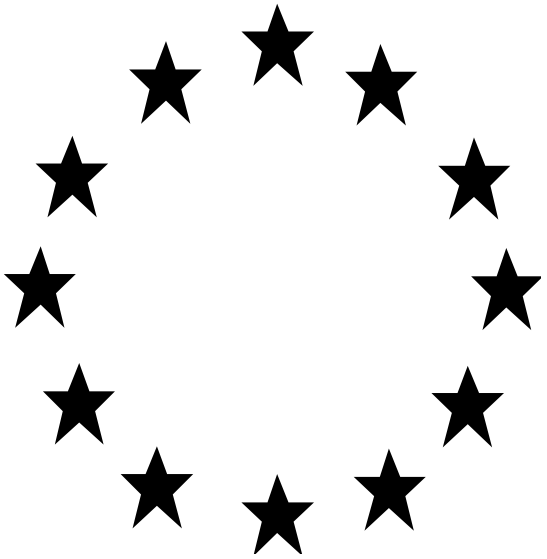


Final addendum to the

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State Italy for the new active substance

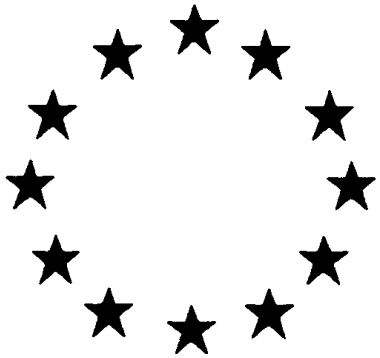
PENOXSULAM

as referred to in Article 8(1) of Council Directive 91/414/EEC

January 2009

Table of contents

Ministry of Health
P.le Marconi, 25
00144 Rome
Italy



PENOXSULAM

Addendum B.7
Residue data

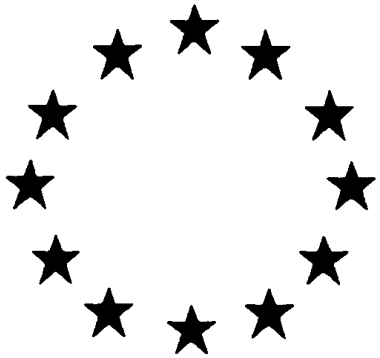
August 2006

Table B.7.9-1: Total Radioactive Residues in Rotational Crops Planted 90 Days after the Application of ^{14}C-DE-638, Phenyl (PH) Label and Triazolopyrimidine (TP) Labels, at 1X and 2X Rates (50 and 100 g a.s./ha) to Soil

Specimen	DAT[a]	DAP[b]	Total Residue, mg/kg[c] PH	Total Residue, mg/kg[c] TP
1X Application:				
Wheat				
Forage	187	97	0.004	<LOQ[d]
Hay	252	162	0.021	0.009
Straw	294	204	0.024	0.011
Grain	294	204	<LOD[e]	<LOD[e]
Kale	238	148	0.005	0.003
Potato				
Foliage	298	208	0.047	0.024
Tuber	305	215	<LOQ[d]	<LOQ[d]
2X Application:				
Wheat				
Forage	187	97	0.007	0.005
Hay	252	162	0.032	0.022
Straw	294	204	0.028	0.030
Grain	294	204	<LOQ[d]	<LOQ[d]
Kale	238	148	0.008	-
	291[f]	201[f]	-	0.014
Potato				
Foliage	298	208	0.062	0.038
Tuber	305	215	0.003	0.003

[a] DAT = Days After Treatment of the soil with test substance (test substance applied 20-Jul-00).
[b] DAP = Days After Planting of the rotational crops (crops planted 18-Oct-00).
[c] Total Residue expressed as mg DE-638 equivalents per kilogram (or ppm or µg/g).
[d] The limit of quantification (LOQ) was either 0.002 mg/kg (wheat forage, kale and potato tubers) or 0.005 mg/kg (all other samples).
[e] The limit of detection (LOD) was 0.001 mg/kg for all samples.
[f] The maturity of the TP-2X kale was delayed and it was harvested at a later time than the other kale samples.

Ministry of Health
P.le Marconi, 25
00144 Rome
Italy



PENOXSULAM

ADDENDUM TO THE MONOGRAPH

October 2006 rev/2 December 2006

ANNEX B

PENOXSULAM

B.6 TOXICOLOGY AND METABOLISM

TOXICOLOGICAL ADDENDA TO DAR

Foreword: this addendum refers to open point identified in the Evaluation Table of August 2006 and discussed at the PRAPeR meetings held at EFSA during September 2006.

Section 2: Mammalian toxicology

Open point 2.1

Selection of Appropriate AOEL for Penoxsulam

Based on the initial evaluation, the NOAEL selected by the RMS for AOEL derivation was 10 mg/kg bw/day from the 90-day study in mice (the lowest NOAEL from subchronic studies)

The notifier argued that the appropriate NOAEL to be used in establishing the AOEL should be 18 mg/kg bw/day from the 90-day dog study.

The RMS has reviewed the data from 90-day studies on penoxsulam, and identified NOAELs of 50 mg/kg bw/day in rats, 18 mg/kg bw/day in dogs, and 10 mg/kg bw/day in mice. Considering that the lowest NOEL value in mice is mainly determined by the dose spacing in the 90-day study and taking into account that a comparison of the data from 90 day studies in rats, mice and dogs indicates the dog as the most sensitive species tested, the RMS supports the use of 18 mg/kg bw/day from the 90-day dog study as the most appropriate NOAEL in establishing the AOEL by applying an uncertainty factor of 100.

RMS observes that that the issue has already been solved: indeed, in the current version of the monograph as well as in the list of end-point which has been discussed at the evaluation meeting and at PRAPeR meeting an AOEL of 0.18 mg/kg bw/day has been proposed.

Detailed explanation

Considering that an Acceptable Operator Exposure Level is the maximum amount of active substance to which an operator (including re-entry workers and bystanders) may be exposed without any adverse effect, the AOEL may be calculated as an internal (systemic) Reference Dose and/or an external Reference Dose. In either case, the AOEL is based on the highest dose at which no adverse effect (i.e. the NOAEL) is observed in appropriate tests in the most sensitive, relevant animal species.

This is just in line to what stated by the EC Guidance for the setting of acceptable operator exposure levels: "*…only one AOEL should be established for an exposure period appropriate to the frequency and duration of exposure of operators (including contractors) and re-entry workers. This is typically short-term exposure, e.g. repeated exposure during a total of up to 3 months per year*." In addition, "*as a default procedure, an AOEL will be based on the NOAEL from an oral short-term toxicity study (28- or 90-day study; rarely 1-year dog study) provided that:*

- *the critical endpoint(s) of the substance (e.g. reproductive/developmental toxicity) are covered;*
- *no irreversible effects occur at lower dose levels after chronic exposure*
- *the number and type of parameters studied are considered adequate; and*
- *the number of animals examined and the animal species is adequate.*"

On this basis, considering that Penoxsulam did not show reproductive or developmental effects, nor is it mutagenic, carcinogenic or neurotoxic and taking into account that the critical end-points of the substance, notably reversible liver and kidney toxicity, are all covered in test guideline compliant 13-week studies in the rat, mouse and dog RMS has the considered that the NOAEL from the 90-day dog study is more appropriate for establishing of the AOEL mainly because the hepatocellular hypertrophy observed at 100 mg/kg bw/day (the LOAEL in the 90-day study in mice), is of low magnitude. So the true NOAEL is likely higher than 10 mg/kg bw/day. The NOAEL from the 90-day dog study (18 mg/kg bw/day) is less than 2-fold higher than that identified in the mouse, while the LOAEL is approximately half (49-57 mg/kg bw/day in dogs) of that in the mouse (100 mg/kg bw/day). See Table 1 for details.

Table 1

Short-term toxicity					
Study	**Species/ Strain**	**Dosage**[†]	**NOAEL**[†]	**LOAEL**[†]	**Target Organ/Major Effect at LOAEL**
90-day dietary	Rat/F344	0, 5, 50, 250 & 500	50 (males) 250 (females)	250 (males) 500 (females)	**Males** Decreased body weight gain & feed consumption; reduced RBC indices; elevated blood albumin & cholesterol; increased liver weight) **Females** Very slight to slight mineralization & hyperplasia of renal pelvic epithelial
90-day dietary	Rat/CD	0, 100, 250, 500 & 1000	100	250	**Kidney**: Very slight hyperplasia of pelvic epithelium in 1 male & 1 female
90-day dietary	Dog/Beagle	0, 6, 18 & 49 for males 0, 6, 20 & 57 for females	18 - 20	49 - 57	**Kidney**: Very slight hyperplasia of pelvic epithelium; crystal deposition in pelvis and collecting ducts. **Liver**: Increased weight
90-day dietary	Mouse/CD-1	0, 10, 100, 500 & 1000	10	100	**Liver** Slight hepatocellular hypertrophy

[†] mg/kg bw/day.

The problem in dose spacing may be due to the fact that one use of the results of the 90-day studies in rodents is to select dose levels for oncogenicity studies based on what is considered an MTD. However, the criteria that determine an appropriate MTD are not globally harmonized and these studies must identify both an MTD and an NOAEL/NOEL. As a result, the spread between dose levels in these studies are frequently 10-fold, even though guidelines recommend intervals of 2-4 fold between doses. Closer spacing of dose levels in rodent studies while attempting to satisfy global criteria would result in studies that could contain up to 5-6 treatment groups in addition to the controls. This results in the use of an excessive number of animals, and is counter to global efforts to reduce the number of animals in studies.
With selection of 100 mg/kg bw/day as the LOAEL, the next lower dose level in the mouse 90-day study is 10 mg/kg bw/day, a 10-fold difference: the magnitude of these effects are such as to suggest that the truly expected NOAEL is higher than 10 mg/kg bw/day.

In the 90-day dog study, dose levels selected were separated by half-log intervals (150, 450 and 1500 ppm). From this study, the LOAEL was 450 ppm (49-57 mg/kg bw/day) based on very slight hyperplasia and inflammation of the renal pelvic epithelium with crystals in the pelvis and collecting ducts of males and females. The NOAEL from the dog study was 150 ppm (18 mg/kg bw/day). The LOAEL for the dog in this study was lower than that for either the rat (250 mg/kg bw/day) or the mouse.
Thus, the 90-day dog study can be regarded as the most appropriate study for establishing the AOEL, and the low dose level of 18 mg/kg bw/day to be the NOAEL.

So the conclusion is that an AOEL of 0.18 mg/kg bw/day, based on a NOAEL of 18 mg/kg bw/day from the 90-day dog study, could be proposed.

<u>Evaluation Table Open point 2.2</u>
Dermal Absorption Value for Penoxsulam

The RMS proposal for dermal absorption in the DAR was 2% for the concentrate and 0.4% (which, due to a printing error, was reported as 0.04%) for the dilution over a 24-h period. The RMS apologizes for the error, and at the same time considers the proposal still valid and continues to support it, based on the results coming from the in vivo study. The RMS agrees that a more detailed description of the above mentioned results would help in taking final decision..
The notifier has submitted, on 30 June 2006, a position paper on dermal absorption value for pexoxsulam, giving all the necessary details and explanation which reported in the following.

Overview
In the EFSA Reporting Table, there is apparently conflicting opinions between Member States on the selection of the appropriate dermal absorption values. As a result dermal absorption has been referred to an Experts Meeting.
The notifier had then submitted the above mentioned position paper to argue that *in vivo* dermal absorption values of 2%/day for the undiluted formulation (GF-657) and 0.4%/day for the 1:100 dilution of the formulation are the appropriate values.
To address the potential concerns raised, it has been considered that in the draft Guidance Document for Dermal Absorption (Sanco 222/2000 Rev 7.2004), the

following statements regarding the residue remaining at the *in vivo* application site are made:*" In case excretion of the substance and/or its metabolites has not come to an end within the sampling period, but there are indications of a clear decrease in excretion, only a part of the skin bound dose may be included in the absorption by expert judgment (Thongsinthusak, 1999 ; De Heer, 1999). In case the experiment is terminated before serial non-detects in excreta are observed and/or no clear decline in excreta is measured, the amount located in the skin should be considered as being absorbed (Chu, 1996)"* .

Based on a review of the original source papers cited in the Guidance, the following points could be considered with regards to Penoxsulam.

Chu, 1996

1."E*xposure calculations for risk assessments could therefore be in error by the failure to measure and include absorbed test compounds remaining in the skin in short-term (i.e. 24-hr) studies***".** So it is clear that data generated post -24 hours should be used to mitigate the need for inclusion of the skin residue. In the case of Penoxsulam data was generated to 72 hours.

2."A *reservoir in the skin after dermal application may enter the body over time, as demonstrated by the decrease in radiolabel in skin and the increase in radiolabel in the body/excreta***".** This criterion is not met in the case of the Penoxsulam data.

and

3. It is also important to note that this paper does not link serial non- detects in excreta with inclusion of the skin residue.

Thongsinthusak, 1999

"T*he question of whether the total Bound Skin Residue should be included in a dermal absorption estimate , the study should be extended in length until there is a minimum dose in the excreta".* There is no mention of serial non –detects in this paper.

De Heer, 1999

"E*liminate (the major part) of the absorbed dose. This latter point is considered to be reached if at least 2 serial non detects occurs in excreta or if the excretion rate has clearly declined".* This criterion is linked to inclusion of application site residue with the caveat –"*unless kinetics demonstrate that this is a clear over estimation".* This caveat is not specified in Guidance Document for Dermal Absorption (Sanco 222/2000 Rev 7.2004).

In general, the Sanco guidance identifies continued excretion as evidence of continued or potentially continuing absorption. Continued excretion is not definitive evidence of continued absorption. Excretion may occur from the already absorbed systemic dose which is by definition already included in the estimation of absorbed dose. Therefore, the use of continuing excretion of the dose to decide on the automatic inclusion of the application site residue, as absorbed dose, is flawed. A more

rationale approach is to consider the difference in the systemic absorbed dose over the duration of the study and compare this to the excreted dose.

The levels of radioactivity in the systemic dose from the Penoxsulam study are so low that it is difficult to determine the trend over the 72 hours.

However, in this case, the variation in the application site residue over the duration of the study should be considered. Initially, there appears to be a decrease in the application site residue. However the decrease is not related to absorption but to transfer to the frame covering the application site. Also the decrease in the application site is not matched by the increase in body/excreta residue as identified by Thongsinthusak, 1999

Therefore, expert judgment indicates that the dose remaining at the application site should not be included in the calculation of dermal absorption.

Summary of the Dermal Absorption Study

The absorption of [^{14}C]-Penoxsulam was assessed *in vivo* in rats following a single topical application of either undiluted GF-237 (25 g Penoxsulam/l) or a spray dilution (0.03 g/l), chosen to represent a concentration that approximates that which will be applied to field crops. GF-237 is a suspension concentrate in oil that contains a nominal concentration of 25 g Penoxsulam/l. Both, the undiluted formulation and the diluted end-use spray concentration (735-fold dilution), were applied at a rate of 10 µl/cm^2 to the clipped dorsal area of approximately 12 cm^2 of male rats. Approximately 1.5-mm thick Teflon® frames, 4 cm × 5 cm with a 3 cm × 4 cm cut-out opening, were positioned intrascapularly as far anterior as possible and attached to the animal using Permabond® Industrial Grade 910 adhesive. The application area was covered with Teflon Spectra/Mesh® macroporous filter material. In order to minimize chewing of the frames and potential oral ingestion of the undiluted GF-237 formulation, rats from Groups 1, 2, and 3 were fitted with small jackets with dermal inserts. Following application, the rats were individually housed in glass Roth-type metabolism cages for the collection of urine and feces. The dose remained in contact with the skin for 24 hr, and then the application site was washed with a soap solution to remove any material remaining on the skin. For those animals sacrificed at 48- and 72-hr post-application, a new Teflon® mesh covering was applied after washing. Each test substance was administered to a group of 12 animals, and four animals per group were sacrificed at 24, 48 and 72 hr after initiation of dosing.
Radioactivity was determined in urine, feces, cage wash, untreated skin, liver, kidneys, carcass, whole blood, treated skin, application apparatus with dressings, and skin wash swabs by liquid scintillation counting.

Following a single topical application of undiluted GF-237 containing ^{14}C-Penoxsulam, minimal amounts of Penoxsulam were absorbed after 24, 48 and 72 hr. The total amount of absorbed Penoxulam was essentially the same in groups sampled over a 72-hr period at approximately 2% of the applied dose. The majority of the radioactivity recovered was located in the skin wash (56-62%), the skin at the application site (8-11%) and the frame and covering (16-23%).

Following a single application of a diluted GF-237 solution to the dorsal skin of male rats, very little dose was absorbed through 72 hr. The actual absorbed dose was equivalent to the levels excreted in the urine, and ranged from <0.04% at 24-hr to 0.71% at 72-hr. The majority of the dose was recovered in the skin wash at 24-hr, ranging from 62-66%, with the frame and covering accounting for 7.2-14.1% of the dose, and the skin at the application site accounting for 8.7-17.3% of the applied dose.

The results are presented in detail in Appendix 1.

Approximately 2% of the radiolabel from the undiluted GF-237 formulation, and < 0.4% of the radiolabel from the spray dilution was absorbed over a 24-hr period. After 72 hours the absorbed radioactivity from the spray dilution increased up to 0.7% of the administered dose, whereas the absorption from the undiluted formulation was higher at 48 hours (2.6%).

Application Site

The low absorption is associated with significant amounts of material retained at the application site. The variation in the amount of material at the application site can be summarized as:

Summary of residue at application site

Undiluted GF-237	% of applied Dose		
Duration (hrs)	24	48	72
Cover	3.28	4.43	7.22
Frame	19.97	11.51	18.5
application site	10.06	11.44	8.21
total	33.31	27.38	33.93

Dilution GF-237	% of applied Dose		
Duration (hrs)	24	48	72
Cover	5.53	8.42	12.8
Frame	1.72	2.47	1.33
application site	17.3	12.19	8.74
total	24.55	23.08	22.87

The variation in the amount of material retained at the application site can be accounted for by the differing distribution in the residue between the application site, the frame retaining the dose and the cover over the frame. Indeed the increase in the material on the cover confirms that the material at the application site is being lost from the skin rather than being absorbed into the skin.

The data in the table above shows that the amount associated with the application site/ frame and cover has not varied over a 72 hour period, i.e. there is no statistical difference between the total values at 24, 48 or 72 hours. This evidence for lack of absorption from the application site is consistent with the lack of any significant increase in the absorbed dose over the 48 hrs.

Absorption from Formulation and Spray Dilution

It has been noted that an inverse relation between concentration and % of absorption is typically observed with most of the plant protection products tested *in vitro* and *in vivo*. At variance, GF-237 showed the opposite behavior, with the undiluted formulation being absorbed more than the spray dilution at any time after an adequate (24h) period of exposure. The relative percentage absorption for the formulation and spray solution of 2% and 0.4% can be rationalized by considering the formulation type (i.e. Oil Dispersion) which is a stable suspension of active ingredient(s) in a water-immiscible fluid (organic phase). This is similar to a Suspension Concentrate where the particles are suspended in water, but the water is replaced with in this formulation with a vegetable oil. The Oil Dispersion formulation is fundamentally different from an Emulsion Concentrate where the active ingredient is dissolved in the organic phase.

The concept that a more dilute solution results in higher percentage absorption is based on the premise that the matrix from which the active substance is absorbed does not fundamentally change on dilution. In the case of GF-657, the active substance is suspended in the organic phase. The spray dilution is *ca*. one-in-one hundred dilution in water. When the matrix of the formulation and the spray solution are compared, it can be concluded that because the oil phase has been diluted. i.e: the percentage of the oil reaching the skin is much lower, the matrices are fundamentally different. In addition, because the active ingredient is not solubilized, and because there is less oil available to aid absorption, the lower percentage absorption from the spray solution can be rationalized.

Thus, the 24-hr *in vivo* dermal absorption values of 2.0% for the undiluted formulation (GF-657) and 0.4% of the 1:100 dilution of the formulation are the appropriate values.

B.6.12 Dermal absorption (Annex IIIA 7.3)

Findings: Following a single topical application of undiluted GF-237 containing ^{14}C-Penoxsulam, l amounts of Penoxsulam were absorbed after 24, 48 and 72 hr. (Table B.6.12.1). The total amount of absorbed Penoxsulam was essentially the same in groups sampled over a 72-hr period at approximately 2% of the applied dose. The majority of the radioactivity recovered was located in the skin wash (56-62%), the skin at the application site (8-11%) and the frame and covering (16-23%).

Table B.6.12.1 : *Percentage of radiochemical material and mg equivalents of Penoxsulam absorbed, remaining in the treated skin and removed from the application site following a single topical application of radiolabelled GF-237 formulation (approximately 0.26 mg Penoxsulam/cm^2)*

	Percentage of Applied Radioactivity		
Sacrifice Time	Group 1 24 Hour Mean ± SD	Group 2 48 Hour Mean ± SD	Group 3 72 Hour Mean ± SD
Covering	3.28 ± 2.41	4.43 ± 1.76	7.22 ± 1.61
Frame	19.97 ± 6.07	11.51 ± 5.41	15.50 ± 6.88
Skin,Wash (at 24hr post application)[1]	56.30 ± 5.26	61.84 ± 8.52	57.87 ± 5.90
Application-site Skin	10.06 ± 3.55	11.44 ± 3.59	8.21 ± 1.77
[µg-eq. DE-638[2]]	[331.638 ± 116.984]	[361.743 ± 113.619]	[254.795 ± 54.776]
Skin[3]	<0.55 ± >0.98	<0.48 ± >0.65	0.32 ± 0.26
Carcass	0.47 ± 0.42	0.62 ± 0.12	<0.24 ± >0.17
Kidneys	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Liver	0.31 ± 0.23	0.16 ± 0.08	0.06 ± 0.01
Blood	0.04 ± 0.03	0.03 ± 0.01	0.05 ± 0.04
Total Tissues	<1.38 ± >1.65	<1.29 ± >0.87	<0.67 ± >0.35
Feces			
0-24 hr	0.13 ± 0.07	0.08 ± 0.01	0.12 ± 0.03
24-48 hr		0.54 ± 0.21	0.40 ± 0.20
48-72 hr			0.45 ± 0.20
Urine			
0-24 hr	0.11 ± 0.03	0.17 ± 0.06	0.14 ± 0.06
24-48 hr		0.25 ± 0.11	0.16 ± 0.03
48-72 hr			0.01 ± 0.00

Rinse			
0-24 hr	0.03 ± 0.04	0.02 ± 0.01	0.02 ± 0.01
24-48 hr		0.07 ± 0.08	0.03 ± 0.02
48-72 hr			0.03 ± 0.01
Final cage wash	<0.09 ± 0.12	0.22 ± 0.20	0.15 ± 0.14
Total Recovery	<91.35 ± >1.25	<91.87 ± >1.29	<90.98 ± >1.73
Percent Absorption[4] [µg-eq. DE-638 absorbed[5]]	<1.74 ± >1.91 [<57.495 ± >62.918]	<2.66 ± >1.37 [<83.957 ± >43.229]	<2.18 ± >0.29 [<67.701 ± >9.094]

Values represent mean ± SD for four rats.

[1] Skin wash total includes other dislodgable radioactivity. Random animals (2 from group 1 and 1 each from Groups 2 and 3) had their dosed skin area and perimeter skin (non-dosed area) outside their frames wiped with a dry Q-tip post-washing at 24-hr post-dosing. These values analyzed via LSC for radioactivity. See appropriate Appendix Table for individual results.

[2] µg-eq Penoxsulam = percentage associated with application-site skin x µg DE-638 applied.

[3] Skin remote from application site

[4] Percent absorbed based on radioactivity found in urine, rinse, feces, carcass, tissues (excluding application-site skin) and final cage wash (FCW).

[5] µg-eq Penoxulam absorbed = percentage x µg Penoxulam applied.

Following a single application of a diluted GF-237 solution to the dorsal skin of male rats, very little dose was absorbed through 72 hr (Table B.6.12/.2). The actual absorbed dose was equivalent to the levels excreted in the urine, and ranged from <0.04% at 24-hr to 0.71% at 72-hr. The majority of the dose was recovered in the skin wash at 24-hr, ranging from 62-66%, with the frame and covering accounting for 7.2-14.1% of the dose, and the skin at the application site accounting for 8.7-17.3% of the applied dose.

Table B.6.12/.2. Disposition of Radioactivity Recovered After Dermal Application of Test Preparation #2, Diluted GF-237

	Percentage of Applied Radioactivity		
Sacrifice Time	Group 4 24 Hour Mean ± SD	Group 5 48 Hour Mean ± SD	Group 6 72 Hour Mean (Range)
Covering	5.53 ± 2.76	8.42 ± 1.48	12.80 (5.24 – 20.36)
Frame	1.72 ± 0.39	2.47 ± 2.27	1.33 (0.88 – 1.78)
Skin,Wash (at 24hr post application)	65.73 ± 2.43	66.06 ± 6.80	62.02 (58.65 – 65.38)
Application-site Skin	17.30 ± 2.56	12.19 ± 3.79	8.74 (8.01 – 9.46)

[µg-eq. DE-638[1]]	[0.220 ± 0.029]	[0.146 ± 0.038]	[0.069 (0.05 – 0.083)]
Skin[2]	NQ (0.05)	NQ (0.06)	NQ (0.08)
Carcass	NQ (0.04)	NQ (0.04)	NQ (0.06)
Kidneys	NQ (0.05)	NQ (0.05)	<0.11 (NQ (0.07) – 0.16)
Liver	NQ (0.05)	NQ (0.05)	NQ (0.08)
Blood	NQ (0.05)	NQ (0.05)	NQ (0.06)
Total Tissues	NQ (0.24)	NQ (0.25)	<0.40 (NQ(0.07) – 0.16)
Feces			
0-24 hr	NQ	NQ	NQ
24-48 hr		NQ	NQ
48-72 hr			NQ
Urine			
0-24 hr	<0.04 ± >0.06	0.11 ± 0.04	NQ
24-48 hr		0.21 ± 0.11	0.23 ± (0.14 – 0.32)
48-72 hr			0.29 ± (0.27 – 0.31)
Rinse			
0-24 hr	NQ	NQ	NQ
24-48 hr		<0.05 ± >0.06	NQ
48-72 hr			<0.08 (NQ(0.00) -0.15)
Final cage wash	NQ	NQ	NQ
Total Recovery	<90.56 ± >0.44	<89.76 ± >1.03	<85.87 (<81.66 - <90.07)
Percent Absorption including NQs[3]	<0.27 ± >0.05	<0.63 ± >0.18	<0.99 (<0.72 - <1.25)
[µg-eq. DE-638 absorbed[4]]	[<0.012 ± >0.002]	[<0.027 ± >0.008]	[<0.032 (<0.023 - <0.040)
Percent Absorption Detected	<0.04 ± >0.06	0.38 ± 0.18	0.67 (0.40 – 0.94)
[µg-eq. DE-638 absorbed]	[<0.002 ± >0.003]	[0.016 ± 0.008]	[0.020 (0.015 - 0.026)]

Values represent mean ± SD for 3 and 4 rats in Groups 4 and 5, respectively. Mean (Range) for Group 6 are based on 2 animals.

[1] µg-eq Penoxsulam = percentage associated with application-site skin x µg DE-638 applied.

[2] Skin remote from application site

[3] Percent absorbed based on radioactivity found in urine, rinse, feces, carcass, tissues (excluding application-site skin) and final cage wash (FCW). Values for NQs are figured in total.

[4] µg-eq Penoxulam absorbed = percentage x µg Penoxulam applied.

Conclusion: Approximately 2% of the radiolabel from the undiluted GF-237 formulation, and < 0.4% of the radiolabel from the spray dilution was absorbed over a 24-hr period. After 72 hours the absorbed radioactivity from the spray dilution

increased up to 0.7% of the administered dose, whereas the absorption from the undiluted formulation was higher at 48 hours (2.6%).

The low absorption coincides with significant amounts of material retained at the application site. The variation in the amount of material at the application site can be summarized as:

Table B.6.12.3 Summary of residue at application site

GF-237	% of applied Dose		
Duration (hrs)	24	48	72
Cover	3.28	4.43	7.22
Frame	19.97	11.51	18.5
application site	10.06	11.44	8.21
total	33.31	27.38	33.93

Dilution GF-237	% of applied Dose		
Duration (hrs)	24	48	72
Cover	5.53	8.42	12.8
Frame	1.72	2.47	1.33
application site	17.3	12.19	8.74
total	24.55	23.08	22.87

The apparent decrease in the amount of material retained at the application site can be accounted for by the differing distribution in the residue between the application site, the frame retaining the dose and the cover over the frame. Indeed the increase in the material on the cover confirms that the material at the application site is being lost from the skin rather than being absorbed into the skin .

The data in Table B.6.12.3 shows that the amount associated with the application site/ frame and cover has not varied over a 72 hour period, i.e. there is no statistical difference between the total values at 24, 48 or 72 hours. This evidence for the lack of absorption from the application site is totally consistent with the lack of any significant increase in the absorbed dose over the period 48 hrs to 72 hrs. In the case of the formulation 2.66% and 2.18%, and for the spray dilution 0.38±0.18 to 0.67 (range 0.4 to 0.96).

In deed in the case of the formulation there is further evidence (Table B.6.12.4)**.** that absorption has ceased. This is shown by the decreasing systemic exposure (i.e. excluding dose that has already been exerted) This effect is not apparently evident in the low amounts absorbed from the spray dilution, this is because of the very low amounts absorbed and the confidence levels i.e there is no statistical significance between the data points.

Table B.6.12.4. Summary of systemic exposure per 24 hours as % of applied dose

Systemic Exposure * over each 24 hr period	0-24	24-48	48-72
Formulation	1.74	2.39	1.31
Dilution	0.04	0.27	0.44

* This excludes material excreta from the body in the previous days

Therefore the most appropriate values for dermal absorption per day are 2% for the formulation and 0.4% for the spray dilution.. There is no evidence that the application site residue will contribute significantly to future absorption
This study is acceptable. It has to be noted it has been demonstrated an inverse relation between concentration and % of absorption with that most of the plant protection products tested in vitro and in vivo. At variance, the GF-237 showed the opposite behavior, with the undiluted formulation being absorbed more than the spray dilution at any time after an adequate (24h) period of exposure.

RMS conclusion

Based on results from an in vivo study in rats, a 2.0% dermal absorption for the undiluted formulation (GF-657) and 0.4% of the 1:100 dilution of the formulation over a 24-hr *in vivo* are the appropriate values for dermal absorption.

Evaluation Table Open point 2.5

Relevance of Large Granular Lymphocytic (LGL) Leukemia in Fischer 344 Rats Treated with Penoxsulam

In the monograph some results from literature papers on molecules which may be considered similar to penoxsulam, are cited which were not submitted. On July 2006 the notifier had submitted these papers. These papers are summarized below, but the RMS stresses on the fact that they should be regarded as confirmatory, so they add nothing to the overall risk assessment.

Summary

In the chronic toxicity/oncogenicity study in Fischer 344 rats with penoxsulam, statistically significant, non-dose related increases in the incidence of large granular lymphocytic (LGL) leukemia were observed in male rats at all dose levels tested when compared to concurrent controls. These data were submitted to an independent Pathology Working Group (PWG) for evaluation. Due to the lack of a dose-response in both the incidence and severity of the LGL leukemia in males over a 50-fold increase in dose, the lack of any increase in this tumor in female rats, the lack of an increase in any other tumors in rats or mice, the lack of mutagenic activity of

penoxsulam, the lack of any increase in the incidence of LGL leukemia in rats administered structural analogs of penoxsulam, and the high spontaneous incidence reported the literature in control animals, the LGL leukemia found in this study was considered spontaneous in origin and unrelated to exposure to penoxsulam. In line with the scientific literature, the finding of an increase in LGL leukemia in one sex in a non-dose related incidence, even when statistically significantly identified, is not considered toxicologically relevant for human risk assessment.

LGL Leukemia

Large Granular Lymphocytic (LGL) leukemia (also known as mononuclear cell leukemia, or MNCL, or Fischer rat leukemia (FRL)) is a lymphoreticular neoplasia, characterized by splenomegaly, increased white blood cells, and infiltration of the spleen, liver, lungs, lymph nodes and bone marrow (Stefanski, *et al*., 1990). The background incidence of this neoplasm in F344 rats is higher in males than females, higher in the F344 rat than any other strain and is not seen in mice or hamsters (Sher, 1982; Frith, *et al*., 1993). The incidence of LGL leukemia in F344 rats reported from studies conducted by the US National Toxicology Program (NTP) average 50.5% in males and 28.1% in females, with frequencies ranging from 10 to 72% in individual studies (Haseman, *et al*., 1998).

LGL leukemia has been well characterized. Dunning and Curtis (1957) were the first to describe this tumor in Fischer 344 rats, classifying it as a monocytic leukemia. Subsequent work by others (Moloney *et al*., 1970; Losco and Ward, 1984; Stefanski *et al*., 1990; Ward and Reynolds, 1990) further characterized the pathology and incidence. LGL leukemia is a tumor of aged rats, and is seldom seen in rats less than 18-20 months of age (Stromberg and Vogtsberger, 1983). However, in older Fischer 344 rats it occurs at a high spontaneous incidence as noted above and is the most common life-threatening neoplasm in this rat strain, accounting for up to 40% of deaths in rats dying before 26 months (Haseman *et al.* 1990, 1998). Clinically and pathologically, this disease progresses quickly and is categorized as an acute leukemia that is well differentiated and highly malignant, progressing from first clinical signs to morbidity and mortality in a period of 2-3 weeks. Histologically, there is no precursor of this tumor. LGL leukemia is easily transplanted and appears to arise in the spleen and spread to other organs, with low and variable involvement of the bone marrow. In fact, the NTP uses criteria to characterize the stage of leukemia based on organ involvement, with Stage 1 characterized by enlargement of the spleen with neoplastic cells in the red pulp, Stage 2 characterized by moderate splenic enlargement and minimal to moderate involvement of the liver, and Stage 3 considered advanced disease with involvement of multiple organs (Dunnick, *et al*., 1989).

The exact mechanism of tumor initiation is unknown, but attempts at linking it to a viral etiology have not been successful. The spleen appears to play a key role in the development and progression of the disease. Moloney and King (1973) demonstrated that splenectomy of young (1-2 month old) Fischer 344 rats greatly reduced the spontaneous incidence of LGL leukemia. Elwell *et al*. (1996) reported that treatment-related decreases in LGL leukemia incidence was seen in 2-year studies with a number of free aromatic amine or nitro compounds that could be metabolized to amines, most of which produced splenic effects in 13-week studies.

Other factors have also been shown to modulate the spontaneous incidence of LGL leukemia in rats. The use of corn oil as a vehicle has been shown to reduce the incidence in male F344 by as much as 50%, though females were unaffected. Dietary restriction in 2-year studies which produced reductions in weight gain has also been shown to decrease the incidence compared to *ad libitum* fed rats. (Stefanski *et al.*, 1990).

The cell of origin of Fischer rat LGL leukemia appears to predominantly be a natural killer (NK) cell based on morphologic and immunologic characterization (Ward and Reynolds, 1983), though there is some heterogeneity within the cell population. The tumor cells are not classified as B-Cell or αβT-Cell, and a good proportion of cells are positive for OX-8 and Asialo GM-1 surface markers (Losco and Ward, 1984; Stromberg *et al*., 1983a, b & c; Ward and Reynolds, 1983).

In humans, two types of LGL leukemias have been identified that account for approximately 10-15% of non-Hodgkin's lymphomas; however, there are clinical and morphologic features of these leukemias that are different from rodent LGL leukemia that make a direct comparison difficult. One type, T-cell Large Granular Lymphocytic leukemia (T-LGL) accounts for 85% of human LGL leukemia and is predominantly found in elderly patients, but is a slow developing disease with involvement of the bone marrow and has been associated with human T-cell lymphotrophic virus (HTLV-1). The other, an NK-cell LGL leukemia, accounts for approximately 15% of human LGL leukemia, but is an aggressive disease found predominantly in young patients. This type of leukemia also involves a high degree of bone marrow infiltration, and has been associated with Epstein-Barr virus (EBV) and HTLV-1 (Greer *et al*., 2001; Reynolds and Foon, 1984; Rose and Berliner, 2004; Stromberg, 1985).

Penoxsulam

In the 2-year chronic toxicity and oncogenicity study with penoxsulam, statistically significant increases in the incidence of LGL leukemia were observed in male Fischer 344 rat in all treatment groups but not in females when compared to the concurrent controls. An independent Pathology Working Group (PWG) was convened to confirm the incidence of leukemia and determine the stage of involvement (Hardisty, 2002). A panel of experienced pathologists reexamined histologic sections of the spleen, liver and lungs from all animals on study, and the stage of development of leukemia was determined using the diagnostic criteria established by the NTP (Dunnick *et al*., 1989). Additional organs were examined from selected animals categorized as having Stage 3 leukemia. The incidence and stage of development of LGL leukemia in this study is summarized in Table 1 below.

Table 1: Incidence and Stage of LGL Leukemia in Fischer 344 Rats (PWG Consensus)

	Males				**Females**			
Dose (mg/kg/day)	0	5	50	250	0	5	50	250
No. Animals	50	50	50	50	50	50	50	50
LGL Leukemia[†]	12	30[*]	29[*]	30[*]	11	11	6	9

Stage 1	1	1	1	6	0	1	0	2
Stage 2	6	9	9	3	2	4	2	1
Stage 3	5	20	19	21	9	6	4	6
Lymphosarcoma	0	0	0	0	0	1	0	0

*Statistically significantly different from control by Yates Chi Square Test, $\alpha = 0.05$.
†Staging according to Dunnick et al. (1989).

The results of the PWG confirmed the statistically significantly higher incidence of LGL leukemia in male F344 rats that was initially diagnosed by the study pathologist. However, there was no dose-response in the incidence as would be expected from a treatment-related effect over a 50-fold increase in dose. In addition, staging of the LGL leukemia with respect to the extent of organ involvement showed no dose-response relationship. In fact, the incidence of Stage 1 LGL (lowest grade) was higher in top-dose males than in controls, while the incidence of Stage 2 LGL was lower in the top-dose males than in controls. In females, the incidence and stage of LGL leukemia was similar in control and treated animals.

The mean historical incidence of LGL leukemia in control animals from 8 studies conducted prior to the study with penoxsulam in the submitting laboratory (The Dow Chemical Company) was 28.5% in males (range 16-40%) and 17.8% in females (range 10-28%). However, the incidence of LGL leukemia in control male Fischer 344 rats from studies conducted by the NTP indicate a mean of 50.5% with a range of 32-74% (Haseman *et al*., 1998). (It should be pointed out that all studies conducted by NTP undergo a peer review to confirm neoplastic diagnoses prior to study finalization.). Thus, although the incidence of LGL leukemia in male F344 rats is outside the historical control range of studies conducted within The Dow Chemical Company, the incidence in treated male rats is close to the mean reported by the NTP, and well within the range of expected values.

There were no treatment-related increases in any other tumor type in either male or female F344 rats in the oncogenicity study with penoxsulam. In addition, no increases in LGL leukemia were observed in carcinogenicity studies conducted with other chemicals in the triazolopyrimidine class of chemistry (Hanley and Billington, 2001). Penoxsulam was also found not to be carcinogenic in male and female mice, and there was no evidence of a mutagenic potential following a battery of *in vitro* and *in vivo* assays.

Therefore, based on:
Therefore, based on:

- the high spontaneous incidence of LGL leukemia unique to Fischer rats, especially males
- the increase in LGL leukemia being limited to one sex (male) and one species (rat)

- the lack of a dose-response in both incidence and severity (degree and extent of organ involvement)
- the lack of any other tumors in either rats or mice
- the lack of genotoxicity
- the lack any increases in LGL leukemia with other compounds in this class of chemistry

the increases in LGL leukemia in male rats following exposure to penoxsulam were considered spontaneous in origin and not treatment-related. In line with the scientific literature, the finding of an increase in LGL leukemia in one sex in a non-dose related incidence, even when statistically significantly identified, is not considered toxicologically relevant for human risk assessment.

References

PSD (2005). PSD Guidance Document: Interpretation of Liver Enlargement in Regulatory Toxicity Studies. 26 May 2005

Sanco/10182/2005 rev.9, Guidance for the Setting of Acceptable Operator Exposure Levels (AOELs), 5 July 2005

US EPA (2004). Federal Register Notice: Penoxsulam, 2-(2,2-difluoroethoxy)-N-(5,8-dimethoxy[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl)benzenesulfonamide; Pesticide Tolerance (http://www.epa.gov/EPA-PEST/2004/September/Day-24/p21502.htm

Position paper on dermal absorption values of penoxsulam
(J M Perkins Msc C.Chem MRSC, Global Leader Dermal Absorption, Human Health Assessment, Dow AgroSciences, **24 July 2006)**

Dunning, W.F., and Curtis, M.R. (1957). A transplantable acute leukemia in an inbred line of rats. *J. Natl. Cancer Inst.* **19**, 845-852.

Dunnick, J.K., Eustis, S.L., Huff, J.E. and Haseman, J.K. (1989). Two-year toxicity and carcinogenicity studies of ampicillin trihydrate and penicillin VK in rodents. *Fundam. Appl. Toxicol.* **12**, 252-257.

Elwell, M.R., Dunnick, J.K., Hailey, J.R., abd Haseman, J.F. (1996). Chemicals associated with decreases in the incidence of mononuclear cell leukemia in the Fischer rat. *Tox. Pathol.* **24**, 238-245.

Frith, C.H., Jerold, M.W. and Chandra, M. (1993). The Morphology, Immunohistochemistry and Incidence of Hematopoietic Neoplasms in Mice and Rats. *Toxicol. Pathol.* **21**, 206-218.

Greer, J.P., Kinney, M.C. and Loughran, T.P., Jr. (2001). T-cell and NK cell lymphoproliferative disorders. *Hematology (Am. Soc. Hematol. Educ. Prog.)* **2001**, 259-281.

Hanley, T.R., Jr. and Billington, R. (2001). "Toxicology of triazolopyrimidine herbicides" in Handbook of Pesticide Toxicology, R. Krieger, ed., Academic Press, Inc., San Diego, CA, pp. 1653-2001.

Hardisty, J.F. (2002). Pathology peer review and pathology working group (PWG) review of large granular lymphocyte leukemia (LGL) in a two-year chronic toxicity/oncogenicity and chronic neurotoxicity study of XDE-638 in Fischer 344 rats. Unpublished report of The Dow Chemical Company prepared by EPL, Inc. DECO HET DR-0357-6203-068a.

Haseman, J.K., Arnold, J. and Eustis, S.L. (1990). "Tumor incidences in Fischer 344 Rats: NTP Historical Data" in Pathology of the Fischer Rat. Boorman, G.A., Eustis, S.L., Elwell, M.R., Montgomery, C.A., Jr. and McKenzie, W.F., eds. Academic Press, Inc., San Diego, CA, pp. 369-393.

Haseman, J.K., Hailey, J.R. and Morris, R.W. (1998). Spontaneous neoplasm incidences in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: A National Toxicology Program update. *Toxicol. Pathol.* **26**, 428-441.

Losco, P.E. and Ward, J.M. (1984). The early stage of larger granular lymphocyte leukemia in the F344 rat. *Vet. Pathol.* **21**, 286-291.

