5	1.5	2.0	0.5
		2011.0	802	119.	() 1.5 () 1.5 ()	1.0	
		4022.0	9 703	149.8 A		2.0 1.0 1.5 1.5	0.5
D	18 hrs	Solvent control	\$\frac{\partial \text{900.0}}{\text{00.0}}	100.0 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	22.50 1.5 1.5 30.0 1.0	J. .5	0.5
		Positive control ⁴	ad,	0	30.0	30.0 ⁸ / ₂	9.0
		100505	30.1	9004.4		1.0	0.0
		2011.0	97.1	106.0	1.0	√ <mark>1.0</mark>	0.0
		4022.0	97.1 97.1 97.1 97.1 97.1 97.1 97.1 97.1	9 <mark>8.1</mark>	1.0 5 1.0 5 1.5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	, 1.5	0.5
# Evalu	ding cells carrying tation of 50 metap	g excepanges de la constant de la co				30.0% 1.0 1.5	
## Eva	lustion of 200 me	somhacae nar cultura					
S Aberr	ration frequency s	tatismally significant hig	her than corresponding	ng contro values	Ø1		
2 EMS	1000.0 N/g/mL			ng control values			
	600 Mg/mL 2.0 Mg/mL						
. 0171	2.0						

III. Conclusions

In conclusion, when tested that under the experimental conditions reported, the test item BCS-CV14885 did not induce structural chromosomes aberrations in V79 cells of the Chinese hamster in vitro, when tested up to the highest required concentrations.



Report:	ö; ;2013;M-449010-02; Amended: 2015-01-19
Title:	Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT) - BCS-CV1985
Report No:	1490203
Document No:	M-449010-02-1
Guidelines:	This study was conducted according to the procedures indicated by the following internationally accepted guidelines and recommendations: Winth Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 476; In vitro Mammalian Calv Gene Mutation Test, adopted July 21, 1997; Commission Regulation (EC) No. 440/2008 B17 dated May 30, 2008; United States Environmental Protection Agence Health Effects Test Guidelines, OPPTS 870.5300, In Vitro Mammalian Cell Cone Mutation Test, EPA-712-C-98-221, August 1998;not specifical
GLP/GEP:	yes & & & & & & & & & & & & & & & & & & &

Executive summary:

The study was performed to investigate the potential of BCS-CV 4885 to induce gene mutations at the HPRT locus in V79 cells of the Chinese Ramster.

The assay was performed in two independent experiments, using two parallel cultures each. The first main experiment was performed with and without liver inicrosomal activation and a freatment period of 4 hours. The second experiment was performed with a treatment time of 4 hours with and 24 hours without metabolic activation.

The highest concentration of 4020 µg/mL applied in the pre-experiment and in the main experiments was equal to a molar concentration of approximately 10mM

No substantial and reproducible dose dependent increase of the mutation frequency exceeding the historical range of solvent controls was observed in both main experiments.

Appropriate reference mutagens, used as positive controls, induced a distinct increase in mutant colonies and thus, showed the sensitivity of the test system and the activity of the metabolic activation system. In conclusion it can be stated that under the experimental conditions reported the test item did not induce gene mutations at the HPRT local in VD cells.

Therefore, BCS-CV14885 is considered to be non-matagenic in this HPRT assay.

"J." Mat@rial and methods

A. Materials

1. Test material:

B&S-CV14885

Description:

≟white powder

Ot/Batch

BC&CV14885-01-01

Purity:

978 % www, dose calculation will be adjusted to purity

Stability of test compound:

Not performed as part of this study

Solven Gused: .

culture medium, (MEM)

2 Control materials:

(Negative:

Tissue Culture medium

Positive: now activation (-S9 mix):

Ethyl methanesulphonate (EMS) dissolved in nutrient medium.

Final concentration 0.15 mg/mL (1.2 mM)

Positive: with activation (+S9 mix):



> DMBA; 7,12-dimethylbenz(a)anthracene dissolved in DMSQ. Final concentration 1.1 µg/mL (4.3 µM)

3 Metabolic activation:

S9 mix from male Wistar rats

Phenobarbital/β-Naphthoflavone induced rat liver S9 was used as the metabolic activation system. was prepared from 8 – 12 weeks old male Wistar rats (Hsd Cob: WU, weight approx. 220 – 320 x, The Netherlands) induced by intraperitones applications of 80 mg/kg b.w. pherobarbital and by peroral administrations of β -naphthoflavone each, on three consecutive days:

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 of ofactors were added to the \$9 supernatant to reach following concentrations in the S9 mix were: 8 mM MgGl₂, 33 mM KQr, 5 mW glucose-6phosphate and 4 mM NADP in 100 mM sodicin-ortho-phosphate-buffer, pH 7.4. During the experiment, the S9 mix was stored in an ice bath.