Moloney, W.C., Boschetti, A.E. and King, V.P. (1970). Spontaneous leukemia in Fischer rats. *Cancer Res.* **30**, 41-43.

Moloney, W.C. and King, V.P. (1973). Reduction of leukemia incidence following splenectomy in the rat. *Cancer Res.* **33**, 573-574.

Reynolds, C.W. and Foon, K.A. (1984). T gamma lymphoproliferative disease and related disorders in humans and experimental animals: a review of the clinical, cellular, and functional characteristics. *Blood* **64**, 1146-1158.

Rose, M.G. and Berliner, N. (2004). T-cell large granular lymphocyte leukemia and related disorders. *The Oncologist* **9**, 247-258.
Sher, S.P. (1982). Tumors in control hamsters, rats, and mice: Literature tabulation. *CRC Crit. Rev. Toxicol.* 49 – 77.

Stefanski, S.A., Elwell, M.R. and Stromber, P.C. (1990). "Spleen, Lymph Nodes and Thymus" in Pathology of the Fischer Rat, Boorman, G.A., Eustis, S.L., Elwell, M.R., Montgomery, C.A., Jr. and McKenzie, W.F., eds. Academic Press, Inc., San Diego, CA, pp. 369-393.

Stromberg, P.C. (1985). Large granular lymphocyte leukemia in F344 rats: Model for human t lymphoma, malignant histiocytosis and T-cell chronic lymphocytic leukemia. *Am. J. Pathol.* **119**, 517-519.

Stromberg, P.C. and Vogstberger, L.M. (1983). Pathology of the mononuclear cell leukemia of Fischer rats. I. Morphologic studies. *Vet Pathol.* **20**, 698-708.

Stromberg, P.C., Rojko, J.L., Vogstberger, L.M., Cheney, C. and Berman, R. (1983a). Immunologic, biochemical, and ultrastructural characterization of the leukemia cell in F344 rats. *J. Nat. Cancer Inst.* **71**, 173-179

Stromberg, P.C., Vogstberger, L.M., Marsh, L.R. and Wilson, F.D. (1983b). Pathology of the mononuclear cell leukemia of Fischer rats. II. Hematology. *Vet Pathol.* **20**, 709 - 717.

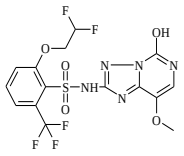
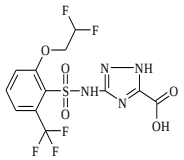
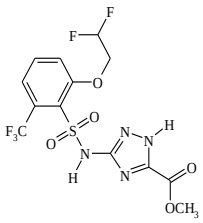
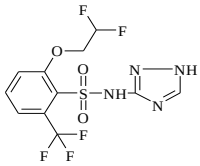
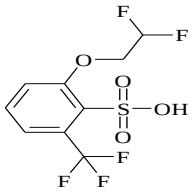
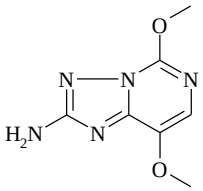
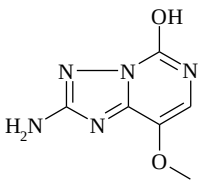
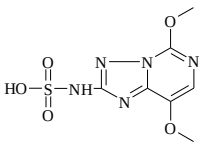
Stromberg, P.C., Vogstberger, L.M. and Marsh, L.R. (1983c). Pathology of the mononuclear cell leukemia of Fischer rats. III. Clinical chemistry. *Vet Pathol.* **20**, 718 – 726.

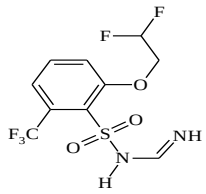
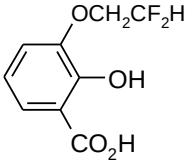
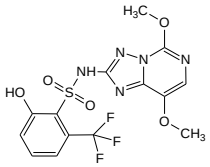
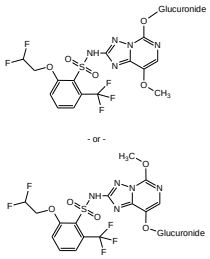
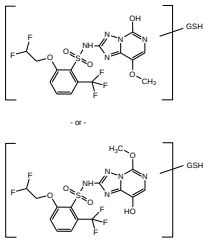
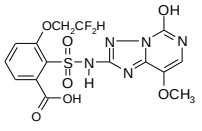
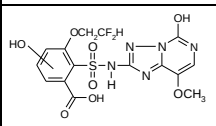
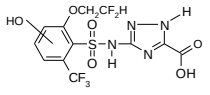
Ward, J.M. and Reynolds, C.W. (1983). Large granular lymphocyte leukemia: A heterogeneous lymphocytic leukemia in F344 rats. *Am. J. Pathol.* **111**, 1-10.

Open point 2.7 (metabolites BST & BSTCA)

Two metabolites were found at high levels in rotational crops: BST and BSTCA (see the table listing the metabolites of DE-638 found in soil, water, crops and animals, based on the metabolites identified in Section 4 (6.1 and 6.2) and Section 5 (7.1 and 7.2) of the original dossier).. It has been requested if they are of toxicological concern. In the following the evaluation is reported.

Table 5.7.1-1: Metabolites identified in soil, water, crops and animals

Metabolite identification	Compartment											
	Structure	Soil			Water			Crops		Animal		
		Aer	An	Phot	Gr	Phot	SW	Rice	Cereals	Rat	Hen	Goat
5-OH-DE-638		X	X		X[1]			X		X		X
BSTCA		X	X	X	X[1]							
Me-BSTCA			< 10%									
BST		< 10%	< 10%		X[1]							
BSA				< 10%		X						
2-AMINO-TP				X		X						
5-OH-2-AMINO-TP						X						
TPSA						X						

Metabolite identification	Compartment											
	Structure	Soil			Water			Crops		Animal		
		Aer	An	Phot	Gr	Phot	SW	Rice	Cereals	Rat	Hen	Goat
SFA												
di-FESA						< 10%						
2-OH-DE-638										X		
Glucuronide conjugate of OH-DE-638										X		
Glutathione conjugate of OH-DE-638										X		
PCA-5-OH-DE-638			< 10%					< 10%				
PHCA-5-OH-DE-638			< 10%									
OH-BSTCA			< 10%					< 10%				

x = present
SW = surface water
[1] PECgw value >0.1 µg/L and <0.75 µg/L based on modelling

Based on recent environmental modeling using revised values for the maximum amount of metabolite formed in the MEDRICE scenario for application in rice, the PECgw for BSTCA is calculated to exceed 0.1 μg/L in groundwater (see Draft Assessment Report, Section B.8.5.1). Based on the results of further environmental modeling using PELMO 3 for application in cereals, the PECgw for 5-OH-DE-638, BSTCA and BST are calculated to exceed 0.1 μg/L (see Annex IIIA 9.6.2).

The EC Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater (SANCO/221/2000 – rev.10; 25 Feb 2003), a scheme is described to determine whether a metabolite is relevant (and thus subject to the 0.1 μg/L limit for drinking water) or not relevant. In this scheme metabolites are assessed stepwise with increasing complexity using criteria of chemical structure, estimated concentrations in groundwater, biological activity, genotoxicity, toxicological hazard and toxicological risk assessment. It is stated that before conducting additional tests it should be checked "...whether metabolites or breakdown products under consideration have already been covered by studies required for the active substance ..."
The two metabolites under consideration pass step 1 and 2 , arriving at step3, at which the Guidance Document indicates *"all metabolites, which might be expected to exceed the limits laid down in Annex VI, point C.2.5.1.2 of Directive 91/414/EEC should be further assessed".* This assessment involves a process, which includes:

- Screening for biological activity
- Screening for genotoxicity
- Screening for toxicity
- Exposure assessment- threshold of concern

Biological Activity: The stated goal of this screening is "*to identify metabolites, which have a comparable target activity as the parent active ingredient, and to deal with cases where the parent molecule is a precursor*". SANCO/221/2000 further states "*(a)s a line of orientation, it should be sufficient to demonstrate that the biological activity of a metabolite is clearly less than 50% of the activity of the parent molecule*".
All major DE-638 soil and water metabolites have been assessed for pesticidal activity and the results of these studies are considered in Annex II, 8.6. With the possible exception of 5-OH-DE-638 at high concentrations, these screening studies demonstrated a lack of herbicidal activity of all metabolites on a wide array of grass and broadleaf plants and sensitive indicator species. These results also showed that DE-638 is not a "pro-herbicide". The 5-OH-DE-638 metabolite was shown to clearly have less than 50% of the activity of the parent in all species tested, demonstrating only 20% injury to redroot pigweed when applied at a rate approximately 4-fold above a rate with DE-638 which produced 100% injury. Based on these data, all metabolites pass this stage.

Genotoxicity: According to SANCO/221/2000, "*all metabolites that have passed…stage 1 of step 3 should be screened for their genotoxic activity*" using a battery of tests to include the "*Ames test, gene mutation test with mammalian cells, and chromosome aberration test"*. 5-OH-DE-638, BSTCA and BST have been evaluated in a battery of tests which include an Ames test, a Chinese hamster ovary HGPRT test (gene mutation) and a rat lymphocyte chromosomal aberration test. The

results of these studies indicate no genotoxic activity of any metabolite in any of these tests. These studies are reported below.

Screening for Toxicity: This step is *"aimed at the question of whether a metabolite has certain toxicological properties, which - from a regulatory perspective – qualify for considering it "relevant*". For pragmatic reasons, the toxicity classification of the parent is used as a starting point to focus the screening activity. Additional testing of metabolites for toxicity is required if the parent active substance:

- is classified as acutely or chronically toxic or very toxic (T followed by R25, R24, R23 or R48, or T+ followed by R28, R27, R26 or R39)
- is classified for reproductive toxicity (any category with R60, R61, R62 or R63)
- is classified as a category 1 or category 2 carcinogen (Carc. Cat. 1; R45) or (Carc. Cat. 2; R45)

Penoxsulam fits into none of the above categories. The toxicity of penoxsulam is low, there was no indication of any effects in developmental or reproductive toxicity tests to suggest classification as a reproductive hazard, and penoxsulam is not classified as a category 1 or category 2 carcinogen. Based on the data on the parent active, there is no reason to suspect that the metabolites would satisfy the requirements for classification in any of these categories.
Based on (1) a lack of biological activity of the metabolites, (2) the negative results of mutagenicity studies conducted with these metabolites, and (3) the lack of toxicological properties of the parent active ingredient that would require classification and labeling as toxic or very toxic, a reproductive toxin, or a carcinogen, the metabolites of penoxsulam are not identified as being relevant.

Exposure Assessment:
The final step in the evaluation of metabolites involves an exposure assessment. SANCO/221/2000 indicates that "*metabolites which have not been identified as being relevant according to the hazard screening outlined in Step 3 should be further tested in an exposure assessment to make sure that any contamination of groundwater will not lead to unacceptable exposure of consumers via their drinking water*". Using a "threshold of concern" approach, SANCO/221/200 states that "*an acceptable exposure level relates to an acceptable upper limit for the concentration of a metabolite of 0.75µg/L.*" It goes on to further state that "*such a threshold can only be considered acceptable if the metabolite in question*

- *does not exceed 0.75 µg/L (or a lower level, if consumers are exposed also via other routes)*
- *and has passed Step 3 i.e.*
    - *has a lower biological activity than the parent*
    - *is not genotoxic and*
    - *is not defined as toxic"*

Exposure estimates for BSTCA based on MEDRICE for uses with rice (see Draft Assessment Report, Section B.8.5.1) and for 5-OH-638, BSTCA and BST using PELMO 3 for cereals use (see Annex IIIA 9.6.2) indicate the PECgw values for these metabolites will not exceed 0.75 µg/L and thus further assessment is not necessary. No detectable residues of parent or metabolites have been identified in rice, wheat or

barley grain following treatment with penoxsulam (see Draft Assessment Report, Section and MIII) to suggest any exposure is to be expected from dietary consumption.

Conclusion: As demonstrated above, metabolites of penoxsulam which have passed Step 3 (Hazard Assessment) can be tolerated without further testing, being the threshold of concern of estimated or actual concentrations in ground water of 0.75 µg/l not exceeded.
Thus, penoxsulam meets the criteria for consideration for Annex I inclusion.

In the following the new studies submitted in order to support the absence of genotoxic potential of the two metabolites under consideration are reported.

5.7.1.1 Mutagenicity studies on 5-OH-XDE-638

There is ample evidence from the rat metabolism study to demonstrate that, as a metabolite, 5-OH-XDE-638 has already been included in the studies conducted on the parent (XDE-638), and separate mutagenicity testing on this metabolite is not warranted. 5-OH-XDE-638 results from the dealkylation of the parent compound. In the rat metabolism study, though 5-OH-XDE-638 was not detected, significant amounts of metabolites derived from 5-OH-XDE-638 have been identified.
Quantifiable levels of downstream metabolites that have been identified include the glucuronide (Metabolite S) and glutathione (Metabolite U) conjugates of the dealkylated-XDE-638, as well as Metabolite Y, which has been identified as the glutathione conjugate of dealkylated XDE-638, which has undergone further hydroxylation, sulfation and loss of the glycine moiety of glutathione. Combined, the amounts of these metabolites in urine and feces account for approximately 17-20% of an administered dose (single or multiple) of 5 mg/kg in males, and approximately 3-6% in females. At a high dose level of 250 mg/kg, the levels in the urine and feces of these metabolites accounted for approximately 4% of the dose in males and 1% in females. In bile duct-cannulated rats given 5 mg/kg, metabolites S, U and Y combined were found to account for 20% of the administered dose in males and 3% in females, consistent with the amounts seen in the urine and feces at this dose level. The proposed metabolic pathway for penoxsulam in the rat (MII, Section 5.1.1) clearly demonstrates the formation of 5-OH-XDE-638.
The presence of metabolites S, U and Y in the bile at levels comparable to those found in the feces of non-cannulated animals suggests that these are formed in the liver. However, the liver was not considered a primary target organ in the rat, with only slight hepatocellular hypertrophy noted at very high dose levels (500 mg/kg/day for 13 weeks) and no increases in mixed function oxidase enzyme (PROD, EROD, MROD and p-NP) levels observed. In addition, these metabolites were all more polar than the parent, enhancing their excretion. Thus, the *in vivo* studies in rats indicate that the formation of these metabolites does not apparently involve reactive species that would be considered more toxic than the parent.
Based on the presence of 5-OH-XDE-638 in the metabolism study in rats, the toxicity of this metabolite has been adequately addressed in the battery of tests conducted with the parent (XDE-638), and that separate mutagenicity studies with this metabolite were not warranted. However, a battery of mutagenicity studies was conducted with

5-OH-XDE-638 in fulfilment of the requirements as laid out in SANCO/221/2000, and the results of these studies confirm the lack of genotoxicity as predicted from the data available from the parent active ingredient.

5.7.1.1a *In Vitro* genotoxicity testing (Reference IIA5.7.1.1a/01)

STUDY TYPE: *(Bacterial system, Salmonella typhimurium; Escherichia coli)*/ mammalian activation gene mutation assay; OPPTS 870.5100[1]; OECD 471 (formerly OECD 471 & 472).

TEST MATERIAL (PURITY): X689643 (6-(2,2-difluoroethoxy)-N-(5,6-dihydro-8-methoxy-5-oxo-s-triazolo[1,5-c]pyrimidin-2-yl)-α,α,α-trifluoro-o-toluenesulfonamide) (99%)

SYNONYMS: 5-OH metabolite of penoxsulam; DES ME-638, (5-Hydroxy-XDE-638)

CITATION: M.S. Mecchi (18 October 2006). *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay Preincubation Method with a Confirmatory Assay with X689643 (5-OH Metabolite of Penoxsulam). Covance Laboratories Inc., Vienna, Virginia. Study ID: 6736-161, 06 June 2006 – 18 October 2006. Unpublished.

SPONSOR: The Dow Chemical Company

SUMMARY:

In a reverse gene mutation assay in bacteria, tester strains TA98, TA100, TA1535, and TA1537 of *S. typhimurium* and tester strain WP2*uvr*A of *E. coli* were exposed to X689643 (5-OH Metabolite of Penoxsulam) (99% a.i.), in dimethylsulfoxide using the preincubation method at concentrations of 100, 333, 1000, 3330, and 5000 µg per plate in the presence and absence of mammalian metabolic activation.

X689643 (5-OH Metabolite of Penoxsulam) was tested up to the limit concentration (5000 µg per plate) with all strains. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as acceptable. This study satisfies the requirement for Test Guideline OPPTS 870.5100[1]; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

---

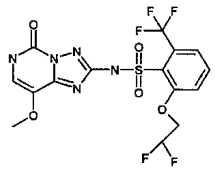
[1]870.5100 - Reverse mutation *E. coli* WP2 and WP2uvrA; *S. typhimurium* TA 97, TA98, TA100, TA1535, TA1537

870.5140 - Gene mutation *Aspergillus nidulans*

870.5250 - Gene mutation *Neurospora crassa*

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	X689643 (5-OH Metabolite of Penoxsulam)
	Description:	White powder
	Lot #:	200000221-139A
	Purity:	99% a.i.
	CAS #:	not provided
		STRUCTURE: 
	Solvent Used:	Dimethylsulfoxide

2.	**Control Materials:**	
	Negative:	NA
	Solvent (final conc'n):	Dimethylsulfoxide at 50 µL per plate
	Positive:	Non-activation: sodium azide 2.0 µg/plate TA100, TA1535 2-nitrofluorene 1.0 µg/plate TA98 ICR-191 2.0 µg/plate TA1537 4-nitroquinoline-N-oxide 0.4 µg/plate WP2*uvr*A
		Activation: benzo[a]pyrene 2.5 µg/plate TA98 2-aminoanthracene 2.5 µg/plate TA100, TA1535, and TA1537 2-aminoanthracene 25.0 µg/plate WP2*uvr*A

3.	**Activation**: S9 derived from									
		x	induced		x	Aroclor 1254	x	Rat	x	Liver
			non-induced			Phenobarbitol		Mouse		Lung
						None		Hamster		Other (name)
						Other (name)		Other (name)		

S9 mix composition: H_2O (70%), 1M NaH_2PO_4/Na_2HPO_4, pH 7.4 (10%), 0.25M Glucose-6-phosphate (2%), 0.10M NADP (4%), 0.825M KCl/0.2M $MgCl_2$ (4%), and S9 Homogenate (10%).

4.	**Test organisms:** *S. typhimurium* strains										
			TA97	x	TA98	x	TA100		TA102		TA104
		x	TA1535	x	TA1537		TA1538	x	WP2*uvr*A (*E. coli*)		
Properly maintained?								x	Yes		No
Checked for appropriate genetic markers (*rfa* mutation, R factor)?								x	Yes		No

5. Test compound concentrations used:

Non-activated conditions:

Cytotoxicity test: TA100 and WP2*uvr*A at 10.0, 33.3, 100, 333, 1000, 3330, and 5000 µg per plate; two replicates.

Mutagenicity assay: TA98, TA100, TA1535, TA1537, and WP2*uvr*A at 100, 333, 1000, 3330, and 5000 µg per plate; three replicates.

Activated conditions:

- Cytotoxicity test: TA100 and WP2*uvr*A at 10.0, 33.3, 100, 333, 1000, 3330, and 5000 µg per plate; two replicates.
- Mutagenicity assay: TA98, TA100, TA1535, TA1537, and WP2*uvr*A at 100, 333, 1000, 3330, and 5000 µg per plate; three replicates.

B. TEST PERFORMANCE

1. Type of *Salmonella* assay: pre-incubation (20 minutes)

2. Protocol:

Tester strains were exposed to the test article via the preincubation modification of the Ames Test originally described by Yahagi *et al.* (1975) and Maron and Ames (1983). This methodology has been shown to detect a wide range of classes of chemical mutagens. In the preincubation methodology, S9 mix (or phosphate buffer, where appropriate), tester strain, and test article were preincubated for approximately 20 minutes prior to addition of molten agar. The agar and preincubation reaction mixture were mixed and then overlaid onto a minimal agar plate. Following incubation, revertant colonies were counted. Test article, vehicle controls, and positive controls were plated in triplicate. The condition of the bacterial background lawn was evaluated both macroscopically and microscopically (using a dissecting microscope) for indications of cytotoxicity and test article precipitate. Evidence of cytotoxicity was scored relative to the vehicle control plate and was recorded along with the revertant counts for all plates at that concentration. Revertant colonies were counted either by automated colony counter or by hand.

3. Statistical Analysis: Mean and standard deviation were calculated for each replicate.

4. Evaluation Criteria: Before assay data were evaluated, criteria for a valid assay had to be met. The following criteria were used to determine a valid assay:

Salmonella typhimurium tester strain cultures exhibited sensitivity to crystal violet to

demonstrate the presence of the *rfa* wall mutation. Cultures of tester strains TA98 and TA100 exhibited resistance to ampicillin to demonstrate the presence of the pKM101 plasmid.

Demonstrating the requirement for histidine (*Salmonella typhimurium*) or tryptophan (*Escherichia coli*), tester strain cultures exhibited a characteristic number of spontaneous revertants per plate when plated along with the vehicle under selective conditions. Acceptable ranges for mean vehicle controls were as follows:

TA98	8	-	60
TA100	60	-	240
TA1535	4	-	45
TA1537	2	-	25
WP2*uvr*A	5	-	40

Demonstrating that appropriate numbers of bacteria were plated, density of tester strain cultures were greater than or equal to 1.0×10^9 bacteria per mL and/or had reached a target density demonstrated to produce cultures with at least 1.0×10^9 bacteria per mL (see Study Deficiencies).

Demonstrating that tester strains were capable of identifying a mutagen, the mean value of a positive control for a respective tester strain exhibited at least a 3-fold increase over the mean value of the vehicle control for that strain. An acceptable positive control in the presence of S9 mix for a specific strain was evaluated as having demonstrated both integrity of the S9 mix and ability of the tester strain to detect a mutagen.

A minimum of three non-toxic concentrations was required to evaluate assay data. Cytotoxicity was detectable as a decrease in the number of revertant colonies per plate and/or by a thinning or disappearance of the bacterial background lawn. Thinning of the bacterial background lawn not accompanied by a reduction in the number of revertants per plate was not evaluated as an indication of cytotoxicity.

Once criteria for a valid assay had been met, responses observed in the assay were evaluated.

For a test article to be considered positive, it had to produce at least a 2-fold (TA100) or 3-fold (TA98, TA1535, TA1537, and WP2*uvr*A) concentration-related and reproducible increase in the mean revertants per plate over the mean revertants per plate of the appropriate vehicle control. A response that did not meet all three of the above criteria (magnitude, concentration-responsiveness, reproducibility) was not evaluated as positive.

II. RESULTS

A. Preliminary cytotoxicity assay

Seven concentrations of test article, from 10.0 to 5000 μg per plate, were tested in Trials 28393-A1 with tester strains TA100 and WP2*uvr*A. No cytotoxicity was observed with either tester strain in the presence or absence of S9 mix as evidenced by no decreases in the number of revertants per plate and normal bacterial background lawns.

B. Mutagenicity assay

The data for the mutagenicity assay were generated in Trials 28393-B1 and 28393-C1.

The tester strains used in the preincubation mutagenicity assay were *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* tester strain WP2*uvr*A.

The assay was conducted with five concentrations of the test article (100, 333, 1000, 3330, and 5000 μg per plate) in the presence and absence of S9 mix along with concurrent vehicle and positive controls using three plates per concentration.

In the initial mutagenicity assay (Trial 28393-B1), all data were acceptable and no positive increases in the mean number of the revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix.

In the confirmatory mutagenicity assay (Trial 28393-C1), all data were acceptable and no positive increases in the mean number of the revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix.

III. CONCLUSION

Under the conditions of this study, X689643 (5-OH metabolite of penoxsulam) did not show evidence of mutagenic potential

5.7.1.1b *In Vitro* genotoxicity testing (Reference IIA5.7.1.1b/01)

STUDY TYPE: Mammalian cells in culture gene assay in CHO-K_1-BH_4 cells; USEPA OPPTS 870.5300; OECD Guideline 476; EC, B.17.

TEST MATERIAL (PURITY): X689643 (5-OH Metabolite of Penoxsulam); (Benzenesulfonamide,2-(2,2-difluoroethoxy)-N-[6-[[2-(2,2-difluoroethoxy)-6-(trifluoromethyl)phenyl]sulfonyl]-5,6-dihydro-8-methoxy-5-oxo-[1,2,4]-triazolo[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl)) (99%)

SYNONYMS: 5-OH metabolite of XDE-638, DES ME-638, (5-Hydroxy-XDE-638)

CITATION: M. R. Schisler, and K. M., Kleinert, (November 17, 2006). EVALUATION OF X689643 (5-OH METABOLITE OF PENOXSULAM) IN THE CHINESE HAMSTER OVARY CELL/HYPOXANTHINE-GUANINE-PHOSPHORIBOSYL TRANSFERASE (CHO/HGPRT) FORWARD MUTATION ASSAY. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, 48674. Study ID: 061093 (November 17, 2006). Unpublished

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

SUMMARY: X689643 (benzenesulfonamide,2-(2,2-difluoroethoxy)-N-[6-[[2-(2,2-difluoroethoxy)-6-(trifluoromethyl)phenyl]sulfonyl]-5,6-dihydro-8-methoxy-5-oxo-[1,2,4]-triazolo[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl), the 5-OH metabolite of penoxsulam, was evaluated in the *in vitro* Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward gene mutation assay. The genotoxic potential of the test material was assessed in two independent assays in the absence and presence of an externally supplied metabolic activation (S9) system. The concentrations ranged from 100 to 2000 µg/ml in the absence and presence of S9. The highest concentration was based on the limit of solubility of the test material in the solvent. The adequacy of the experimental conditions for detection of induced mutation was confirmed by employing positive control chemicals, ethyl methanesulfonate for assays without S9 and 20-methylcholanthrene for assays with S9. Solvent control cultures were treated with the solvent used to dissolve the test material (*i.e.* dimethyl sulfoxide). Based upon the frequency of TG^r mutants recovered in cultures treated with the test material, it was concluded that X689643, the 5-OH metabolite of penoxsulam, was non-mutagenic in the assay system employed.

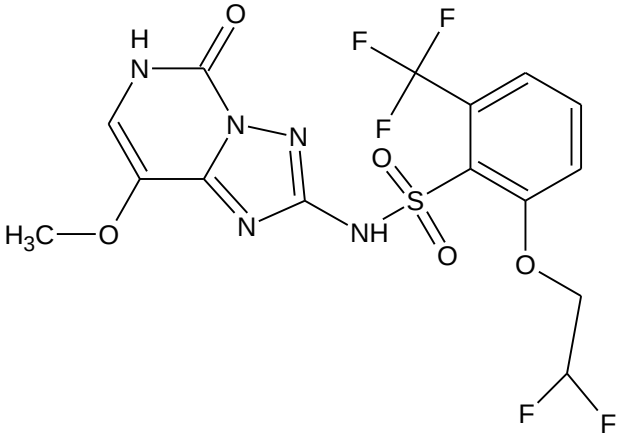
This study is acceptable and satisfies the guideline requirement for a Mammalian cells in culture gene assay in CHO-K_1-BH_4 cells; USEPA OPPTS 870.5300; OECD Guideline 476; EC, B.17

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	X689643 5-OH Metabolite of Penoxsulam
	Description:	White solid
	Lot/Batch #:	200000221-139A, TSN102471

	Purity:	The purity of the test material was determined to be 99% by liquid chromatography with ultra violet detection and identification by infrared spectroscopy.
	CAS #:	Not Applicable
	Chemical Structure:	
	Solvent Used:	Dimethyl sulfoxide (DMSO, Sigma, St. Louis, Missouri)

2.	**<u>Control Materials</u>:**	
	Solvent control (final conc'n):	Dimethyl sulfoxide (DMSO) 1%
	Positive control:	Non-activation: Ethyl methanesulfonate (EMS, CAS No. 62-50-0), 621 μg/ml
		Activation: 20-methylcholanthrene (20-MCA, CAS No. 56-49-5), 4 and 6 μg/ml

3.	**<u>Activation</u>**: S9 derived from								
	X	induced		X	Aroclor 1254	X	Rat	X	Liver
		non-induced			Phenobarbitol		Mouse		Lung
					None		Hamster		Other
					Other		Other		

The S9 mix consisted of the following co-factors: 10 mM $MgCl_2 \cdot 6H_2O$, 5 mM glucose-6-phosphate, 4 mM nicotinamide adenine dinucleotide phosphate, 10 mM $CaCl_2$, 30 mM KCl, and 50 mM sodium phosphate (pH 8.0). The reconstituted mix was added to the culture medium to obtain the desired final concentration of S9 in the culture, *i.e.*, 2% v/v. Hence, the final concentration of the co-factors in the culture medium is 1/5 of the concentrations stated above.

4.	**Test cells:** mammalian cells in culture				
			mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
		X	Chinese hamster ovary (CHO) cells		list any others

Media: The cells were routinely maintained in Ham's F-12 nutrient mix (GIBCO, Grand Island, New York) supplemented with 5% (v/v) heat-inactivated (56°C, 30 minutes), dialyzed fetal bovine serum (GIBCO), antibiotics and antimycotics (penicillin G, 100 units/ml; streptomycin sulfate, 0.1 mg/ml; fungizone, 25 µg/ml; GIBCO) and an additional 2 mM L-glutamine (GIBCO). The selection medium used for the detection of $HGPRT^-$ mutants was Ham's F-12 nutrient mix without hypoxanthine, supplemented with 10 µM 6-thioguanine (GIBCO) and 5% serum and the above-mentioned antibiotics.

Properly maintained?	X	Yes		No
Periodically checked for Mycoplasma contamination?	X	Yes		No
Periodically checked for karyotype stability?	X	Yes		No
Periodically "cleansed" against high spontaneous background?	X	Yes		No

5.	**Locus Examined:**		Thymidine kinase (TK)	X	Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)		Na^+/K^+ ATPase
	Selection agent:		bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		ouabain
			fluorodeoxyuridine (FdU)	X	6-thioguanine (6-TG)		
			trifluorothymidine (TFT)				

6.	**Test compound concentrations used:**	
	Non-activated conditions:	Assay A1: 0 (solvent control), 15.625, 31.25, 62.5, 125, 250, 500, 1000, 1500, and 2000.µg/mL Assay B1: 0 (solvent control), 125, 250, 500, 1000, and 2000 µg/mL Assay C1: 0 (solvent control), 100, 300, 600, 1200, and 2000 µg/mL
	Activated conditions:	Assay A1: 0 (solvent control), 15.625, 31.25, 62.5, 125, 250, 500, 1000, 1500, and 2000.µg/mL Assay B1: 0 (solvent control), 125, 250, 500, 1000, and 2000 µg/mL Assay C1: 0 (solvent control), 100, 300, 600, 1200, and 2000 µg/mL

B. TEST PERFORMANCE

1. Cell treatment:

a. Cells were exposed to test compound, negative/solvent or positive controls for 4 hours (non-activated), 4 hours (activated).

b. After washing, cells were cultured for 8 days (expression period) before cell selection.

c. After expression, 2 x 10^5 cells/dish (10 dishes/group) were cultured for 9 days in selection medium to determine numbers of mutants and 200 cells/dish (3 dishes/group) were cultured for 7 days without selective agent to determine cloning efficiency.

2. Statistical Methods: The frequency of mutants per 10^6 clonable cells was statistically evaluated using a weighted analysis of variance; weights were derived from the inverse of the mutation frequency variance. The actual plate counts are assumed to follow a Poisson distribution, therefore the mean plate count was used as an estimate of variance.

If the analysis of variance was significant at alpha = 0.05, a Dunnett's t-test was conducted, comparing each treated group and the positive control to the negative control (alpha = 0.05, one-sided). An additional comparison of the positive control to the negative control (alpha = 0.05) was conducted using a linear contrast statement. Linear dose-related trend tests were performed if any of the pairwise comparisons of test material with the negative control yielded significant differences.

3. Evaluation Criteria: For an assay to be acceptable, the mutant frequency in positive controls should have been significantly higher than the solvent controls. An additional criteria, was that the mutant frequency in the solvent controls should have been within reasonable limits of the laboratory historical control values and literature values. The test chemical was considered positive if it induced a statistically significant, dose related, reproducible increase in mutant frequency. The final interpretation of the data took into consideration such factors as the mutant frequency and cloning efficiencies in the solvent controls.

II. RESULTS

A. Preliminary cytotoxicity

pH and Osmolality: The pH and osmolality of treatment medium containing approximately 1975 µg/ml of the test material and medium containing approximately 1% DMSO were determined using a Denver Basic pH meter (Denver Instrument Co., Arvada, Colorado) and an OSMETTE A freezing point osmometer (Precision Systems, Inc., Natick, Massachusetts). Alterations in the pH and osmolality of the culture medium have been shown to induce false positive responses in *in vitro* genotoxicity assays. There was no appreciable change in either the pH or osmolality at this concentration as compared to the culture medium with solvent alone (culture medium with the test material, pH = 7.41, osmolality = 490 mOsm/kgH_2O; culture medium with 1% DMSO, pH = 7.73, osmolality = 444 mOsm/kgH_2O).

Assay A1 – Preliminary Toxicity Assay: In a preliminary toxicity assay, the test material was assayed at concentrations of 0 (solvent control), 15.625, 31.25, 62.5, 125, 250, 500, 1000, 1500, and 2000 µg/ml in the absence and presence of an externally supplied metabolic activation system (S9). The highest concentration

evaluated was based upon solubility limitations of the test material in the solvent. The test material also formed precipitate in the culture medium at this concentration. The treated cultures without S9 activation showed no signs of toxicity with relative cell survival (RCS) values ranging from 90.5 to 134.9%. In the presence of S9 activation, no toxicity was observed with RCS values ranging from 83.1 to 116.7%. Based upon the results of this assay, concentration levels of 0 (solvent control), 125, 250, 500, 1000, and 2000 µg/ml of the test material were selected for the initial gene mutation assay in the absence and presence of S9.

B. Mutagenicity assay

Assay B1 – Initial Mutagenicity Assay: In the initial mutagenicity assay (Assay B1), in the absence and presence of S9, no toxicity was observed with RCS values ranging from 110.1 to 129.5% in the absence of S9 and 82.2 to 108.0% in the presence of S9. The mutant frequencies observed in all cultures treated with the test material were not significantly different from the concurrent solvent control values. All mutant frequencies were within the range of historical background values.

Assay C1 – Confirmatory Mutagenicity Assay: In a confirmatory assay (Assay C1), the concentrations ranged from 100 to 2000 µg/ml without and with S9. There were no signs of toxicity observed in the absence of S9 activation, as indicated by RCS values (92.6 to 114.0%). In the presence of S9, RCS values showed varying toxicity as measured by RCS values ranging from 75.6 to 103.4%. The mutant frequencies observed in cultures treated with the test material in the absence and presence of S9 were not significantly different from the concurrent solvent control values and were within the range of the laboratory historical background values.

In both the initial and confirmatory mutagenicity assays, the positive control chemicals induced significant increases in mutant frequencies and these data confirmed the adequacy of the experimental conditions for detecting induced mutations.

The analytically observed concentrations of the test material in the stock solutions in Assay B1 ranged from 76.6 to 107.6% of target. In Assay C1, the observed concentration of the test material in the stock solutions ranged from 107.3 to 110.0% of target.

Table 1. Results of Initial Mutagenicity Assay (assay B1)

	Initial Mutagenicity Assay (B1)			
Concentration	-S9		+S9	
μg/ml	%RCS	MF	%RCS	MF
Solvent Control	97.8	6.0	90.7	0.6
Solvent. Control	102.2	7.0	109.3	6.5
125	118.1	4.6	90.5	5.7
125	121.3	7.5	84.9	5.1
250	116.3	6.5	107.1	5.7
250	128.4	6.1	108.0	12.1
500	124.2	13.8	106.4	7.9
500	110.1	5.1	103.5	5.5
1000	124.2	1.2	96.0	5.0
1000	115.9	9.0	99.8	8.3
2000[a]	117.4	4.3	95.7	7.5
2000[a]	129.5	3.4	82.2	5.8
Pos. Control[b]	35.6	278.2*	79.4	261.4*
Pos. Control[b]	16.3	387.6*	69.9	250.9*
Pos. Control[c]	ND	ND	74.7	205.1*
Pos. Control[c]	ND	ND	73.7	191.1*

RCS = Relative Cell Survival
MF = TG^r Mutants per 10^6 clonable cells
ND = Not done
[a]Precipitate in treatment medium
[b]Positive control = 621 μg/ml EMS (-S9); 4 μg/ml 20-MCA (+S9)
[c]Positive control = 6 μg/ml 20-MCA (+S9)
*The frequency of TG^r mutants is significantly higher than the concurrent solvent control value.

Table 2. Results of Confirmatory Mutagenicity Assay (assay C1)

	Confirmatory Mutagenicity Assay (C1)			
Concentration	-S9		+S9	
µg/ml	%RCS	MF	%RCS	MF
Solvent Control	105.2	11.1	99.4	7.5
Solvent. Control	94.8	7.1	100.9	7.5
100	94.0	5.7	101.9	7.9
100	100.4	7.3	95.7	7.3
300	92.6	3.3	103.4	1.3
300	94.0	3.0	101.9	7.0
600	103.3	7.1	98.8	9.4
600	107.2	8.1	96.3	2.1
1200	95.3	7.6	93.0	3.9
1200	114.0	21.1	94.9	4.8
2000[a]	93.6	13.8	75.6	10.0
2000[a]	113.0	19.1	95.7	1.5
Pos. Control[b]	29.5	382.1*	92.2	188.5*
Pos. Control[b]	29.9	228.0*	96.3	157.4*
Pos. Control[c]	ND	ND	80.6	180.7*
Pos. Control[c]	ND	ND	83.1	112.0*

RCS = Relative Cell Survival
MF = TG^r Mutants per 10^6 clonable cells
ND = Not done
[a]Precipitate in treatment medium
[b]Positive control = 621 µg/ml EMS (-S9); 4 µg/ml 20-MCA (+S9)
[c]Positive control = 6 µg/ml 20-MCA (+S9)
*The frequency of TG^r mutants is significantly higher than the concurrent solvent control value.

Conclusions

Based upon the frequency of TG^r mutants recovered in cultures treated with the test material, it was concluded that X689643 (5-OH-XDE-638) was not mutagenic in the CHO/HGPRT gene mutation assay.

5.7.1.1c *In Vitro* genotoxicity testing (Reference IIA5.7.1.1c/01)

STUDY TYPE: *In vitro* mammalian cytogenetics OPPTS 870.5375; OECD 473

TEST MATERIAL (PURITY): X689643 (Benzenesulfonamide,2-(2,2-difluoroethoxy)-N-[6-[[2-(2,2-difluoroethoxy)-6-(trifluoromethyl)phenyl]sulfonyl]-5,6-dihydro-8-methoxy-5-oxo-[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl)) (99%)

SYNONYMS: 5-OH metabolite of XDE-638, DES ME-638, (5-Hydroxy-XDE-638)

CITATION: M. R. Schisler and K. M. Kleinert, (2006). EVALUATION OF X689643 (5-OH METABOLITE OF PENOXSULAM) IN AN *IN VITRO* CHROMOSOMAL ABERRATION ASSAY UTILIZING RAT LYMPHOCYTES. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, 48674. Study ID: 061092 (November 8, 2006). Unpublished

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

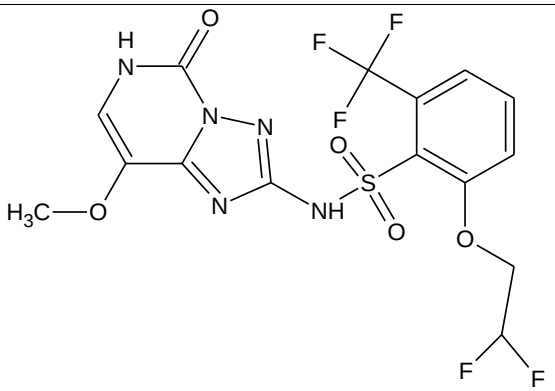
SUMMARY: X689643 (benzenesulfonamide,2-(2,2-difluoroethoxy)-N-[6-[[2-(2,2-difluoroethoxy)-6-(trifluoromethyl)phenyl]sulfonyl]-5,6-dihydro-8-methoxy-5-oxo-[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl)), the 5-OH metabolite of penoxsulam, was evaluated in an *in vitro* chromosomal aberration assay utilizing rat lymphocytes. Approximately 48 hours after the initiation of whole blood cultures, cells were treated either in the absence or presence of S9 activation with concentrations ranging from 0 (solvent control) to 2000 μg X689643 per ml of culture medium. The duration of treatment was 4 or 24 hours without S9 and 4 hours with S9. The highest concentration was based on the solubility limitation of the test material in the solvent. Based upon the mitotic indices, cultures treated for 4 hours with targeted concentrations of 0 (solvent control), 500, 1500, and 2000 μg/ml in the absence and 0 (solvent control), 500, 1000, and 2000 μg/ml in the presence of S9 activation were selected for determining the incidence of chromosomal aberrations. In cultures treated for 24 hours in the absence of metabolic activation, concentrations of 0 (solvent control), 500, 1000, and 2000 μg/ml were selected for determining the incidence of chromosomal aberrations. There were no significant increases in the frequencies of cells with aberrations in either the presence or absence of S9 activation. Cultures treated with the positive control chemicals (*i.e.*, mitomycin C without S9 and cyclophosphamide with S9) had significantly higher incidences of abnormal cells in all assays. Based upon these results, X689643, the 5-OH metabolite of penoxsulam, was considered to be non-genotoxic in this *in vitro* chromosomal aberration assay utilizing rat lymphocytes.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	X689643 (5-OH metabolite of penoxsulam)
	Description:	White solid
	Lot/Batch #:	lot # 200000221-139A, TSN102471
	Purity:	The purity of the test material was determined to be 99% by liquid chromatography with ultra violet detection and identification by infrared spectroscopy.

	CAS #:	Not applicable
	Chemical Structure:	
	Solvent Used:	Dimethyl sulfoxide (DMSO)

2.	**Control Materials:**	
	Solvent Control (final concentration):	Dimethyl sulfoxide (DMSO) 1%
	Positive Control:	Non-activation: Mitomycin C (MMC, Sigma, CAS No. 50-07-7), 0.5 µg/ml (4 hour) or 0.05 and 0.075 µg/ml (24 hour) Activation: Cyclophosphamide monohydrate (CP, Sigma, CAS No. 6055-19-2), 4 and 6 µg/ml

3.	**Activation**: S9 derived from							
	X	Induced	X	Aroclor 1254	X	Rat	X	Liver
		Not-induced		Phenobarbitol		Mouse		Lung
				None		Hamster		Other (name)
				Other (name)		Other (name)		

The S9 mix consisted of 10 mM $MgCl_2 \cdot 6H_2O$ (Sigma), 5 mM glucose-6-phosphate (Sigma), 4mM nicotinamide adenine dinucleotide phosphate (Sigma), 10mM $CaCl_2$ (Fisher, Fair Lawn, New Jersey), 30 mM KCl (Sigma), and 50 mM sodium phosphate (pH 8.0, Sigma and Fisher). The reconstituted mix was added to the culture medium to obtain the desired final concentration of S9 in the culture, *i.e.*, 2% v/v. Hence, the final concentration of the co-factors in the culture medium was 1/5 of the concentrations stated above.

4.	**Test cells:** Peripheral lymphocytes male CD ISG (Outbred Crl:CD (SD)) rats			
Media: RPMI 1640 medium (with 25 mM HEPES, GIBCO, Grand Island, New York) supplemented with 10% heat-inactivated dialyzed fetal bovine serum (GIBCO), antibiotics and antimycotics (Fungizone 0.25 µg/ml; penicillin G, 100 U/ml; and streptomycin sulfate, 0.1 mg/ml; GIBCO), 30 µg/ml PHA (HA16, Murex Diagnostics Ltd., Dartford, England), and an additional 2 mM L-glutamine (GIBCO).				
Properly maintained?	X	Yes		No
Periodically checked for *Mycoplasma* contamination?		Yes	X	No
Periodically checked for karyotype stability?	X	Yes		No

5.	**Test compound concentrations used:**	
	Non-activated conditions:	Assay A1 – 4 hours: 0 (solvent control), 62.5, 125, 250, 500, 1000, 1500, and 2000 μg/ml Assay A1 – 24 hours: 0 (solvent control), 31.25, 62.5, 125, 250, 500, 1000, 1500, and 2000 μg/ml
	Activated conditions:	Assay A1 – 4 hours: 0 (solvent control), 62.5, 125, 250, 500, 1000, 1500, and 2000 μg/ml

B. TEST PERFORMANCE

1. Preliminary Cytotoxicity Assay: Not performed

2. Cytogenetic Assay:

a.	**Cell exposure time:**	Test Material	Solvent Control	Positive Control
	Non-activated: Activated:	4 h & 24 h 4h	4 h & 24 h 4h	4 h & 24 h 4h

b.	**Spindle inhibition**	
	Inhibition used/concentration:	Colcemid (1μg/culture)
	Administration time:	2-3 hours (before cell harvest)

c.	**Cell harvest time after termination of treatment:**	Test Material	Solvent Control	Positive Control
	Non-activated: Activated:	0 and 20h 20h	0 and 20h 20h	0 and 20h 20h

d. Details of slide preparation: The cells were swollen by hypotonic treatment (0.075 M KCl), fixed with methanol:acetic acid (3:1), dropped on microscope slides, and stained in Giemsa.

e. Metaphase analysis:

No. of cells examined per dose:				
Scored for structural?	X	Yes		No
Scored for numerical?	X	Yes (polyploidy)		No
Coded prior to analysis?	X	Yes		No

f. Evaluation criteria: For a test to be acceptable, the chromosomal aberration frequency in the positive control cultures should be significantly higher than the solvent controls. The aberration frequency in the solvent control should be within reasonable limits of the laboratory historical values. A test chemical is considered positive in this assay if it induces a significant dose-related and reproducible increase in the frequency of cells with aberrations.

g. Statistical analysis: The proportions of cells with aberrations (excluding gaps) were compared by the following statistical methods. At each dose level, data from the replicates was pooled. A two-way contingency table was constructed to analyze the frequencies of aberrant cells. An overall Chi-square statistic, based on the table, was partitioned into components of interest. Specifically, statistics were generated to test the global hypotheses: 1) no difference in the average number of cells with aberrations among the dose groups, and 2) no linear trend of increasing number of cells with aberrations with increasing dose. An ordinal metric (0, 1, 2, etc.) was used for the doses in the statistical evaluation. If either statistic was found to be significant at alpha = 0.05, versus a one-sided increasing alternative, pairwise tests (*i.e.* control vs. treatment) were performed at each dose level and evaluated at alpha = 0.05, again versus a one-sided alternative.

Polyploid cells were analyzed by the Fisher Exact probability test. The number of polyploid cells were pooled across replicates for the analysis and evaluated at alpha = 0.05. The data was analyzed separately based on the presence or absence of S-9 and based on the exposure time.

II. RESULTS

pH and Osmolality

The pH and osmolality of the treatment medium containing approximately 1975 µg/ml of the test material and medium containing approximately 1% solvent (DMSO) was determined using a Denver Basic pH meter (Denver Instrument Co., Arvada, Colorado) and an OSMETTE A freezing point osmometer (Precision Systems, Inc., Natick, Massachusetts), respectively. There was no appreciable change in either the pH or osmolality at this concentration as compared to the culture medium with solvent alone (culture medium with the test material, pH = 7.03, osmolality = 450 mOsm/kg H_20; culture medium with 1% DMSO, pH = 7.24, osmolality = 467 mOsm/kg H_20).

Assay A1

In the initial assay, cultures were treated with the test material in the absence and presence of S9 activation for 4 hours at concentrations of 0 (solvent control), 62.5, 125, 250, 500, 1000, 1500 and 2000 µg/ml. Cultures were also treated continuously for 24 hours in the absence of S9 with the above concentrations plus an additional lower concentration of 31.25 µg/ml. The highest concentration evaluated was based upon solubility limitations of the test material in the solvent. The test material also formed a precipitate in the culture medium at this concentration. The analytically detected concentrations of the test material in the stock solutions (Assay A1) varied from 100.7 to 110.9% of the target.

Short Treatment

Without and with metabolic activation (4 hour treatment), the cultures displayed varying levels of toxicity as measured by mitotic indices. In the absence of S9, the mitotic indices for the treated cultures ranged from 66.7 to 91.5% relative to the solvent control values. In the presence of S9, the mitotic indices of the treated cultures ranged from 69.5 to 98.1% as compared to the solvent control values. Based upon these results, cultures treated with the top three concentrations (*i.e.*, 500, 1500 and 2000 µg/ml) in the absence of metabolic activation and 500, 1000 and 2000 µg/ml in the presence of metabolic activation were chosen for the determination of chromosomal aberration frequencies and incidence of polyploidy.

Among the cultures treated with the positive control chemicals for 4 hour, 0.5 µg/ml of MMC and 4 µg/ml of CP were selected for evaluation of aberrations in the absence and presence of S9, respectively.

There were no significant increases in the incidence of polyploidy cells in any of the test material treated cultures as compared to the solvent control values.

In the 4 hour non-activation assay, the frequency of cells with aberrations in the solvent control was 0.5% and the corresponding values at treatment levels of 500, 1500, and 2000 µg/ml were 1.0, 0.5, and 1.0%, respectively. In the activation assay, cultures treated with the test material at concentrations of 500, 1000, and 2000 µg/ml had aberrant cell frequencies of 2.0, 1.0 and 1.0%, respectively as compared to the solvent control value of 2.0%. Statistical analyses of these data did not identify significant differences between the solvent control and any of the treated cultures without or with S9 activation. The frequencies of aberrant cells observed in the test material treated cultures were within the laboratory historical background range.

Significant increases in the frequency of cells with aberrations were observed in cultures treated with the positive control chemicals. Aberrant cell frequencies in MMC (- S9, 4 hour treatment), and CP (+ S9) cultures were 27%, and 31%, respectively.

Continuous Treatment

Upon completion of the slide evaluation for the 4 hour treated cultures, and after concluding that the results were negative, slides from the continuous 24 hour treatment were evaluated. Varying signs of toxicity were observed in the treated cultures as determined by relative mitotic indices ranging from 56.3 to 89.3%. Based upon these results, 0 (solvent control), 500, 1000 and 2000 µg/ml were chosen for the determination of chromosomal aberration frequencies and incidence of polyploidy in the non-activation assay treated continuously for 24 hours. Cultures treated with 0.05 µg/ml MMC were selected for evaluation to serve as the positive control for the 24 hour assay in the absence of metabolic activation.

There were no significant increases in the incidence of polyploidy cells in any of the test material treated cultures as compared to the solvent control values.

The frequencies of aberrant cells in the solvent control was 2.5% and the corresponding values at concentration levels of 500, 1000, and 2000 µg/ml were 1.5, 2.5, and 2.0%, respectively. There were no statistically significant differences between the test material treated cultures and the solvent control values and all values were within the laboratory historical background range.

Significant increases in the frequency of cells with aberrations were observed in cultures treated with the positive control chemical. Aberrant cell frequency in MMC treated cultures was 29%.

A second assay with treatment of cultures in the presence of S9 was not considered necessary since the results of the initial test were clearly negative.

Table 1. Mitotic Indices and Polypoidy Incidence in Rat Lymphocyte Cultures

	Mean mitotic index [a]			Incidence of polyploidy (%)		
	Assay A1			Assay A1		
Conc. µg/ml	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)	-S9 (4 hr)	+S9 (4 hr)	-S9 (24hr)
Solvent control	11.7 (100.0)	10.5 (100.0)	10.3 (100.0)	0.0	0.5	0.5
31.25	ND	ND	9.2 (89.3)	ND	ND	ND
62.5	10.4 (88.9)	10.0 (95.2)	8.2 (79.6)	ND	ND	ND
125	10.5 (89.7)	10.3 (98.1)	7.4 (71.8)	ND	ND	ND
250	10.7 (91.5)	9.0 (85.7)	6.7 (65.0)	ND	ND	ND
500	10.6 (90.6)	9.1 (86.7)	7.4 (71.8)	0.5	0.5	0.5
1000	10.2 (87.2)	8.8 (83.8)	5.8 (56.3)	ND	0.5	0.0
1500	8.6 (73.5)	7.3 (69.5)	5.9 (57.3)	0.0	ND	ND
2000[b]	7.8 (66.7)	7.4 (70.5)	6.5 (63.1)	0.5	0.0	0.5
Positive control [c]	5.2 (44.4)	3.1 (29.5)	5.3 (51.5)	0.0	0.0	0.0
Positive control [d]	ND	0.9 (8.6)	3.6 (35.0)	ND	ND	ND

[a] values in parenthesis are % relative mitotic index
[b] Precipitation in culture medium
[c] Positive control = 0.5 µg/ml MMC (-S9, 4 hr); 4 µg/ml CP (+S9, 4 hr); 0.05 µg/ml MMS (-S9, 24 hr)
[d] Positive control = 6 µg/ml CP (+S9, 4hr); 0.075 µg/ml MMS (-S9, 24 hr)
ND = not done

Table 2. Results of the Cytogenetic Assay

	Total aberrations (excluding gaps)			No. of cells with aberrations (excluding gaps) [a]		
	Assay A1			Assay A1		
Conc. µg/ml	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 h
Solvent control	1	3	5	0.5	2.0	2.5
500	2	4	3	1.0	2.0	1.5
1000	ND	2	5	ND	1.0	2.5
1500	1	ND	ND	0.5	ND	ND
2000[b]	2	2	3	1.0	1.0	2.0
Positive control [c]	23	26	30	27*	31*	29*

[a] Values are percentages
[b] Precipitation in culture medium
[c] Positive control = 0.5 µg/ml MMC (-S9, 4 hr); 4 µg/ml CP (+S9, 4 hr); 0.05 µg/ml MMC (-S9, 24 hr)
*Significantly different from solvent controls, $\alpha = 0.05$.
ND = not done

It was concluded that under the experimental conditions used, X689643, the 5-OH metabolite of penoxsulam, was non-genotoxic in this *in vitro* chromosomal aberration test.

5.7.1.2 Mutagenicity studies on BSTCA

5.7.1.2a *In Vitro* genotoxicity testing (Reference IIA5.7.1.2a/01)

STUDY TYPE: *(Bacterial system, Salmonella typhimurium; Escherichia coli)*/ mammalian activation gene mutation assay; OPPTS 870.5100[2]; OECD 471 (formerly OECD 471 & 472).

TEST MATERIAL (PURITY): X768359 (5-[2,2-difluoroethoxy)-6-trifluoromethyl-benzensulfonylamino]-1H-1,2,4-triazole-3-carboxylate) (99%TEA salt, 80% acid equivalent)

SYNONYMS: BSTCA metabolite of penoxsulam,

CITATION: M. S. Mecchi (29 August 2006). *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay Preincubation Method with a Confirmatory Assay with X768359 (BSTCA Metabolite of Penoxsulam). Covance Laboratories Inc., Vienna, Virginia. Study ID: 6736-159, 06 March 2006 – 29 August 2006. Unpublished.

SPONSOR: The Dow Chemical Company

SUMMARY:

In a reverse gene mutation assay in bacteria, tester strains TA98, TA100, TA1535, and TA1537 of *S. typhimurium* and tester strain WP2*uvr*A of *E. coli* were exposed to X768359 (BSTCA metabolite of penoxsulam) (99% TEA salt, 80% acid equivalent), in dimethylsulfoxide using the preincubation method at concentrations of 100, 333, 1000, 3330, and 5000 µg per plate in the presence and absence of mammalian metabolic activation.

X768359 (BSTCA Metabolite of Penoxsulam) was tested up to the limit concentration (5000 µg per plate) with all strains. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as acceptable. This study satisfies the requirement for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

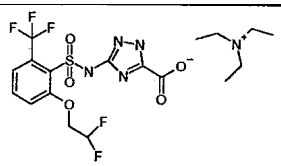
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	X768359 (BSTCA Metabolite of Penoxsulam)
	Description:	White powder
	Lot #:	035298-79

[2]870.5100 - Reverse mutation *E. coli* WP2 and WP2uvrA; *S. typhimurium* TA 97, TA98, TA100, TA1535, TA1537
870.5140 - Gene mutation *Aspergillus nidulans*
870.5250 - Gene mutation *Neurospora crassa*

	Purity:	99% TEA salt, 80% acid equivalent.
	CAS #:	not provided
		STRUCTURE: 
	Solvent Used:	Dimethylsulfoxide

2.	**Control Materials:**	
	Negative:	NA
	Solvent (final conc'n):	Dimethylsulfoxide at 50 µL per plate
	Positive:	Non-activation: sodium azide 2.0 µg/plate TA100, TA1535 2-nitrofluorene 1.0 µg/plate TA98 ICR-191 2.0 µg/plate TA1537 4-nitroquinoline-N-oxide 0.4 µg/plate WP2*uvr*A
		Activation: benzo[a]pyrene 2.5 µg/plate TA98 2-aminoanthracene 2.5 µg/plate TA100, TA1535, and TA1537 2-aminoanthracene 25.0 µg/plate WP2*uvr*A

3.	**Activation**: S9 derived from								
	x	induced		x	Aroclor 1254	x	Rat	x	Liver
		non-induced			Phenobarbitol		Mouse		Lung
					None		Hamster		Other (name)
					Other (name)		Other (name)		

S9 mix composition: H_2O (70%), 1M NaH_2PO_4/Na_2HPO_4, pH 7.4 (10%), 0.25M Glucose-6-phosphate (2%), 0.10M NADP (4%), 0.825M KCl/0.2M $MgCl_2$ (4%), and S9 Homogenate (10%).

4.	**Test organisms:** *S. typhimurium* strains								
		TA97	x	TA98	x	TA100		TA102		TA104
	x	TA1535	x	TA1537		TA1538	x	WP2*uvr*A (*E. coli*)		
Properly maintained?							x	Yes		No
Checked for appropriate genetic markers (*rfa* mutation, R factor)?							x	Yes		No

5. Test compound concentrations used:
Non-activated conditions:

Cytotoxicity test: TA100 and WP2*uvr*A at 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, and 5000 µg per plate; one plate per concentration

Mutagenicity assay: TA98, TA100, TA1535, TA1537, and WP2*uvr*A at 100, 333, 1000, 3330, and 5000 µg per plate; three replicates.

Activated conditions:

- Cytotoxicity test: TA100 and WP2*uvr*A at 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, and 5000 µg per plate; one plate per concentration.
- Mutagenicity assay: TA98, TA100, TA1535, TA1537, and WP2*uvr*A at 100, 333, 1000, 3330, and 5000 µg per plate; three replicates.

B. TEST PERFORMANCE

1. Type of *Salmonella* assay: pre-incubation (20 minutes)

2. Protocol:

Tester strains were exposed to the test article via the preincubation modification of the Ames Test originally described by Yahagi *et al.* (1975) and Maron and Ames (1983). This methodology has been shown to detect a wide range of classes of chemical mutagens. In the preincubation methodology, S9 mix (or phosphate buffer, where appropriate), tester strain, and test article were preincubated for approximately 20 minutes prior to addition of molten agar. The agar and preincubation reaction mixture were mixed and then overlaid onto a minimal agar plate. Following incubation, revertant colonies were counted. Test article, vehicle controls, and positive controls were plated in triplicate. The condition of the bacterial background lawn was evaluated both macroscopically and microscopically (using a dissecting microscope) for indications of cytotoxicity and test article precipitate. Evidence of cytotoxicity was scored relative to the vehicle control plate and was recorded along with the revertant counts for all plates at that concentration. Revertant colonies were counted either by automated colony counter or by hand.

3. Statistical Analysis: Mean and standard deviation were calculated for each replicate.

4. Evaluation Criteria: Before assay data were evaluated, criteria for a valid assay had to be met. The following criteria were used to determine a valid assay:

Salmonella typhimurium tester strain cultures exhibited sensitivity to crystal violet to demonstrate the presence of the *rfa* wall mutation. Cultures of tester strains TA98 and TA100 exhibited resistance to ampicillin to demonstrate the presence of the pKM101 plasmid.

Demonstrating the requirement for histidine (*Salmonella typhimurium*) or tryptophan (*Escherichia coli*), tester strain cultures exhibited a characteristic number of spontaneous revertants per plate when plated along with the vehicle under selective conditions. Acceptable ranges for mean vehicle controls were as follows:

TA98	8	-	60
TA100	60	-	240
TA1535	4	-	45
TA1537	2	-	25
WP2*uvr*A	5	-	40

Demonstrating that appropriate numbers of bacteria were plated, density of tester strain cultures were greater than or equal to 1.0×10^9 bacteria per mL and/or had reached a target density demonstrated to produce cultures with at least 1.0×10^9 bacteria per mL.

Demonstrating that tester strains were capable of identifying a mutagen, the mean value of a positive control for a respective tester strain exhibited at least a 3-fold increase over the mean value of the vehicle control for that strain. An acceptable positive control in the presence of S9 mix for a specific strain was evaluated as having demonstrated both integrity of the S9 mix and ability of the tester strain to detect a mutagen.

A minimum of three non-toxic concentrations was required to evaluate assay data. Cytotoxicity was detectable as a decrease in the number of revertant colonies per plate and/or by a thinning or disappearance of the bacterial background lawn. Thinning of the bacterial background lawn not accompanied by a reduction in the number of revertants per plate was not evaluated as an indication of cytotoxicity.

Once criteria for a valid assay had been met, responses observed in the assay were evaluated.

For a test article to be considered positive, it had to produce at least a 2-fold (TA100) or 3-fold (TA98, TA1535, TA1537, and WP2*uvr*A) concentration-related and reproducible increase in the mean revertants per plate over the mean revertants per plate of the appropriate vehicle control. A response that did not meet all three of the above criteria (magnitude, concentration-responsiveness, reproducibility) was not evaluated as positive.

II. RESULTS

A. Preliminary cytotoxicity assay

Ten concentrations of test article, from 6.67 to 5000 μg per plate, were tested in Trials 28134-A1 with tester strains TA100 and WP2*uvr*A. No cytotoxicity was observed with either tester strain in the presence or absence of S9 mix as evidenced by no decreases in the number of revertants per plate and normal bacterial background lawns.

B. Mutagenicity assay

The data for the mutagenicity assay were generated in Trials 28134-B1 28134-C1, and 28134-D1.

The tester strains used in the preincubation mutagenicity assay were *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* tester strain WP2*uvr*A.

The assay was conducted with five concentrations of the test article (100, 333, 1000, 3330, and 5000 μg per plate) in the presence and absence of S9 mix along with concurrent vehicle and positive controls using three plates per concentration.

In the initial mutagenicity assay (Trial 28134-B1), all data were acceptable and no positive increases in the mean number of the revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix. In the initial mutagenicity assay, no valid data were generated for tester strain TA1537 due to unacceptable mean vehicle control values in the presence and absence of S9 mix. For this reason, the test article was re-tested with TA1537 in the presence and absence of S9 mix in Trial 28134-D1.

In the confirmatory mutagenicity assay (Trial 28134-C1), all data were acceptable and no positive increases in the mean number of the revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix.

In the repeat mutagenicity assay, (Trial 28134-D1), all data were acceptable and no positive increases in the mean number of revertants per plate were observed with TA1537 in either the presence or absence of S9 mix.

III. CONCLUSION

Under the conditions of this study, X768359 (BSTCA metabolite of penoxsulam) did not show evidence of mutagenic potential

5.7.1.2b *In Vitro* genotoxicity testing (IIA5.7.1.2b/01)

STUDY TYPE: Mammalian cells in culture gene assay in CHO-K_1-BH_4 cells; USEPA OPPTS 870.5300; OECD Guideline 476; EC, B.17.

TEST MATERIAL (PURITY): X768359 (5-[2,2-difluoroethoxy)-6-trifluoromethyl-benzensulfonylamino]-1H-1,2,4-triazole-3-carboxylate) (99%)

SYNONYMS: BSTCA metabolite of penoxsulam

CITATION: S. D. Seidel, M. R. Schisler, and K. M. Kleinert. (August 16, 2006). EVALUATION OF X768359 (BSTCA METABOLITE OF PENOXSULAM) IN THE CHINESE HAMSTER OVARY CELL/HYPOXANTHINE-GUANINE-PHOSPHORIBOSYL TRANSFERASE (CHO/HGPRT) FORWARD MUTATION ASSAY. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, 48674. Study ID: 061037, (August 16, 2006). Unpublished

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

SUMMARY: X768359 (5-[2,2-difluoroethoxy)-6-trifluoromethyl-benzensulfonylamino]-1H-1,2,4-triazole-3-carboxylate), the BSTCA metabolite of penoxsulam, was evaluated in the *in vitro* Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward gene mutation assay. The genotoxic potential of the test material was assessed in two independent assays in the absence and presence of an externally supplied metabolic activation (S9) system with concentrations ranging from 216.25 to 5175 μg/ml. The highest concentration was based on the limit dose of 10 mM for this assay system. The adequacy of the experimental conditions for detection of induced mutations was confirmed by employing positive control chemicals, ethyl methanesulfonate for assays without S9 and 20-methylcholanthrene for assays with S9. Solvent control cultures were treated with the solvent used to dissolve the test material (*i.e.* dimethyl sulfoxide). Based upon the frequency of TG^r mutants recovered in cultures treated with the test material, it was concluded that X768359, the BSTCA metabolite of penoxsulam, was non-mutagenic in the assay system employed.

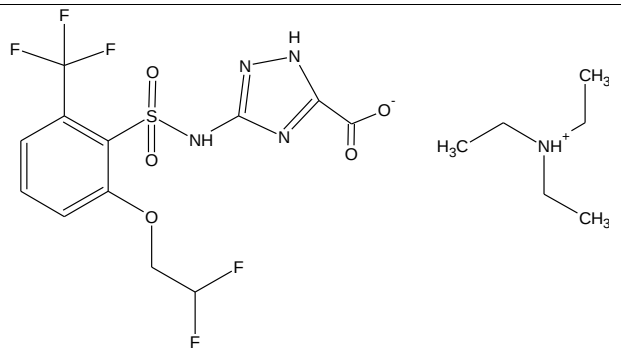
This study is acceptable and satisfies the guideline requirement for an Mammalian cells in culture gene assay in CHO-K_1-BH_4 cells; USEPA OPPTS 870.5300; OECD Guideline 476; EC, B.17.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	X768359 (BSTCA metabolite of penoxsulam)
	Description:	White, solid
	Lot/Batch #:	035298-79, TSN105480

	Purity:	The BSTCA metabolite was isolated and provided as the triethylamine salt. The purity of the BSTCA metabolite of penoxsulam TEA salt was determined to be 99% (80% acid equivalent) as measured by liquid chromatography with ultraviolet detection. Structural identify was confirmed by infrared spectroscopy, mass spectroscopy, along with proton and carbon-13 nuclear magnetic resonance.
	CAS #:	N/A
	Chemical Structure:	
	Solvent Used:	Dimethyl sulfoxide (DMSO, Sigma, St. Louis, Missouri)

2.	**<u>Control Materials</u>:**	
	Negative control	(e.g. 1% DMSO)
	Solvent control (final conc'n):	Dimethyl sulfoxide (DMSO) 1%
	Positive control:	Nonactivation: Ethyl methanesulfonate (EMS, CAS No. 62-50-0), 621 μg/ml
		Activation: 20-methylcholanthrene (20-MCA, CAS No. 56-49-5), 4, 6 and 8 μg/ml

3.	**<u>Activation</u>**: S9 derived from									
	X	induced		X	Aroclor 1254	X	Rat	X	Liver
		non-induced			Phenobarbitol		Mouse		Lung
					None		Hamster		Other
					Other		Other		

The S9 mix consisted of the following co-factors: 10 mM $MgCl_2 \cdot 6H_2O$, 5 mM glucose-6-phosphate, 4 mM nicotinamide adenine dinucleotide phosphate, 10 mM $CaCl_2$, 30 mM KCl, and 50 mM sodium phosphate (pH 8.0). The reconstituted mix was added to the culture medium to obtain the desired final concentration of S9 in the culture, *i.e.*, 2% v/v. Hence, the final concentration of the co-factors in the culture medium was 1/5th of the concentrations stated above.

4.	**Test cells:** mammalian cells in culture				
			mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
		X	Chinese hamster ovary (CHO) cells		

Media: The cells were routinely maintained in Ham's F-12 nutrient mix (GIBCO, Grand Island, New York) supplemented with 5% (v/v) heat-inactivated (56°C, 30 minutes), dialyzed fetal bovine serum (GIBCO), antibiotics and antimycotics (penicillin G, 100 units/ml; streptomycin sulfate, 0.1 mg/ml; fungizone, 25 µg/ml; GIBCO) and an additional 2 mM L-glutamine (GIBCO). The selection medium used for the detection of $HGPRT^-$ mutants was Ham's F-12 nutrient mix without hypoxanthine, supplemented with 10 µM 6-thioguanine (GIBCO) and 5% serum and the above-mentioned antibiotics.

Properly maintained?	X	Yes		No
Periodically checked for Mycoplasma contamination?	X	Yes		No
Periodically checked for karyotype stability?	X	Yes		No
Periodically "cleansed" against high spontaneous background?	X	Yes		No

5.	**Locus Examined:**		Thymidine kinase (TK)	X	Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)		Na^+/K^+ ATPase
	Selection agent:		bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		ouabain
			fluorodeoxyuridine (FdU)	X	6-thioguanine (6-TG)		
			trifluorothymidine (TFT)				

6.	**Test compound concentrations used**	
	Nonactivated conditions:	Assay A1 0, 13.5, 27, 54.1, 108.13, 216.25, 432.5, 865, 1730 and 3460 µg/mL Assay B1 0, 216.25, 432.5, 865, 1730, and 3460 µg/mL Assay C1 0, 1000, 2000, 3000, 4000, and 5175 µg/mL
	Activated conditions:	Assay A1 0, 13.5, 27, 54.1, 108.13, 216.25, 432.5, 865, 1730 and 3460 µg/mL Assay B1 0, 216.25, 432.5, 865, 1730, and 3460 µg/mL Assay C1 0, 1000, 2000, 3000, 4000, and 5175 µg/mL

B. TEST PERFORMANCE

2. Cell treatment:

a. Cells were exposed to test compound, negative/solvent or positive controls for 4 hours (non-activated), 4 hours (activated).

b. After washing, cells were cultured for 8 days (expression period) before cell

selection.

c. After expression, 2 x 10^5 cells/dish (10 dishes/group) were cultured for 9 days in selection medium to determine numbers of mutants and 200 cells/dish (3 dishes/group) were cultured for 7 days without selective agent to determine cloning efficiency.

2. **Statistical Methods:** The frequency of mutants per 10^6 clonable cells was statistically evaluated using a weighted analysis of variance; weights were derived from the inverse of the mutation frequency variance. The actual plate counts are assumed to follow a Poisson distribution therefore the mean plate count was used as an estimate of variance.

If the analysis of variance was significant at alpha = 0.05, a Dunnett's t-test was conducted, comparing each treated group and the positive control to the negative control (alpha = 0.05, one-sided). An additional comparison of the positive control to the negative control (alpha = 0.05) was conducted using a linear contrast statement. Linear dose-related trend tests were performed if any of the pairwise comparisons of test material with the negative control yielded significant differences.

3. **Evaluation Criteria:** For an assay to be acceptable, the mutant frequency in positive controls should have been significantly higher than the solvent controls. An additional criteria, was that the mutant frequency in the solvent controls should have been within reasonable limits of the laboratory historical control values and literature values. The test chemical was considered positive if it induced a statistically significant, dose-related, reproducible increase in mutant frequency. The final interpretation of the data took into consideration such factors as the mutant frequency and cloning efficiencies in the solvent controls.

II. RESULTS

C. Preliminary cytotoxicity

pH and Osmolality

The pH and osmolality of treatment medium containing approximately 5140 μg/ml of the test material (limit dose of 10 mM) and medium containing 1% DMSO were determined using a Denver Basic pH meter (Denver Instrument Co., Arvada, Colorado) and an OSMETTE A freezing point osmometer (Precision Systems, Inc., Natick, Massachusetts). Alterations in the pH and osmolality of the culture medium have been shown to induce false positive responses in *in vitro* genotoxicity assays. There was no appreciable change in pH at this concentration as compared to the culture medium with solvent alone; however, there was an appreciable change in osmolality. When the osmolality was compared to culture medium alone, there was no appreciable change and therefore, the change in osmolality was deemed inconsequential to the assay (culture medium with the test material, pH = 7.61, osmolality = 371 mOsm/kgH_2O; culture medium with 1% DMSO, pH = 7.70, osmolality = 452 mOsm/kgH_2O; culture medium alone, pH 7.69, osmolality = 324 mOsm/kgH_2O).

Assay A1 – Preliminary Toxicity Assay

In a preliminary toxicity assay, the test material was assayed at concentrations of 0 (solvent control), 13.5, 27, 54.1, 108.13, 216.25, 432.5, 865, 1730 and 3460 μg/ml in the absence and presence of an externally supplied metabolic activation system (S9). The highest concentration tested was based upon the initial solubility determination of the test material in the culture medium. In the absence of S9 activation, little to no toxicity was observed, with the relative cell survival (RCS) values ranging from 86.5 to 116.7%. However, in the highest concentration (*i.e.* 3460 μg/ml) the cells were inadvertently not plated due to a technical error. In the presence of S9 activation, all treated cultures showed little to no toxicity with RCS values ranging from 92.0 to 111.7%. Based upon the results of this assay, concentration

levels of 0, 216.25, 432.5, 865, 1730, and 3460 μg/ml of the test material were selected for the initial gene mutation assay in the absence and presence of S9.

D. Mutagenicity assay

Assay B1 – Initial Mutagenicity Assay

In the initial mutagenicity assay (Assay B1), in the absence and presence of S9, little to no toxicity was observed based on RCS values. These values ranged from 82.7 to 118.7% in the absence of S9 and 88.2 to 104.1% in the presence of S9. The mutant frequencies observed in cultures treated with the test material in the absence and presence of S9 were not statistically different from the concurrent solvent control values and all mutant frequencies were within a reasonable range of historical background values.

Table 5.7.1.2-1: Results of Initial Mutagenicity Assay (assay B1)

	Initial Mutagenicity Assay (B1)			
Concentration	-S9		+S9	
μg/ml	%RCS	MF	%RCS	MF
Solvent Control	101.8	4.5	93.9	2.7
Solvent. Control	98.2	7.3	106.1	6.4
216.25	92.2	2.1	100.0	9.5
216.25	118.7	3.1	103.3	6.3
432.5	109.3	6.4	98.0	-[a]
432.5	100.5	12.4	100.6	4.4
865	82.7	9.1	88.2	5.7
865	90.2	14.2	90.0	8.3
1730	92.0	3.6	98.4	10.0
1730	90.0	19.0	95.7	3.4
3460	87.0	7.3	104.1	5.8
3460	90.6	4.2	89.2	11.3
Pos. Control[b]	36.9	428.8*	92.5	132.0*
Pos. Control[b]	36.2	395.5*	92.7	55.6*
Pos. Control[c]	ND	ND	101.2	92.2*
Pos. Control[c]	ND	ND	91.0	51.6*

RCS = Relative Cell Survival
MF = TG^r Mutants per 10^6 clonable cells
ND = Not done
[a]lost due to contamination
[b]Positive control = 621 μg/ml EMS (-S9); 4 μg/ml 20-MCA (+S9)
[c]Positive control = new lot of 20-MCA at 4 μg/ml (+S9)
*The frequency of TG^r mutants is significantly higher than the concurrent solvent control value.

Assay C1 – Confirmatory Mutagenicity Assay

In a confirmatory assay (Assay C1), the concentrations ranged from 1000 to 5175 μg/ml both in the absence and presence of S9. This increase in treatment concentration was due to a re-evaluation of the solubility of the test material, which indicated that the test material could be added to the treatment media at the limit concentration of 10 mM. There was little to no toxicity observed, as indicated by RCS values, in the absence of S9 activation (range from 86.6 to 108.6%) or the presence of S9 activation (range from 88.3 to 115.1%). The mutant frequencies observed in cultures treated with the test material in the absence of S9 and presence of S9 were

not statistically different from the concurrent solvent control values and were within the range of the laboratory historical background.

Table 5.7.1.2-2: Results of Confirmatory Mutagenicity Assay (assay B1)

	Confirmatory Mutagenicity Assay (C1)			
Concentration	-S9		+S9	
μg/ml	%RCS	MF	%RCS	MF
Solvent Control	92.6	11.4	99.1	5.3
Solvent. Control	107.4	10.2	100.9	6.4
1000	95.1	8.3	88.3	7.5
1000	108.6	8.4	103.4	13.3
2000	92.9	3.6	107.2	10.6
2000	104.3	2.8	112.3	8.5
3000	86.6	8.0	102.1	10.2
3000	102.5	10.6	106.6	4.8
4000	89.0	10.7	115.1	5.4
4000	105.9	12.3	109.8	3.7
5175	89.8	14.4	101.5	12.6
5175	101.7	5.1	104.5	7.7
Pos. Control[a]	24.6	510.2*	89.1	208.8*
Pos. Control[a]	27.6	342.3*	104.5	238.4*
Pos. Control[b]	ND	ND	108.3	101.5*
Pos. Control[b]	ND	ND	102.1	130.3*
Pos. Control[c]	ND	ND	105.5	86.7*
Pos. Control[c]	ND	ND	114.0	119.1*

RCS = Relative Cell Survival
MF = TG^r Mutants per 10^6 clonable cells
ND = Not done
[a]Positive control = 621 μg/ml EMS (-S9); 4 μg/ml 20-MCA (+S9)
[b]Positive control = 6 μg/ml 20-MCA (+S9)
[c]Positive control = 8 μg/ml 20-MCA (+S9)
*The frequency of TG^r mutants is significantly higher than the concurrent solvent control value.

In both the initial and confirmatory mutagenicity assays, the positive control chemicals induced significant increases in mutant frequencies and these data confirmed the adequacy of the experimental conditions for detecting induced mutations.

The analytically observed concentrations of the test material in the stock solutions in Assay B1 ranged from 102.3 to 122.3% of target. In Assay C1, the observed concentration of the test material in the stock solutions ranged from 114.8 to 119.0% of target.

Based upon the frequency of TG^r mutants recovered in cultures treated with the test material, it was concluded that X768359 (BSTCA metabolite of penoxsulam) was not mutagenic in the CHO/HGPRT gene mutation assay.

5.7.1.2c *In Vitro* genotoxicity testing (IIA5.7.1.2c/01)

STUDY TYPE: *In vitro* mammalian cytogenetics OPPTS 870.5375; OECD 473

TEST MATERIAL (PURITY): X768359 (5-[2,2-difluoroethoxy)-6-trifluoromethyl-benzensulfonylamino]-1H-1,2,4-triazole-3-carboxylate) (99% (80% acid equivalent))

SYNONYMS: BSTCA metabolite of penoxsulam

CITATION: M. R. Schisler, and K. M. Kleinert (12 October 2006). EVALUATION OF X768359 (BSTCA METABOLITE OF PENOXSULAM) IN AN *IN VITRO* CHROMOSOMAL ABERRATION ASSAY UTILIZING RAT LYMPHOCYTES. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, 48674. Study ID: 061038, (12 October 2006). Unpublished

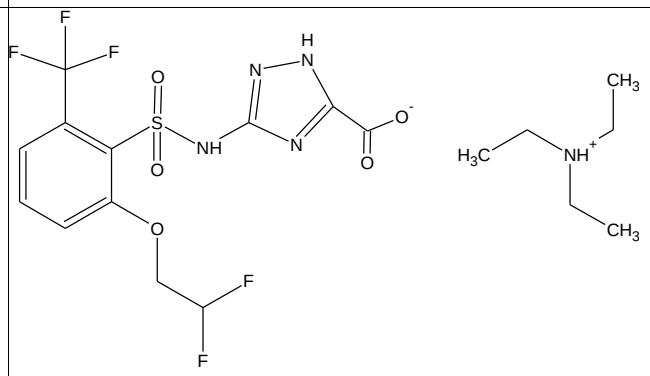
SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

SUMMARY: X768359 (5-[2,2-difluoroethoxy)-6-trifluoromethyl-benzensulfonylamino]-1H-1,2,4-triazole-3-carboxylate), the BSTCA metabolite of penoxsulam, was evaluated in an *in vitro* chromosomal aberration assay utilizing rat lymphocytes. Approximately 48 hours after the initiation of whole blood cultures, cells were treated in the absence and presence of S9 activation with concentrations ranging from 0 (solvent control) to 5175 μg/ml of X768359. The duration of treatment was 4 or 24 hours without S9 and 4 hours with S9. The highest concentration was based on the limit dose of 10 mM in this assay system. Based upon the mitotic indices, cultures treated for 4 hours with targeted concentrations of 0 (solvent control), 3000, 4000, and 5175 μg/ml in the absence and presence of S9 activation and cultures treated for 24 hours with 0 (solvent control), 865, 1730, and 3460 μg/ml were selected for determining the incidence of chromosomal aberrations. There were no significant increases in the frequencies of cells with aberrations in either the presence or absence of S9 activation. Cultures treated with the positive control chemicals (*i.e.*, mitomycin C without S9 and cyclophosphamide with S9) had significantly higher incidences of abnormal cells in all assays. Based upon these results, X768359, the BSTCA metabolite of penoxsulam, was considered to be non-genotoxic in this *in vitro* chromosomal aberration assay utilizing rat lymphocytes.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	X768359 (BSTCA metabolite of Penoxsulam)
	Description:	White solid
	Lot/Batch #:	lot # 035298-79, TSN105480
	Purity:	The BSTCA metabolite was isolated and provided as the triethylamine (TEA) salt. The purity of the BSTCA metabolite of penoxsulam TEA salt was determined to be 99% (80% acid equivalent) as measured by liquid chromatography with ultra violet detection. Structural identity was confirmed by infrared spectroscopy, mass spectroscopy, along with proton and carbon-13 nuclear magnetic resonance.
	CAS #:	Not Applicable
	Chemical Structure:	
	Solvent Used:	Dimethyl sulfoxide (DMSO)

2.	**Control Materials:**	
	Solvent Control (final concentration):	Dimethyl sulfoxide (DMSO) 1%
	Positive Control:	Nonactivation: Mitomycin C (MMC, Sigma, CAS No. 50-07-7), 0.5 µg/ml (4 hour) or 0.05 and 0.075 µg/ml (24 hour) Activation: Cyclophosphamide monohydrate (CP, Sigma, CAS No. 6055-19-2), 4 and 6 µg/ml

3.	**Activation**: S9 derived from							
	X	Induced	X	Aroclor 1254	X	Rat	X	Liver
		Not-induced		Phenobarbitol		Mouse		Lung

				None		Hamster		Other (name)
				Other (name)		Other (name)		

The S9 mix consisted of 10 mM $MgCl_2 \cdot 6H_2O$ (Sigma), 5 mM glucose-6-phosphate (Sigma), 4mM nicotinamide adenine dinucleotide phosphate (Sigma), 10mM $CaCl_2$ (Fisher, Fair Lawn, New Jersey), 30 mM KCl (Sigma), and 50 mM sodium phosphate (pH 8.0, Sigma and Fisher). The reconstituted mix was added to the culture medium to obtain the desired final concentration of S9 in the culture, *i.e.*, 2% v/v. Hence, the final concentration of the co-factors in the culture medium was 1/5 of the concentrations stated above.

4.	**Test cells:** Peripheral lymphocytes male CD ISG (Outbred Crl:CD (SD)IGSBR rats				
Media: RPMI 1640 medium (with 25 mM HEPES, GIBCO, Grand Island, New York) supplemented with 10% heat-inactivated dialyzed fetal bovine serum (GIBCO), antibiotics and antimycotics (Fungizone 0.25 µg/ml; penicillin G, 100 U/ml; and streptomycin sulfate, 0.1 mg/ml; GIBCO), 30 µg/ml PHA (HA16, Murex Diagnostics Ltd., Dartford, England), and an additional 2 mM L-glutamine (GIBCO).					
Properly maintained?	X	Yes		No	
Periodically checked for *Mycoplasma* contamination?		Yes	X	No	
Periodically checked for karyotype stability?	X	Yes		No	

5.	**Test compound concentrations used:**	
	Nonactivated conditions:	Assay A1 – 4 hours 0, 54.1, 108.13, 216.25, 432.5, 865, 1730, and 3460 µg/ml Assay A1 – 24 hours 0, 27, 54.1, 108.13, 216.25, 432.5, 865, 1730, and 3460 µg/ml Assay B1 – 4 hours 0, 250, 500, 1000, 2000, 3000, 4000, and 5175 µg/ml
	Activated conditions:	Assay A1 – 4 hours 0, 54.1, 108.13, 216.25, 432.5, 865, 1730, and 3460 µg/ml Assay B1 – 4 hours 0, 250, 500, 1000, 2000, 3000, 4000, and 5175 µg/ml

C. TEST PERFORMANCE

1. Preliminary Cytotoxicity Assay: not performed

2. Cytogenetic Assay:

a.	**Cell exposure time:**	Test Material	Solvent Control	Positive Control
	Non-activated: Activated:	4 h & 24 h 4 h	4 h & 24 h 4 h	4 h & 24 h 4 h

b.	**Spindle inhibition**	

	Inhibition used/concentration:	Colcemid (1 µg/culture)
	Administration time:	2-3 hours (before cell harvest)

c.	**Cell harvest time after termination of treatment:**	Test Material	Solvent Control	Positive Control
	Non-activated: Activated:	0 and 20h 20 h	0 and 20h 20 h	0 and 20h 20 h

d. Details of slide preparation: The cells were swollen by hypotonic treatment (0.075 M KCl), fixed with methanol:acetic acid (3:1), dropped on microscope slides, and stained in Giemsa.

e. Metaphase analysis:

No. of cells examined per dose: 200 cells/treatment, positive controls 100 cells				
Scored for structural?	X	Yes		No
Scored for numerical?	X	Yes If Y, list *(polyploidy),*		No
Coded prior to analysis?	X	Yes		No

f. Evaluation criteria: For a test to be acceptable, the chromosomal aberration frequency in the positive control cultures should be significantly higher than the solvent controls. The aberration frequency in the solvent control should be within reasonable limits of the laboratory historical values. A test chemical is considered positive in this assay if it induces a significant dose-related and reproducible increase in the frequency of cells with aberrations.

g. Statistical analysis: The proportions of cells with aberrations (excluding gaps) were compared by the following statistical methods. At each dose level, data from the replicates was pooled. A two-way contingency table was constructed to analyze the frequencies of aberrant cells. An overall Chi-square statistic, based on the table, was partitioned into components of interest. Specifically, statistics were generated to test the global hypotheses: 1) no difference in the average number of cells with aberrations among the dose groups, and 2) no linear trend of increasing number of cells with aberrations with increasing dose. An ordinal metric (0, 1, 2, etc.) was used for the doses in the statistical evaluation. If either statistic was found to be significant at alpha = 0.05, versus a one-sided increasing alternative, pairwise tests (*i.e.* control vs. treatment) were performed at each dose level and evaluated at alpha = 0.05, again versus a one-sided alternative.