4 Test cells:

4 Test 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 han stock cultures) and good froning 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 kary otype with a modal chromosome number of 22.

5 Test compound consentrations used (µg/mL):

exposure period	S9 nãx			concentry in µg	amI. √√	. V	
		y «p		Experin	nent F	j	
4 hours	" - [©]	125.6	× 244.5	× 502	0 ₁₀₀₅	2010	4020
4 hoors	+	_@ 0125.6	251.3	502.5	1605	2010	4020
· "/	~O		4, Q"	Experin	ngast II		
24 hours		125.6	² 251.3 6	50205	1005	2010	4020
4 hours	@ + @	\$\\\125 ₆ \\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	251.3	\$02.5	1005	2010	4020

Concentrations given in bold lefters were chosen for the mutation rate analysis

In experiment I and II the cultures at the lowest concentration of 125.6 μg/mL were not continued since a minimum of only four analysable concentrations or required by the guidelines.

B. Study design and methods

1 Test performance,

The study was conducted at

/Germany). The experimental start and completion dates of the study were November 22nd, 2012 and January 24th 2013, respectively.

2 Culture Medium:

For seeding and treatment of the cell cultures the complete culture medium was MEM (minimal essential medium) containing Hank's salts, neomycin (5 μg/mL) and amphotericin B (1 %). For the selection of



mutant cells the complete medium was supplemented with 11 μ g/mL 6-thioguanine. All cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % CO₂ (98.5 % air)

3 Seeding of the cultures:
Two to three days after sub-cultivation stock cultures were trypsinized at 37 for 5 minutes. Then the enzymatic digestion was stopped by adding complete culture medium with 90 % FBS and a single cell suspension was prepared. The trypsin concentration for all sub-culturing steps was 0.2% in PBS.

Prior to the trypsin treatment the cells were ripsed with 10 % FBS. EDTA (ethylene diamine tetraacetic acid). Approximately 1.5×10 single culture and 5 00 cells (in duplicate) were seeded in plastic culture flasks. The cells were gown for 24 hours prior to treatment.

4 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 stops with "saline G". In the second experiment the cells were exposed to the test them for 24 hours in complete medium, supplemented with 10 % FBS, in the absence of metabolic activation. The pil was adjusted to 7.2 The colonies used to determine the cloning efficiency (survival) were fixed and stained approx. 7 days after treatment.

Three or four days after treatment 1.5×106 cells per experimental point were sub-cultivated in 175 cm² flasks containing 30 mg/medium. Following the expression time of 7 days five 800m² cell culture flasks were seeded with about 3 - \$\times 10^5 cell's each in medium containing 6-TOP 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% CO₂ for about 8 days. The colonies were stained with 10% methylene blue in 0.01 & KOI solution.

The stained colonies with more than 50 cets were counted. In doubt the colony size was checked with a preparation microscope.

6 Acceptability of the Assay:

The gene initiation assay:

The gene potation assay is considered deceptable if it meets the following criteria:

- The numbers of mutam colonies per 100 cells found in the solvent controls falls within the Laboratory historical control data
- the positive control substances abould produce a significant increase in mutant colony frequencies.
- the clouding efficiency of (absolute value) of the solvent controls should exceed 50 %.

7 Statistical analysis:

A linear regression (least squares) was performed to assess a possible dose dependent increase of mutant frequencies. The numbers 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 was considered together.



8 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

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 contraction of the concentration in the experiment. mutation frequency. Such evaluation may be considered also in the case that a three fold increase of the mutant frequency is not observed.

However, in a case by case evaluation this decision depends on the level of the corresponding sowent & " control data. If there is by chance a low sportaneous mutation rate within the Japoratory'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 solvers controls within all experiments of his study was also taken into consideration.

J. Hesults and discussion

The test item BCS-CV14885 was assessed for its oftential to induce gene mutations at the HPRT locus

using V79 cells of the Chinese hanster. The assay was performed in two independent experiments, using two parallel cultures each. The first main experiment was performed with and without liver microsomal activation and a treatment period of 4 hours. The second experiment was performed with a treatment time of 4 hours with and 24 hours without metabolic activation.