Polyploid cells were analyzed by the Fisher Exact probability test. The number of polyploid cells were pooled across replicates for the analysis and evaluated at alpha = 0.05. The data was analyzed separately based on the presence or absence of S9 and based on the exposure time.

II. RESULTS

A. Preliminary cytotoxicity assay: Not performed

B. Cytogenetic assay:

pH and Osmolality

The pH and osmolality of treatment medium containing approximately 5150 µg/ml of the test material (approximately the limit dose of 10 mM limit) and medium containing 1% solvent (DMSO) was determined using a Denver Basic pH meter (Denver Instrument Co., Arvada, Colorado) and an OSMETTE A freezing point osmometer (Precision Systems, Inc., Natick, Massachusetts), respectively. There was no appreciable change in the pH or osmolality in the treatment medium containing the test material compared to culture medium alone (culture medium containing the test material, pH = 7.26, osmolality = 392 mOsm/kg H_20; culture medium with 1% DMSO, pH = 7.32, osmolality = 422 mOsm/kg H_20).

Assay A1

In the initial assay, cultures were treated with the test material in the absence and presence of S9 activation for 4 hours at concentrations of 0 (solvent control), 54.1, 108.13, 216.25, 432.5, 865, 1730, and 3460 µg/ml. Cultures were also treated continuously for 24 hours in the absence of S9 with the above concentrations plus an additional lower concentration of 27.0 µg/ml. The highest concentration tested was based upon the initial solubility determination of the test material in the culture medium. The analytically detected concentrations of the test material in the stock solutions (Assay A1) varied from 93.0 to 102.6% of the target.

With and without metabolic activation (4 hour treatment), the cultures did not display any toxicity as measured by relative mitotic indices. In the absence of S9, the mitotic indices for the treated cultures ranged from 71.4 to 102.0% relative to the solvent control values. In the presence of S9, the mitotic indices of the treated cultures ranged from 86.4 to 119.7% as compared to the solvent control values. Cultures treated continuously for 24 hours in the absence of S9 activation showed signs of toxicity at the highest concentration (*i.e.*, 3460 µg/ml) as measured by a relative mitotic index of 45.1%. The remaining concentrations had relative mitotic indices ranging from 73.6 to 104.4%.

A re-evaluation of the solubility of the test material indicated that the test material could be added to the treatment medium up to the limit concentration of 10 mM. Because the test material did not induce the desired level of toxicity in Assay A1 following the short-treatment, a repeat assay was conducted in the absence and presence of S9 with the limit concentration of 5175 µg/ml as the highest concentration (Assay B1). A repeat of the continuous treatment in the absence of S9 was deemed unnecessary due to the desired level of toxicity seen at the highest concentration in Assay A1.

Assay B1

In Assay B1, cultures were treated with the test material in the absence and presence of S9 activation for 4 hours at concentrations of 0 (solvent control), 250, 500, 1000, 2000, 3000, 4000, and 5175 µg/ml. The highest concentration tested represented the 10 mM limit for this assay system. The analytically detected concentrations of the test material in the stock solutions (Assay B1) varied from 102.8 to 113.9% of the target.

With and without metabolic activation (4 hour treatment), the cultures did not display any toxicity as measured by relative mitotic indices. In the absence of S9, the mitotic indices for the treated cultures ranged from 79.8 to 109.6% relative to the solvent control values. In the presence of S9, the mitotic indices of the treated cultures ranged from 85.1 to 105.3% as compared to the solvent control values. Based upon these results, the three highest concentrations (*i.e.*, 3000, 4000, and 5175 µg/ml) were chosen for the determination of chromosomal aberration frequencies and incidence of polyploidy in the absence and presence of S9 with a 4 hour treatment.

Among the cultures treated with the positive control chemicals for 4 hour, 0.5 µg/ml of MMC and 4 µg/ml of CP were selected for evaluation of aberrations in the absence and presence of S9, respectively.

There were no significant increases in the incidence of polyploidy cells in any of the test material treated cultures as compared to the solvent control values.

In the 4 hour non-activation assay, the frequency of cells with aberrations in the solvent control was 0.5% and the corresponding values at treatment levels of 3000, 4000, and 5175 µg/ml were 0.5, 2.0, 2.5%, respectively. Although, the statistical analysis of the chromosomal frequency data identified an increasing linear trend among the treatment groups, the pairwise comparisons of the treatment groups to the solvent controls did not identify a significant response. The frequencies of aberrant cells observed in all of the test material treated cultures were within the range of the solvent control values reported in this study, as well as, the laboratory historical control values. Hence, this apparent linear trend was deemed to be a chance occurrence unrelated to the treatment and biologically insignificant.

Table 5.7.1.2-3. Mitotic Indices in Rat Lymphocyte Cultures Assay A1

	Mean mitotic index [a]		
	Assay A1		
Conc. µg/ml	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)
Solvent control	9.8 (100.0)	6.6 (100.0)	9.1 (100.0)
27	ND	ND	8.3 (91.2)
54.1	8.9 (90.8)	6.7 (101.5)	9.2 (101.1
108.13	10.0 (102.0)	5.9 (89.4)	8.2 (90.1)
216.25	8.0 (81.6)	5.7 (86.4)	9.5 (104.4)
432.5	7.0 (71.4)	7.2 (109.1)	8.0 (87.9)
865	9.5 (96.9)	7.7 (116.7)	9.4 (103.3)

1730	8.6 (87.8)	7.9 (119.7)	6.7 (73.6)
3460	7.2 (73.5)	7.2 (109.1)	4.1 (45.1)
Positive control [b]	2.9 (29.6)	2.5 (37.9)	5.4 (59.3)
Positive control [c]	ND	0.9 (13.6)	3.1 (34.1)

[a] values in parenthesis are % relative mitotic index

[b] Positive control = 0.5 µg/ml MMC (-S9, 4 hr); 4 µg/ml CP (+S9, 4 hr); 0.05 µg/ml MMS (-S9, 24 hr)

[c] Positive control = 6 µg/ml CP (+S9, 4hr); 0.075 µg/ml MMS (-S9, 24 hr)

ND = not done

Table 5.7.1.2-4. Mitotic Indices in Rat Lymphocyte Cultures Assay B1

	Mean mitotic index [a]	
	Assay B1	
Conc. µg/ml	-S9 (4 hr)	+S9 (4 hr)
Solvent control	10.4 (100.0)	9.4 (100.0)
250	11.3 (108.7)	9.9 (105.3)
500	11.4 (109.6)	8.7 (92.6)
1000	9.9 (95.2)	8.8 (93.6)
2000	9.7 (93.3)	9.4 (100.0)
3000	8.6 (82.7)	9.0 (95.7)
4000	8.3 (79.8)	8.9 (94.7)
5175	9.0 (86.5)	8.0 (85.1)
Positive control [b]	3.3 (31.7)	4.2 (44.7)
Positive control [c]	ND	1.9 (20.2)

[a] values in parenthesis are % relative mitotic index

[b] Positive control = 0.5 µg/ml MMC (-S9, 4 hr); 4 µg/ml CP (+S9, 4 hr)

[c] Positive control = 6 µg/ml CP (+S9, 4hr)

ND = not done

Table 5.7.1.2-5. Results of the Cytogentic Assay

	Total aberrations (excluding gaps)			No. of cells with aberrations (excluding gaps) [a]			Incidence of polyploidy (%)		
Conc. µg/ml	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)
Solvent control	1	5	4	0.5	2.5	2.0	0.0	0.5	0.5
865	ND	ND	3	ND	ND	1.0	ND	ND	0.0
1730	ND	ND	4	ND	ND	2.0	ND	ND	0.5
3000	0	0	ND	0.5	0.0	ND	0.5	0.0	ND
3460	ND	ND	2	ND	ND	1.0	ND	ND	0.0
4000	4	5	ND	2.0	2.5	ND	0.0	0.0	ND
5175	5	4	ND	2.5	2.0	ND	0.5	0.5	ND

Positive control [b]	23	36	28	34.0*	36.0*	16.6*	0.0	0.0	0.0

[a] Values are percentages

[b] Positive control = 0.5 µg/ml MMC (-S9, 4 hr); 4 µg/ml CP (+S9, 4 hr); 0.05 µg/ml MMC (-S9, 24 hr)

*Significantly different from solvent controls, $\alpha = 0.05$.

ND = not done

It was concluded that under the experimental conditions used, X768359, the BSTCA metabolite of penoxsulam, was non-genotoxic in this *in vitro* chromosomal aberration test.

5.7.1.3. Mutagenicity studies on BST

5.7.1.3a *In Vitro* genotoxicity testing (Reference IIA5.7.1.3a/01)

STUDY TYPE: *(Bacterial system, Salmonella typhimurium; Escherichia coli)*/ mammalian activation gene mutation assay; OPPTS 870.5100[3]; OECD 471 (formerly OECD 471 & 472).

TEST MATERIAL (PURITY): X697134 (3-([{2,2-difluoroethyoxy)-6-(trifluormethyl)phenyl]sulfonyl}-amino-1H-1,2,4-triazole) (99%)

SYNONYMS: BST metabolite of penoxsulam

CITATION: M. S. Mecchi (21 July 2006). *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay Preincubation Method with a Confirmatory Assay with X697134 (BST Metabolite of Penoxsulam). Covance Laboratories Inc., Vienna, Virginia. Study ID 6736-157, 13 February 2006 – 21 July 2006. Unpublished.

SPONSOR: The Dow Chemical Company

SUMMARY:

In a reverse gene mutation assay in bacteria, tester strains TA98, TA100, TA1535, and TA1537 of *S. typhimurium* and tester strain WP2*uvr*A of *E. coli* were exposed to X697134 (BST Metabolite of Penoxsulam) (99% a.i.), in dimethylsulfoxide using the preincubation method at concentrations of 33.3, 100, 333, 1000, 3330, and 5000 µg per plate in the presence and absence of mammalian metabolic activation.

X697134 (BST Metabolite of Penoxsulam) was tested up to the limit concentration (5000 µg per plate) with all strains. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as acceptable. This study satisfies the requirement for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

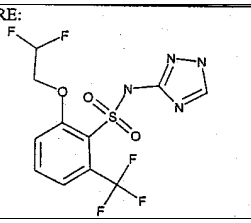
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	X697134 (BST Metabolite of Penoxsulam)
	Description:	White powder

[3]870.5100 - Reverse mutation *E. coli* WP2 and WP2uvrA; *S. typhimurium* TA 97, TA98, TA100, TA1535, TA1537
870.5140 - Gene mutation *Aspergillus nidulans*
870.5250 - Gene mutation *Neurospora crassa*

	Lot #:	035434-5
	Purity:	99% a.i.
	CAS #:	not provided
		STRUCTURE: 
	Solvent Used:	Dimethylsulfoxide

2.	**Control Materials:**	
	Negative:	NA
	Solvent (final conc'n):	Dimethylsulfoxide at 50 µL per plate
	Positive:	Nonactivation: sodium azide 2.0 µg/plate TA100, TA1535 2-nitrofluorene 1.0 µg/plate TA98 ICR-191 2.0 µg/plate TA1537 4-nitroquinoline-N-oxide 0.4 µg/plate WP2*uvr*A
		Activation: benzo[a]pyrene 2.5 µg/plate TA98 2-aminoanthracene 2.5 µg/plate TA100, TA1535, and TA1537 2-aminoanthracene 25.0 µg/plate WP2*uvr*A

3.	**Activation**: S9 derived from								
	x	induced		x	Aroclor 1254	x	Rat	x	Liver
		non-induced			Phenobarbitol		Mouse		Lung
					None		Hamster		Other (name)
					Other (name)		Other (name)		

S9 mix composition: H_2O (70%), 1M NaH_2PO_4/Na_2HPO_4, pH 7.4 (10%), 0.25M Glucose-6-phosphate (2%), 0.10M NADP (4%), 0.825M KCl/0.2M $MgCl_2$ (4%), and S9 Homogenate (10%).

4.	**Test organisms:** *S. typhimurium* strains									
		TA97	x	TA98	x	TA100		TA102		TA104
	x	TA1535	x	TA1537		TA1538	x	WP2*uvr*A (*E. coli*)		
Properly maintained?							x	Yes		No

Checked for appropriate genetic markers (*rfa* mutation, R factor)?	x	Yes		No

5. <u>Test compound concentrations used</u>:

Nonactivated conditions:
Cytotoxicity test: TA100 and WP2*uvr*A at 10.0, 33.3, 100, 333, 1000, 3330, and 5000 µg per plate; two replicates.
Mutagenicity assay: TA98, TA100, TA1535, TA1537, and WP2*uvr*A at 33.3, 100, 333, 1000, 3330, and 5000 µg per plate; three replicates.

Activated conditions:
Cytotoxicity test: TA100 and WP2*uvr*A at 10.0, 33.3, 100, 333, 1000, 3330, and 5000 µg per plate; two replicates.
Mutagenicity assay: TA98, TA100, TA1535, TA1537, and WP2*uvr*A at 33.3, 100, 333, 1000, 3330, and 5000 µg per plate; three replicates.

B. <u>TEST PERFORMANCE:</u>
1. <u>Type of *Salmonella* assay</u>: pre-incubation (20 minutes)

2. <u>Protocol</u>:

Tester strains were exposed to the test article via the preincubation modification of the Ames Test originally described by Yahagi *et al.* (1975) and Maron and Ames (1983). This methodology has been shown to detect a wide range of classes of chemical mutagens. In the preincubation methodology, S9 mix (or phosphate buffer, where appropriate), tester strain, and test article were preincubated for approximately 20 minutes prior to addition of molten agar. The agar and preincubation reaction mixture were mixed and then overlaid onto a minimal agar plate. Following incubation, revertant colonies were counted. Test article, vehicle controls, and positive controls were plated in triplicate. The condition of the bacterial background lawn was evaluated both macroscopically and microscopically (using a dissecting microscope) for indications of cytotoxicity and test article precipitate. Evidence of cytotoxicity was scored relative to the vehicle control plate and was recorded along with the revertant counts for all plates at that concentration. Revertant colonies were counted either by automated colony counter or by hand.

3. <u>Statistical Analysis</u>: Mean and standard deviation were calculated for each replicate.

4. <u>Evaluation Criteria</u>: Before assay data were evaluated, criteria for a valid assay had to be met. The following criteria were used to determine a valid assay:

Salmonella typhimurium tester strain cultures exhibited sensitivity to crystal violet to demonstrate the presence of the *rfa* wall mutation. Cultures of tester strains TA98 and TA100 exhibited resistance to ampicillin to demonstrate the presence of the pKM101 plasmid.

Demonstrating the requirement for histidine (*Salmonella typhimurium*) or tryptophan (*Escherichia coli*), tester strain cultures exhibited a characteristic number of spontaneous

revertants per plate when plated along with the vehicle under selective conditions. Acceptable ranges for mean vehicle controls were as follows:

TA98	8	-	60
TA100	60	-	240
TA1535	4	-	45
TA1537	2	-	25
WP2*uvr*A	5	-	40

Demonstrating that appropriate numbers of bacteria were plated, density of tester strain cultures were greater than or equal to 1.0×10^9 bacteria per mL and/or had reached a target density demonstrated to produce cultures with at least 1.0×10^9 bacteria per mL (see Study Deficiencies).

Demonstrating that tester strains were capable of identifying a mutagen, the mean value of a positive control for a respective tester strain exhibited at least a 3-fold increase over the mean value of the vehicle control for that strain. An acceptable positive control in the presence of S9 mix for a specific strain was evaluated as having demonstrated both integrity of the S9 mix and ability of the tester strain to detect a mutagen.

A minimum of three non-toxic concentrations was required to evaluate assay data. Cytotoxicity was detectable as a decrease in the number of revertant colonies per plate and/or by a thinning or disappearance of the bacterial background lawn. Thinning of the bacterial background lawn not accompanied by a reduction in the number of revertants per plate was not evaluated as an indication of cytotoxicity.

Once criteria for a valid assay had been met, responses observed in the assay were evaluated.

For a test article to be considered positive, it had to produce at least a 2-fold (TA100) or 3-fold (TA98, TA1535, TA1537, and WP2*uvr*A) concentration-related and reproducible increase in the mean revertants per plate over the mean revertants per plate of the appropriate vehicle control. A response that did not meet all three of the above criteria (magnitude, concentration-responsiveness, reproducibility) was not evaluated as positive.

II. RESULTS

A. Preliminary cytotoxicity assay

Seven concentrations of test article, from 10.0 to 5000 μg per plate, were tested in Trials 28097-A1 with tester strains TA100 and WP2*uvr*A. No cytotoxicity was observed with either tester strain in the presence or absence of S9 mix as evidenced by no decreases in the number of revertants per plate and normal bacterial background lawns.

B. Mutagenicity assay

The data for the mutagenicity assay were generated in Trials 28097-B1 and 28097-C1.

The tester strains used in the preincubation mutagenicity assay were *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* tester strain WP2*uvr*A.

The assay was conducted with six concentrations of the test article (33.3, 100, 333, 1000, 3330, and 5000 μg per plate) in the presence and absence of S9 mix along with concurrent vehicle and positive controls using three plates per concentration.

In the initial mutagenicity assay (Trial 28096-B1), all data were acceptable and no positive increases in the mean number of the revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix.

In the confirmatory mutagenicity assay (Trial 28096-C1), all data were acceptable and no positive increases in the mean number of the revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix.

III. CONCLUSION

Under the conditions of this study, X697134 (BST metabolite of penoxsulam) did not show evidence of mutagenic potential.

5.7.1.3b *In Vitro* genotoxicity testing (Reference IIA5.7.1.3b/01)

STUDY TYPE: Mammalian cells in culture gene assay in CHO-K_1-BH_4 cells; USEPA OPPTS 870.5300; OECD Guideline 476; EC, B.17.

TEST MATERIAL (PURITY): X697134; (3-([{2,2-difluoroethyoxy)-6-(trifluormethyl)phenyl]sulfonyl}-amino-1H-1,2,4-triazole) (99%)

SYNONYMS: BST metabolite of penoxsulam

CITATION: S. D. Seidel, M. R.., Schisler, and. K. M. Kleinert. (August 28, 2006). EVALUATION OF X697134 (BST METABOLITE OF PENOXSULAM) IN THE CHINESE HAMSTER OVARY CELL/HYPOXANTHINE-GUANINE-PHOSPHORIBOSYL TRANSFERASE (CHO/HGPRT) FORWARD MUTATION ASSAY. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, 48674. Study ID: 061019, (August 28, 2006). Unpublished

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

SUMMARY:

X697134 (3-([{2,2-difluoroethyoxy)-6-(trifluormethyl)phenyl]sulfonyl}-amino-1H-1,2,4-triazole), the BST metabolite of penoxsulam, was evaluated in the *in vitro* Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward gene mutation assay. The genotoxic potential of the test material was assessed in at least two independent assays in the absence and presence of an externally supplied metabolic activation (S9) system at concentrations ranging from 153.13 to 2450 µg/ml. The highest concentration was based on limit of solubility of the test material in the treatment medium. The adequacy of the experimental conditions for detection of induced mutation was confirmed by employing positive control chemicals, ethyl methanesulfonate for assays without S9 and 20-methylcholanthrene for assays with S9. Solvent control cultures were treated with the solvent used to dissolve the test material (*i.e.* dimethyl sulfoxide). Based upon the frequency of TG^r mutants recovered in cultures treated with the test material, it was concluded that X697134, the BST metabolite of penoxsulam, was non-mutagenic in the assay system employed.

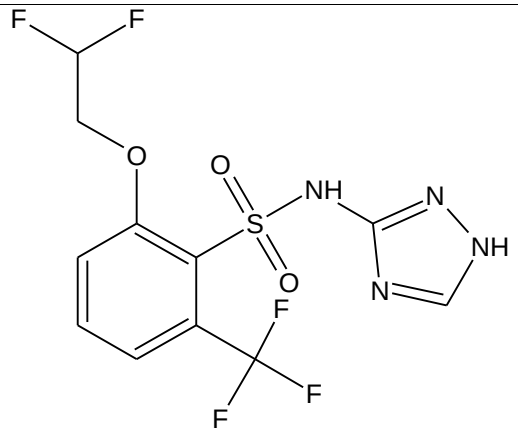
This study is acceptable and satisfies the guideline requirement for an Mammalian cells in culture gene assay in CHO-K_1-BH_4 cells; USEPA OPPTS 870.5300; OECD Guideline 476; EC, B.17.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	X697134 (BST metabolite of penoxsulam)
	Description:	White solid
	Lot/Batch #:	# 035434-5, TSN105514
	Purity:	99%
	CAS #:	Not applicable

	Chemical Structure:	
	Solvent Used:	Dimethyl sulfoxide (DMSO, Sigma, St. Louis, Missouri)

2.	**<u>Control Materials</u>:**	
	Solvent control (final conc'n):	Dimethyl sulfoxide (DMSO) 1%
	Positive control:	Nonactivation: Ethyl methanesulfonate (EMS, CAS No. 62-50-0), 621 μg/ml
		Activation: 20-methylcholanthrene (20-MCA, CAS No. 56-49-5), 4, 6, and 8μg/ml

3.	**<u>Activation</u>**: S9 derived from								
	X	induced		X	Aroclor 1254	X	Rat	X	Liver
		non-induced			Phenobarbitol		Mouse		Lung
					None		Hamster		Other
					Other		Other		

The S9 mix consisted of the following co-factors: 10 mM $MgCl_2 \cdot 6H_2O$, 5 mM glucose-6-phosphate, 4 mM nicotinamide adenine dinucleotide phosphate, 10 mM $CaCl_2$, 30 mM KCl, and 50 mM sodium phosphate (pH 8.0). The reconstituted mix was added to the culture medium to obtain the desired final concentration of S9 in the culture, *i.e.*, 2% v/v. Hence, the final concentration of the co-factors in the culture medium was 1/5th of the concentrations stated above.

4.	**<u>Test cells</u>:** mammalian cells in culture				
		mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)	
	X	Chinese hamster ovary (CHO) cells		list any others	

Media: The cells were routinely maintained in Ham's F-12 nutrient mix (GIBCO, Grand Island, New York) supplemented with 5% (v/v) heat-inactivated (56°C, 30 minutes), dialyzed fetal bovine serum (GIBCO), antibiotics and antimycotics (penicillin G, 100 units/ml; streptomycin sulfate, 0.1 mg/ml; fungizone, 25 µg/ml; GIBCO) and an additional 2 mM L-glutamine (GIBCO). The selection medium used for the detection of HGPRT$^-$ mutants was Ham's F-12 nutrient mix without hypoxanthine, supplemented with 10 µM 6-thioguanine (GIBCO) and 5% serum and the above-mentioned antibiotics.				
Properly maintained?	X	Yes		No
Periodically checked for Mycoplasma contamination?	X	Yes		No
Periodically checked for karyotype stability?	X	Yes		No
Periodically "cleansed" against high spontaneous background?	X	Yes		No

5.	**Locus Examined:**		Thymidine kinase (TK)	X	Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)		Na^+/K^+ ATPase
	Selection agent:		bromodeoxyuridine (BrdU		8-azaguanine (8-AG)		ouabain
			fluorodeoxyuridine (FdU)	X	6-thioguanine (6-TG)		
			trifluorothymidine (TFT)				

6.	**Test compound concentrations used:**	
	Nonactivated conditions:	Assay A1 0, 9.6, 19.1, 38.3, 76.6, 153.13, 306.25, 612.5, 1225, and 2450 µg/mL Assay B1 0, 153.13, 306.25, 612.5, 1225, and 2450 µg/mL Assay C1 0, 153.13, 306.25, 612.5, 1225, and 2450 µg/mL
	Activated conditions:	Assay A1 0, 9.6, 19.1, 38.3, 76.6, 153.13, 306.25, 612.5, 1225, and 2450 µg/mL Assay B1 0, 153.13, 306.25, 612.5, 1225, and 2450 µg/mL Assay C1 0, 153.13, 306.25, 612.5, 1225, and 2450 µg/mL

B. TEST PERFORMANCE

3. Cell treatment:

a. Cells were exposed to test compound, negative/solvent or positive controls for 4 hours (nonactivated) and 4 hours (activated).

b. After washing, cells were cultured for 8 days (expression period) before cell selection.

c. After expression,2 x 10^5cells/dish (10 dishes/ group) were cultured for 9 days in selection medium to determine numbers of mutants and 200 cells/dish (3 dishes/group) were cultured for 7 days without selective agent to determine cloning efficiency.

2. **Statistical Methods:** The frequency of mutants per 10^6 clonable cells was statistically evaluated using a weighted analysis of variance; weights were derived from the inverse of the mutation frequency variance. The actual plate counts are assumed to follow a Poisson distribution therefore the mean plate count was used as an estimate of variance.

   If the analysis of variance was significant at alpha = 0.05, a Dunnett's t-test was conducted, comparing each treated group and the positive control to the negative control (alpha = 0.05, one-sided). An additional comparison of the positive control to the negative control (alpha = 0.05) was conducted using a linear contrast statement. Linear dose-related trend tests were performed if any of the pairwise comparisons of test material with the negative control yielded significant differences.

3. **Evaluation Criteria:** For an assay to be acceptable, the mutant frequency in positive controls should have been significantly higher than the solvent controls. An additional criteria, was that the mutant frequency in the solvent controls should have been within reasonable limits of the laboratory historical control values and literature values. The test chemical was considered positive if it induced a statistically significant, dose related, reproducible increase in mutant frequency. The final interpretation of the data took into consideration such factors as the mutant frequency and cloning efficiencies in the solvent controls.

II. RESULTS

E. Preliminary cytotoxicity

pH and Osmolality

The pH and osmolality of treatment medium containing approximately 2450 µg/ml of the test material (limit of solubility in culture medium) and medium containing 1% DMSO were determined using a Denver Basic pH meter (Denver Instrument Co., Arvada, Colorado) and an OSMETTE A™ freezing point osmometer (Precision Systems, Inc., Natick, Massachusetts). Alterations in the pH and osmolality of the culture medium have been shown to induce false positive responses in *in vitro* genotoxicity assays. The changes in both the pH and osmolality at this concentration as compared to the culture medium with solvent alone (culture medium with the test material, pH = 7.08, osmolality= 432 mOsm/kg H_20; culture medium with 1% DMSO, pH = 7.34, osmolality = 467 mOsm/kg H_20) were deemed to be inconsequential to the assay conduct.

™ Trademark of Precision Systems Inc.

Assay A1 – Preliminary Toxicity Assay

In a preliminary toxicity assay, the test material was assayed at concentrations of 0, 9.6, 19.1, 38.3, 76.6, 153.13, 306.25, 612.5, 1225 and 2450 µg/ml in the absence and presence of an externally supplied metabolic activation system (S9). The highest concentration tested was based upon the limited solubility of the test material in the culture medium. The cultures treated without S9 activation showed no toxicity, as determined by, relative cell survival (RCS) values ranging from 100.9 to 127.0%. In the presence of S9 activation, little to no toxicity was observed with the RCS values ranging from 63.8 to 115.1%. Based upon the results of this assay, concentration levels of 0, 153.13, 306.25, 612.5, 1225, and 2450 µg/ml of the test material were selected for the initial gene mutation assay in the absence and presence of S9.

F. Mutagenicity assay

Assay B1 – Initial Mutagenicity Assay

In the initial mutagenicity assay (Assay B1), in the absence and presence of S9, little to no toxicity was observed. The RCS values ranged from 87.8 to 118.1% in the absence of S9 and 72.8 to 93.7% in the presence of S9. The mutant frequencies in the test material treated cultures were not statistically different from the concurrent solvent control values and all mutant frequencies were within a reasonable range of historical background values. The positive control chemicals induced significant increases in mutant frequencies in both the absence and presence of S9 and these data confirmed the adequacy of the experimental conditions for detecting induced mutations.

Table 5.7.1.3-1: Results of Initial Mutagenicity Assay (Assay B1)

	Initial Mutagenicity Assay (B1)			
Concentration	-S9		+S9	
µg/ml	%RCS	MF	%RCS	MF
Solvent Control	104.8	3.4	106.2	7.1
Solvent. Control	95.2	6.5	93.8	6.0
153.13	88.6	1.5	76.6	10.8
153.13	87.8	7.0	93.7	1.6
306.25	106.0	10.9	90.2	10.4
306.25	97.3	5.8	86.9	6.2
612.5	118.1	7.0	81.6	6.3
612.5	109.0	7.1	93.4	3.1
1225	104.3	1.8	83.2	2.6
1225	111.0	5.1	76.9	11.1
2450[a]	111.8	7.5	84.0	2.0
2450 [a]	101.5	9.7	72.8	10.0
Pos. Control[b]	38.4	572.0*	92.2	40.9*
Pos. Control[b]	39.0	450.0*	97.6	31.8*

RCS = Relative Cell Survival
MF = TG^r Mutants per 10^6 clonable cells
ND = Not done
[a]Precipitation in treatment medium
[b]Positive control = 621 µg/ml EMS (-S9); 4 µg/ml 20-MCA (+S9)
*The frequency of TG^r mutants is significantly higher than the concurrent solvent control value.

Assay C1 – Confirmatory Mutagenicity Assay

In a confirmatory assay (Assay C1), the concentrations tested ranged from 153.13 to 2450 µg/ml without and with S9. In the absence and presence of S9, no toxicity was observed as indicated by RCS values, ranging from 100.1 to 120.8% (-S9) and 91.9 to 101.2% (+S9). The mutant frequencies observed in cultures treated with the test material in the absence of S9 and presence of S9 were not significantly different from the concurrent solvent control values and were within the range of the laboratory historical background. The positive control chemical in the absence of metabolic activation (*i.e.*, EMS) induced statistically significant increases in mutant frequency compared to the concurrent solvent control values. However, the positive control in the presence of metabolic activation (*i.e.*, 20-MCA) failed to induce a statistically significant response compared to the concurrent solvent control values. Therefore, the assay was repeated in the presence of metabolic activation with higher concentrations of 20-MCA (Assay D1).

Table 5.7.1.3-2: Results of Confirmatory Mutagenicity Assay (assay C1)

	Confirmatory Mutagenicity Assay (C1)			
Concentration	-S9		+S9	
µg/ml	%RCS	MF	%RCS	MF
Solvent Control	105.1	10.9	97.3	20.7
Solvent. Control	94.9	12.5	102.7	13.2
153.13	108.7	12.7	97.7	5.7
153.13	111.5	8.4	101.2	11.5
306.25	110.2	4.3	99.0	9.5
306.25	120.8	11.0	92.3	12.0
612.5	113.7	15.1	95.8	10.9
612.5	100.1	8.0	97.5	15.5
1225	113.7	10.6	91.9	11.9
1225	102.5	3.4	96.3	11.9
2450[a]	102.0	4.7	95.4	13.4
2450 [a]	103.8	18.4	93.5	16.5
Pos. Control[b]	25.6	292.6*	100.2	41.1
Pos. Control[b]	35.5	352.5*	92.3	22.5

RCS = Relative Cell Survival
MF = TG^r Mutants per 10^6 clonable cells
ND = Not done
[a]Precipitation in treatment medium
[b]Positive control = 621 µg/ml EMS (-S9); 4 µg/ml 20-MCA (+S9)
*The frequency of TG^r mutants is significantly higher than the concurrent solvent control value.

Assay D1 – Repeat Confirmatory Mutagenicity Assay in the Presence of S9

In the repeat confirmatory assay (Assay D1), in the presence of S9, the concentrations tested ranged from 153.13 to 2450 µg/ml. There was little to no toxicity observed, as indicated by RCS values ranging from 65.3 to 102.2%. The mutant frequencies observed in cultures treated with the test material in the presence of S9 were not significantly different from the concurrent solvent

control values and were within the range of the laboratory historical background. The positive control chemical, 20-MCA, induced statistically significant increases in mutant frequency and this data confirmed the adequacy of the experimental conditions for detecting induced mutations.

Table 5.7.1.3-3: Repeat Confirmatory Mutagenicity Assay in the Presence of S9 (Assay D1)

Confirmatory Mutagenicity Assay (D1)		
Concentration	+S9	
µg/ml	%RCS	MF
Solvent Control	102.2	5.5
Solvent. Control	97.8	5.5
153.13	89.7	4.8
153.13	96.8	-[a]
306.25	87.1	1.5
306.25	102.2	3.0
612.5	91.7	1.3
612.5	89.1	0.8
1225	89.6	12.5
1225	79.8	7.9
2450[b]	70.7	7.0
2450 [b]	65.3	3.4
Pos. Control[c]	85.7	155.0[*]
Pos. Control[c]	83.0	231.3[*]
Pos. Control[d]	67.4	131.4[*]
Pos. Control[d]	91.2	157.9[*]
Pos. Control[e]	72.7	86.7[*]
Pos. Control[e]	69.7	115.0[*]

RCS = Relative Cell Survival
MF = TG^r Mutants per 10^6 clonable cells
ND = Not done
[a]Lost due to contamination
[b]Precipitation in treatment medium
[c]Positive control = 4 µg/ml 20-MCA (+S9)
[d]Positive control = 6 µg/ml 20-MCA (+S9)
[e]Positive control = 8 µg/ml 20-MCA (+S9)
*The frequency of TG^r mutants is significantly higher than the concurrent solvent control value.

The analytically observed concentrations of the test material in the stock solutions in Assay B1 ranged from 109.6 to 113.1% of target. In Assay C1, the observed concentration of the test material in the stock solutions ranged from 104.9 to 109.6% of target. In Assay D1, the observed concentration of the test material in the stock solutions ranged from 109.8 to 112.7% of target.

Conclusions

Based upon the frequency of TG^r mutants recovered in cultures treated with the test material, it was concluded that XDE-638 BST Metabolite was not mutagenic in the CHO/HGPRT gene mutation assay.

5.7.1.3c *In Vitro* genotoxicity testing (Reference IIA5.7.1.3c/01)

STUDY TYPE: *In vitro* mammalian cytogenetics OPPTS 870.5375; OECD 473

TEST MATERIAL (PURITY): X697134; (3-([{2,2-difluoroethyoxy)-6-(trifluormethyl)phenyl]sulfonyl}-amino-1H-1,2,4-triazole) (99%)

SYNONYMS: BST metabolite of penoxsulam

CITATION: G. D. Charles, M. R. Schisler, and K. M. Kleinert. (2006). EVALUATION OF X697134 (BST METABOLITE OF PENOXSULAM) IN AN *IN VITRO* CHROMOSOMAL ABERRATION ASSAY UTILIZING RAT LYMPHOCYTES. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, 48674. Study ID: 061018, (June 28, 2006). . Unpublished

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

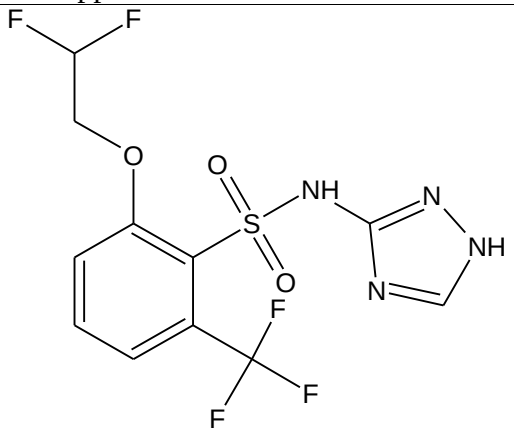
SUMMARY: X697134, the BST metabolite of penoxsulam (3-([{2,2-difluoroethyoxy)-6-(trifluormethyl)phenyl]sulfonyl}-amino-1H-1,2,4-triazole) was evaluated in an *in vitro* chromosomal aberration assay utilizing rat lymphocytes. Approximately 48 hours after the initiation of whole blood cultures, cells were treated either in the absence or presence of S9 activation with concentrations ranging from 0 (solvent control) to 2450 μg of X697134, the BST metabolite of penoxsulam, per ml of culture medium. The duration of treatment was 4 or 24 hours without S9 and 4 hours with S9. The highest concentration was based on the solubility of the test material in the vehicle. Based upon the mitotic indices, cultures treated for 4 hours with targeted concentrations of 0 (solvent control), 612.5, 1225, and 2450 μg/ml in the absence and presence of S9 activation and cultures treated for 24 hours with 0 (solvent control), 76.6, 153.13, and 306.25 μg/ml were selected for determining the incidence of chromosomal aberrations. There were no significant increases in the frequencies of cells with aberrations in either the absence or presence of S9 activation. Cultures treated with the positive control chemicals (*i.e.*, mitomycin C without S9 and cyclophosphamide with S9) had significantly higher incidences of abnormal cells in all assays. Based upon these results, X697134, the BST metabolite of penoxsulam, was considered to be non-genotoxic in this *in vitro* chromosomal aberration assay utilizing rat lymphocytes.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	X697134 (BST METABOLITE OF PENOXSULAM)
	Description:	White solid
	Lot/Batch #:	035434-5, TSN105514

	Purity:	The purity of the test material was determined to be 99% by liquid chromatography with ultra violet detection. Structural identity was confirmed by infrared spectroscopy, mass spectroscopy, along with proton and carbon-13 nuclear magnetic resonance.
	CAS #:	Not applicable
	Chemical Structure:	
	Solvent Used:	Dimethyl sulfoxide, DMSO

2.	**Control Materials:**	
	Solvent Control (final concentration):	Dimethyl sulfoxide (DMSO) 1%
	Positive Control:	Nonactivation: Mitomycin C (MMC, Sigma, CAS No. 50-07-7), 0.5 µg/ml (4 hour) or 0.05 and 0.075 µg/ml (24 hour) Activation: Cyclophosphamide monohydrate (CP, Sigma, CAS No. 6055-19-2), 4 and 6 µg/ml

3.	**Activation**: S9 derived from							
	X	Induced	X	Aroclor 1254	X	Rat	X	Liver
		Not-induced		Phenobarbitol		Mouse		Lung
				None		Hamster		Other (name)
				Other (name)		Other (name)		

The S9 mix consisted of 10 mM $MgCl_2 \cdot 6H_2O$ (Sigma), 5 mM glucose-6-phosphate (Sigma), 4mM nicotinamide adenine dinucleotide phosphate (Sigma), 10mM $CaCl_2$ (Fisher, Fair Lawn, New Jersey), 30 mM KCl (Sigma), and 50 mM sodium phosphate (pH 8.0, Sigma and Fisher). The reconstituted mix was added to the culture medium to obtain the desired final concentration of S9 in the culture, *i.e.*, 2% v/v. Hence, the final concentration of the co-factors in the culture medium was 1/5 of the concentrations stated above.

4.	**Test cells:** Peripheral lymphocytes from male CD ISG (Outbred Crl:CD (SD)) rats

Media: RPMI 1640 medium (with 25 mM HEPES, GIBCO, Grand Island, New York) supplemented with 10% heat-inactivated dialyzed fetal bovine serum (GIBCO), antibiotics and antimycotics (Fungizone 0.25 µg/ml; penicillin G, 100 U/ml; and streptomycin sulfate, 0.1 mg/ml; GIBCO), 30 µg/ml PHA (HA16, Murex Diagnostics Ltd., Dartford, England), and an additional 2 mM L-glutamine (GIBCO).				
Properly maintained?	X	Yes		No
Periodically checked for *Mycoplasma* contamination?		Yes	X	No
Periodically checked for karyotype stability?	X	Yes		No

5.	**Test compound concentrations used:**	
	Nonactivated conditions:	Assay A1 – 4 hours 0, 38.3, 76.6, 153.13, 306.25, 612.5, 1225, and 2450 µg/ml Assay A1 – 24 hours 0, 19.1, 38.3, 76.6, 153.13, 306.25, 612.5, 1225 and 2450 µg/ml
	Activated conditions:	Assay A1 – 4 hours 0, 38.3, 76.6, 153.13, 306.25, 612.5, 1225, and 2450 µg/ml

D. TEST PERFORMANCE

1. Preliminary Cytotoxicity Assay: Not performed

2. Cytogenetic Assay:

a.	**Cell exposure time:**	Test Material	Solvent Control	Positive Control
	Non-activated:	4 h & 24 h	4 h & 24 h	4 h & 24 h
	Activated:	4 h	4 h	4 h

b.	**Spindle inhibition**	
	Inhibition used/concentration:	Colcemid (1 µg/culture)
	Administration time:	2-3 hours (before cell harvest)

c.	**Cell harvest time after termination of treatment:**	Test Material	Solvent Control	Positive Control
	Non-activated:	0 and 20h	0 and 20h	0 and 20h
	Activated:	20 h	20 h	20 h

d. Details of slide preparation: The cells were swollen by hypotonic treatment (0.075 M KCl), fixed with methanol:acetic acid (3:1), dropped on microscope slides, and stained in Giemsa.

e. Metaphase analysis:

No. of cells examined per dose: 200 cells/treatment, positive controls 100 cells				
Scored for structural?	X	Yes		No
Scored for numerical?	X	Yes If Y, list *(polyploidy),*		No
Coded prior to analysis?	X	Yes		No

f. Evaluation criteria: For a test to be acceptable, the chromosomal aberration frequency in the positive control cultures should be significantly higher than the solvent controls. The aberration frequency in the solvent control should be within reasonable limits of the laboratory historical values. A test chemical is considered positive in this assay if it induces a significant dose-related and reproducible increase in the frequency of cells with aberrations.

h. Statistical analysis: The proportions of cells with aberrations (excluding gaps) were compared by the following statistical methods. At each dose level, data from the replicates was pooled. A two-way contingency table was constructed to analyze the frequencies of aberrant cells. An overall Chi-square statistic, based on the table, was partitioned into components of interest. Specifically, statistics were generated to test the global hypotheses: 1) no difference in the average number of cells with aberrations among the dose groups, and 2) no linear trend of increasing number of cells with aberrations with increasing dose. An ordinal metric (0, 1, 2, etc.) was used for the doses in the statistical evaluation. If either statistic was found to be significant at alpha = 0.05, versus a one-sided increasing alternative, pairwise tests (*i.e.* control vs. treatment) were performed at each dose level and evaluated at alpha = 0.05, again versus a one-sided alternative.

Polyploid cells were analyzed by the Fisher Exact probability test. The number of polyploid cells were pooled across replicates for the analysis and evaluated at alpha = 0.05. The data was analyzed separately based on the presence or absence of S-9 and based on the exposure time.