No precipitation of the test item was observed up to the maximum concentration in all experiments. No cytotoxic effects indicated by a cloning relative efficiency. I and/or relative cell density below 50 % in both parallel cultures occurred by to the maximum soncentration of 4020 μg/mL in experiment I and II with and without metabolic activation following A and 24 hours treatment.

No relevant and reproducible increase of mutant colon numbers/106 cells was observed in the main experiments up to the maximum concentration with and without metabolic activation. The induction factor did not exceed the threshold of three times the corresponding solvent control at any experimental data point.

In experiment I the murant coonies/10% cells exceeded the range of the historical solvent control data (3.4) - 36.6 mutant colonies/ 106 cells) in the presence of metabolic activation at 252.3 μg/mL (culture II) and at 2010 and 4020 μg/mL (opture 1) 36.9, 40.9 and 38.3 mutant colonies/106 cells). However, the threshold of three times the mutation frequence of the corresponding solvent control was neither reached nor exceeded.

A linear regression analysis (least squares) was performed to assess a possible dose dependent increase of mutant frequency. A significant dose dependent trend of the mutation frequency indicated by a probability value of <0,05 was determined in both cultures of the second experiment without metabolic activation. However, the trend was judged as biologically irrelevant as the mutation frequency did not reach or exceed the threshold described above and all of the individual values of the mutation frequency remained within the historical range of solvent controls in the second culture.



The viability of the solvent control of the second culture of the first experiment with metabolic activation reached but did not exceed the lower limit of 50%. The data are a second culture.

In eviability of the solvent control of the second culture of the first experiment with metabolic activation reached but did not exceed the lower limit of 50%. The data are acceptable however as the lower limit was exceeded in the parallel culture under identical conditions.

EMS (150 μg/ml.) and DMBA (1.1 μg/ml.) were used as positive controls and showed a district inergase in induced mutant colonies.

The results are summarized in the following table: The state of the s the low wed a district the state of the stat de little de lit



												 @j°
	Conc. µg/ml	S9- mi x	Relative cloning efficienc	Relativ e cell density	Relative cloning efficienc	Mutant colonies / 10 ⁶ cells	Inductio n factor	Relative cloning efficienc	4	Relative claming officienc y II	Mutant colonies	Inductio In factor
<u> </u>		-	<mark>%</mark>	<mark>%</mark>	<mark>%</mark>			<mark>%</mark>	% ()	<mark>%</mark>		
Column	1	2	3	<mark>4</mark>	<mark>5</mark>	<mark>6</mark>	7	8	9	10	11 0	12,9
Experimen treatment	t I / 4 h	•	Culture I			♠		Culture	Ö, °		C C	
Solvent		-	100.0	100.0	100.0	29,9	1.0 。	100.0 %	\$\frac{\circ}{100.0} \times	100.0 \O	37.6°	100
control (29.9 0			700.0	100.0 N		
with medium					Š	29,9		Q .		· O		Sy °
Positice	150.0	_	119.3	<mark>78.7</mark>	76.6°	(13¥.7	4 .5	100.6	98.8	684,	186.3	\$.0
control		•				, Ç		100.6			Ç C	§ .0
(EMS)				4	Y '0			Ž 2				
Test item	125.6	-	<mark>119.8</mark>	Cult@re v	vas net contin	nuæd .	0.3	111.8	Culture v	vas no conti	nued [#]	
Test item	251.3	-	<mark>96.4</mark>	₹00.8	A 3	≽ <mark>9.0</mark>			110.5		¥ <mark>23.3</mark>	0.6
Test item	<mark>502.5</mark>	-	113.4 Q	117.4	82.7	31.0	1. P	101.2	141.4	85.4	33.3	0.9
Test item	1005. 0	•	76.7	1364	104.5 ()	86.1	\$0.9 \$\int \text{3.9}	87.7 W		. 789	19.8	0.5
Test item	2010. 0	- Ĉ		101.6	92.6			000	139.0 📡	<mark>85.3</mark>	37.3	1.0
Test item	4020.		889 ×	0 <mark>107.8</mark>	89.2 &	21.6	0.7 N	10¥.5	2 <mark>37.9</mark>	89.2	15.4	0.4
Experimen treatment			Cultifie I	Ž,			'O" _	Culture II				
Solvent		+ 🍣	9 <mark>100.0</mark> &	100.0	1000	21,9	1.0	100.0	100.0	100.0	<mark>35.7</mark>	1.0
<mark>control</mark>			9 <mark>180.0</mark> &		~ ×							
with medium	~\$	D .			1000	21, y						
Positice		+	126.8	92 <mark>9</mark> ″		356.4 356.4	^{25.4}	<mark>92.0</mark>	<mark>89.5</mark>	100.7	<mark>718.9</mark>	20.1
(DMBA)	*	\$ \$/	S A			~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~						
Test item	125.6	+,	135%	Gullure v	vas Rot conti	pwed#		<mark>96.0</mark>	Culture v	vas not conti	nued [#]	1
Test item	251.3	4	₄ 159.5	119.1	113.3	32.7	<mark>1.5</mark>	104.5	98.0	116.8	36.9	1.0
Test item	502	+ ^	140.2	109.7	103,8	24.4	1.1	<mark>97.8</mark>	96.2	111.6	36.1	1.0
Test item	1 905.		16 0,4	0.8±0	∞ 9 .8	24.2	1.1	117.0	95.0	107.2	33.6	0.9
Test item	2000.	, +,\$	133.9	111.9	99.2	40.9	1.9	102.3	93.2	104.7	24.3	0.7
Test item	4020.	+	130.0	104.6	108.1	38.3	1.7	115.7	<mark>74.0</mark>	104.0	<mark>27.7</mark>	0.8