II. RESULTS

pH and Osmolality:

The pH and osmolality of treatment medium containing approximately 2450 µg/ml of the test material (solubility limitations in the solvent) and medium containing 1% solvent (DMSO) was determined using a Denver Basic pH meter (Denver Instrument Co., Arvada, Colorado) and an OSMETTE A freezing point osmometer (Precision Systems, Inc., Natick, Massachusetts), respectively. The changes in both the pH or osmolality at this concentration as compared to the culture medium with solvent alone (culture medium with the test material, pH = 7.18, osmolality = 394 mOsm/kg H_20; culture medium with 1% DMSO, pH = 7.41, osmolality = 424 mOsm/kg H_20) were deemed to be inconsequential to the assay conduct.

Assay A1

In the initial assay, cultures were treated with the test material in the absence and presence of S9 activation for 4 hours at concentrations of 0 (solvent control), 38.3, 76.6, 153.13, 306.25, 612.5, 1225, and 2450 µg/ml. Cultures were also treated continuously for 24 hours in the absence of S9 with the above concentrations plus an additional lower concentration of 19.1 µg/ml. The highest concentration evaluated was based on solubility limitations of the test material in the solvent. Furthermore, there was also a slight precipitate in the treatment medium at the highest concentration level (2450 µg/ml). The analytically detected concentrations of the test material in the stock solutions (Assay A1) varied from 107.5 to 111.5% of the target.

Short Treatment

Without and with metabolic activation (4 hour treatment), the cultures displayed varying levels of toxicity as measured by relative mitotic indices. In the absence of S9, the mitotic indices for the treated cultures ranged from 47.7 to 111.0% relative to the solvent control values. In the presence of S9, the relative mitotic indices of the treated cultures ranged from 58.4 to 106.7% as compared to the solvent control values. Based upon these results, cultures treated with the top three concentrations (*i.e.*, 612.5, 1225, and 2450 µg/ml) were chosen for the determination of chromosomal aberration frequencies and incidence of polyploidy both in the absence and presence of S9 activation for the 4 hour treatment

Among the cultures treated with the positive control chemicals for 4 hour, 0.5 µg/ml of MMC and 4 µg/ml of CP were selected for evaluation of aberrations in the absence and presence of S9, respectively.

There were no significant increases in the incidence of polyploidy cells in any of the test material treated cultures as compared to the solvent control values.

In the 4 hour non-activation assay, the frequency of cells with aberrations in the solvent control was 0.5% and the corresponding values at treatment levels of 612.5, 1225 and 2450 µg/ml were 2.0, 1.5, and 2.5%, respectively. In the activation assay, cultures treated with the test material at concentrations of 612.5, 1225 and 2450 µg/ml had aberrant cell frequencies of 2.0, 1.5, and 1.0%, respectively as compared to the solvent control value of 1.0%. Statistical analyses of these data did not identify significant differences between the solvent control and any of the treated cultures without or with S9 activation. The frequencies of aberrant cells observed in the test material treated cultures were within the laboratory historical background range.

Significant increases in the frequency of cells with aberrations were observed in cultures treated with the positive control chemicals. Aberrant cell frequencies in MMC (- S9, 4 hour treatment), and CP (+ S9, 4 hour treatment) cultures were 48%, and 30%, respectively.

Continuous Treatment

Based upon the clearly negative findings in the 4 hour treatment in the absence of metabolic activation, slides from the continuous 24 hour treatment were evaluated. Excessive toxicity was evident at the highest treatment level (*i.e.*, 2450 µg/ml) as determined by a relative mitotic index of 5%. The remaining treatment levels had mitotic indices ranging from 17.5 to 100.0% relative to the solvent control values. Based on these results, cultures treated with 76.6, 153.13, and 306.25 µg/ml of the test material were chosen for the determination of chromosomal aberration frequencies and incidence of polyploidy in the 24 hour continuous treatment in the absence of metabolic activation. Cultures treated with 0.05 µg/ml MMC were selected for evaluation to serve as the positive control for the 24 hour assay in the absence of metabolic activation.

There were no significant increases in the incidence of polyploidy cells in any of the test material treated cultures as compared to the solvent control values.

The frequency of aberrant cells in the solvent control was 4.0% and this value was within the range of the laboratory historical negative controls. The corresponding values at treatment levels of 76.6, 153.13, and 306.25 μg/ml were 3.0, 3.0, and 5.0%, respectively. There were no statistically significant differences between the test material treated cultures and the solvent control values, and all values were within the laboratory historical background range.

Significant increases in the frequency of cells with aberrations were observed in cultures treated with the positive control chemical. Aberrant cell frequency in MMC treated cultures was 32%.

A second assay with treatment of cultures in the presence of S9 was not considered necessary in this study since the results of the initial test yielded clearly negative results.

Table 5.7.1.3-4. Mitotic Indices and Polyploidy Incidence in Rat Lymphocyte Cultures

	Mean mitotic index [a]			Incidence of polyploidy (%)		
	Assay A1			Assay A1		
Conc. µg/ml	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)	-S9 (4 hr)	+S9 (4 hr)	-S9 (24hr)
Solvent control	10.9 (100.0)	8.9 (100.0)	8.0 (100.0)	0.5	0.5	0.0
19.1	ND	ND	7.6 (95.0)	ND	ND	ND
38.3	9.1 (83.5)	9.5 (106.7)	8.0 (100.0)	ND	ND	ND
76.6	12.1 (111.0)	6.4 (71.9)	6.4 (80.0)	ND	ND	0.5
153.13	9.7 (89.0)	6.5 (73.0)	5.4 (67.5)	ND	ND	0.0
306.25	10.0 (91.7)	6.8 (76.4)	3.4 (42.5)	ND	ND	0.0
612.5	7.0 (64.2)	5.2 (58.4)	1.7 (21.3)	0.0	0.0	ND
1225	6.2 (56.9)	5.9 (66.3)	1.4 (17.5)	0.0	0.0	ND
2450	5.2 (47.7)	5.8(65.2)	0.4 (5.0)	0.0	0.5	ND
Positive control [b]	2.7 (24.8)	3.5 (39.3)	2.8 (35.0)	0.0	0.0	0.5
Positive control [c]	ND	1.5 (16.9)	2.4 (30.0)	ND	ND	ND

[a] values in parenthesis are % relative mitotic index

[b] Positive control = 0.5 µg/ml MMC (-S9, 4 hr); 4 µg/ml CP (+S9, 4 hr); 0.05 µg/ml MMS (-S9, 24 hr)

[c] Positive control = 6 µg/ml CP (+S9, 4hr); 0.075 µg/ml MMS (-S9, 24 hr)

ND = not done

Table 5.7.1.3-5. Results of the Cytogentic Assay

	Total aberrations (excluding gaps)			No. of cells with aberrations (excluding gaps) [a]		
	Assay A1			Assay A1		
Conc. µg/ml	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)
Solvent control	1	2	8	0.5	1.0	4.0
76.6	ND	ND	6	ND	ND	3.0
153.13	ND	ND	5	ND	ND	3.0
306.25	ND	ND	11	ND	ND	5.0
612.5	4	4	ND	2.0	2.0	ND
1225	3	3	ND	1.5	1.5	ND
2450	5	2	ND	2.5	1.0	ND
Positive control [b]	33	35	37	48.0*	30.0*	32.0*

[a] Values are percentages

[b] Positive control = 0.5 µg/ml MMC (-S9, 4 hr); 4 µg/ml CP (+S9, 4 hr); 0.05 µg/ml MMC (-S9, 24 hr)

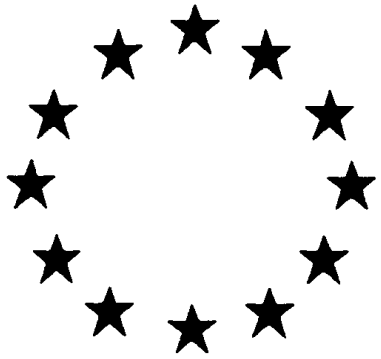
*Significantly different from solvent controls, $\alpha = 0.05$.

ND = not done

Based on the results of mutagenicity testing with 5-OH-638, BSTCA and BST, there was no evidence of a genotoxic potential for penoxsulam metabolites.

==========

Ministry of Health
P.le Marconi, 25
00144 Rome
Italy



PENOXSULAM

Addendum B.6
Toxicology and Metabolism

January 2007

THE GERMAN MODEL (GEOMETRIC MEAN VALUES)

Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles				
Product	**GF-657**		Active substance	**Penoxsulam**	
Formulation type	Liquid		a.s. concentration	**20.4**	g/l
Dermal absorption from product	**10**	%	Dermal absorption from spray	**10**	%
RPE during mix/loading	None		RPE during application	None	
PPE during mix/loading	None				
PPE during application: Head	None	Hands	None	Body	None
Dose	**2**	l product/ha	Work rate/day	**20**	ha

DERMAL EXPOSURE DURING MIXING AND LOADING

Hand contamination/kg a.s.	2.4	mg/kg a.s.
Hand contamination/day	1.9584	mg/day
Protective clothing	none	
Transmission to skin	100	%
Dermal exposure to a.s.	1.9584	mg/day

INHALATION EXPOSURE DURING MIXING AND LOADING

Inhalation exposure/kg a.s.	0.0006	mg/kg a.s.
Inhalation exposure/day	0.0004896	mg/day
RPE	none	
Transmission through RPE	100	%
Inhalation exposure to a.s.	0.0004896	mg/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique Tractor-mounted/trailed boom sprayer: hydraulic nozzles

	Head	Hands	Rest of body
Dermal contamination/kg a.s.	0.06	0.38	1.6

Dermal contamination/day	0.04896	0.31008		1.3056	
Protective clothing	none	none		none	
Transmission to skin	100	100		100	%
Total dermal exposure to a.s.	1.66464	mg/day			

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure/kg a.s.	0.001	mg/kg a.s.
Inhalation exposure/day	0.000816	mg/day
RPE	none	
Transmission through RPE	100	%
Inhalation exposure to a.s.	0.000816	mg/day

ABSORBED DOSE

	Mix/load		Application		
Dermal exposure to a.s.	1.9584	mg/day		1.66464	mg/day
Percent absorbed	10	%		10	%
Absorbed dose (dermal route)	0.19584	mg/day		0.166464	mg/day
Inhalation exposure to a.s.	0.0004896	mg/day		0.000816	mg/day
Total systemic exposure	0.1963296	mg/day		0.16728	mg/day

PREDICTED EXPOSURE

Total systemic exposure	0.36	mg/day
Operator body weight	70.00	kg
		mg/kg
Operator exposure	0.01	bw/day

TOXICITY vs EXPOSURE

AOEL	0.18	mg/kg bw/day
Operator exposure	0.01	mg/kg bw/day
Exposure as % of AOEL	2.89	%

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Product	**GF-657**	Active substance (a.s.)	**Penoxsulam**	
Formulation type	organic solvent-based	a.s. concentration	**20.4**	mg/ml
Dermal absorption - product	**10** %	Dermal absorption - spray	**10**	%
Container	5 litres 45 or 63 mm closure			
PPE during mix/loading	None	PPE during application	None	
Dose	**2** l/ha	Work rate/day	**50**	ha
Application volume	**150** l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	5	litres
Hand contamination/operation	0.01	ml
Application dose	2	litres product/ha
Work rate	50	ha/day
Number of operations	20	/day
Hand contamination	0.2	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.2	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Application volume	150	litres spray/ha		
Volume of surface contamination	10	ml/h		
Distribution	Hands	Trunk	Legs	
	65%	10%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	5%	15%	
Dermal exposure	6.5	0.05	0.375	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	41.55	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.2	ml/day	41.55	ml/day
Conc. of a.s. product or spray	20.4	mg/ml	0.2720	mg/ml
Dermal exposure to a.s.	4.08	mg/day	11.3016	mg/day

Percent absorbed	10	%	10	%
Absorbed dose	0.408	mg/day	1.1302	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.01	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.272	mg/ml
Inhalation exposure to a.s.	0.0163	mg/day
Percent absorbed	100	%
Absorbed dose	0.0163	mg/day

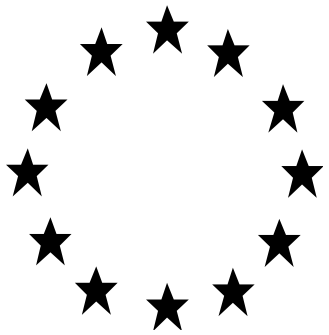
PREDICTED EXPOSURE

Total absorbed dose	1.5545	mg/day
Operator body weight	60	kg
Operator exposure	0.026	mg/kg bw/day

TOXICITY vs EXPOSURE

AOEL	0.18	mg/kg bw/day
Operator exposure	0.026	mg/kg bw/day
Exposure as % of AOEL	14.39	%

Council Directive 91/414/EEC



PENOXSULAM

Addendum/Corrigendum to the Draft Assessment Report
made to the European Commission
under 91/414/EEC

Foreword:

This document reflects the discussion reported in the Evaluation Table which were not covered by previous addenda and contains both the assessments of new information and the corrections which were necessary to amend the DAR.

Section 1 Identity, Physical and chemical properties, Details of uses and further information, Methods of analysis

Open point 1.1:To amend the list of end points with respect to classification and labelling.

with regard to physical/chemical data on penoxsulam/638: none
with regard to physical/chemical data on Viper/GF-657 : none
Fulfilled

Open point 1.2: To amend the list of end points with respect to the list of representative uses.

Fulfilled

Open point 1.3:To provide a corrigendum or revised Volume 4 to clarify the used codes.

Active substance	Code	Used between
penoxsulam	X638177	Started in 1998
penoxsulam	XR-638	March 1998 to November 1998
penoxsulam	XDE-638	November 1998 to November 2001
penoxsulam	DE-638	November 2001 to now

Open point 1.4: to amend the list of end points with respect to method for the determination of Bis-CHYMP.

Fulfilled

Identity of relevant impurities (of toxicological, environmental and/or other significance) in the active substance as manufactured (g/kg)	Bis-CHYMP 4(1H)-pyrimidinone, 2-chloro-5-methoxy-, 2-chloro-5-methoxy-4-pyrimidinylhydrazone Maximum content 0.5 g/kg
Impurities in technical as (principle of method)	**Moreland, J., Fonquerne, C. (2002) Validation of Analytical Method DAS-AM-01-051 for the Determination of Impurities in Technical Grade DE-638** For impurities 1-11 and 13, - reversed phase liquid chromatography using a C8 column, o-toluic acid internal standard and UV detection at 285 nm. Samples are dissolved in internal standard solution and diluted with eluent prior to analysis. Impurity 12 - reversed phase liquid chromatography using a C18 column, phthalimide internal standard and UV detection at 265 nm. Samples are dissolved in internal standard solution and diluted with acetonitrile prior to analysis. Bis-CHYMP – reversed phase liquid chromatography using a C18 column, external

	standard calibration and UV detection at 360 nm. Samples are dissolved in acetonitrile and mobile phase. The LOQ for Bis-CHYMP is 86 ppm.

Open point 1.5: To amend the list of end points to indicate that a method for blood and tissues (Annex point 4.2.5) is not required.

Fulfilled

Body fluids and tissues (principle of method and LOQ)	**Chickering, C.D. (2002): Determination of Residues of XDE-638 in Whole Blood and Urine by Liquid Chromatography with Tandem Mass Spectrometry Detection** As Penoxsulam is not classified as a toxic or highly toxic compound no method for the determination of residues is relevant. However, a method for body fluids and tissues does exist. Urine. Sample is diluted with water and then transferred to an autosampler vial for analysis by LC/MS/MS. Whole human blood samples. An aliquot of the sample is diluted with water and extracted with acetonitrile. After centrifugation, the extract is diluted with water and then transferred to an autosampler vial for analysis by LC/MS/MS. LOQ = 0.01 g/ml. It was calculated as the lowest level of fortification for recovery samples.

Open point 1.6: to amend the list of end points with respect to the validated matrices in food of plant origin.

Fulfilled

Food/feed of plant origin (principle of method and LOQ for methods for monitoring purposes) on rice and rice processed products (polished rice, rice bran and rice hulls)	**Hastings, M.J., Schelle, G.E. (2002): Determination of Residues of XDE-638 in Rice and Rice Processed Products by Liquid Chromatography with Tandem Mass Spectrometry** Extraction: acetonitrile: water (80:20 v/v). Clean up: Solid phase extraction (SPE) plate; elution with acetonitrile:formic acid (100:0.1). Liquid chromatography with positive ion electrospray tandem mass spectrometry (LC/MS/MS). Ion: Q1 484.0 m/z; Q3 = 195.0

m/z.
LOQ = 0.01 mg/kg
Chickering, C.D., (2002): Independent Laboratory Validation of Dow AgroSciences LLC, Method GRM01.25 – Determination of Residues of XDE-638 in Rice and Rice Processed Products by Liquid Chromatography with Tandem Mass Spectrometry Detection.

Extraction: acetonitrile:water (80:20 v/v).
Clean up: Solid phase extraction (SPE) plate; elution with acetonitrile:formic acid (100:0.1).
Liquid chromatography with positive ion electrospray tandem mass spectrometry (LC/MS/MS). Ion: Q1 484.0 m/z; Q3 = 195.0 m/z
LOQ = 0.01 mg/kg

1.1

To provide a shelf-life study as well as data on the relative density.

Report:	**Lindsay, D. A. (2004): Frozen Storage Stability of XDE-638 in Rice (Raw Agricultural Commodities: Grain, Straw, Immature Forage) and its Processed Products (Bran, Hulls, Polished Rice), Dow AgroSciences unpublished report number 010100.01. Ref. A26**
Guidelines:	This study reflects the guidance for storage stability described in Lundehn (7028/VI/95 EN rev.2.6/1/97) and in EC Directive 96/68 amending Annex II and II of 91/414.
GLP:	Yes (certified laboratory)
Methodology:	Control samples of rice grain, straw, immature forage, and the rice processed products bran, hulls, and polished rice were fortified at 0.10 mg/kg with DE-638 and stored in polypropylene containers. This concentration is 10X the validated analytical method limit of quantitation of 0.01 mg/kg. The fortified samples were stored frozen at –20 ± 5°C. On the day of fortification (0 day) and at each subsequent sampling interval, storage stability samples were analysed in triplicate along with at least three freshly fortified controls (for procedural recoveries) and an unfortified control sample. Analysis was conducted using Dow AgroSciences analytical method GRM 01.25. Sample extracts were analyzed immediately after preparation and therefore no separate storage stability study on sample extracts was conducted.

Findings: No degradation of DE-638 in rice grain, straw or immature forage was observed during the storage period of up to 732 days. No degradation of DE-638 in rice bran, hulls, or polished rice was observed during the storage period of 390 days. Recovery of DE-638 in each matrix at each timepoint is summarized in **Table 1** and **Table 2**.

Table 1: DE-638 recoveries in rice grain, straw, and immature forage fortified at 0.10 mg/kg following frozen storage

Storage period (days)	**Mean recovery (%)**		
	Grain	Straw	Immature Forage
0	90	100	100
210	98	114	118
732	94	93	94

Note: each time point is an average of 3 replicate samples; recoveries from stored samples have not been corrected for procedural recovery.

Table 6.0.1-2: DE-638 recoveries in rice bran, hulls, and polished rice fortified at 0.10 mg/kg following frozen storage

Storage period (days)	**Mean recovery (%)**		
	Bran	Hulls	Polished Rice
0	83	104	95
42	79	93	98
83	90	103	101
197	99	101	110
390	84	110	114

Note: each time point is an average of 3 replicate samples; recoveries from stored samples have not been corrected for procedural recovery.

Conclusions: Residues of DE-638 are stable in rice grain, straw, and immature forage when stored frozen at -20°C for up to 732 days. Residues of DE-638 are stable in rice bran, hulls and polished rice when stored frozen at -20^{o}C for up to 390 days.

Open point 1.12 (new) to summarise and evaluate the shelf life study for the representative formulation in an addendum and remove the study for the GF-237 formulation from the references relied on.

See above

1.2

To provide data on the oxidising properties of the formulation based on a theoretical assessment or on the EEC method A21.

Data on the oxidising properties of the formulation based on a theoretical assessment or on the EEC method A21 provided, summarized as followed:

The current EU test method A21 for determining oxidizing properties is applicable to liquids. The test need not be performed when examination of the structural formula establishes beyond reasonable doubt that the substance is incapable of reacting exothermically with a combustible material. GF-657 is an oil dispersion liquid formulation containing 20 g/litre penoxsulam and the information shown below assesses, by reviewing the individual components of the formulation, whether this product is likely to show any oxidising properties.

An oxidising agent is defined as a substance which, although alone may not be combustible, may generally cause or contribute to the combustion of other materials by yielding oxygen.

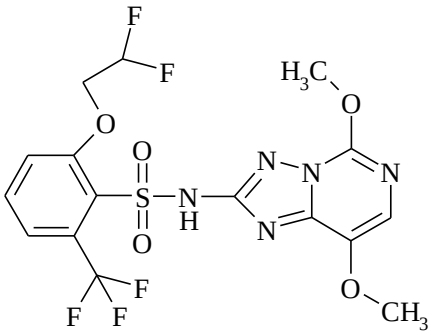
Table 1 attached provides a realistic assessment of the oxidising potential of the components of GF-657 formulation based on their chemical nature, safety data sheet information, and other reactivity data.

None of the components of the formulation contain reactive groups that may yield substances of oxidising potential, e.g. organo nitro compounds, N-halogen compounds, oxyhalogen compounds.

The conclusion from this assessment is that none of the components of GF-657 demonstrate oxidising potential. Since GF-657 is a simple blend of these components, and shows good chemical and physical stability on storage, it is reasonable to conclude that this formulation will not demonstrate any oxidising properties.

Table 1: Assessment of GF-657 Oxidising Potential

Penoxsulam chemical structure:



Ingredient Name and description	Amount present (%w/w)	Reactive chemistry information	Conclusion
Penoxsulam	2.15%	Chemical structure shows no potential for peroxide formation. Penoxsulam technical was tested for oxidising potential according to EU Test A17 and it was not found to show any oxidising properties (Reference 1).	Substance is not classified as an oxidising agent.
Aerosil R974	3.0%	Substance is a hydrophobic amorphous fumed silica (SiO_2). The product is non-flammable and stable at elevated temperatures. MSDS states that the product is inert and will not undergo hazardous polymerization.	Substance is not classified as an oxidising agent.
Atlox LP1	3.0%	Substance is a proprietary polymeric dispersant. The flash point is 230°C (PMCC). The product is stable under normal conditions, and is incompatible with oxidizing agents. Hazardous polymerization will not occur.	Substance is not classified as an oxidising agent.
Break-Thru OE 441	5.0%	Substance is a proprietary modified polyether organo siloxane. The flash point is 85°C (TAG CC). MSDS states that the product has no explosion hazards, the product is stable, and hazardous polymerization will not occur.	Substance is not classified as an oxidising agent.
Emery 2033C	15.0%	Substance is a proprietary blend of esters. The flash point is > 93°C (PMCC). The MSDS states that the product is normally stable, and that hazardous polymerization will not occur. The product is incompatible with strong oxidizing agents.	Substance is not classified as an oxidising agent.
Emery 2224 (Balance ingredient)	71.85%	Substance is a methylated soybean oil. The flash point is 190°C. The substance is incompatible with strong acids, bases and oxidizing agents.	Substance is not classified as an oxidising agent.
GF-657 Blend of the components in nominal ratios as listed above.	100	All components do not possess any oxidising potential. Product is simple blend of these components which demonstrates good long term physical and chemical stability.	Product is not an oxidising substance.

Open point 1.13 (new) The evaluation in column 3 of the evaluation table to be transferred to an addendum.

Same as above (1.2)

<u>Open point 1.7</u> To remove confidential data form the box "Impurities in technical as" from the list of end points.

<u>Fulfilled</u>

Open point 1.8

Purity of Starting Materials for Penoxsulam Manufacturing

Information on raw material submitted and available in Annex C, confidential information.

1.3 Applicant to provide actual batch analysis of the large scale production or a justification that specified limits above the maximum value found in the batch analyses is acceptable in respect to the toxicological and ecotoxicological assessment.

Notifier informs large scale 5 batches will be ready in June , but meanwhile provided a 6 batches, summarized below.

Six typical batches of DE-638 technical were analyzed for active ingredient, DE-638 related impurities, 3,5-lutidine, water and BIS-CHYMP. The analysis of DE-638 technical for active ingredient was determined by HPLC as described in DAS-AM-02-003. (for detailed assessment please refer to Annex C, confidential information)

September 2007 note: Annex C confidential information summarizes the report

Penoxsulam: Batch analysis C 1.4.2.

Six batcht of DE-638 Technical Grade of active ingredient were analysed for the active ingredient level, DE-638 related impurities, residual 3,5-litudine, water and low level of BIS-CHYMP. For the determination of the active ingredient and DE-638 related impurities, approximately 100 mg of DE-638 technical was weighed into 100 ml volumetric flask and 5 ml of o-toluic acid solution (internal standard) was added. The flask was diluted to volume with mobile phase, sonicated to dissolve solids and mixed well. The components were determined by HPLC/UV

Open point 1.9 to provide the specified maximum value of the relevant impurity

See 1.8

Message to tox and ecotox meeting of experts.

The ecotoxicology and toxicology experts should carry out an assessment comparing impurity levels in the pilot plant production batches with the material used in their studies as well as those in the proposed specification.

Note for the relevant impurity Bis-CHYMP though the specification proposes a level of 0.5 g/kg in the pilot batches it was not determined (<26 mg/kg, method not fully validated)

Based on a comparison of the impurity profile in the manufacturing lots with those used for toxicity testing, the toxicology and ecotoxicology studies are judged to accurately reflect the toxicity of the manufacturing specifications.
The Bis-CHYMP has been found to be <0.1 g/kg

Open point 1.10 to provide CAS numbers of formulants

CAS numbers for penoxsulam and non-proprietary co-formulants are listed in Vol 4 of DAR. Notifier is not allowed access to CAS numbers for Trade Secret co-formulants, which must be obtained directly from suppliers. No CAS number is available for Bis-CHYMP impurity.

Open point 1.11 To provide validation data (incl. the used UV wavelength) for the analytical method used for the determination of the relevant impurity Bis-CHYMP

Identity of relevant impurities (of toxicological, environmental and/or other significance) in the active substance as manufactured (g/kg)	Bis-CHYMP 4(1H)-pyrimidinone, 2-chloro-5-methoxy-, 2-chloro-5-methoxy-4-pyrimidinylhydrazone Maximum content 0.5 g/kg
Impurities in technical as (principle of method)	Moreland, J., Fonquerne, C. (2002) Validation of Analytical Method DAS-AM-01-051 for the Determination of Impurities in Technical Grade DE-638 For impurities 1-11 and 13, - reversed phase liquid chromatography using a C8 column, o-toluic acid internal standard and UV detection at 285 nm. Samples are dissolved in internal standard solution and diluted with eluent prior to analysis. Impurity 12 - reversed phase liquid chromatography using a C18 column, phthalimide internal standard and UV detection at 265 nm. Samples are dissolved in internal standard solution and diluted with acetonitrile prior to analysis. Bis-CHYMP – reversed phase liquid chromatography using a C18 column, external standard calibration and UV detection at 360 nm. Samples are dissolved in acetonitrile and mobile

phase. The LOQ for Bis-CHYMP is 86 ppm.

1.3: (new) Applicant to clarify what happened to batches out of specification with respect to the specified minimum purity.

As indicated in the up dated version JII (April 08) Document JII, sect. 1.8, pag. 4, batches which were out of specification were re-purified to meet specifications using procedures outlined in the manufacturing description, assessed in the original Annex C, Confidential information.

Open point 1.14 to submit the updated versions of the end points and the evaluation table to the EFSA for distribution

Submitted as requested by EPCO manual

Open point 1.15 (new) RMS to amend the list of end points
Fulfilled

2. Section 2. Mammalian toxicology

Open point 2.1

Selection of Appropriate AOEL for Penoxsulam

Based on the initial evaluation, the NOAEL selected by the RMS for AOEL derivation was 10 mg/kg bw/day from the 90-day study in mice (the lowest NOAEL from subchronic studies)
The notifier argued that the appropriate NOAEL to be used in establishing the AOEL should be 18 mg/kg bw/day from the 90-day dog study.
The RMS has reviewed the data from 90-day studies on penoxsulam, and identified NOAELs of 50 mg/kg bw/day in rats, 18 mg/kg bw/day in dogs, and 10 mg/kg bw/day in mice. Considering that the lowest NOEL value in mice is mainly determined by the dose spacing in the 90-day study and taking into account that a comparison of the data from 90 day studies in rats, mice and dogs indicates the dog as the most sensitive species tested, the RMS supports the use of 18 mg/kg bw/day from the 90-day dog study as the most appropriate NOAEL in establishing the AOEL by applying an uncertainty factor of 100.
RMS observes that that the issue has already been solved: indeed, in the current version of the monograph as well as in the list of end-point which has been discussed at the evaluation meeting and at PRAPeR meeting an AOEL of 0.18 mg/kg bw/day has been proposed.

For details see separate Annex B addenda/corrigenda discussed on Jan. 2007

Evaluation Table Open point 2.2
Dermal Absorption Value for Penoxsulam

The RMS proposal for dermal absorption in the DAR was 2% for the concentrate and 0.4% (which, due to a printing error, was reported as 0.04%) for the dilution over a 24-h period. The RMS apologizes for the error, and at the same time considers the proposal still valid and continues to support it, based on the results coming from the in vivo study. The RMS agrees that a more detailed description of the above mentioned results would help in taking final decision.
The notifier has submitted, on 30 June 2006, a position paper on dermal absorption value for pexoxsulam, giving all the necessary details and explanation which reported in a *separate Annex B addenda/corrigenda discussed on Jan. 2007.* RMS conclusion
Based on results from an in vivo study in rats, a 2.0% dermal absorption for the undiluted formulation (GF-657) and 0.4% of the 1:100 dilution of the formulation over a 24-hr *in vivo* are the appropriate values for dermal absorption.

Evaluation Table Open point 2.5

Relevance of Large Granular Lymphocytic (LGL) Leukemia in Fischer 344 Rats Treated with Penoxsulam

In the monograph some results from literature papers on molecules which may be considered similar to penoxsulam, are cited which were not submitted. On July 2006 the notifier had submitted these papers. These papers are summarized in a *separate Annex B addenda/corrigenda discussed on Jan. 2007* but the RMS stresses on the fact that they should be regarded as confirmatory, so they add nothing to the overall risk assessment. RMS concluded the increases in LGL leukemia in male rats following exposure to penoxsulam were considered spontaneous in origin and not treatment-related. In line with the scientific literature, the finding of an increase in LGL leukemia in one sex in a non-dose related incidence, even when statistically significantly identified, is not considered toxicologically relevant for human risk assessment.

Open point 2.7 (metabolites BST & BSTCA)

Two metabolites were found at high levels in rotational crops: BST and BSTCA (see the table listing the metabolites of DE-638 found in soil, water, crops and animals, based on the metabolites identified in Section 4 (6.1 and 6.2) and Section 5 (7.1 and 7.2) of the original dossier).. It has been requested if they are of toxicological concern.

The evaluation is reported in a *separate Annex B addenda/corrigenda discussed on Jan. 2007.*

RMS concluded that based on (1) a lack of biological activity of the metabolites, (2) the negative results of mutagenicity studies conducted with these metabolites, and (3) the lack of toxicological properties of the parent active ingredient that would require classification and labeling as toxic or very toxic, a reproductive toxin, or a carcinogen, the metabolites of penoxsulam are not identified as being relevant. Furthermore as demonstrated in the same *Addenda/corrigenda,* metabolites of penoxsulam which have passed Step 3 (Hazard Assessment) can be tolerated without further testing, being the

threshold of concern of estimated or actual concentrations in ground water of 0.75 μg/l not exceeded.

Thus, penoxsulam meets the criteria for consideration for Annex I inclusion.

To support the absence of genotoxic potential of the two metabolites under consideration new studies were submitted as described in a *separate Annex B addenda/corrigenda discussed on Jan. 2007*nd based on the results of mutagenicity testing with 5-OH-638, BSTCA and BST, there was no evidence of a genotoxic potential for penoxsulam metabolites

3. Section 3 Residue

Open point 3.1

To include TMDI according to WHO/FAO European diet and worst case national diet in the listing of end-points: RMS included to the end points

TMDI (European Diet) (% ADI) 0.003 (Portuguese Diet – Worst Case) 0.022

Open point 3.2

To provide consideration of possible impacts of a later application in practice on the qualitative and quantitative findings in the rice metabolism study.

^{14}C-Penoxsulam, radiolabeled in the 2-position on the sulfonamide ring (TP) or uniformly labeled in the phenyl ring (Ph), was applied in a single application to Japonica rice at a rate of approximately 100 g ai/ha or equivalent to 2.5x the maximum use rate on rice (40 g/ha). Penoxsulam was formulated as a suspension concentrate (SC) and applied to both the paddy water and the rice foliage at the 5 to 6 leaf stage of development.
This application method is covered in the cGAP and is considered the worst-case scenario for several reasons. Due to the relatively small size of the plants at the time of application, penoxsulam was liberally applied to the water of the plot where the rice was grown as well as to the rice foliage. Since penoxsulam is rapidly photodegraded in aquatic systems, this application timing maximized the potential exposure of the rice plants to parent penoxsulam and the possibility of photoproduct uptake—both through the root system and through the leaf surface. The primary photodegradation route involves breaking the sulfonamide bridge, however, no photoproducts were observed in the plant tissues (extracted) or the surface rinses conducted at 0 DAT, 3, 7, 14 and 30 DAT.

Immature samples were analyzed at 0, 3, 7, 14 and 30 days after treatment (DAT) to elucidate the metabolic pathway while mature samples were harvested at 134 DAT. The same residue profile was observed in the immature samples and at harvest. The residue profile consisted primarily of parent penoxsulam, the 5-OH metabolite of penoxsulam and two other minor metabolites. The 5-OH metabolite reached levels of 30% of the TRR at 30 DAT and remained at 30% of the TRR in the mature straw. However, the concentration of 5-OH decreased to less than 0.010 µg/g in mature straw. The other two metabolites reached levels of about 10-20% of the TRR in the mature straw; corresponding to approximately 0.005 µg/g. Due to their low levels in the mature samples, no attempt was made to identify either of these components.

In addition to maximizing the potential uptake of photoproducts, the early application timing also increased the time for the rice plants to metabolize penoxsulam. Less than

10% of the TRR remained parent penoxsulam in the mature straw. The remainder of the TRR was equally divided between the 5-OH metabolite and the two minor metabolites. Neither penoxsulam nor its metabolites were present in the mature straw at levels greater than 0.010 µg/g (penoxsulam equivalents) and no compounds were detected at greater than 0.001 µg/g in the rice grain.

Panicle initiation, the latest application timing, is approximately 30 days later than the application timing in the current study (5 to 6 leaf stage). Since the pathway of penoxsulam rice metabolism is well established, the qualitative results of a later application would be the same. No differences would be expected in the residue profiles for rice treated with penoxsulam at either an early (5 to 6 leaf stage) or a later growth stage (panicle initiation).

Based the current study, total residue levels in rice tissues rapidly decline during the first 30 days after application—parent and metabolite concentrations at 30 DAT were all less than 0.02 µg/g. Application at panicle initiation should not result in a significant increase in residue levels at harvest. This is confirmed by magnitude of residue studies that showed rice grain contained no detectable residues of penoxsulam following either application scenario.

The conditions chosen for the rice metabolism study provided the maximum possibility of identifying all potentially significant components of the crop residue. Different application timings would not yield significantly different qualitative or quantitative results.

Open point 3.3

To propose a residue definition for risk assessment in an addendum

Plants: DE-638
Animal: DE-638
Soil: DE-638
Surface water: DE-638
Ground water: DE-638
Sediment: DE-638
Air: DE-638
Fulfilled

Open point 3.4

To summarize additional storage stability data covering a period of 24 month:

Report:	**Lindsay, D. A. (2004): Frozen Storage Stability of XDE-638 in Rice (Raw Agricultural Commodities: Grain, Straw, Immature Forage) and its Processed Products (Bran, Hulls, Polished Rice), unpublished report number 010100.01.**
Guidelines:	This study reflects the guidance for storage stability described in Lundehn (7028/VI/95 EN rev.2.6/1/97) and in EC Directive 96/68 amending Annex II and II of 91/414.
GLP:	Yes (certified laboratory)
Methodology:	Control samples of rice grain, straw, immature forage, and the rice processed products bran, hulls, and polished rice were fortified at 0.10 mg/kg with DE-638 and stored in polypropylene containers. This concentration is 10X the validated analytical method limit of quantitation of 0.01 mg/kg. The fortified samples were stored frozen at –20 ± 5°C. On the day of fortification (0 day) and at each subsequent sampling interval, storage stability samples were analysed in triplicate along with at least three freshly fortified controls (for procedural recoveries) and an unfortified control sample. Analysis was conducted using Dow AgroSciences analytical method GRM 01.25. Sample extracts were analyzed immediately after preparation and therefore no separate storage stability study on sample extracts was conducted.
Findings:	No degradation of DE-638 in rice grain, straw or immature forage was observed during the storage period of up to 732 days. No degradation of DE-638 in rice bran, hulls, or polished rice was observed during the storage period of 390 days. Recovery of DE-638 in each matrix at each timepoint is summarized in **Table 1** and **Table 2**.

End points amended

Open point: 3.5

To present total radioactive residues (TRR) in rotational crops

Table B.7.9-1: Total Radioactive Residues in Rotational Crops Planted 90 Days after the Application of ^{14}C-DE-638, Phenyl (PH) Label and Triazolopyrimidine (TP) Labels, at 1X and 2X Rates (50 and 100 g a.s./ha) to Soil

Specimen	DAT[a]	DAP[b]	Total Residue, mg/kg[c] PH	TP
1X Application:				
Wheat				
Forage	187	97	0.004	<LOQ[d]
Hay	252	162	0.021	0.009
Straw	294	204	0.024	0.011
Grain	294	204	<LOD[e]	<LOD[e]
Kale	238	148	0.005	0.003
Potato				
Foliage	298	208	0.047	0.024
Tuber	305	215	<LOQ[d]	<LOQ[d]
2X Application:				
Wheat				
Forage	187	97	0.007	0.005
Hay	252	162	0.032	0.022
Straw	294	204	0.028	0.030
Grain	294	204	<LOQ[d]	<LOQ[d]
Kale	238	148	0.008	-
	291[f]	201[f]	-	0.014
Potato				
Foliage	298	208	0.062	0.038
Tuber	305	215	0.003	0.003

[a] DAT = Days After Treatment of the soil with test substance (test substance applied 20-Jul-00).

[b] DAP = Days After Planting of the rotational crops (crops planted 18-Oct-00).

[c] Total Residue expressed as mg DE-638 equivalents per kilogram (or ppm or µg/g).

[d] The limit of quantification (LOQ) was either 0.002 mg/kg (wheat forage, kale and potato tubers) or 0.005 mg/kg (all other samples).

[e] The limit of detection (LOD) was 0.001 mg/kg for all samples.

[f] The maturity of the TP-2X kale was delayed and it was harvested at a later time than the other kale samples.

Open point 3.6 To discuss the role of metabolites BST and BSTCA not found in primary but in rotational crops

BST and BSTCA have been evaluated and determined to be of no toxicological concern (see January 2007 Toxicology Addendum, Section 5.7.1). Based on these data, there are no concerns for these metabolites in rotational crops.

Open point 3.7 to perform a new consumer exposure/risk assessment in an addendum by taking into account the most recent GEMS/food diet

Estimates of the potential and actual exposure through diet and other means (Annex IIA 6.9; Annex IIIA 8.6)

B.7.15.1 Diet. Estimates of the potential and actual exposure through diet (Annex IIA 6.9/01)

Report:	Tiu, C (2002): Estimation of the potential and actual exposure through diet and other means (Annex IIA.6.9).
Guidelines:	"Guidelines for Predicting Dietary Intake of Pesticide Residues" (World Health Organisation, Geneva, 1989).
GLP:	No
Methodology:	To assess the potential risk to consumers resulting from the use of penoxsulam on rice, an estimate of the intake of the residue must be made for average consumers of this crop. Consumption data may be based on a hypothetical global diet or national food consumption surveys. In the following calculation, the intakes have been estimated using data from the WHO 1998 Standard European Diet also published by FAO (updated 2003). The Theoretical Maximum Daily Intake (TMDI) for WHO is calculated by summing the estimates of mean consumption for all crop commodities that have the potential to contain residues. assuming that: all produce has been treated and contains residues at the MRL, and there is no loss of residue during transport, storage, processing or preparation of foods prior to consumption. In addition, the intake of toddler/young children is also considered using the UK Consumer model (2005). Here, the TMDI calculation takes the 97.5th percentile intake value for the 2 highest intakes and the mean values for the remainder. As this assessment only considers rice, the assessment will be for the 97.5th percentile intake of rice. Using food consumption data and the proposed MRL of 0.01 mg/kg, the theoretical annual daily intake of penoxsulam per person was calculated and compared to the ADI. For the determination of acceptable daily intake (ADI), the lowest NOAEL from chronic studies was established from the rat 2-year chronic toxicity/carcinogenicity study at 5.0 mg/kg body weight/day. A safety factor of 100 (*i.e.* a factor of 10 for intra-species variation and a factor of 10 for inter-species variation) is considered appropriate. Thus, the ADI for penoxsulam is 0.05 mg/kg

body weight/day (Annex II, Point 5.10.2.1).

Findings: The theoretical maximum daily intake of penoxsulam for a person with a 60 kg body weight, was calculated to be 0.0001 mg/day, according to 1998 FAO Standard European Diet (updated 2003). It is equivalent to 0.003 % of the ADI. Based on the UK TMDI assessment, the worst case exposure would be for toddlers and 7-10 year olds at 0.1% of the ADI.

Table 7.15.1-1. Total Maximum Daily Intake (TMDI) calculated for two different European consumption standards (WHO and UK)

TMDI (FAO)

DE-638 ADI = 0 - 0.0500 mg/kg bw Diets: g/person/day

		Intake = daily intake: µg/person		
Codex Code	Commodity	MRL mg/kg	European diet	intake
GC 0649	Rice	0.01	11.8	0.1
	Total intake (µg/person)=			0.1
	Bodyweight per region (kg bw) =			60
	ADI (µg/person)=			3000
	%ADI=			0.003%

UK Consumer Model TMDI Assessment

Active substance: DE-638 **ADI:** 0.05 mg/kg bw/day

	TOTAL INTAKE based on 97.5th percentile									
	ADULT	**INFANT**	**TODDLER**	**4-6 YEARS**	**7-10 YEARS**	**11-14 YEARS**	**15-18 YEARS**	**VEGETARIAN**	**ELDERLY (OWN HOME)**	**ELDERLY (RESIDENTIAL)**
% of ADI	0.04	**0.06**	**0.1**	**0.08**	**0.1**	**0.08**	0.06	0.04	0.02	0

	MRL	**COMMODITY INTAKES**									
Commodity	**(mg/kg)**	**(mg/kg bw/day)**									
Rice	0.01	0.00002	0.00003	0.00005	0.00004	0.00005	0.00004	0.00003	0.00002	0.00001	0.00000

* 0.00000 corresponds to <0.000005 mg/kg bw/day (any value ≥0.000005 is rounded to 0.00001

L/C Low consumption (<0.1 g/day) or low number of consumers (<4)

Conclusions: The proposed import tolerance for penoxsulam residue in rice grain is 0.01 ppm and the same value is the proposed EU MRL potential dietary exposure from imported rice and for rice produced in the EU. Therefore, no separate dietary exposure estimate was calculated based on the proposed import tolerance.

Based on the findings of the assessment conducted, it is extremely unlikely that any European diet will contain residues of penoxsulam that exceeds the ADI, therefore, the risk to consumers can be regarded as low. Since the TMDI does not reach 100% of the ADI, NEDI calculations are not necessary.

The assessment is acceptable.

4. Section 4 Fate and Behavior in the Environment

4.1 To provide argumentation on their selection of Koc values used to calculate a mean value for use in PEC calculations.

Route of degradation (aerobic) in soil (Annex IIA, point 7.1.1.1.1)

Mineralisation after 100 days	Phenyl ring label: Average = 0.9% AR (120 days) (range 0.5 – 1.4% AR) Triazolopyrimidine ring label: 0.3% AR
Non-extractable residues after 100 days	Phenyl ring label: Average = 12.7% AR (120 days) (range 10.2 – 15.6% AR) Triazolopyrimidine ring label: 11.9% AR
Major metabolites - name and/or code, % of applied (range and maximum)	5-OH-DE-638 (range 15.3 – 40.5% AR at 14-58 days) BSTCA (range 29.4 – 53.0% AR at 14-120 days)

Route of degradation in soil - Supplemental studies (Annex IIA, point 7.1.1.1.2)

Anaerobic degradation	Mineralization: ≤1% AR (120 days, both TP and PH radiolabels) NER: Average = 64.1% AR (120 days, 65.3% TP radiolabel, 62.9% PH radiolabel) Major metabolites: 5-OH-DE-638 (33.4% AR at 14 days), BSTCA (19.1% AR at 120 days)
Soil photolysis	Photolysis on moist soil, 25 °C, 40 °N latitude, summer sunlight Mineralization: <1% AR (37 days, both TP and PH radiolabels) NER: Average = 24.4% AR (37 days, 30.9% TP radiolabel, 17.9% PH radiolabel) Major metabolites: BSTCA (11.1% AR at 30 days), 2-amino-TP (10.4% AR at 37 days)

Rate of degradation in soil (Annex IIA, point 7.1.1.2, Annex IIIA, point 9.1.1)

Method of calculation	Linear first-order kinetics for parent compound. Non-linear first-order compartment modelling used for 5-OH-DE-638 and BSTCA metabolites.
Laboratory studies (range or median, with n value, with r^2 value)	DT_{50lab} (20 °C, aerobic): Average = 32 days (range 22 - 58 days, n = 4, r^2 = 0.96 - 0.99) 5-OH-XDE-638 DT_{50lab} (20 °C, aerobic): Average = 26 days (range 19 - 37 days, n = 4, r^2 = 0.97 - 0.99) BSTCA DT_{50lab} (20 °C, aerobic): Average = 79 days (range 61 – 118 days, n = 4, r^2 = 0.97 - 0.99)
	DT_{90lab} (20 °C, aerobic): Average = 107 days (range 74 - 192 days, n = 4, r^2 = 0.96 - 0.99)
	DT_{50lab} (6 °C, aerobic): 137 days (n = 1, r^2 = 0.95)
	DT_{50lab} (20 °C, anaerobic): 6.6 days (total system, n = 1, r^2 = 0.98), 5.3 days (water only, n = 1, r^2 = 1.00), 8.8 days (soil only, n = 1, r^2 = 0.91) 5-OH-XDE-638 DT_{50lab} (20 °C, anaerobic): 5.1days (total system, n = 1, r2 = 0.80)
	DT_{50lab} (photolysis on moist soil, 25 °C, 40 °N latitude, summer sunlight): 19 days (n = 1, r^2=0.90)
	Degradation in the saturated zone: Data not submitted, not required.
Field studies (state location, range or median with n value)	DT_{50f} (Italy, Spain, water): median 5.9 days (n = 2, range 5.6 - 6.1 days) DT_{50f} (Italy, Spain, soil): <1 day
	DT_{90f} (Italy, Spain, water): median 19.5 days (n = 2, range 19 - 20 days)
Soil accumulation and plateau concentration	Data not submitted, not required.

Soil adsorption/desorption (Annex IIA, point 7.1.2)

K_f /K_{oc}
K_d
pH dependence (yes / no)
(if yes type of dependence)

	DE-638			
Soil	K_f	K_d (L/kg)	K_{oc} (L/kg)	1/n
Greggio (Italy)	1.96	2.50	253	0.90
Ottobaiano (Italy)	0.32	0.38	45	0.89
Charentilly (France)	0.48	0.63	65	0.88
Marcham (UK)	0.16	20	12	0.93
Average[1]	0.73	5.88	94	0.90
Median[1]	0.40	1.57	55	0.90
Supplemental Information				
Wagram-Troup (USA)	0.27	0.31	77	1.02
Amagon (USA)	0.30	0.39	40	0.91
Oswald (USA)	0.49	0.47	19	0.94
Glyndon-Tiffany (USA)	0.45	57	21	0.88
AR Sediment (USA)	nm[2]	1.39	1130	nm
Chernozemic (Canada)	nm	1.44	72	nm
Ryerson (Canada)	nm	0.67	19	nm
Glu Humid (Brazil)	nm	0.63	14	nm
Red Latisoil (Brazil)	nm	0.13	13	nm
Purple Latisoil (Brazil)	nm	0.50	35	nm
Volcanic/Upland (Japan)	0.59	0.81	22	0.86
Non-Volcanic/Upland (Japan)	0.56	0.85	39	0.86
Volcanic/Rice (Japan)	4.69	10.40	305	0.80
Non-Volcanic/Rice (Japan)	1.55	2.49	194	0.83
Average[3]	0.99	1.38	104	0.89
Median[3]	0.49	0.63	40	0.89

[1] Average and median values for 4 European soils

[2] nm = Freundlich coefficients were not measured for these soils

[3] Average and median values for all reported soils

pH dependence: Yes. Sorption increases with decreasing pH. As soil pH decreases, sorption of DE-638 is increasingly dependent on the soil organic carbon content.

K_f / K_{oc}

$\underline{K_d}$

pH dependence (yes / no)

(if yes type of dependence)

	5-OH-DE-638			
Soil	K_f	K_d (L/kg)	K_{oc} (L/kg)	1/n
Greggio (Italy)	nm[1]	1.42	144	nm
Ottobaiano (Italy)	nm	0.40	41	nm
Charentilly (France)	nm	0.28	17	nm
Marcham (UK)	nm	0.30	34	nm
Average[2]	n/a	0.60	59	n/a
Median[2]	n/a	0.35	37	n/a
Supplemental Information				
Wagram-Troup (USA)	nm	0.14	34	nm
Amagon (USA)	nm	0.32	33	nm
Oswald (USA)	nm	0.46	19	nm
Glyndon-Tiffany (USA)	nm	1.03	38	nm
Average[3]	n/a	0.54	45	n/a
Median[3]	n/a	0.36	34	n/a

[1] nm = Freundlich coefficients were not measured for these soils

[2] Average and median values for 4 European soils

[3] Average and median values for all reported soils

pH dependence: No.

K_f /K_{oc}

K_d

pH dependence (yes / no)
(if yes type of dependence)

	BSTCA			
Soil	K_f	K_d (L/kg)	K_{oc} (L/kg)	1/n
Greggio (Italy)	nm[1]	4.39	444	nm
Ottobaiano (Italy)	nm	0.72	74	nm
Charentilly (France)	nm	0.09	5	nm
Average[2]	n/a	1.73	174	n/a
Median[2]	n/a	0.72	74	n/a
Supplemental Information Wagram-Troup (USA)	nm	0.18	46	nm
Amagon (USA)	nm	1.52	156	nm
Oswald (USA)	nm	0.60	25	nm
Average[3]	n/a	1.25	125	n/a
Median[3]	n/a	0.66	60	n/a
[1] nm = Freundlich coefficients were not measured for these soils				
[2] Average and median values for 3 European soils [3] Average and median values for all reported soils				
pH dependence: No.				

Mobility in soil (Annex IIA, point 7.1.3, Annex IIIA, point 9.1.2)

Column leaching	Sandy loam soil (free draining), leached for 2 d at 10 cm water/d 9.7% 0-5 cm soil layer 95.8% total in soil (0-30 cm) 3.1% in leachate Sandy loam soil (saturated), leached for 2 d at 10 cm water/d 20.9% 0-5 cm soil layer 97.5% total in soil (0-30 cm) 0.2% in leachate Clay loam soil (free draining), leached for 2 d at 10 cm water/d 102.9% 0-5 cm soil layer 102.9% total in soil (0-30 cm) <LOD in leachate Clay loam soil (saturated), leached for 2 d at 10 cm water/d 35.7% 0-5 cm soil layer 97.1% total in soil (0-30 cm) 1.0% in leachate Sandy silt loam soil (free draining), leached for 2 d at 10 cm water/d 40.2% 0-5 cm soil layer 100.2% total in soil (0-30 cm) <LOD in leachate Silt loam soil (free draining), leached for 2 d at 20 cm water/d 86.1% 0-5 cm 98.2% total in soil (0-30 cm) <LOD in leachate
Aged residues leaching	Data not submitted, not required.
Lysimeter/ field leaching studies	Data not submitted, not required.

PEC (soil) (Annex IIIA, point 9.1.3)

Active substance

Method of calculation	Modelling as per Guidance Document for Environmental Risk Assessment of Active Substances used on Rice in the EU (Sanco/1090/2000-rev0) No crop interception, Koc 94 L/kg (average from 4 European soils), DT_{50} 8.8 days (soil phase of anaerobic study - worst case compared to the field study where the DT50 was <1 day).
Application rate	40 g a.s./ha

PEC$_{(s)}$	Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial	29.83 µg/kg (clay) 20.70 µg/kg (sand)	not applicable not applicable	Not calculated . One application per growing season	Not calculated . One application per growing season
Short term 24h	27.57 µg/kg (clay) 19.14 µg/kg (sand)	28.68 µg/kg (clay) 19.91 µg/kg (sand)		
2d	25.48 µg/kg (clay) 17.69 µg/kg (sand)	27.60 µg/kg (clay) 19.16 µg/kg (sand)		
4d	21.77 µ/kg (clay) 15.11 µg/kg (sand)	25.59 µg/kg (clay) 17.76 µg/kg (sand)		
Long term 7d	17.19 µg/kg (clay) 11.93 µg/kg (sand)	22.93 µg/kg (clay) 15.91 µg/kg (sand)		
28d	3.29 µg/kg (clay) 2.28 µg/kg (sand)	12.03 µg/kg (clay) 8.35 µg/kg (sand)		
50d	0.58 µg/kg (clay) 0.40 µg/kg (sand)	7.43 µg/kg (clay) 5.15 µg/kg (sand)		
100d	0.01 µg/kg (clay) 0.01 µg/kg (sand)	3.79 µg/kg (clay) 2.63 µg/kg (sand)		

Major metabolites

Method of calculation	5-OH-DE-638: max formation from parent = 33% (from anaerobic aquatic study) and correction for molecular weight BSTCA: max formation from parent = 53% (from aerobic soil study) and correction for molecular weight

Maximum PEC$_{(s)}$	Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
5-OH-DE-638	9.56 µg/kg (clay) 6.63 µg/kg (sand)	not applicable not applicable	Not calculated . One application per growing season	Not calculated . One application per growing season
BSTCA	13.62 µg/kg (clay) 9.45 µg/kg (sand)	not applicable not applicable		

Route and rate of degradation in water (Annex IIA, point 7.2.1)

Hydrolysis of active substance and relevant metabolites (DT_{50}) (state pH and temperature)		pH 5, 25 °C: stable
		pH 7, 25 °C: stable
		pH 9, 25 °C: stable
Photolytic degradation of active substance and relevant metabolites		DT_{50} = 2 days (summer, 40 °N latitude, sterile pH 7 buffer and natural water) Major metabolites: TPSA: 56% AR at 1 day, DT_{50} = 4.7 days (buffer), 2.0 days (natural water) 2-amino-TP: 18% AR at 1 day, DT_{50} = 0.6 days (buffer), 0.6 days (natural water) 5-OH-2-amino-TP: 23% AR at 14 days (natural water) BSA: 36% at 1.5 days, DT_{50} = 0.9 days (buffer), 0.7 days (natural water)
Readily biodegradable (yes/no)		No (Penoxsulam) Yes (GF-657)
Degradation in water/sediment	DT_{50} water	Average = 15 days (n = 2, range 10 – 20 days, r^2 = 0.90 - 0.96)
	DT_{90} water	Average = 50 days (n = 2, range 34 – 65 days, r^2 = 0.90 - 0.96
	DT_{50} whole system	DE-638 Average = 23 days (n = 2, range 11 – 34 days, r^2 = 0.78 - 0.91) 5-OH-DE-638 Average = 50 days (n = 2, range 24 – 75 days, r^2 = 0.89 - 0.93)
	DT_{90} whole system	Average = 76 days (n = 2, range 37 – 114 days, r^2 = 0.78 - 0.91
Mineralisation		Average = 1.6% AR (n = 2, range 0.8 – 2.4% AR, 99 days)
Non-extractable residues		Average = 39.4% AR (n = 2, range 20.8 – 57.9% AR, 99 days)
Distribution in water / sediment systems (active substance)		Water Phase: Maximum of 87.5 – 91.5% AR at 0 DAT (days after treatment) decreasing to 0.4 – 3.9% AR at 99 DAT Sediment Phase: 0% AR at 0 DAT, maximum of 2.5 – 20.9% AR at 4 DAT

Distribution in water / sediment systems (metabolites)	Major Metabolites (water): 5-OH-DE-638 (18.6% AR), BSTCA (23.7% AR) Major Metabolites (sediment): 5-OH-DE-638 (15.6% AR)

PEC (surface water) (Annex IIIA, point 9.2.3)

Active substance

Method of calculation	Modelling as per Guidance Document for Environmental Risk Assessment of Active Substances used on Rice in the EU (Sanco/1090/2000-rev1) No crop interception, Koc 94 L/kg (average from 4 European soils), DT50 in paddy of 6.6 days (anaerobic whole system) and DT50 in surface water 15 days (aerobic aquatic water phase). Dilution factor of 10 for outflow from paddy to drainage channel after 5 days. Calculation was refined using measured paddy water concentrations from field studies 5 days after application.
Application rate	40 g a.s./ha
Main routes of entry	Outflow of paddy water to drainage canals 5 days after application (major route). Spray drift at application (minor route).

PEC$_{(sw)}$	Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial	1.14 µg/L	Not applicable	Not calculated . One application per growing season	Not calculated . One application per growing season
Short term 24h 2d 4d	1.09 µg/L 1.04 µg/L 0.95 µg/L	1.11 µg/L 1.09 µg/L 1.04 µg/L		

Long term 7d 14d 21d 28d 42d	0.82 µg/L 0.60 µg/L 0.43 µg/L 0.31 µg/L 0.16 µg/L	0.97 µg/L 0.84 µg/L 0.73 µg/L 0.64 µg/L 0.50 µg/L		

Major Metabolites

Method of calculation	Calculated using maximum PEC_{SW} for parent at Step 1b corrected for molecular weight and max amount formed in aerobic aquatic study (5-OH-DE-638 = 30% and BSTCA = 24%) or aqueous photolysis study (BSA = 36%, TPSA= 56%, 2-amino-TP = 18% and 5-OH-2-amino-TP = 23%)

Max $PEC_{(sw)}$	Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
5-OH-DE-638	0.55 µg/L	Not applicable	Not calculated . One application per growing season	Not calculated . One application per growing season
BSTCA	0.39 µg/L	Not applicable		
BSA	0.43 µg/L	Not applicable		
TPSA	0.60 µg/L	Not applicable		
2-amino-TP	0.14 µg/L	Not applicable		
5-OH-2-amino-TP	0.18 µg/L	Not applicable		

PEC (sediment)

Active substance

Method of calculation	Modelling as per Guidance Document for Environmental Risk Assessment of Active Substances used on Rice in the EU (Sanco/1090/2000-rev1). Calculated using outflow and drift PEC_{SW} for parent at Step 1b Partitioning to sediment with Koc of 94 L/kg and DT50 of 23 days (aerobic aquatic whole system).
Application rate	40 g a.s./ha

PEC$_{(sed)}$	Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial	2.08 µg/kg	Not applicable	Not calculated . One application per growing season	Not calculated . One application per growing season
Short term 24h 2d 4d	2.02 µg/kg 1.96 µg/kg 1.84 µg/kg	2.05 µg/kg 2.02 µg/kg 1.96 µg/kg		
Long term 7d 14d 21d 28d 42d	1.68 µg/kg 1.36 µg/kg 1.10 µg/kg 0.89 µg/kg 0.59 µg/kg	1.88 µg/kg 1.70 µg/kg 1.54 µg/kg 1.40 µg/kg 1.18 µ/kg		

Major Metabolites

Method of calculation	Calculated using maximum PEC_{SW} for parent at Step 1b corrected for molecular weight and max amount formed in sediment in aerobic aquatic study (5-OH-DE-638 = 16%, BSTCA = 2.2%), PCA-5-OH = 16%, OH-BSTCA = 16%

Max $PEC_{(SED)}$	Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
5-OH-DE-638	0.33 µg/kg	Not applicable	Not calculated . One application per growing season	Not calculated . One application per growing season
BSTCA	0.05 µg/kg	Not applicable		
PCA-5-OH	0.33 µg/kg	Not applicable		
OH-BSTCA	0.33 µg/kg	Not applicable		

PEC (ground water) (Annex IIIA, point 9.2.1)

Method of calculation and type of study (*e.g.* modelling, monitoring, lysimeter)	Modelling as per Guidance Document for Environmental Risk Assessment of Active Substances used on Rice in the EU (Sanco/1090/2000-rev0). Parent: Koc = 94 L/kg (average from 4 European soils) and DT50 = 5.3 days (anaerobic water phase value, comparable to that seen in the water phase of the field study). 5-OH: formation 33%, DT50 = 5.1 days (anaerobic whole system value, worst case compared to field study where water concentrations were not detectable, <3µg/L) and Koc = 59 L/kg. BSTCA: formation 50% (conservative estimate), DT50 = 100 days (estimated value) and Koc = 174 L/kg
Application rate	40 g a.s./ha

$PEC_{(gw)}$

Maximum concentration	Not evaluated
Average annual concentration (active substance)	< 0.0001 µg/L (clay soil) 0.0013 µg/L (sandy soil)
Average annual concentration (5-OH metabolite)	< 0.0001 µg/L (clay soil) 0.0001 µg/L (sandy soil)
Average annual concentration (BSTCA metabolite)	< 0.0001 µg/L (clay soil) 0.234 µg/L (sandy soil)

Fate and behaviour in air (Annex IIA, point 7.2.2, Annex III, point 9.3)

Direct photolysis in air		Not evaluated		
Quantum yield of direct phototransformation		0.180 (measured in water)		
Photochemical oxidative degradation in air (DT_{50})		Latitude: N/A	Season: N/A	DT_{50}: 2.1 hours
Volatilisation	from plant surfaces:	Not evaluated		
	from soil:	Not evaluated		

PEC (air)

Method of calculation	No method of calculation available

$PEC_{(a)}$

Maximum concentration	No method of calculation available

Open point 4.1 Endpoints for definition of the residue to be updated to include a list of all major residues that require risk assessments as well a relevant residues for monitoring

Definition of the Residue (Annex IIA, point 7.3)

Relevant to the environment	Soil: DE-638 Surface water: DE-638 Ground water: DE-638 Sediment: DE-638 Air: DE-638

Monitoring data, if available (Annex IIA, point 7.4)

Soil (indicate location and type of study)	None
Surface water (indicate location and type of study)	None
Ground water (indicate location and type of study)	None
Air (indicate location and type of study)	None

4.2 To clarify all assumptions used to calculate metabolite PECgw, to include clear information on how $TWA_{pw,t(close)}$ for both 5-OH and BSTCA were estimated and to present new calculations that use a realistic worst case formation fraction of BSTCA.

Clarification provided into the revised fate section attached to point 4.1

Open point 4.2 For phenyl and triazolopyrimidine ring radiolabels' still needs to be added to the endpoints to put the mineralization and NER values in context.

Clarification provided into the revised fate section attached to point 4.1

Open point 4.3 For phenyl and triazolopyrimidine ring radiolabels' and 'moist soil first order DT50 19 days at 25°C summer sunlight at 40°N (r^2=0.9)' still need to be added to the endpoints.

Clarification provided into the revised fate section attached to point 4.1

Open point 4.3 Applicant to provide an audited corrigendum to the original report to correct the Kf, 1/n and Kfoc values for the Amagon soil.

Applicant has stated in its comments that the Kf and 1/n values for the Amagon soil reported in the original study were incorrectly calculated and submitted the report enclosed below

A batch equilibrium study investigated the sorptive behavior of XDE-638 on seventeen soils and one sediment. XDE-638 adsorption K_d values ranged from 0.12 to 10.67 L/kg and averaged 1.38 ± 2.32 L/kg with an application rate of 0.2 mg/L. As soil pH decreases, XDE-638 is predominantly found as the neutral form and sorption increases. Adsorption K_{oc} values ranged from 12 to 1141 L/kg and averaged 104 ± 260 L/kg. Freundlich analysis for one adsorption and two desorption steps resulted in 1/n values close to 1. Desorption coefficients were generally higher than the adsorption coefficient for the same soil.

abstract

A batch equilibrium study investigated the sorptive behavior of XDE-638, a herbicide under development for control of broadleaf weeds and barnyard grass in rice. Adsorption and desorption partition coefficients were determined on 17 soils and one sediment at a nominal concentration of 0.2 ppm to the aqueous phase. Additionally, Freundlich analyses were conducted at four concentrations, 0.04, 0.2, 1 and 5 mg/L on twelve of the 17 soils. The soil: solution ratio was 1:2; 10 mL 0.01 M $CaCl_2$ containing XDE-638-het-2-^{14}C was added to 5 g (oven dry weight) moist soil. Sorption equilibrium was reached after 24 hours on a horizontal shaker. Several screening tests were run at a 1:5 soil: solution ratio and an overnight (approximately 20-hour) equilibrium.

XDE-638 adsorption K_d values ranged from 0.12 to 10.67 L/kg and averaged 1.38 ± 2.32 L/kg at an aqueous concentration of 0.2 mg/L. Adsorption K_{oc} values ranged from 12 to 1141 L/kg, indicating XDE-638 may be considered potentially mobile to immobile. A correlation between soil pH and K_{oc} was elucidated; as soil pH decreases, XDE-638 exists as predominantly the neutral form and is more highly sorbed, depending upon soil organic carbon content. The adsorption K_{oc} values for the neutral and anionic forms of XDE-638 were calculated as approximately 4000 and 11 L/kg, respectively. Freundlich analysis for one adsorption and two desorption steps resulted in 1/n values close to 1; signifying that XDE-638 sorption is not dependent upon concentration. Desorption coefficients were generally higher than the adsorption coefficient for the same soil, suggesting that there is irreversible sorption of XDE-638 to soil. Material balance for each sample set averaged between 90 and 110%.

Open point 4.4 Non linear first order Modelmaker compartment modelling' still need to be added to the endpoints in the context of the metabolites.

Clarification provided into the revised fate section attached to point 4.1

Open point 4.5 The DT50 for the major metabolites (5-OH and BSTCA for aerobic studies and 5-OH for anaerobic studies) need to be added to the endpoints.

Clarification provided into the revised fate section attached to point 4.1

4.3 to provide an audited corrigendum to the original report to correct the Kf, 1/n and Kfoc values for the Amagon soil.

The DAR reflects the corrected numbers. The Amagon soil is non EU and therefore considered supplementary. With reference to data requirement 4.1 the value is the average of 4 European soils. The notifier will submit the final report in April.

Open point 4.6

To check that Koc were not used to calculate the metabolite PEC. If they were used the values should be added to the method of calculation box.

The values are not used for calulation of PECsoil. No change to endpoints list required

Fulfilled

Open point 4.7

To check that Koc were not used to calculate the metabolite PEC. If they were used the values should be added to the method of calculation box.

The values are not used for calulation of PECsoil. No change to endpoints list required

Fulfilled

Open point 4.8

After data requirement 4.2 has been addressed the endpoints will need appropriately updating with the necessary information in the method of calculation box.

Clarification provided into the revised fate section attached to point 4.1

Open point 4.9 to update LoEP as indicated.

Replace the code for the active with penoxsulam and consistently use the same codes for all metabolites.

The column header for Koc should be changed to Kdoc for penoxsulam and metabolites.

The product name in the list of representative uses should be changed to Viper.

Viper® Herbicide is readily biodegradable by OECD Method 301B (therefore not triggering R53), since the aquatic toxicity values for Viper® Herbicide are all greater than 1 mg/L (therefore not triggering R50);

- 96 hr LC50 for fish >100 mg product/L
- 48 hr EC50 for *Daphnia magna* >100 mg product/L
- 96 hr EC50 for algae 4 mg product/L

.

Classification and labelling: as for the up dated EPCO manual version of LoEP

5.Ecotoxicology

Open point 5.1
To present the revised assessment in a revised DAR/corrigendum.

The list of end points has been amended

Open point 5.2

Risk to aquatic plants

The risk to aquatic plants has been discussed. The RMS explained the derivation of the EC50 for *Lemna gibba* on the basis of the current guidance.

A standard toxicity study is available with the active substance.

The RMS proposed to refine the risk assessment for *Lemna gibba* using the results from another study available with the formulation (with *Lemna gibba*, sediment, prolonged observation period). This study was an outdoor study The study gives as well some indication of recovery (compensation) or "re-colonisation".

Growth rate should be the appropriate parameter to conclude on recovery instead of biomass. (comment from DE)

The appropriate end point to be selected was discussed (EC50 or NOEC).

The appropriate end point could be the EC50 based on bio-mass derived from the study with the formulation. (DE)

The option to use the NOEC was discussed and what trigger value should be used in that case.

The trigger value of 10 was discussed related to this study, which is not a standard study. The approach was considered to be too conservative. Therefore a trigger value of less than 10 should be applied, if a NOEC is chosen (proposal from GR).

The results from the two studies at 14 days have been compared, which result in 3.3 ug/L (a.s) and 4.74 ug/L (formulated product). Except for the potential of re-colonisation the second study does not provide results being so different.

It was concluded that these studies are comparable and therefore the lowest value at 14 days was chosen (3.29 ug/L).

A TER of 2.9 was calculated based on this end point. The effects of recovery observed for one species does not necessarily indicate a recovery for other species. Therefore further refinement of recovery potential and variability of sensitivity between species should be explored.

5.1 Applicant to provide data to demonstrate the recovery potential also for other aquatic plants besides *Lemna*

The notifier submitted a model for refined exposure assessment for the rice-herbicide penoxsulam performed at the basin scale level, summarized below. (Ref. K 23)

Report: Karpouzas D. G., E. Capri (2007): Report on higher tier risk assesment of the herbicide Penoxsulam applied in rice crop for calculating exposure in surface water systems

An additional refinement risk assessment for aquatic plants was provided by a higher-tier exposure assessment, using the RICEWQ and RIVWQ modelling systems, in order to evaluate the realistic PECsw after application of Penoxsulam to rice crops in european countries. Rice paddies were assumed to be drained when treated with penoxsulam (40 g a.s./ha), then flooded the next day. Water was held in the paddy for 5 days after which water was released from the paddy to the immediately adjacent drainage ditches. Water in drainage ditches flow from each paddy into larger canals and then into rivers. Since rice is cultivated in large river basin a more realistic exposure assessment was performed at the basin-scale level using scenarios representative of rice cultivation at member-state level. Thus, basin-scale scenarios representative of rice cultivation in three main rice producing countries of EU, Greece, Italy and Spain were developed. More specifically, a single scenario for Greece (Axios river basin), five scenarios for Italy (Vercelli, Lomellina settedrionale, Lomellina meridionale, Ticino, Mantova) and four scenarios for Spain (Sevilla, Extremadura, Taragona-Ebro Delta, Valencia) were developed. All scenarios comprised a simulated rice cultivated basin of varying area consisting of rice paddy blocks associated with ditches or drainage canals receiving water from the paddies, which in turn, discharged their waters into secondary larger canals and subsequently into larger river systems. Concentrations of Penoxsulam were calculated in each segment of the water flow regime (paddy, ditch, canal and river) over several years of weather simulations. A detailed summary of the refined modelling report provided as Reference K23. The TER values for all modelled scenarios were calculated using the 95^{th} percentile PEC_{sw} values of ditchs, canals and rivers and the standard laboratory E_bC_{50} of *Lemna gibba*, 14 day EC50 = 3.29 µg a.s./L (Tab. 10.2.10)

Tab. 10.2.10 (M3S6 final version up date oct. 2007) ***Refined Toxicity Exposure Ratios for the higher aquatic plant Lemna in surface water: RICEWQ/RIVWQ modelling***

Refined Exposure Scenario[(a)]	**rice area treated %**	**E_bC_{50} (µg a.s./L)**	**95th Percentile PEC_{sw}**[(b)] **(µg a.s./L)**	**TER**	**Annex VI Trigger**
Greece- Drainage canals	30	3.29	0.078	42	10
Greece-River			<0.001	>3000	
Italy-Lomellina meridionale-Ditches	30		0.159	20.7	10
Italy-Lomellina meridionale-Ditches	100		0.231	14.2	
Italy-Lomellina meridionale-Drainage canals	30		0.013	253	
Italy-Lomellina meridionale-Drainage canals	100		0.017	194	
Italy-Lomellina meridionale-River	30 – 100		<0.001	>3000	
Italy-Lomellina settentrionale-Ditches	30		0.311	10.6	10
Italy-Lomellina settentrionale-Ditches	100		0.348	**9.5**	
Italy-Lomellina settentrionale-Drainage canals	30		0.019	170	
Italy-Lomellina settentrionale-Drainage canals	100		0.030	109	
Italy-Lomellina settentrionale-River	30 – 100		<0.001	>3000	
Italy-Mantova-Ditches	30		0.148	22.2	10
Italy-Mantova-Ditches	100		0.283	11.6	
Italy-Mantova -Drainage canals	30		0.012	274	
Italy-Mantova -Drainage canals	100		0.016	200	
Italy-Mantova-River	30 – 100		<0.001	>3000	
Italy-Ticino-Ditches	30		0.231	14.2	10
Italy-Ticino-Ditches	100		0.373	**8.8**	
Italy-Ticino-Drainage canals	30		0.058	56	
Italy-Ticino-Drainage canals	100		0.090	36	
Italy-Ticino-River	30 – 100		0.001	3200	
Italy-Vercelli-Ditches	30		0.292	11.3	10
Italy-Vercelli-Ditches	100		0.327	10.1	
Italy-Vercelli -Drainage canals	30		0.023	140	
Italy-Vercelli -Drainage canals	100		0.059	55	
Italy-Vercelli-River	30 – 100		<0.001	>3000	
Spain-Sevilla-Ditches	25		0.304	10.8	10
Spain-Sevilla-Drainage canals			0.021	156	
Spain-Sevilla-River			<0.001	>3000	
Spain-Extremadura-Ditches	25		0.177	18.6	10
Spain-Extremadura-Drainage canals			0.011	299	
Spain-Extremadura-River			<0.001	>3000	
Spain-Taragona-Ditches	25		0.057	57.7	10
Spain-Taragona-Drainage canals			0.006	548	
Spain-Taragona-River			<0.001	>3000	
Spain-Valencia-Ditches	25		0.137	24	10
Spain-Valencia-Drainage canals			0.019	173	
Spain-Valencia-River			0.001	3000	

(a) **Greece**: Axios river basin, 30% of the total rice cultivated area treated with Penoxsulam during a 20 day period, PECsw based on 20 years simulation

Italy: 30% of the total rice cultivated area in each scenario treated with Penoxsulam on the same day, or 100% of the total rice cultivated area in each scenario treated with Penoxsulam during two week period, PECsw based on 5 years simulation.

Spain: 25% of the total rice cultivated area in each scenario treated with Penoxsulam during a week period, PECsw based on 5 years simulation (Sevilla, Valencia) and 6 years simulation (Extremadura, Taragona)

(b) Number of simulation years depends from meteorological data availability.

Findings

The TER values for ditches, for all modelled scenarios, are above or very close to the trigger value. All TER values for canals and rivers result above the trigger value. Ditches immediately adjacent to rice paddies are intimately associated with rice production, and are not independent water body that can sustain viable aquatic plant communities over the long term. Furthermore, proper functioning of drainage ditches requires that they be periodically managed to allow unimpeded water flow from the rice paddies. Considered that drainage canals and rivers should be considered the ecologically relevant aquatic habitats requiring protection, the risk for aquatic macrophytes can be considered acceptable in all modelled scenarios.

Conclusion

An adequate risk assessment for penoxsulam in all modelled scenarios was achieved using the 95th percentile PEC_{sw} values derived from higher tier modelling in association with the standard laboratory-derived E_bC_{50} in Lemna gibba (14 day E_bC_{50} = 3.29 µg a.s./L) and evaluated against the TER trigger value of 10.

A possible safe use of penoxsulam was demonstrated for aquatic macrophytes. Realistic national scenarios should be considered at member state level.

Open point 5.3

To clearly indicate in the list of intended uses that the assessment only covers tractor application technology, list of intended use.

Supported intended uses for Annex I listing only covers tractor application technology. Additional application technologies could be examined at Annex III level depending on local uses.

5.2 Notifier to provide the composition of the batches in order to assess the relevance of the impurities

Open point 5.4 (New) proposed at PRAPeR 03: RMS to check the comparability of the profiles

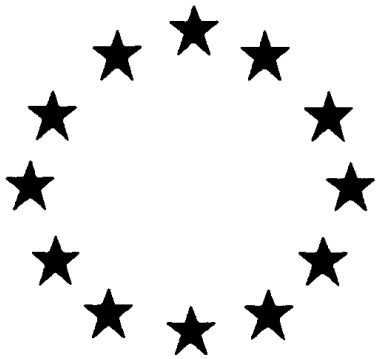
Based on a comparison of the impurity profile in the manufacturing lots with those used for toxicity testing, the toxicology and ecotoxicology studies are judged to accurately reflect the toxicity of the manufacturing specifications (see details on Annex C confidential information)

Ministry of Health

P.le Marconi, 25

00144 Rome

Italy



PENOXSULAM

DAR up dated sections (march 2008)

ANNEX B

B.6 TOXICOLOGY

B.6.12 Dermal absorption (Annex IIIA 7.3)

Findings: Following a single topical application of undiluted GF-237 containing ^{14}C-Penoxsulam, l amounts of Penoxulam were absorbed after 24, 48 and 72 hr. (Table B.6.12.1). The total amount of absorbed Penoxsulam was essentially the same in groups sampled over a 72-hr period at approximately 2% of the applied dose. The majority of the radioactivity recovered was located in the skin wash (56-62%), the skin at the application site (8-11%) and the frame and covering (16-23%).

Table B.6.12.1 : *Percentage of radiochemical material and mg equivalents of Penoxsulam absorbed, remaining in the treated skin and removed from the application site following a single topical application of radiolabelled GF-237 formulation (approximately 0.26 mg Penoxsulam/cm^2)*

	Percentage of Applied Radioactivity		
Sacrifice Time	Group 1 24 Hour Mean ± SD	Group 2 48 Hour Mean ± SD	Group 3 72 Hour Mean ± SD
Covering	3.28 ± 2.41	4.43 ± 1.76	7.22 ± 1.61
Frame	19.97 ± 6.07	11.51 ± 5.41	15.50 ± 6.88
Skin,Wash (at 24hr post application)[1]	56.30 ± 5.26	61.84 ± 8.52	57.87 ± 5.90
Application-site Skin	10.06 ± 3.55	11.44 ± 3.59	8.21 ± 1.77
[µg-eq. DE-638[2]]	[331.638 ± 116.984]	[361.743 ± 113.619]	[254.795 ± 54.776]
Skin[3]	<0.55 ± >0.98	<0.48 ± >0.65	0.32 ± 0.26
Carcass	0.47 ± 0.42	0.62 ± 0.12	<0.24 ± >0.17
Kidneys	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Liver	0.31 ± 0.23	0.16 ± 0.08	0.06 ± 0.01
Blood	0.04 ± 0.03	0.03 ± 0.01	0.05 ± 0.04
Total Tissues	<1.38 ± >1.65	<1.29 ± >0.87	<0.67 ± >0.35
Feces			
0-24 hr	0.13 ± 0.07	0.08 ± 0.01	0.12 ± 0.03
24-48 hr		0.54 ± 0.21	0.40 ± 0.20
48-72 hr			0.45 ± 0.20
Urine			
0-24 hr	0.11 ± 0.03	0.17 ± 0.06	0.14 ± 0.06
24-48 hr		0.25 ± 0.11	0.16 ± 0.03
48-72 hr			0.01 ± 0.00
Rinse			
0-24 hr	0.03 ± 0.04	0.02 ± 0.01	0.02 ± 0.01
24-48 hr		0.07 ± 0.08	0.03 ± 0.02
48-72 hr			0.03 ± 0.01

Final cage wash	<0.09 ± 0.12	0.22 ± 0.20	0.15 ± 0.14
Total Recovery	<91.35 ± >1.25	<91.87 ± >1.29	<90.98 ± >1.73
Percent Absorption[4]	<1.74 ± >1.91	<2.66 ± >1.37	<2.18 ± >0.29
[µg-eq. DE-638 absorbed[5]]	[<57.495 ± >62.918]	[<83.957 ± >43.229]	[<67.701 ± >9.094]

Values represent mean ± SD for four rats.

[6] Skin wash total includes other dislodgable radioactivity. Random animals (2 from group 1 and 1 each from Groups 2 and 3) had their dosed skin area and perimeter skin (non-dosed area) outside their frames wiped with a dry Q-tip post-washing at 24-hr post-dosing. These values analyzed via LSC for radioactivity. See appropriate Appendix Table for individual results.

[7] µg-eq Penoxsulam = percentage associated with application-site skin x µg DE-638 applied.

[8] Skin remote from application site

[9] Percent absorbed based on radioactivity found in urine, rinse, feces, carcass, tissues (excluding application-site skin) and final cage wash (FCW).

[10] µg-eq Penoxulam absorbed = percentage x µg Penoxulam applied.

Following a single application of a diluted GF-237 solution to the dorsal skin of male rats, very little dose was absorbed through 72 hr (Table B.6.12/.2). The actual absorbed dose was equivalent to the levels excreted in the urine, and ranged from <0.04% at 24-hr to 0.71% at 72-hr. The majority of the dose was recovered in the skin wash at 24-hr, ranging from 62-66%, with the frame and covering accounting for 7.2-14.1% of the dose, and the skin at the application site accounting for 8.7-17.3% of the applied dose.

Table B.6.12/.2. Disposition of Radioactivity Recovered After Dermal Application of Test Preparation #2, Diluted GF-237

	Percentage of Applied Radioactivity		
Sacrifice Time	Group 4 24 Hour Mean ± SD	Group 5 48 Hour Mean ± SD	Group 6 72 Hour Mean (Range)
Covering	5.53 ± 2.76	8.42 ± 1.48	12.80 (5.24 – 20.36)
Frame	1.72 ± 0.39	2.47 ± 2.27	1.33 (0.88 – 1.78)
Skin,Wash (at 24hr post application)	65.73 ± 2.43	66.06 ± 6.80	62.02 (58.65 – 65.38)
Application-site Skin	17.30 ± 2.56	12.19 ± 3.79	8.74 (8.01 – 9.46)
[µg-eq. DE-638[1]]	[0.220 ± 0.029]	[0.146 ± 0.038]	[0.069 (0.05 – 0.083)]
Skin[2]	NQ (0.05)	NQ (0.06)	NQ (0.08)
Carcass	NQ (0.04)	NQ (0.04)	NQ (0.06)
Kidneys	NQ (0.05)	NQ (0.05)	<0.11 (NQ (0.07) – 0.16)
Liver	NQ (0.05)	NQ (0.05)	NQ (0.08)
Blood	NQ (0.05)	NQ (0.05)	NQ (0.06)
Total Tissues	NQ (0.24)	NQ (0.25)	<0.40 (NQ(0.07) – 0.16)
Feces			
0-24 hr	NQ	NQ	NQ

24-48 hr		NQ	NQ
48-72 hr			NQ
Urine			
0-24 hr	<0.04 ± >0.06	0.11 ± 0.04	NQ
24-48 hr		0.21 ± 0.11	0.23 ± (0.14 – 0.32)
48-72 hr			0.29 ± (0.27 – 0.31)
Rinse			
0-24 hr	NQ	NQ	NQ
24-48 hr		<0.05 ± >0.06	NQ
48-72 hr			<0.08 (NQ(0.00) -0.15)
Final cage wash	NQ	NQ	NQ
Total Recovery	<90.56 ± >0.44	<89.76 ± >1.03	<85.87 (<81.66 - <90.07)
Percent Absorption including NQs[3]	<0.27 ± >0.05	<0.63 ± >0.18	<0.99 (<0.72 - <1.25)
[µg-eq. DE-638 absorbed[4]]	[<0.012 ± >0.002]	[<0.027 ± >0.008]	[<0.032 (<0.023 - <0.040)
Percent Absorption Detected	<0.04 ± >0.06	0.38 ± 0.18	0.67 (0.40 – 0.94)
[µg-eq. DE-638 absorbed]	[<0.002 ± >0.003]	[0.016 ± 0.008]	[0.020 (0.015 - 0.026)]

Values represent mean ± SD for 3 and 4 rats in Groups 4 and 5, respectively. Mean (Range) for Group 6 are based on 2 animals.

[5] µg-eq Penoxsulam = percentage associated with application-site skin x µg DE-638 applied.

[6] Skin remote from application site

[7] Percent absorbed based on radioactivity found in urine, rinse, feces, carcass, tissues (excluding application-site skin) and final cage wash (FCW). Values for NQs are figured in total.

[8] µg-eq Penoxulam absorbed = percentage x µg Penoxulam applied.