			I									
Experimen 24 h treatn			Culture I					Culture II		ð	(T)	
Solvent control with		-	100.0	100.0	100.0	27.2	1.0	100.0	100.0	P00.0	12.9	1.0 C7
ositice control EMS)	150.0	-	92.0	105.5	85.8	450.6	\$ 150 to	105.3	W .	Ý , Ó	39935 Z	60.4 60.4 6.5
Γest item	125.6	<u> </u>	101.5	Culture v	vas not conti	nued 🗸		گ <mark>ـ 102.9</mark> پ	Culture v	vas no Contin	nued	Ţ
Γest item	251.3	<u> </u>	99.4	83.4	104.1		0.6	100.40	1204.5	104 .9	16.5 17.9	1.3
Test item	502.5		97.4	98.8	103.7	15,1	0.6	<u>≈100 3</u>	125.7	≫ <mark>96.1</mark>	17.9	1.4
Test item	1005. 0	-	101.7	<mark>86.1</mark>	98.70	(19%) (19%)	9 <mark>4.7</mark>	102.5	118.6		19.9	27.5
<mark>Γest item</mark>	2010. 0	-	<mark>101.2</mark>	113.3 «	93.6	28.5 ©	1.0 ()	9.6 0	7122.1 6	93.4 98.0	21.8 ⁽²⁾	1.7
Test item	4020. 0	-	99.5	102.1 G	88.4 ~	* 43.1	1.6 * ¥	10/20	122.1	88.0	₽ <mark>26.7</mark>	2.1
Experiment treatment						15,45						
Solvent		+	@ V	100.0	100.0	15,4	1.6	100.0 O	100.0	100.0	18.6	1.0
control with medium			\$60.0 \$\int\tag{\frac{1}{2}} \times						Š			
Positice control (1.3	+	100.6	942 5 7 8	92 .	20.8 (014.4 27	95.8	89.1	82.3	224.0	12.0
Γest item	125.6	+,3	102.2	Cutzvire v	vas not contin	naed [#]		98.3	Culture v	vas not conti	nued [#]	l
est item	251.3	n <mark>+</mark>	1.000 " .	306.6	© 5.7 ° ~	20.7	1.3	<mark>99.7</mark>	<mark>93.4</mark>	<mark>95.4</mark>	22.1	1.2
est item	502.3	+	902.0	108.9	12f:3	M	<mark>0.9</mark>	<mark>97.4</mark>	109.1	<mark>98.8</mark>	17.0	0.9
<mark>Γest item</mark>	100s. So	+	101.1	<mark>136</mark> 25 Q	1400/4 0	21.8 21.8	1.4	<mark>98.0</mark>	106.5	<mark>101.0</mark>	13.1	<mark>0.7</mark>
Test item	2010. 0	+ 🔏	102.9	102.2	99.8 Q	, <u>19</u>	0.8	98.0	105.1	91.7	<mark>22.4</mark>	1.2
<mark>Γest item</mark>	4020. 0	Q ₁	101.6	\$6.7 \$6.7	V90.2	<mark>21.7</mark>	1.4	<mark>99.6</mark>	<mark>99.8</mark>	<mark>99.0</mark>	<mark>16.0</mark>	0.9
# culture w	as hot con	ntimued	since mini	mu‱of on	lŷ Q ur analy	sabel conce	ntrations is r	equired	ı		1	
			since Ominin									



III. Conclusions

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-CV14885 is considered to be now mutagenic in this HPRT assay.