Conclusion: Approximately 2% of the radiolabel from the undiluted GF-237 formulation, and < 0.4% of the radiolabel from the spray dilution was absorbed over a 24-hr period. After 72 hours the absorbed radioactivity from the spray dilution increased up to 0.7% of the administered dose, whereas the absorption from the undiluted formulation was higher at 48 hours (2.6%).
The low absorption coincides with significant amounts of material retained at the application site. The variation in the amount of material at the application site can be summarized as:

Table B.6.12.3 Summary of residue at application site

GF-237	% of applied Dose		
Duration (hrs)	24	48	72
Cover	3.28	4.43	7.22
Frame	19.97	11.51	18.5
application site	10.06	11.44	8.21
total	33.31	27.38	33.93

Dilution GF-237	% of applied Dose		
Duration (hrs)	24	48	72
Cover	5.53	8.42	12.8
Frame	1.72	2.47	1.33
application site	17.3	12.19	8.74
total	24.55	23.08	22.87

The apparent decrease in the amount of material retained at the application site can be accounted for by the differing distribution in the residue between the application site, the frame retaining the dose and the cover over the frame. Indeed the increase in the material on the cover confirms that the material at the application site is being lost from the skin rather than being absorbed into the skin .
The data in Table B.6.12.3 shows that the amount associated with the application site/ frame and cover has not varied over a 72 hour period, i.e. there is no statistical difference between the total values at 24, 48 or 72 hours. This evidence for the lack of absorption from the application site is totally consistent with the lack of any significant increase in the absorbed dose over the period 48 hrs to 72 hrs. In the case of the formulation 2.66% and 2.18%, and for the spray dilution 0.38±0.18 to 0.67 (range 0.4 to 0.96).
In deed in the case of the formulation there is further evidence (Table B.6.12.4)**.** that absorption has ceased. This is shown by the decreasing systemic exposure (i.e. excluding dose that has already been exerted) This effect is not apparently evident in the low amounts absorbed from the spray dilution, this is because of the very low amounts absorbed and the confidence levels i.e there is no statistical significance between the data points.
Table B.6.12.4. Summary of systemic exposure per 24 hours as % of applied dose

Systemic Exposure * over each 24 hr period	0-24	24-48	48-72
Formulation	1.74	2.39	1.31
Dilution	0.04	0.27	0.44

* This excludes material excreta from the body in the previous days

Therefore the most appropriate values for dermal absorption per day are 2% for the formulation and 0.4% for the spray dilution.. There is no evidence that the application site residue will contribute significantly to future absorption
This study is acceptable. It has to be noted it has been demonstrated an inverse relation between concentration and % of absorption with that most of the plant protection products tested in vitro and in vivo. At variance, the GF-237 showed the opposite behavior, with the undiluted formulation being absorbed more than the spray dilution at any time after an adequate (24h) period of exposure.

B.6.5 Relevance of Large Granular Lymphocytic (LGL) Leukemia in Fischer 344 Rats Treated with Penoxsulam

Results from literature submitted papers on molecules which may be considered similar to penoxsulam, are summarized below.

Summary

In the chronic toxicity/oncogenicity study in Fischer 344 rats with penoxsulam, statistically significant, non-dose related increases in the incidence of large granular lymphocytic (LGL) leukemia were observed in male rats at all dose levels tested when compared to concurrent controls. These data were submitted to an independent Pathology Working Group (PWG) for evaluation. Due to the lack of a dose-response in both the incidence and severity of the LGL leukemia in males over a 50-fold increase in dose, the lack of any increase in this tumor in female rats, the lack of an increase in any other tumors in rats or mice, the lack of mutagenic activity of penoxsulam, the lack of any increase in the incidence of LGL leukemia in rats administered structural analogs of penoxsulam, and the high spontaneous incidence reported the literature in control animals, the LGL leukemia found in this study was considered spontaneous in origin and unrelated to exposure to penoxsulam. In line with the scientific literature, the finding of an increase in LGL leukemia in one sex in a non-dose related incidence, even when statistically significantly identified, is not considered toxicologically relevant for human risk assessment.

LGL Leukemia

Large Granular Lymphocytic (LGL) leukemia (also known as mononuclear cell leukemia, or MNCL, or Fischer rat leukemia (FRL)) is a lymphoreticular neoplasia, characterized by splenomegaly, increased white blood cells, and infiltration of the spleen, liver, lungs, lymph nodes and bone marrow (Stefanski, *et al*., 1990). The background incidence of this neoplasm in F344 rats is higher in males than females, higher in the F344 rat than any other strain and is not seen in mice or hamsters (Sher, 1982; Frith, *et al*., 1993). The incidence of LGL leukemia in F344 rats reported from studies conducted by the US National Toxicology Program (NTP) average 50.5% in males and 28.1% in females, with frequencies ranging from 10 to 72% in individual studies (Haseman, *et al*., 1998).

LGL leukemia has been well characterized. Dunning and Curtis (1957) were the first to describe this tumor in Fischer 344 rats, classifying it as a monocytic leukemia. Subsequent work by others (Moloney *et al*., 1970; Losco and Ward, 1984; Stefanski *et al*., 1990; Ward and Reynolds, 1990) further characterized the pathology and incidence. LGL leukemia is a tumor of aged rats, and is seldom seen in rats less than 18-20 months of age (Stromberg and Vogtsberger, 1983). However, in older Fischer 344 rats it occurs at a high spontaneous incidence as noted above and is the most common life-threatening neoplasm in this rat strain, accounting for up to 40% of deaths in rats dying before 26 months (Haseman *et al*. 1990, 1998). Clinically and pathologically, this disease progresses quickly and is categorized as an acute leukemia that is well differentiated and highly malignant, progressing from first clinical signs to morbidity and mortality in a period of 2-3 weeks. Histologically, there is no precursor of this tumor. LGL leukemia is easily transplanted and appears to arise in the spleen and spread to other organs, with low and variable involvement of the bone marrow. In fact, the NTP uses

criteria to characterize the stage of leukemia based on organ involvement, with Stage 1 characterized by enlargement of the spleen with neoplastic cells in the red pulp, Stage 2 characterized by moderate splenic enlargement and minimal to moderate involvement of the liver, and Stage 3 considered advanced disease with involvement of multiple organs (Dunnick, *et al*., 1989).

The exact mechanism of tumor initiation is unknown, but attempts at linking it to a viral etiology have not been successful. The spleen appears to play a key role in the development and progression of the disease. Moloney and King (1973) demonstrated that splenectomy of young (1-2 month old) Fischer 344 rats greatly reduced the spontaneous incidence of LGL leukemia. Elwell *et al*. (1996) reported that treatment-related decreases in LGL leukemia incidence was seen in 2-year studies with a number of free aromatic amine or nitro compounds that could be metabolized to amines, most of which produced splenic effects in 13-week studies.

Other factors have also been shown to modulate the spontaneous incidence of LGL leukemia in rats. The use of corn oil as a vehicle has been shown to reduce the incidence in male F344 by as much as 50%, though females were unaffected. Dietary restriction in 2-year studies which produced reductions in weight gain has also been shown to decrease the incidence compared to *ad libitum* fed rats. (Stefanski *et al.*, 1990).

The cell of origin of Fischer rat LGL leukemia appears to predominantly be a natural killer (NK) cell based on morphologic and immunologic characterization (Ward and Reynolds, 1983), though there is some heterogeneity within the cell population. The tumor cells are not classified as B-Cell or αβT-Cell, and a good proportion of cells are positive for OX-8 and Asialo GM-1 surface markers (Losco and Ward, 1984; Stromberg *et al*., 1983a, b & c; Ward and Reynolds, 1983).

In humans, two types of LGL leukemias have been identified that account for approximately 10-15% of non-Hodgkin's lymphomas; however, there are clinical and morphologic features of these leukemias that are different from rodent LGL leukemia that make a direct comparison difficult. One type, T-cell Large Granular Lymphocytic leukemia (T-LGL) accounts for 85% of human LGL leukemia and is predominantly found in elderly patients, but is a slow developing disease with involvement of the bone marrow and has been associated with human T-cell lymphotrophic virus (HTLV-1). The other, an NK-cell LGL leukemia, accounts for approximately 15% of human LGL leukemia, but is an aggressive disease found predominantly in young patients. This type of leukemia also involves a high degree of bone marrow infiltration, and has been associated with Epstein-Barr virus (EBV) and HTLV-1 (Greer *et al*., 2001; Reynolds and Foon, 1984; Rose and Berliner, 2004; Stromberg, 1985).

Penoxsulam

In the 2-year chronic toxicity and oncogenicity study with penoxsulam, statistically significant increases in the incidence of LGL leukemia were observed in male Fischer 344 rat in all treatment groups but not in females when compared to the concurrent controls. An independent Pathology Working Group (PWG) was convened to confirm the incidence of leukemia and determine the stage of involvement (Hardisty, 2002). A panel of experienced pathologists reexamined histologic sections of the spleen, liver and lungs from all animals on study, and the stage of development of leukemia was determined using the diagnostic criteria established by the NTP (Dunnick *et al*., 1989). Additional organs were examined from selected animals categorized as having Stage 3 leukemia. The incidence and stage of development of LGL leukemia in this study is summarized in Table 1 below.

Table 1: Incidence and Stage of LGL Leukemia in Fischer 344 Rats
(PWG Consensus)

	Males	**Females**

Dose (mg/kg/day)	0	5	50	250	0	5	50	250
No. Animals	50	50	50	50	50	50	50	50
LGL Leukemia[†]	12	30[*]	29[*]	30[*]	11	11	6	9
Stage 1	1	1	1	6	0	1	0	2
Stage 2	6	9	9	3	2	4	2	1
Stage 3	5	20	19	21	9	6	4	6
Lymphosarcoma	0	0	0	0	0	1	0	0

[*]Statistically significantly different from control by Yates Chi Square Test, $\alpha = 0.05$.
[†]Staging according to Dunnick et al. (1989).

The results of the PWG confirmed the statistically significantly higher incidence of LGL leukemia in male F344 rats that was initially diagnosed by the study pathologist. However, there was no dose-response in the incidence as would be expected from a treatment-related effect over a 50-fold increase in dose. In addition, staging of the LGL leukemia with respect to the extent of organ involvement showed no dose-response relationship. In fact, the incidence of Stage 1 LGL (lowest grade) was higher in top-dose males than in controls, while the incidence of Stage 2 LGL was lower in the top-dose males than in controls. In females, the incidence and stage of LGL leukemia was similar in control and treated animals.

The mean historical incidence of LGL leukemia in control animals from 8 studies conducted prior to the study with penoxsulam in the submitting laboratory (The Dow Chemical Company) was 28.5% in males (range 16-40%) and 17.8% in females (range 10-28%). However, the incidence of LGL leukemia in control male Fischer 344 rats from studies conducted by the NTP indicate a mean of 50.5% with a range of 32-74% (Haseman *et al*., 1998). (It should be pointed out that all studies conducted by NTP undergo a peer review to confirm neoplastic diagnoses prior to study finalization.). Thus, although the incidence of LGL leukemia in male F344 rats is outside the historical control range of studies conducted within The Dow Chemical Company, the incidence in treated male rats is close to the mean reported by the NTP, and well within the range of expected values.

There were no treatment-related increases in any other tumor type in either male or female F344 rats in the oncogenicity study with penoxsulam. In addition, no increases in LGL leukemia were observed in carcinogenicity studies conducted with other chemicals in the triazolopyrimidine class of chemistry (Hanley and Billington, 2001). Penoxsulam was also found not to be carcinogenic in male and female mice, and there was no evidence of a mutagenic potential following a battery of *in vitro* and *in vivo* assays.

Therefore, based on:

- the high spontaneous incidence of LGL leukemia unique to Fischer rats, especially males
- the increase in LGL leukemia being limited to one sex (male) and one species (rat)
- the lack of a dose-response in both incidence and severity (degree and extent of organ involvement)
- the lack of any other tumors in either rats or mice
- the lack of genotoxicity
- the lack any increases in LGL leukemia with other compounds in this class of chemistry

the increases in LGL leukemia in male rats following exposure to penoxsulam were considered spontaneous in origin and not treatment-related. In line with the scientific literature, the finding of an increase in LGL leukemia in one sex in a non-dose related incidence, even when statistically significantly identified, is not considered toxicologically relevant for human risk assessment.

References

PSD (2005). PSD Guidance Document: Interpretation of Liver Enlargement in Regulatory Toxicity Studies. 26 May 2005

Sanco/10182/2005 rev.9, Guidance for the Setting of Acceptable Operator Exposure Levels (AOELs), 5 July 2005

US EPA (2004). Federal Register Notice: Penoxsulam, 2-(2,2-difluoroethoxy)-N-(5,8-dimethoxy[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl)benzenesulfonamide; Pesticide Tolerance (http://www.epa.gov/EPA-PEST/2004/September/Day-24/p21502.htm

Position paper on dermal absorption values of penoxsulam
(J M Perkins Msc C.Chem MRSC, Global Leader Dermal Absorption, Human Health Assessment, Dow AgroSciences, **24 July 2006)**

Dunning, W.F., and Curtis, M.R. (1957). A transplantable acute leukemia in an inbred line of rats. *J. Natl. Cancer Inst.* **19**, 845-852.

Dunnick, J.K., Eustis, S.L., Huff, J.E. and Haseman, J.K. (1989). Two-year toxicity and carcinogenicity studies of ampicillin trihydrate and penicillin VK in rodents. *Fundam. Appl. Toxicol.* **12**, 252-257.

Elwell, M.R., Dunnick, J.K., Hailey, J.R., abd Haseman, J.F. (1996). Chemicals associated with decreases in the incidence of mononuclear cell leukemia in the Fischer rat. *Tox. Pathol.* **24**, 238-245.

Frith, C.H., Jerold, M.W. and Chandra, M. (1993). The Morphology, Immunohistochemistry and Incidence of Hematopoietic Neoplasms in Mice and Rats. *Toxicol. Pathol.* **21**, 206-218.

Greer, J.P., Kinney, M.C. and Loughran, T.P., Jr. (2001). T-cell and NK cell lymphoproliferative disorders. *Hematology (Am. Soc. Hematol. Educ. Prog.)* **2001**, 259-281.

Hanley, T.R., Jr. and Billington, R. (2001). "Toxicology of triazolopyrimidine herbicides" in Handbook of Pesticide Toxicology, R. Krieger, ed., Academic Press, Inc., San Diego, CA, pp. 1653-2001.

Hardisty, J.F. (2002). Pathology peer review and pathology working group (PWG) review of large granular lymphocyte leukemia (LGL) in a two-year chronic toxicity/oncogenicity and chronic neurotoxicity study of XDE-638 in Fischer 344 rats. Unpublished report of The Dow Chemical Company prepared by EPL, Inc. DECO HET DR-0357-6203-068a.

Haseman, J.K., Arnold, J. and Eustis, S.L. (1990). "Tumor incidences in Fischer 344 Rats: NTP Historical Data" in Pathology of the Fischer Rat. Boorman, G.A., Eustis, S.L., Elwell, M.R., Montgomery, C.A., Jr. and McKenzie, W.F., eds. Academic Press, Inc., San Diego, CA, pp. 369-393.

Haseman, J.K., Hailey, J.R. and Morris, R.W. (1998). Spontaneous neoplasm incidences in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: A National Toxicology Program update. *Toxicol. Pathol.* **26**, 428-441.

Losco, P.E. and Ward, J.M. (1984). The early stage of larger granular lymphocyte leukemia in the F344 rat. *Vet. Pathol.* **21**, 286-291.

Moloney, W.C., Boschetti, A.E. and King, V.P. (1970). Spontaneous leukemia in Fischer rats. *Cancer Res.* **30**, 41-43.

Moloney, W.C. and King, V.P. (1973). Reduction of leukemia incidence following splenectomy in the rat. *Cancer Res.* **33**, 573-574.

Reynolds, C.W. and Foon, K.A. (1984). T gamma lymphoproliferative disease and related disorders in humans and experimental animals: a review of the clinical, cellular, and functional characteristics. *Blood* **64**, 1146-1158.

Rose, M.G. and Berliner, N. (2004). T-cell large granular lymphocyte leukemia and related disorders. *The Oncologist* **9**, 247-258.
Sher, S.P. (1982). Tumors in control hamsters, rats, and mice: Literature tabulation. *CRC Crit. Rev. Toxicol.* 49 – 77.

Stefanski, S.A., Elwell, M.R. and Stromber, P.C. (1990). "Spleen, Lymph Nodes and Thymus" in Pathology of the Fischer Rat, Boorman, G.A., Eustis, S.L., Elwell, M.R., Montgomery, C.A., Jr. and McKenzie, W.F., eds. Academic Press, Inc., San Diego, CA, pp. 369-393.

Stromberg, P.C. (1985). Large granular lymphocyte leukemia in F344 rats: Model for human t lymphoma, malignant histiocytosis and T-cell chronic lymphocytic leukemia. *Am. J. Pathol.* **119**, 517-519.

Stromberg, P.C. and Vogstberger, L.M. (1983). Pathology of the mononuclear cell leukemia of Fischer rats. I. Morphologic studies. *Vet Pathol.* **20**, 698-708.

Stromberg, P.C., Rojko, J.L., Vogstberger, L.M., Cheney, C. and Berman, R. (1983a). Immunologic, biochemical, and ultrastructural characterization of the leukemia cell in F344 rats. *J. Nat. Cancer Inst.* **71**, 173-179

Stromberg, P.C., Vogstberger, L.M., Marsh, L.R. and Wilson, F.D. (1983b). Pathology of the mononuclear cell leukemia of Fischer rats. II. Hematology. *Vet Pathol.* **20**, 709 - 717.

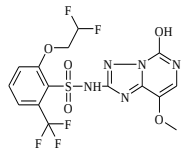
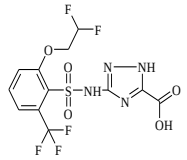
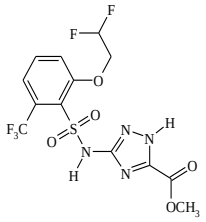
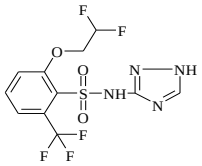
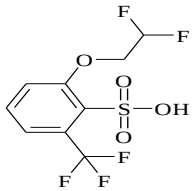
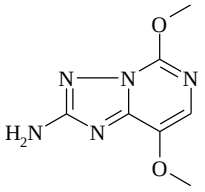
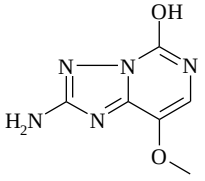
Stromberg, P.C., Vogstberger, L.M. and Marsh, L.R. (1983c). Pathology of the mononuclear cell leukemia of Fischer rats. III. Clinical chemistry. *Vet Pathol.* **20**, 718 – 726.

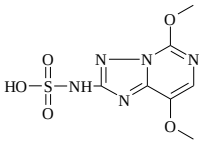
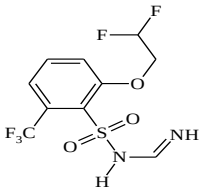
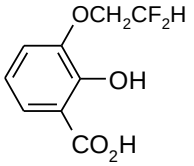
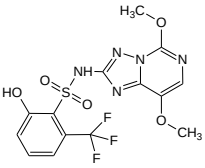
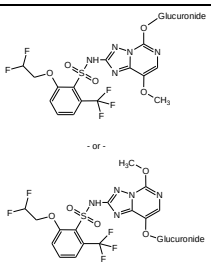
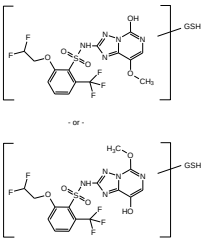
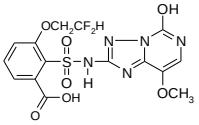
Ward, J.M. and Reynolds, C.W. (1983). Large granular lymphocyte leukemia: A heterogeneous lymphocytic leukemia in F344 rats. *Am. J. Pathol.* **111**, 1-10.

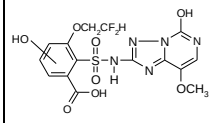
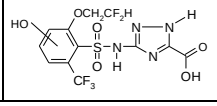
METABOLITES (BST & BSTCA)

Two metabolites were found at high levels in rotational crops: BST and BSTCA (see the table listing the metabolites of DE-638 found in soil, water, crops and animals, based on the metabolites identified in Section 4 (6.1 and 6.2) and Section 5 (7.1 and 7.2) of the original dossier).. It has been requested if they are of toxicological concern. In the following the evaluation is reported.

Table 5.7.1-1: Metabolites identified in soil, water, crops and animals

Metabolite identification	Compartment											
	Structure	Soil			Water			Crops		Animal		
		Aer	An	Phot	Gr	Phot	SW	Rice	Cereals	Rat	Hen	Goat
5-OH-DE-638		X	X		X[1]			X		X		X
BSTCA		X	X	X	X[1]							
Me-BSTCA			< 10%									
BST		< 10%	< 10%		X[1]							
BSA				< 10%		X						
2-AMINO-TP				X		X						
5-OH-2-AMINO-TP						X						

Metabolite identification	Compartment											
	Structure	Soil			Water			Crops		Animal		
		Aer	An	Phot	Gr	Phot	SW	Rice	Cereals	Rat	Hen	Goat
TPSA						X						
SFA												
di-FESA						< 10 %						
2-OH-DE-638										X		
Glucuronide conjugate of OH-DE-638										X		
Glutathione conjugate of OH-DE-638										X		
PCA-5-OH-DE-638			< 10%					< 10%				

Metabolite identification	Compartment											
	Structure	Soil			Water			Crops		Animal		
		Aer	An	Phot	Gr	Phot	SW	Rice	Cereals	Rat	Hen	Goat
PHCA-5-OH-DE-638			< 10%									
OH-BSTCA			< 10%					< 10%				

x = present
SW = surface water
[1] PECgw value >0.1 µg/L and <0.75 µg/L based on modelling

Based on recent environmental modeling using revised values for the maximum amount of metabolite formed in the MEDRICE scenario for application in rice, the PECgw for BSTCA is calculated to exceed 0.1 µg/L in groundwater (see Draft Assessment Report, Section B.8.5.1). Based on the results of further environmental modeling using PELMO 3 for application in cereals, the PECgw for 5-OH-DE-638, BSTCA and BST are calculated to exceed 0.1 µg/L (see Annex IIIA 9.6.2).

The EC Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater (SANCO/221/2000 – rev.10; 25 Feb 2003), a scheme is described to determine whether a metabolite is relevant (and thus subject to the 0.1 µg/L limit for drinking water) or not relevant. In this scheme metabolites are assessed stepwise with increasing complexity using criteria of chemical structure, estimated concentrations in groundwater, biological activity, genotoxicity, toxicological hazard and toxicological risk assessment. It is stated that before conducting additional tests it should be checked "...whether metabolites or breakdown products under consideration have already been covered by studies required for the active substance ..."
The two metabolites under consideration pass step 1 and 2 , arriving at step3, at which the Guidance Document indicates *"all metabolites, which might be expected to exceed the limits laid down in Annex VI, point C.2.5.1.2 of Directive 91/414/EEC should be further assessed"*. This assessment involves a process, which includes:

- Screening for biological activity
- Screening for genotoxicity
- Screening for toxicity
- Exposure assessment- threshold of concern

Biological Activity: The stated goal of this screening is "*to identify metabolites, which have a comparable target activity as the parent active ingredient, and to deal with cases where the parent molecule is a precursor*". SANCO/221/2000 further states "*(a)s a line of orientation, it should be sufficient to demonstrate that the biological activity of a metabolite is clearly less than 50% of the activity of the parent molecule*".
All major DE-638 soil and water metabolites have been assessed for pesticidal activity and the results of these studies are considered in Annex II, 8.6. With the possible exception of 5-OH-DE-638 at high

concentrations, these screening studies demonstrated a lack of herbicidal activity of all metabolites on a wide array of grass and broadleaf plants and sensitive indicator species. These results also showed that DE-638 is not a "pro-herbicide". The 5-OH-DE-638 metabolite was shown to clearly have less than 50% of the activity of the parent in all species tested, demonstrating only 20% injury to redroot pigweed when applied at a rate approximately 4-fold above a rate with DE-638 which produced 100% injury. Based on these data, all metabolites pass this stage.

Genotoxicity: According to SANCO/221/2000, "*all metabolites that have passed…stage 1 of step 3 should be screened for their genotoxic activity*" using a battery of tests to include the "*Ames test, gene mutation test with mammalian cells, and chromosome aberration test".* 5-OH-DE-638, BSTCA and BST have been evaluated in a battery of tests which include an Ames test, a Chinese hamster ovary HGPRT test (gene mutation) and a rat lymphocyte chromosomal aberration test. The results of these studies indicate no genotoxic activity of any metabolite in any of these tests. These studies are reported below.

Screening for Toxicity: This step is *"aimed at the question of whether a metabolite has certain toxicological properties, which - from a regulatory perspective – qualify for considering it "relevant*". For pragmatic reasons, the toxicity classification of the parent is used as a starting point to focus the screening activity. Additional testing of metabolites for toxicity is required if the parent active substance:

- is classified as acutely or chronically toxic or very toxic (T followed by R25, R24, R23 or R48, or T+ followed by R28, R27, R26 or R39)
- is classified for reproductive toxicity (any category with R60, R61, R62 or R63)
- is classified as a category 1 or category 2 carcinogen (Carc. Cat. 1; R45) or (Carc. Cat. 2; R45)

Penoxsulam fits into none of the above categories. The toxicity of penoxsulam is low, there was no indication of any effects in developmental or reproductive toxicity tests to suggest classification as a reproductive hazard, and penoxsulam is not classified as a category 1 or category 2 carcinogen. Based on the data on the parent active, there is no reason to suspect that the metabolites would satisfy the requirements for classification in any of these categories.
Based on (1) a lack of biological activity of the metabolites, (2) the negative results of mutagenicity studies conducted with these metabolites, and (3) the lack of toxicological properties of the parent active ingredient that would require classification and labeling as toxic or very toxic, a reproductive toxin, or a carcinogen, the metabolites of penoxsulam are not identified as being relevant.

Exposure Assessment:
The final step in the evaluation of metabolites involves an exposure assessment. SANCO/221/2000 indicates that "*metabolites which have not been identified as being relevant according to the hazard screening outlined in Step 3 should be further tested in an exposure assessment to make sure that any contamination of groundwater will not lead to unacceptable exposure of consumers via their drinking water*". Using a "threshold of concern" approach, SANCO/221/200 states that "*an acceptable exposure level relates to an acceptable upper limit for the concentration of a metabolite of 0.75µg/L.*" It goes on to further state that "*such a threshold can only be considered acceptable if the metabolite in question*

- *does not exceed 0.75 µg/L (or a lower level, if consumers are exposed also via other routes)*
- *and has passed Step 3 i.e.*
    - *has a lower biological activity than the parent*
    - *is not genotoxic and*
    - *is not defined as toxic"*

Exposure estimates for BSTCA based on MEDRICE for uses with rice (see Draft Assessment Report, Section B.8.5.1) and for 5-OH-638, BSTCA and BST using PELMO 3 for cereals use (see Annex IIIA 9.6.2) indicate the PECgw values for these metabolites will not exceed 0.75 µg/L and thus further assessment is not necessary. No detectable residues of parent or metabolites have been identified in rice, wheat or barley grain following treatment with penoxsulam (see Draft Assessment Report, Section and MIII) to suggest any exposure is to be expected from dietary consumption.

Conclusion: As demonstrated above, metabolites of penoxsulam which have passed Step 3 (Hazard Assessment) can be tolerated without further testing, being the threshold of concern of estimated or actual concentrations in ground water of 0.75 µg/l not exceeded.
Thus, penoxsulam meets the criteria for consideration for Annex I inclusion.

In the following the new studies submitted in order to support the absence of genotoxic potential of the two metabolites under consideration are reported.

B.6.7.1.1 Mutagenicity studies on 5-OH-XDE-638 (IIA 5.7.1.1)

There is ample evidence from the rat metabolism study to demonstrate that, as a metabolite, 5-OH-XDE-638 has already been included in the studies conducted on the parent (XDE-638), and separate mutagenicity testing on this metabolite is not warranted. 5-OH-XDE-638 results from the dealkylation of the parent compound. In the rat metabolism study, though 5-OH-XDE-638 was not detected, significant amounts of metabolites derived from 5-OH-XDE-638 have been identified.
Quantifiable levels of downstream metabolites that have been identified include the glucuronide (Metabolite S) and glutathione (Metabolite U) conjugates of the dealkylated-XDE-638, as well as Metabolite Y, which has been identified as the glutathione conjugate of dealkylated XDE-638, which has undergone further hydroxylation, sulfation and loss of the glycine moiety of glutathione.
MUCombined, the amounts of these metabolites in urine and feces account for approximately 17-20% of an administered dose (single or multiple) of 5 mg/kg in males, and approximately 3-6% in females. At a high dose level of 250 mg/kg, the levels in the urine and feces of these metabolites accounted for approximately 4% of the dose in males and 1% in females. In bile duct-cannulated rats given 5 mg/kg, metabolites S, U and Y combined were found to account for 20% of the administered dose in males and 3% in females, consistent with the amounts seen in the urine and feces at this dose level. The proposed metabolic pathway for penoxsulam in the rat (MII, Section 5.1.1) clearly demonstrates the formation of 5-OH-XDE-638.
The presence of metabolites S, U and Y in the bile at levels comparable to those found in the feces of non-cannulated animals suggests that these are formed in the liver. However, the liver was not considered a primary target organ in the rat, with only slight hepatocellular hypertrophy noted at very high dose levels (500 mg/kg/day for 13 weeks) and no increases in mixed function oxidase enzyme (PROD, EROD, MROD and p-NP) levels observed. In addition, these metabolites were all more polar than the parent, enhancing their excretion. Thus, the *in vivo* studies in rats indicate that the formation of these metabolites does not apparently involve reactive species that would be considered more toxic than the parent.
Based on the presence of 5-OH-XDE-638 in the metabolism study in rats, the toxicity of this metabolite has been adequately addressed in the battery of tests conducted with the parent (XDE-638), and that separate mutagenicity studies with this metabolite were not warranted. However, a battery of mutagenicity studies was conducted with 5-OH-XDE-638 in fulfilment of the requirements as laid out

in SANCO/221/2000, and the results of these studies confirm the lack of genotoxicity as predicted from the data available from the parent active ingredient.

B 6.7.1.1a *In Vitro* genotoxicity testing (Reference IIA5.7.1.1a/01)

STUDY TYPE: *(Bacterial system, Salmonella typhimurium; Escherichia coli)*/ mammalian activation gene mutation assay; OPPTS 870.5100[4]; OECD 471 (formerly OECD 471 & 472).

TEST MATERIAL (PURITY): X689643 (6-(2,2-difluoroethoxy)-N-(5,6-dihydro-8-methoxy-5-oxo-s-triazolo[1,5-c]pyrimidin-2-yl)-α,α,α-trifluoro-o-toluenesulfonamide) (99%)

SYNONYMS: 5-OH metabolite of penoxsulam; DES ME-638, (5-Hydroxy-XDE-638)

CITATION: M.S. Mecchi (18 October 2006). *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay Preincubation Method with a Confirmatory Assay with X689643 (5-OH Metabolite of Penoxsulam). Covance Laboratories Inc., Vienna, Virginia. Study ID: 6736-161, 06 June 2006 – 18 October 2006. Unpublished.

SPONSOR: The Dow Chemical Company

SUMMARY:

In a reverse gene mutation assay in bacteria, tester strains TA98, TA100, TA1535, and TA1537 of *S. typhimurium* and tester strain WP2*uvr*A of *E. coli* were exposed to X689643 (5-OH Metabolite of Penoxsulam) (99% a.i.), in dimethylsulfoxide using the preincubation method at concentrations of 100, 333, 1000, 3330, and 5000 µg per plate in the presence and absence of mammalian metabolic activation.

X689643 (5-OH Metabolite of Penoxsulam) was tested up to the limit concentration (5000 µg per plate) with all strains. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

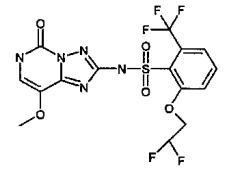
This study is classified as acceptable. This study satisfies the requirement for Test Guideline OPPTS 870.5100[1]; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

[4]870.5100 - Reverse mutation *E. coli* WP2 and WP2uvrA; *S. typhimurium* TA 97, TA98, TA100, TA1535, TA1537
870.5140 - Gene mutation *Aspergillus nidulans*
870.5250 - Gene mutation *Neurospora crassa*

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	X689643 (5-OH Metabolite of Penoxsulam)
	Description:	White powder
	Lot #:	200000221-139A
	Purity:	99% a.i.
	CAS #:	not provided
		STRUCTURE: 
	Solvent Used:	Dimethylsulfoxide

2.	**Control Materials:**	
	Negative:	NA
	Solvent (final conc'n):	Dimethylsulfoxide at 50 µL per plate
	Positive:	Non-activation: sodium azide 2.0 µg/plate TA100, TA1535 2-nitrofluorene 1.0 µg/plate TA98 ICR-191 2.0 µg/plate TA1537 4-nitroquinoline-N-oxide 0.4 µg/plate WP2*uvr*A
		Activation: benzo[a]pyrene 2.5 µg/plate TA98 2-aminoanthracene 2.5 µg/plate TA100, TA1535, and TA1537 2-aminoanthracene 25.0 µg/plate WP2*uvr*A

3.	**Activation**: S9 derived from								
	x	induced		x	Aroclor 1254	x	Rat	x	Liver
		non-induced			Phenobarbitol		Mouse		Lung
					None		Hamster		Other (name)
					Other (name)		Other (name)		

S9 mix composition: H_2O (70%), 1M NaH_2PO_4/Na_2HPO_4, pH 7.4 (10%), 0.25M Glucose-6-phosphate (2%), 0.10M NADP (4%), 0.825M KCl/0.2M $MgCl_2$ (4%), and S9 Homogenate (10%).

4.	**Test organisms:** *S. typhimurium* strains									
		TA97	x	TA98	x	TA100		TA102		TA104
	x	TA1535	x	TA1537		TA1538	x	WP2*uvr*A (*E. coli*)		
Properly maintained?							x	Yes		No
Checked for appropriate genetic markers (*rfa* mutation, R factor)?							x	Yes		No

5. Test compound concentrations used:
Non-activated conditions:

Cytotoxicity test: TA100 and WP2*uvr*A at 10.0, 33.3, 100, 333, 1000, 3330, and 5000 µg per plate; two replicates.

Mutagenicity assay: TA98, TA100, TA1535, TA1537, and WP2*uvr*A at 100, 333, 1000, 3330, and 5000 µg per plate; three replicates.

Activated conditions:

- Cytotoxicity test: TA100 and WP2*uvr*A at 10.0, 33.3, 100, 333, 1000, 3330, and 5000 µg per plate; two replicates.
- Mutagenicity assay: TA98, TA100, TA1535, TA1537, and WP2*uvr*A at 100, 333, 1000, 3330, and 5000 µg per plate; three replicates.

B. TEST PERFORMANCE

1. Type of *Salmonella* assay: pre-incubation (20 minutes)
2. Protocol:
Tester strains were exposed to the test article via the preincubation modification of the Ames Test originally described by Yahagi *et al.* (1975) and Maron and Ames (1983). This methodology has been shown to detect a wide range of classes of chemical mutagens. In the preincubation methodology, S9 mix (or phosphate buffer, where appropriate), tester strain, and test article were preincubated for approximately 20 minutes prior to addition of molten agar. The agar and preincubation reaction mixture were mixed and then overlaid onto a minimal agar plate. Following incubation, revertant colonies were counted. Test article, vehicle controls, and positive controls were plated in triplicate. The condition of the bacterial background lawn was evaluated both macroscopically and microscopically (using a dissecting microscope) for indications of cytotoxicity and test article precipitate. Evidence of cytotoxicity was scored relative to the vehicle control plate and was recorded along with the revertant counts for all plates at that concentration. Revertant colonies were counted either by automated colony counter or by hand.

3. Statistical Analysis: Mean and standard deviation were calculated for each replicate.

4. Evaluation Criteria: Before assay data were evaluated, criteria for a valid assay had to be met. The following criteria were used to determine a valid assay:

Salmonella typhimurium tester strain cultures exhibited sensitivity to crystal violet to demonstrate the presence of the *rfa* wall mutation. Cultures of tester strains TA98 and TA100 exhibited resistance to ampicillin to demonstrate the presence of the pKM101 plasmid.

Demonstrating the requirement for histidine (*Salmonella typhimurium*) or tryptophan (*Escherichia coli*), tester strain cultures exhibited a characteristic number of spontaneous revertants per plate when plated along with the vehicle under selective conditions. Acceptable ranges for mean vehicle controls were as follows:

TA98	8	-	60
TA100	60	-	240
TA1535	4	-	45
TA1537	2	-	25
WP2*uvr*A	5	-	40

Demonstrating that appropriate numbers of bacteria were plated, density of tester strain cultures were greater than or equal to 1.0 x 10^9 bacteria per mL and/or had reached a target density demonstrated to produce cultures with at least 1.0 x 10^9 bacteria per mL (see Study Deficiencies).

Demonstrating that tester strains were capable of identifying a mutagen, the mean value of a positive control for a respective tester strain exhibited at least a 3-fold increase over the mean value of the vehicle control for that strain. An acceptable positive control in the presence of S9 mix for a specific strain was evaluated as having demonstrated both integrity of the S9 mix and ability of the tester strain to detect a mutagen.

A minimum of three non-toxic concentrations was required to evaluate assay data. Cytotoxicity was detectable as a decrease in the number of revertant colonies per plate and/or by a thinning or disappearance of the bacterial background lawn. Thinning of the bacterial background lawn not accompanied by a reduction in the number of revertants per plate was not evaluated as an indication of cytotoxicity.

Once criteria for a valid assay had been met, responses observed in the assay were evaluated.

For a test article to be considered positive, it had to produce at least a 2-fold (TA100) or 3-fold (TA98, TA1535, TA1537, and WP2*uvr*A) concentration-related and reproducible increase in the mean revertants per plate over the mean revertants per plate of the appropriate vehicle control. A response that did not meet all three of the above criteria (magnitude, concentration-responsiveness, reproducibility) was not evaluated as positive.

II.RESULTS

A. <u>Preliminary cytotoxicity assay</u>
Seven concentrations of test article, from 10.0 to 5000 □ g per plate, were tested in Trials 28393-A1 with tester strains TA100 and WP2*uvr*A. No cytotoxicity was observed with either tester strain in the presence or absence of S9 mix as evidenced by no decreases in the number of revertants per plate and normal bacterial background lawns.

B. <u>Mutagenicity assay</u>
The data for the mutagenicity assay were generated in Trials 28393-B1 and 28393-C1.

The tester strains used in the preincubation mutagenicity assay were *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* tester strain WP2*uvr*A.

The assay was conducted with five concentrations of the test article (100, 333, 1000, 3330, and 5000 □ g per plate) in the presence and absence of S9 mix along with concurrent vehicle and positive controls using three plates per concentration.

In the initial mutagenicity assay (Trial 28393-B1), all data were acceptable and no positive increases in the mean number of the revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix.

In the confirmatory mutagenicity assay (Trial 28393-C1), all data were acceptable and no positive increases in the mean number of the revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix.

III. CONCLUSION
Under the conditions of this study, X689643 (5-OH metabolite of penoxsulam) did not show evidence of mutagenic potential

B 6.7.1.1b *In Vitro* genotoxicity testing (Reference IIA5.7.1.1b/01)

STUDY TYPE: Mammalian cells in culture gene assay in CHO-K_1-BH_4 cells; USEPA OPPTS 870.5300; OECD Guideline 476; EC, B.17.

TEST MATERIAL (PURITY): X689643 (5-OH Metabolite of Penoxsulam); (Benzenesulfonamide,2-(2,2-difluoroethoxy)-N-[6-[[2-(2,2-difluoroethoxy)-6-(trifluoromethyl)phenyl]sulfonyl]-5,6-dihydro-8-methoxy-5-oxo-[1,2,4]-triazolo[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl)) (99%)

SYNONYMS: 5-OH metabolite of XDE-638, DES ME-638, (5-Hydroxy-XDE-638)

CITATION: M. R. Schisler, and K. M., Kleinert, (November 17, 2006). EVALUATION OF X689643 (5-OH METABOLITE OF PENOXSULAM) IN THE CHINESE HAMSTER OVARY CELL/HYPOXANTHINE-GUANINE-PHOSPHORIBOSYL TRANSFERASE (CHO/HGPRT) FORWARD MUTATION ASSAY. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, 48674. Study ID: 061093 (November 17, 2006). Unpublished

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

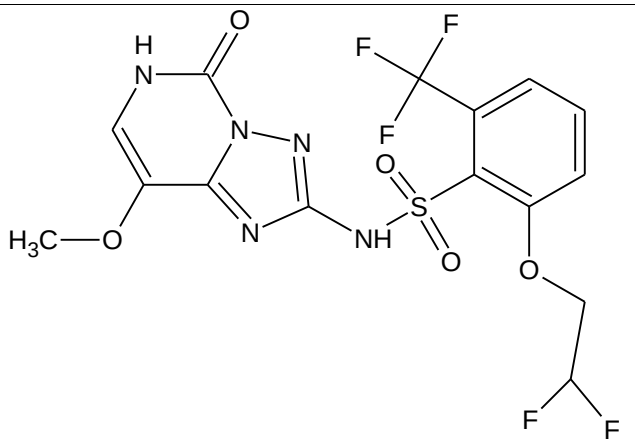
SUMMARY: X689643 (benzenesulfonamide,2-(2,2-difluoroethoxy)-N-[6-[[2-(2,2-difluoroethoxy)-6-(trifluoromethyl)phenyl]sulfonyl]-5,6-dihydro-8-methoxy-5-oxo-[1,2,4]-triazolo[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl), the 5-OH metabolite of penoxsulam, was evaluated in the *in vitro* Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward gene mutation assay. The genotoxic potential of the test material was assessed in two independent assays in the absence and presence of an externally supplied metabolic activation (S9) system. The concentrations ranged from 100 to 2000 µg/ml in the absence and presence of S9. The highest concentration was based on the limit of solubility of the test material in the solvent. The adequacy of the experimental conditions for detection of induced mutation was confirmed by employing positive control chemicals, ethyl methanesulfonate for assays without S9 and 20-methylcholanthrene for assays with S9. Solvent control cultures were treated with the solvent used to dissolve the test material (*i.e.* dimethyl sulfoxide). Based upon the frequency of TG^r mutants recovered in cultures treated with the test material, it was concluded that X689643, the 5-OH metabolite of penoxsulam, was non-mutagenic in the assay system employed.

This study is acceptable and satisfies the guideline requirement for a Mammalian cells in culture gene assay in CHO-K_1-BH_4 cells; USEPA OPPTS 870.5300; OECD Guideline 476; EC, B.17

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	X689643 5-OH Metabolite of Penoxsulam
	Description:	White solid
	Lot/Batch #:	200000221-139A, TSN102471
	Purity:	The purity of the test material was determined to be 99% by liquid chromatography with ultra violet detection and identification by infrared spectroscopy.
	CAS #:	Not Applicable
	Chemical Structure:	
	Solvent Used:	Dimethyl sulfoxide (DMSO, Sigma, St. Louis, Missouri)

2.	**Control Materials:**	
	Solvent control (final conc'n):	Dimethyl sulfoxide (DMSO) 1%
	Positive control:	Non-activation: Ethyl methanesulfonate (EMS, CAS No. 62-50-0), 621 μg/ml
		Activation: 20-methylcholanthrene (20-MCA, CAS No. 56-49-5), 4 and 6 μg/ml

3.	**Activation**: S9 derived from								
	X	induced		X	Aroclor 1254	X	Rat	X	Liver
		non-induced			Phenobarbitol		Mouse		Lung
					None		Hamster		Other
					Other		Other		

The S9 mix consisted of the following co-factors: 10 mM $MgCl_2 \cdot 6H_2O$, 5 mM glucose-6-phosphate, 4 mM nicotinamide adenine dinucleotide phosphate, 10 mM $CaCl_2$, 30 mM KCl, and 50 mM sodium phosphate (pH 8.0). The reconstituted mix

was added to the culture medium to obtain the desired final concentration of S9 in the culture, *i.e.*, 2% v/v. Hence, the final concentration of the co-factors in the culture medium is 1/5 of the concentrations stated above.