CA 5.8.2 Supplementary studies on the active substance

Since the toxicological profile was sufficiently demonstrated, no supple

Endocrine disrupting properti@ **CA 5.8.3**

ne disrupting proporties. It should be noted that to date, no clear criteria are available &

Mesosulfuron-methyl has no effects of reproductive indices nor fertility for reproductive tissues and organs as shown in the multi-generation stud@ Mesosulfuron-methyl is not a developmental toxicant. After a detailed analysis of all these apical texicological soldies onder reclusion of scientific and regulatory hazard principles in Orscussion at present no evidence of endocrine disrupting properties are seen and mesosulfuron-methyl does not all under the interim defination for endocrine disruption. Therefore, based on a complete toxicological data set, there is no evidence of endocrine disrupting properties of mesosulfuron-methyl

RMS has considered the following reference as relevant for

		~~~		~ ./ \$	
Report:	© & CA 5.8.3	6/01;	;		;2013;M-476775-01
Title:			· // //	affect the functio	n of sex hormone receptors
	and aroma	tase enzyme acti			
Report No."	<b>M</b> -4767 <b>\$</b>	-01-1			
Document No:	∾ M-476775		~ Y (		
<b>Guidelines:</b>	not applic	ante;not applica	able o '		
<b>GLP/GEP:</b>					

As mentioned as footnote of ablication, Mesosulfuron was negative in the tests



#### CA 5.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

Report:		000;M-199528-01	Á	, S	
Title:	Medical surveillance of man	ufacturing plant person	nnel Medical 🚳	ia mesosulfuro	on-meth C
	Code: AE F130060		A	Ş	
Report No:	C009926	ĈA		~~	~ Ø ~
Document No:	M-199528-01-1		W.	<b>, , ,</b>	
<b>Guidelines:</b>	Deviation not specified	¥.	Q	. 0 3	
GLP/GEP:	no	4 W	4		\$ \(\alpha\)

The following information (CA 5.9.2 – 5.9.7) was provided by the global medical director of Bayer CropScience, and gives the most corrent facts.

#### **Medical assessment:**

Occupational medical surveillance of workers exposed to Mesosulfuror methyl performed since 2004 annually on a routine basis, not directly related to exposures and not reveal any uncanted effects to the workers. The examinations included the following laboratory parameters and clinical and technical examinations:

No. of workers exposed:

Medical examinations:

Laboratory examinations:

Technical examinations:

History and full physical examination

FBC liver enzymes, urine stick

Long furction testing, Osion testing and audiometry as

meeded for specific ich tasks

During the production period since 2004 neaccidents with Mesosuffuron-methyl occurred in the workers. No further consultations of the Medical Department due to work or confact with Mesosulfuron-methyl were required.

# CA 5.9.2 Data collected on humans

One suicide attempt has been reported from ran. After drinking an unknown amount of a formulation containing mesosulfuron-methyl musea, vomiting and stomach upset were seen. The ingestion was survived without sequelae.

# CA 3.9.3 Direct observations

None.

# CA 5.9.4 Epidemiological studies

No epidemiological studies are available in literature.



#### **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, mesosulfuron-methyl does not interest aid measures, antidotes, medical treatment.

First Aid: Animal experiments with high doses showed unspecific symptoms like irregular breathing, weak

Remove patient from exposure/terminate exposure

Thorough skin decontamination with copions appoints of water and

polyethylenglykol 300 followed by water 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 Jegard of the low toxicity It should only be considered if a large and ount has been swallowed, if the ingestion was less than one hour ago, and if the patient is fully consolous.

Induced vomiting can reprove maximum 50% of the ingested substance. Note: Induction of vomiting is forbidden, if a formulation comaining organic solverits has been intested

## Treatment:

Gastric lavage does not seem to be required in regard of the low toxicity of the compound The application of activated chargoal and sodium sulphate (or other carthartic) might be considered in significant ingestions.

As there is no fritidote treatment has to be symptomatic and supportive.

# Expected effects of poisoning No persistent effects are expected after posoning