4.	**Test cells:** mammalian cells in culture				
			mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
		X	Chinese hamster ovary (CHO) cells		list any others

Media: The cells were routinely maintained in Ham's F-12 nutrient mix (GIBCO, Grand Island, New York) supplemented with 5% (v/v) heat-inactivated (56°C, 30 minutes), dialyzed fetal bovine serum (GIBCO), antibiotics and antimycotics (penicillin G, 100 units/ml; streptomycin sulfate, 0.1 mg/ml; fungizone, 25 µg/ml; GIBCO) and an additional 2 mM L-glutamine (GIBCO). The selection medium used for the detection of $HGPRT^-$ mutants was Ham's F-12 nutrient mix without hypoxanthine, supplemented with 10 µM 6-thioguanine (GIBCO) and 5% serum and the above-mentioned antibiotics.

Properly maintained?	X	Yes		No
Periodically checked for Mycoplasma contamination?	X	Yes		No
Periodically checked for karyotype stability?	X	Yes		No
Periodically "cleansed" against high spontaneous background?	X	Yes		No

5.	**Locus Examined:**		Thymidine kinase (TK)	X	Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)		Na^+/K^+ ATPase
	Selection agent:		bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		ouabain
			fluorodeoxyuridine (FdU)	X	6-thioguanine (6-TG)		
			trifluorothymidine (TFT)				

6.	**Test compound concentrations used:**	
	Non-activated conditions:	Assay A1: 0 (solvent control), 15.625, 31.25, 62.5, 125, 250, 500, 1000, 1500, and 2000.µg/mL Assay B1: 0 (solvent control), 125, 250, 500, 1000, and 2000 µg/mL Assay C1: 0 (solvent control), 100, 300, 600, 1200, and 2000 µg/mL
	Activated conditions:	Assay A1: 0 (solvent control), 15.625, 31.25, 62.5, 125, 250, 500, 1000, 1500, and 2000.µg/mL Assay B1: 0 (solvent control), 125, 250, 500, 1000, and 2000 µg/mL Assay C1: 0 (solvent control), 100, 300, 600, 1200, and 2000 µg/mL

B. TEST PERFORMANCE

4. Cell treatment:

a. Cells were exposed to test compound, negative/solvent or positive controls for 4 hours (non-activated), 4 hours (activated).

b. After washing, cells were cultured for 8 days (expression period) before cell selection.

c. After expression, 2 x 10^5 cells/dish (10 dishes/group) were cultured for 9 days in selection medium to determine numbers of mutants and 200 cells/dish (3 dishes/group) were cultured for 7 days without selective agent to determine cloning efficiency.

2. Statistical Methods: The frequency of mutants per 10^6 clonable cells was statistically evaluated using a weighted analysis of variance; weights were derived from the inverse of the mutation frequency variance. The actual plate counts are assumed to follow a Poisson distribution, therefore the mean plate count was used as an estimate of variance.

If the analysis of variance was significant at alpha = 0.05, a Dunnett's t-test was conducted, comparing each treated group and the positive control to the negative control (alpha = 0.05, one-sided). An additional comparison of the positive control to the negative control (alpha = 0.05) was conducted using a linear contrast statement. Linear dose-related trend tests were performed if any of the pairwise comparisons of test material with the negative control yielded significant differences.

3. Evaluation Criteria: For an assay to be acceptable, the mutant frequency in positive controls should have been significantly higher than the solvent controls. An additional criteria, was that the mutant frequency in the solvent controls should have been within reasonable limits of the laboratory historical control values and literature values. The test chemical was considered positive if it induced a statistically significant, dose related, reproducible increase in mutant frequency. The final interpretation of the data took into consideration such factors as the mutant frequency and cloning efficiencies in the solvent controls.

II. RESULTS

G. Preliminary cytotoxicity

pH and Osmolality: The pH and osmolality of treatment medium containing approximately 1975 µg/ml of the test material and medium containing approximately 1% DMSO were determined using a Denver Basic pH meter (Denver Instrument Co., Arvada, Colorado) and an OSMETTE A freezing point osmometer (Precision Systems, Inc., Natick, Massachusetts). Alterations in the pH and osmolality of the culture medium have been shown to induce false positive responses in *in vitro* genotoxicity assays. There was no appreciable change in either the pH or osmolality at this concentration as compared to the culture medium with solvent alone (culture medium with the test material, pH = 7.41, osmolality = 490 mOsm/kgH_2O; culture medium with 1% DMSO, pH = 7.73, osmolality = 444 mOsm/kgH_2O).

Assay A1 – Preliminary Toxicity Assay: In a preliminary toxicity assay, the test material was assayed at concentrations of 0 (solvent control), 15.625, 31.25, 62.5, 125, 250, 500, 1000, 1500, and 2000 µg/ml in the absence and presence of an externally supplied metabolic activation system (S9). The highest concentration evaluated was based upon solubility limitations of the test material in the solvent. The test material also formed precipitate in the culture medium at this concentration. The treated cultures without S9 activation showed no signs of toxicity with relative cell survival (RCS) values ranging from 90.5 to 134.9%. In the presence of S9 activation, no toxicity was observed with RCS values ranging from 83.1 to 116.7%. Based upon the results of

this assay, concentration levels of 0 (solvent control), 125, 250, 500, 1000, and 2000 µg/ml of the test material were selected for the initial gene mutation assay in the absence and presence of S9.

H. Mutagenicity assay

Assay B1 – Initial Mutagenicity Assay: In the initial mutagenicity assay (Assay B1), in the absence and presence of S9, no toxicity was observed with RCS values ranging from 110.1 to 129.5% in the absence of S9 and 82.2 to 108.0% in the presence of S9. The mutant frequencies observed in all cultures treated with the test material were not significantly different from the concurrent solvent control values. All mutant frequencies were within the range of historical background values.

Assay C1 – Confirmatory Mutagenicity Assay: In a confirmatory assay (Assay C1), the concentrations ranged from 100 to 2000 µg/ml without and with S9. There were no signs of toxicity observed in the absence of S9 activation, as indicated by RCS values (92.6 to 114.0%). In the presence of S9, RCS values showed varying toxicity as measured by RCS values ranging from 75.6 to 103.4%. The mutant frequencies observed in cultures treated with the test material in the absence and presence of S9 were not significantly different from the concurrent solvent control values and were within the range of the laboratory historical background values.

In both the initial and confirmatory mutagenicity assays, the positive control chemicals induced significant increases in mutant frequencies and these data confirmed the adequacy of the experimental conditions for detecting induced mutations.

The analytically observed concentrations of the test material in the stock solutions in Assay B1 ranged from 76.6 to 107.6% of target. In Assay C1, the observed concentration of the test material in the stock solutions ranged from 107.3 to 110.0% of target.

Table 1. Results of Initial Mutagenicity Assay (assay B1)

	Initial Mutagenicity Assay (B1)			
Concentration	-S9		+S9	
µg/ml	%RCS	MF	%RCS	MF
Solvent Control	97.8	6.0	90.7	0.6
Solvent. Control	102.2	7.0	109.3	6.5
125	118.1	4.6	90.5	5.7
125	121.3	7.5	84.9	5.1
250	116.3	6.5	107.1	5.7
250	128.4	6.1	108.0	12.1
500	124.2	13.8	106.4	7.9
500	110.1	5.1	103.5	5.5
1000	124.2	1.2	96.0	5.0
1000	115.9	9.0	99.8	8.3
2000[a]	117.4	4.3	95.7	7.5
2000[a]	129.5	3.4	82.2	5.8
Pos. Control[b]	35.6	278.2*	79.4	261.4*
Pos. Control[b]	16.3	387.6*	69.9	250.9*
Pos. Control[c]	ND	ND	74.7	205.1*
Pos. Control[c]	ND	ND	73.7	191.1*

RCS = Relative Cell Survival
MF = TG^r Mutants per 10^6 clonable cells
ND = Not done
[a]Precipitate in treatment medium
[b]Positive control = 621 µg/ml EMS (-S9); 4 µg/ml 20-MCA (+S9)
[c]Positive control = 6 µg/ml 20-MCA (+S9)
*The frequency of TG^r mutants is significantly higher than the concurrent solvent control value.

Table 2. Results of Confirmatory Mutagenicity Assay (assay C1)

	Confirmatory Mutagenicity Assay (C1)			
Concentration	-S9		+S9	
µg/ml	%RCS	MF	%RCS	MF
Solvent Control	105.2	11.1	99.4	7.5
Solvent. Control	94.8	7.1	100.9	7.5
100	94.0	5.7	101.9	7.9
100	100.4	7.3	95.7	7.3
300	92.6	3.3	103.4	1.3
300	94.0	3.0	101.9	7.0
600	103.3	7.1	98.8	9.4
600	107.2	8.1	96.3	2.1
1200	95.3	7.6	93.0	3.9
1200	114.0	21.1	94.9	4.8
2000[a]	93.6	13.8	75.6	10.0
2000[a]	113.0	19.1	95.7	1.5
Pos. Control[b]	29.5	382.1*	92.2	188.5*
Pos. Control[b]	29.9	228.0*	96.3	157.4*
Pos. Control[c]	ND	ND	80.6	180.7*
Pos. Control[c]	ND	ND	83.1	112.0*

RCS = Relative Cell Survival
MF = TG^r Mutants per 10^6 clonable cells
ND = Not done
[a]Precipitate in treatment medium
[b]Positive control = 621 µg/ml EMS (-S9); 4 µg/ml 20-MCA (+S9)
[c]Positive control = 6 µg/ml 20-MCA (+S9)
*The frequency of TG^r mutants is significantly higher than the concurrent solvent control value.

Conclusions

Based upon the frequency of TG^r mutants recovered in cultures treated with the test material, it was concluded that X689643 (5-OH-XDE-638) was not mutagenic in the CHO/HGPRT gene mutation assay.

B.6.7.1.1c *In Vitro* genotoxicity testing (Reference IIA5.7.1.1c/01)

STUDY TYPE: *In vitro* mammalian cytogenetics OPPTS 870.5375; OECD 473

TEST MATERIAL (PURITY): X689643 (Benzenesulfonamide,2-(2,2-difluoroethoxy)-N-[6-[[2-(2,2-difluoroethoxy)-6-(trifluoromethyl)phenyl]sulfonyl]-5,6-dihydro-8-methoxy-5-oxo-[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl)) (99%)

SYNONYMS: 5-OH metabolite of XDE-638, DES ME-638, (5-Hydroxy-XDE-638)

CITATION: M. R. Schisler and K. M. Kleinert, (2006). EVALUATION OF X689643 (5-OH METABOLITE OF PENOXSULAM) IN AN *IN VITRO* CHROMOSOMAL ABERRATION ASSAY UTILIZING RAT LYMPHOCYTES. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, 48674. Study ID: 061092 (November 8, 2006). Unpublished

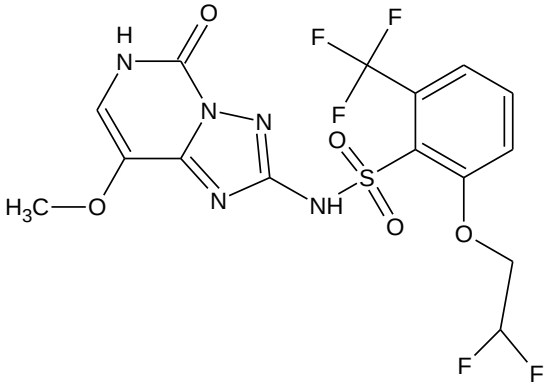
SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

SUMMARY: X689643 (benzenesulfonamide,2-(2,2-difluoroethoxy)-N-[6-[[2-(2,2-difluoroethoxy)-6-(trifluoromethyl)phenyl]sulfonyl]-5,6-dihydro-8-methoxy-5-oxo-[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl)), the 5-OH metabolite of penoxsulam, was evaluated in an *in vitro* chromosomal aberration assay utilizing rat lymphocytes. Approximately 48 hours after the initiation of whole blood cultures, cells were treated either in the absence or presence of S9 activation with concentrations ranging from 0 (solvent control) to 2000 μg X689643 per ml of culture medium. The duration of treatment was 4 or 24 hours without S9 and 4 hours with S9. The highest concentration was based on the solubility limitation of the test material in the solvent. Based upon the mitotic indices, cultures treated for 4 hours with targeted concentrations of 0 (solvent control), 500, 1500, and 2000 μg/ml in the absence and 0 (solvent control), 500, 1000, and 2000 μg/ml in the presence of S9 activation were selected for determining the incidence of chromosomal aberrations. In cultures treated for 24 hours in the absence of metabolic activation, concentrations of 0 (solvent control), 500, 1000, and 2000 μg/ml were selected for determining the incidence of chromosomal aberrations. There were no significant increases in the frequencies of cells with aberrations in either the presence or absence of S9 activation. Cultures treated with the positive control chemicals (*i.e.*, mitomycin C without S9 and cyclophosphamide with S9) had significantly higher incidences of abnormal cells in all assays. Based upon these results, X689643, the 5-OH metabolite of penoxsulam, was considered to be non-genotoxic in this *in vitro* chromosomal aberration assay utilizing rat lymphocytes.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	X689643 (5-OH metabolite of penoxsulam)
	Description:	White solid
	Lot/Batch #:	lot # 200000221-139A, TSN102471
	Purity:	The purity of the test material was determined to be 99% by liquid chromatography with ultra violet detection and identification by infrared spectroscopy.
	CAS #:	Not applicable
	Chemical Structure:	
	Solvent Used:	Dimethyl sulfoxide (DMSO)

2.	**Control Materials:**	
	Solvent Control (final concentration):	Dimethyl sulfoxide (DMSO) 1%
	Positive Control:	Non-activation: Mitomycin C (MMC, Sigma, CAS No. 50-07-7), 0.5 µg/ml (4 hour) or 0.05 and 0.075 µg/ml (24 hour) Activation: Cyclophosphamide monohydrate (CP, Sigma, CAS No. 6055-19-2), 4 and 6 µg/ml

3.	**Activation**: S9 derived from							
	X	Induced	X	Aroclor 1254	X	Rat	X	Liver
		Not-induced		Phenobarbitol		Mouse		Lung
				None		Hamster		Other (name)
				Other (name)		Other (name)		

The S9 mix consisted of 10 mM $MgCl_2 \cdot 6H_2O$ (Sigma), 5 mM glucose-6-phosphate (Sigma), 4mM nicotinamide adenine dinucleotide phosphate (Sigma), 10mM $CaCl_2$ (Fisher, Fair Lawn, New Jersey), 30 mM KCl (Sigma), and 50 mM sodium phosphate (pH 8.0, Sigma and Fisher). The reconstituted mix was added to the culture medium to obtain the desired final

concentration of S9 in the culture, *i.e.*, 2% v/v. Hence, the final concentration of the co-factors in the culture medium was 1/5 of the concentrations stated above.

4.	**Test cells:** Peripheral lymphocytes male CD ISG (Outbred Crl:CD (SD)) rats			
Media: RPMI 1640 medium (with 25 mM HEPES, GIBCO, Grand Island, New York) supplemented with 10% heat-inactivated dialyzed fetal bovine serum (GIBCO), antibiotics and antimycotics (Fungizone 0.25 µg/ml; penicillin G, 100 U/ml; and streptomycin sulfate, 0.1 mg/ml; GIBCO), 30 µg/ml PHA (HA16, Murex Diagnostics Ltd., Dartford, England), and an additional 2 mM L-glutamine (GIBCO).				
Properly maintained?	X	Yes		No
Periodically checked for *Mycoplasma* contamination?		Yes	X	No
Periodically checked for karyotype stability?	X	Yes		No

5.	**Test compound concentrations used:**	
	Non-activated conditions:	Assay A1 – 4 hours: 0 (solvent control), 62.5, 125, 250, 500, 1000, 1500, and 2000 µg/ml Assay A1 – 24 hours: 0 (solvent control), 31.25, 62.5, 125, 250, 500, 1000, 1500, and 2000 µg/ml
	Activated conditions:	Assay A1 – 4 hours: 0 (solvent control), 62.5, 125, 250, 500, 1000, 1500, and 2000 µg/ml

E. TEST PERFORMANCE

1. Preliminary Cytotoxicity Assay: Not performed

2. Cytogenetic Assay:

a.	**Cell exposure time:**	Test Material	Solvent Control	Positive Control
	Non-activated: Activated:	4 h & 24 h 4h	4 h & 24 h 4h	4 h & 24 h 4h

b.	**Spindle inhibition**	
	Inhibition used/concentration:	Colcemid (1µg/culture)
	Administration time:	2-3 hours (before cell harvest)

c.	**Cell harvest time after termination of treatment:**	Test Material	Solvent Control	Positive Control
	Non-activated: Activated:	0 and 20h 20h	0 and 20h 20h	0 and 20h 20h

d. Details of slide preparation: The cells were swollen by hypotonic treatment (0.075 M KCl), fixed with methanol:acetic acid (3:1), dropped on microscope slides, and stained in Giemsa.

e. Metaphase analysis:

No. of cells examined per dose:				
Scored for structural?	X	Yes		No
Scored for numerical?	X	Yes (polyploidy)		No
Coded prior to analysis?	X	Yes		No

f. Evaluation criteria: For a test to be acceptable, the chromosomal aberration frequency in the positive control cultures should be significantly higher than the solvent controls. The aberration frequency in the solvent control should be within reasonable limits of the laboratory historical values. A test chemical is considered positive in this assay if it induces a significant dose-related and reproducible increase in the frequency of cells with aberrations.

g. Statistical analysis: The proportions of cells with aberrations (excluding gaps) were compared by the following statistical methods. At each dose level, data from the replicates was pooled. A two-way contingency table was constructed to analyze the frequencies of aberrant cells. An overall Chi-square statistic, based on the table, was partitioned into components of interest. Specifically, statistics were generated to test the global hypotheses: 1) no difference in the average number of cells with aberrations among the dose groups, and 2) no linear trend of increasing number of cells with aberrations with increasing dose. An ordinal metric (0, 1, 2, etc.) was used for the doses in the statistical evaluation. If either statistic was found to be significant at alpha = 0.05, versus a one-sided increasing alternative, pairwise tests (*i.e.* control vs. treatment) were performed at each dose level and evaluated at alpha = 0.05, again versus a one-sided alternative.

Polyploid cells were analyzed by the Fisher Exact probability test. The number of polyploid cells were pooled across replicates for the analysis and evaluated at alpha = 0.05. The data was analyzed separately based on the presence or absence of S-9 and based on the exposure time.

III. RESULTS

pH and Osmolality

The pH and osmolality of the treatment medium containing approximately 1975 µg/ml of the test material and medium containing approximately 1% solvent (DMSO) was determined using a Denver Basic pH meter (Denver Instrument Co., Arvada, Colorado) and an OSMETTE A freezing point osmometer (Precision Systems, Inc., Natick, Massachusetts), respectively. There was no appreciable change in either the pH or osmolality at this concentration as compared to the culture medium with solvent alone (culture medium with the test material, pH = 7.03, osmolality = 450 mOsm/kg H_20; culture medium with 1% DMSO, pH = 7.24, osmolality = 467 mOsm/kg H_20).

Assay A1

In the initial assay, cultures were treated with the test material in the absence and presence of S9 activation for 4 hours at concentrations of 0 (solvent control), 62.5, 125, 250, 500, 1000, 1500 and 2000 µg/ml. Cultures were also treated continuously for 24 hours in the absence of S9 with the above concentrations plus an additional lower concentration of 31.25 µg/ml. The highest concentration evaluated was based upon solubility limitations of the test material in the solvent. The test material also formed a precipitate in the culture medium at this concentration. The analytically detected concentrations of the test material in the stock solutions (Assay A1) varied from 100.7 to 110.9% of the target.

Short Treatment

Without and with metabolic activation (4 hour treatment), the cultures displayed varying levels of toxicity as measured by mitotic indices. In the absence of S9, the mitotic indices for the treated cultures ranged from 66.7 to 91.5% relative to the solvent control values. In the presence of S9, the mitotic indices of the treated cultures ranged from 69.5 to 98.1% as compared to the solvent control values. Based upon these results, cultures treated with the top three concentrations (*i.e.*, 500, 1500 and 2000 µg/ml) in the absence of metabolic activation and 500, 1000 and 2000 µg/ml in the presence of metabolic activation were chosen for the determination of chromosomal aberration frequencies and incidence of polyploidy.

Among the cultures treated with the positive control chemicals for 4 hour, 0.5 µg/ml of MMC and 4 µg/ml of CP were selected for evaluation of aberrations in the absence and presence of S9, respectively.

There were no significant increases in the incidence of polyploidy cells in any of the test material treated cultures as compared to the solvent control values.

In the 4 hour non-activation assay, the frequency of cells with aberrations in the solvent control was 0.5% and the corresponding values at treatment levels of 500, 1500, and 2000 µg/ml were 1.0, 0.5, and 1.0%, respectively. In the activation assay, cultures treated with the test material at concentrations of 500, 1000, and 2000 µg/ml had aberrant cell frequencies of 2.0, 1.0 and 1.0%, respectively as compared to the solvent control value of 2.0%. Statistical analyses of these data did not identify significant differences between the solvent control and any of the treated cultures without or with S9 activation. The frequencies of aberrant cells observed in the test material treated cultures were within the laboratory historical background range.

Significant increases in the frequency of cells with aberrations were observed in cultures treated with the positive control chemicals. Aberrant cell frequencies in MMC (- S9, 4 hour treatment), and CP (+ S9) cultures were 27%, and 31%, respectively.

Continuous Treatment

Upon completion of the slide evaluation for the 4 hour treated cultures, and after concluding that the results were negative, slides from the continuous 24 hour treatment were evaluated. Varying signs of toxicity were observed in the treated cultures as determined by relative mitotic indices ranging from 56.3 to 89.3%. Based upon these results, 0 (solvent control), 500, 1000 and 2000 µg/ml were chosen for the determination of chromosomal aberration frequencies and incidence of polyploidy in the non-activation assay treated continuously for 24 hours. Cultures treated with 0.05 µg/ml MMC were selected for evaluation to serve as the positive control for the 24 hour assay in the absence of metabolic activation.

There were no significant increases in the incidence of polyploidy cells in any of the test material treated cultures as compared to the solvent control values.

The frequencies of aberrant cells in the solvent control was 2.5% and the corresponding values at concentration levels of 500, 1000, and 2000 µg/ml were 1.5, 2.5, and 2.0%, respectively. There were no statistically significant differences between the test material treated cultures and the solvent control values and all values were within the laboratory historical background range.

Significant increases in the frequency of cells with aberrations were observed in cultures treated with the positive control chemical. Aberrant cell frequency in MMC treated cultures was 29%.

A second assay with treatment of cultures in the presence of S9 was not considered necessary since the results of the initial test were clearly negative.

Table 1. Mitotic Indices and Polypoidy Incidence in Rat Lymphocyte Cultures

	Mean mitotic index [a]			Incidence of polyploidy (%)		
	Assay A1			Assay A1		
Conc. µg/ml	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)	-S9 (4 hr)	+S9 (4 hr)	-S9 (24hr)
Solvent control	11.7 (100.0)	10.5 (100.0)	10.3 (100.0)	0.0	0.5	0.5
31.25	ND	ND	9.2 (89.3)	ND	ND	ND
62.5	10.4 (88.9)	10.0 (95.2)	8.2 (79.6)	ND	ND	ND
125	10.5 (89.7)	10.3 (98.1)	7.4 (71.8)	ND	ND	ND
250	10.7 (91.5)	9.0 (85.7)	6.7 (65.0)	ND	ND	ND
500	10.6 (90.6)	9.1 (86.7)	7.4 (71.8)	0.5	0.5	0.5
1000	10.2 (87.2)	8.8 (83.8)	5.8 (56.3)	ND	0.5	0.0
1500	8.6 (73.5)	7.3 (69.5)	5.9 (57.3)	0.0	ND	ND
2000[b]	7.8 (66.7)	7.4 (70.5)	6.5 (63.1)	0.5	0.0	0.5
Positive control [c]	5.2 (44.4)	3.1 (29.5)	5.3 (51.5)	0.0	0.0	0.0
Positive control [d]	ND	0.9 (8.6)	3.6 (35.0)	ND	ND	ND

[a] values in parenthesis are % relative mitotic index
[b] Precipitation in culture medium
[c] Positive control = 0.5 µg/ml MMC (-S9, 4 hr); 4 µg/ml CP (+S9, 4 hr); 0.05 µg/ml MMS (-S9, 24 hr)
[d] Positive control = 6 µg/ml CP (+S9, 4hr); 0.075 µg/ml MMS (-S9, 24 hr)
ND = not done

Table 2. Results of the Cytogenetic Assay

	Total aberrations (excluding gaps)			No. of cells with aberrations (excluding gaps) [a]		
	Assay A1			Assay A1		
Conc. µg/ml	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)
Solvent control	1	3	5	0.5	2.0	2.5
500	2	4	3	1.0	2.0	1.5
1000	ND	2	5	ND	1.0	2.5
1500	1	ND	ND	0.5	ND	ND
2000[b]	2	2	3	1.0	1.0	2.0
Positive control [c]	23	26	30	27*	31*	29*

[a] Values are percentages
[b] Precipitation in culture medium
[c] Positive control = 0.5 µg/ml MMC (-S9, 4 hr); 4 µg/ml CP (+S9, 4 hr); 0.05 µg/ml MMC (-S9, 24 hr)
*Significantly different from solvent controls, $\alpha = 0.05$.
ND = not done

It was concluded that under the experimental conditions used, X689643, the 5-OH metabolite of penoxsulam, was non-genotoxic in this *in vitro* chromosomal aberration test.

B.6.7.1.2 Mutagenicity studies on BSTCA

B.6.7.1.2a *In Vitro* genotoxicity testing (Reference IIA5.7.1.2a/01)

STUDY TYPE: *(Bacterial system, Salmonella typhimurium; Escherichia coli)*/ mammalian activation gene mutation assay; OPPTS 870.5100[5]; OECD 471 (formerly OECD 471 & 472).

TEST MATERIAL (PURITY): X768359 (5-[2,2-difluoroethoxy)-6-trifluoromethyl-benzensulfonylamino]-1H-1,2,4-triazole-3-carboxylate) (99%TEA salt, 80% acid equivalent)

SYNONYMS: BSTCA metabolite of penoxsulam,

CITATION: M. S. Mecchi (29 August 2006). *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay Preincubation Method with a Confirmatory Assay with X768359 (BSTCA Metabolite of Penoxsulam). Covance Laboratories Inc., Vienna, Virginia. Study ID: 6736-159, 06 March 2006 – 29 August 2006. Unpublished.

SPONSOR: The Dow Chemical Company

SUMMARY:

In a reverse gene mutation assay in bacteria, tester strains TA98, TA100, TA1535, and TA1537 of *S. typhimurium* and tester strain WP2*uvr*A of *E. coli* were exposed to X768359 (BSTCA metabolite of penoxsulam) (99% TEA salt, 80% acid equivalent), in dimethylsulfoxide using the preincubation method at concentrations of 100, 333, 1000, 3330, and 5000 µg per plate in the presence and absence of mammalian metabolic activation.

X768359 (BSTCA Metabolite of Penoxsulam) was tested up to the limit concentration (5000 µg per plate) with all strains. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as acceptable. This study satisfies the requirement for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

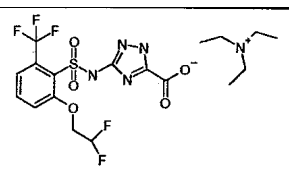
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	X768359 (BSTCA Metabolite of Penoxsulam)
	Description:	White powder
	Lot #:	035298-79
	Purity:	99% TEA salt, 80% acid equivalent.
	CAS #:	not provided

[5]870.5100 - Reverse mutation *E. coli* WP2 and WP2uvrA; *S. typhimurium* TA 97, TA98, TA100, TA1535, TA1537
870.5140 - Gene mutation *Aspergillus nidulans*
870.5250 - Gene mutation *Neurospora crassa*

		STRUCTURE: 
	Solvent Used:	Dimethylsulfoxide

2.	**Control Materials:**	
	Negative:	NA
	Solvent (final conc'n):	Dimethylsulfoxide at 50 µL per plate
	Positive:	Non-activation: sodium azide 2.0 µg/plate TA100, TA1535 2-nitrofluorene 1.0 µg/plate TA98 ICR-191 2.0 µg/plate TA1537 4-nitroquinoline-N-oxide 0.4 µg/plate WP2*uvr*A
		Activation: benzo[a]pyrene 2.5 µg/plate TA98 2-aminoanthracene 2.5 µg/plate TA100, TA1535, and TA1537 2-aminoanthracene 25.0 µg/plate WP2*uvr*A

3.	**Activation**: S9 derived from								
	x	induced		x	Aroclor 1254	x	Rat	x	Liver
		non-induced			Phenobarbitol		Mouse		Lung
					None		Hamster		Other (name)
					Other (name)		Other (name)		

S9 mix composition: H_2O (70%), 1M NaH_2PO_4/Na_2HPO_4, pH 7.4 (10%), 0.25M Glucose-6-phosphate (2%), 0.10M NADP (4%), 0.825M KCl/0.2M $MgCl_2$ (4%), and S9 Homogenate (10%).

4.	**Test organisms:** *S. typhimurium* strains									
		TA97	x	TA98	x	TA100		TA102		TA104
	x	TA1535	x	TA1537		TA1538	x	WP2*uvr*A (*E. coli*)		
Properly maintained?							x	Yes		No
Checked for appropriate genetic markers (*rfa* mutation, R factor)?							x	Yes		No

5. Test compound concentrations used:
Non-activated conditions:

Cytotoxicity test: TA100 and WP2*uvr*A at 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, and 5000 µg per plate; one plate per concentration

Mutagenicity assay: TA98, TA100, TA1535, TA1537, and WP2*uvr*A at 100, 333, 1000, 3330, and 5000 µg per plate; three replicates.

Activated conditions:

- Cytotoxicity test: TA100 and WP2*uvr*A at 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, and 5000 µg per plate; one plate per concentration.
- Mutagenicity assay: TA98, TA100, TA1535, TA1537, and WP2*uvr*A at 100, 333, 1000, 3330, and 5000 µg per plate; three replicates.

B. TEST PERFORMANCE

1. Type of *Salmonella* assay: pre-incubation (20 minutes)

2. Protocol:

Tester strains were exposed to the test article via the preincubation modification of the Ames Test originally described by Yahagi *et al.* (1975) and Maron and Ames (1983). This methodology has been shown to detect a wide range of classes of chemical mutagens. In the preincubation methodology, S9 mix (or phosphate buffer, where appropriate), tester strain, and test article were preincubated for approximately 20 minutes prior to addition of molten agar. The agar and preincubation reaction mixture were mixed and then overlaid onto a minimal agar plate. Following incubation, revertant colonies were counted. Test article, vehicle controls, and positive controls were plated in triplicate. The condition of the bacterial background lawn was evaluated both macroscopically and microscopically (using a dissecting microscope) for indications of cytotoxicity and test article precipitate. Evidence of cytotoxicity was scored relative to the vehicle control plate and was recorded along with the revertant counts for all plates at that concentration. Revertant colonies were counted either by automated colony counter or by hand.

3. Statistical Analysis: Mean and standard deviation were calculated for each replicate.

4. Evaluation Criteria: Before assay data were evaluated, criteria for a valid assay had to be met. The following criteria were used to determine a valid assay:

Salmonella typhimurium tester strain cultures exhibited sensitivity to crystal violet to demonstrate the presence of the *rfa* wall mutation. Cultures of tester strains TA98 and TA100 exhibited resistance to ampicillin to demonstrate the presence of the pKM101 plasmid.

Demonstrating the requirement for histidine (*Salmonella typhimurium*) or tryptophan (*Escherichia coli*), tester strain cultures exhibited a characteristic number of spontaneous revertants per plate when plated along with the vehicle under selective conditions. Acceptable ranges for mean vehicle controls were as follows:

TA98	8	-	60
TA100	60	-	240
TA1535	4	-	45
TA1537	2	-	25
WP2*uvr*A	5	-	40

Demonstrating that appropriate numbers of bacteria were plated, density of tester strain cultures were greater than or equal to 1.0 x 10^9 bacteria per mL and/or had reached a target density demonstrated to produce cultures with at least 1.0 x 10^9 bacteria per mL.

Demonstrating that tester strains were capable of identifying a mutagen, the mean value of a positive control for a respective tester strain exhibited at least a 3-fold increase over the mean value of the vehicle control for that strain. An acceptable positive control in the presence of S9 mix for a specific strain was evaluated as having demonstrated both integrity of the S9 mix and ability of the tester strain to detect a mutagen.

A minimum of three non-toxic concentrations was required to evaluate assay data. Cytotoxicity was detectable as a decrease in the number of revertant colonies per plate and/or by a thinning or disappearance of the bacterial background lawn. Thinning of the bacterial background lawn not accompanied by a reduction in the number of revertants per plate was not evaluated as an indication of cytotoxicity.

Once criteria for a valid assay had been met, responses observed in the assay were evaluated.

For a test article to be considered positive, it had to produce at least a 2-fold (TA100) or 3-fold (TA98, TA1535, TA1537, and WP2*uvr*A) concentration-related and reproducible increase in the mean revertants per plate over the mean revertants per plate of the appropriate vehicle control. A response that did not meet all three of the above criteria (magnitude, concentration-responsiveness, reproducibility) was not evaluated as positive.

II.RESULTS

A. <u>Preliminary cytotoxicity assay</u>

Ten concentrations of test article, from 6.67 to 5000 □ g per plate, were tested in Trials 28134-A1 with tester strains TA100 and WP2*uvr*A. No cytotoxicity was observed with either tester strain in the presence or absence of S9 mix as evidenced by no decreases in the number of revertants per plate and normal bacterial background lawns.

B. <u>Mutagenicity assay</u>

The data for the mutagenicity assay were generated in Trials 28134-B1 28134-C1, and 28134-D1.

The tester strains used in the preincubation mutagenicity assay were *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* tester strain WP2*uvr*A.

The assay was conducted with five concentrations of the test article (100, 333, 1000, 3330, and 5000 □ g per plate) in the presence and absence of S9 mix along with concurrent vehicle and positive controls using three plates per concentration.

In the initial mutagenicity assay (Trial 28134-B1), all data were acceptable and no positive increases in the mean number of the revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix. In the initial mutagenicity assay, no valid data were generated for tester strain TA1537 due to unacceptable mean vehicle control values in the presence and absence of S9 mix. For this reason, the test article was re-tested with TA1537 in the presence and absence of S9 mix in Trial 28134-D1.

In the confirmatory mutagenicity assay (Trial 28134-C1), all data were acceptable and no positive increases in the mean number of the revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix.

In the repeat mutagenicity assay, (Trial 28134-D1), all data were acceptable and no positive increases in the mean number of revertants per plate were observed with TA1537 in either the presence or

absence of S9 mix.

III. CONCLUSION

Under the conditions of this study, X768359 (BSTCA metabolite of penoxsulam) did not show evidence of mutagenic potential

B.6.7.1.2b *In Vitro* genotoxicity testing (IIA5.7.1.2b/01)

STUDY TYPE: Mammalian cells in culture gene assay in CHO-K_1-BH_4 cells; USEPA OPPTS 870.5300; OECD Guideline 476; EC, B.17.

TEST MATERIAL (PURITY): X768359 (5-[2,2-difluoroethoxy)-6-trifluoromethyl-benzensulfonylamino]-1H-1,2,4-triazole-3-carboxylate) (99%)

SYNONYMS: BSTCA metabolite of penoxsulam

CITATION: S. D. Seidel, M. R. Schisler, and K. M. Kleinert. (August 16, 2006). EVALUATION OF X768359 (BSTCA METABOLITE OF PENOXSULAM) IN THE CHINESE HAMSTER OVARY CELL/HYPOXANTHINE-GUANINE-PHOSPHORIBOSYL TRANSFERASE (CHO/HGPRT) FORWARD MUTATION ASSAY. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, 48674. Study ID: 061037, (August 16, 2006). Unpublished

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

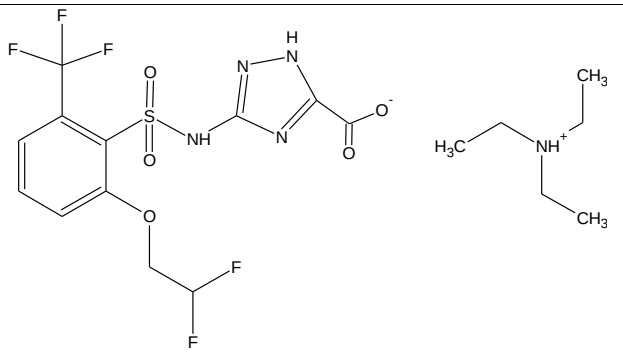
SUMMARY: X768359 (5-[2,2-difluoroethoxy)-6-trifluoromethyl-benzensulfonylamino]-1H-1,2,4-triazole-3-carboxylate), the BSTCA metabolite of penoxsulam, was evaluated in the *in vitro* Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward gene mutation assay. The genotoxic potential of the test material was assessed in two independent assays in the absence and presence of an externally supplied metabolic activation (S9) system with concentrations ranging from 216.25 to 5175 μg/ml. The highest concentration was based on the limit dose of 10 mM for this assay system. The adequacy of the experimental conditions for detection of induced mutations was confirmed by employing positive control chemicals, ethyl methanesulfonate for assays without S9 and 20-methylcholanthrene for assays with S9. Solvent control cultures were treated with the solvent used to dissolve the test material (*i.e.* dimethyl sulfoxide). Based upon the frequency of TG^r mutants recovered in cultures treated with the test material, it was concluded that X768359, the BSTCA metabolite of penoxsulam, was non-mutagenic in the assay system employed.

This study is acceptable and satisfies the guideline requirement for an Mammalian cells in culture gene assay in CHO-K_1-BH_4 cells; USEPA OPPTS 870.5300; OECD Guideline 476; EC, B.17.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	X768359 (BSTCA metabolite of penoxsulam)
	Description:	White, solid
	Lot/Batch #:	035298-79, TSN105480
	Purity:	The BSTCA metabolite was isolated and provided as the triethylamine salt. The purity of the BSTCA metabolite of penoxsulam TEA salt was determined to be 99% (80% acid equivalent) as measured by liquid chromatography with ultraviolet detection. Structural identify was confirmed by infrared spectroscopy, mass spectroscopy, along with proton and carbon-13 nuclear magnetic resonance.
	CAS #:	N/A
	Chemical Structure:	
	Solvent Used:	Dimethyl sulfoxide (DMSO, Sigma, St. Louis, Missouri)

2.	**Control Materials:**	
	Negative control	(e.g. 1% DMSO)
	Solvent control (final conc'n):	Dimethyl sulfoxide (DMSO) 1%
	Positive control:	Nonactivation: Ethyl methanesulfonate (EMS, CAS No. 62-50-0), 621 μg/ml
		Activation: 20-methylcholanthrene (20-MCA, CAS No. 56-49-5), 4, 6 and 8 μg/ml

3.	**Activation**: S9 derived from									
		X	induced		X	Aroclor 1254	X	Rat	X	Liver
			non-induced			Phenobarbitol		Mouse		Lung
						None		Hamster		Other

					Other		Other		

The S9 mix consisted of the following co-factors: 10 mM $MgCl_2 \cdot 6H_2O$, 5 mM glucose-6-phosphate, 4 mM nicotinamide adenine dinucleotide phosphate, 10 mM $CaCl_2$, 30 mM KCl, and 50 mM sodium phosphate (pH 8.0). The reconstituted mix was added to the culture medium to obtain the desired final concentration of S9 in the culture, *i.e.*, 2% v/v. Hence, the final concentration of the co-factors in the culture medium was 1/5th of the concentrations stated above.

4.	**Test cells:** mammalian cells in culture				
			mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
		X	Chinese hamster ovary (CHO) cells		

Media: The cells were routinely maintained in Ham's F-12 nutrient mix (GIBCO, Grand Island, New York) supplemented with 5% (v/v) heat-inactivated (56°C, 30 minutes), dialyzed fetal bovine serum (GIBCO), antibiotics and antimycotics (penicillin G, 100 units/ml; streptomycin sulfate, 0.1 mg/ml; fungizone, 25 µg/ml; GIBCO) and an additional 2 mM L-glutamine (GIBCO). The selection medium used for the detection of $HGPRT^-$ mutants was Ham's F-12 nutrient mix without hypoxanthine, supplemented with 10 µM 6-thioguanine (GIBCO) and 5% serum and the above-mentioned antibiotics.

Properly maintained?	X	Yes		No
Periodically checked for Mycoplasma contamination?	X	Yes		No
Periodically checked for karyotype stability?	X	Yes		No
Periodically "cleansed" against high spontaneous background?	X	Yes		No

5.	**Locus Examined:**		Thymidine kinase (TK)	X	Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)		Na^+/K^+ ATPase
	Selection agent:		bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		ouabain
			fluorodeoxyuridine (FdU)	X	6-thioguanine (6-TG)		
			trifluorothymidine (TFT)				

6.	**Test compound concentrations used**	
	Nonactivated conditions:	Assay A1 0, 13.5, 27, 54.1, 108.13, 216.25, 432.5, 865, 1730 and 3460 µg/mL Assay B1 0, 216.25, 432.5, 865, 1730, and 3460 µg/mL Assay C1 0, 1000, 2000, 3000, 4000, and 5175 µg/mL

	Activated conditions:	Assay A1 0, 13.5, 27, 54.1, 108.13, 216.25, 432.5, 865, 1730 and 3460 μg/mL Assay B1 0, 216.25, 432.5, 865, 1730, and 3460 μg/mL Assay C1 0, 1000, 2000, 3000, 4000, and 5175 μg/mL

B. TEST PERFORMANCE

5. Cell treatment:

a. Cells were exposed to test compound, negative/solvent or positive controls for 4 hours (non-activated), 4 hours (activated).

b. After washing, cells were cultured for 8 days (expression period) before cell selection.

c. After expression, 2 x 10^5 cells/dish (10 dishes/group) were cultured for 9 days in selection medium to determine numbers of mutants and 200 cells/dish (3 dishes/group) were cultured for 7 days without selective agent to determine cloning efficiency.

2. Statistical Methods: The frequency of mutants per 10^6 clonable cells was statistically evaluated using a weighted analysis of variance; weights were derived from the inverse of the mutation frequency variance. The actual plate counts are assumed to follow a Poisson distribution therefore the mean plate count was used as an estimate of variance.

If the analysis of variance was significant at alpha = 0.05, a Dunnett's t-test was conducted, comparing each treated group and the positive control to the negative control (alpha = 0.05, one-sided). An additional comparison of the positive control to the negative control (alpha = 0.05) was conducted using a linear contrast statement. Linear dose-related trend tests were performed if any of the pairwise comparisons of test material with the negative control yielded significant differences.

3. Evaluation Criteria: For an assay to be acceptable, the mutant frequency in positive controls should have been significantly higher than the solvent controls. An additional criteria, was that the mutant frequency in the solvent controls should have been within reasonable limits of the laboratory historical control values and literature values. The test chemical was considered positive if it induced a statistically significant, dose-related, reproducible increase in mutant frequency. The final interpretation of the data took into consideration such factors as the mutant frequency and cloning efficiencies in the solvent controls.

II. RESULTS

I. Preliminary cytotoxicity

pH and Osmolality

The pH and osmolality of treatment medium containing approximately 5140 μg/ml of the test material (limit dose of 10 mM) and medium containing 1% DMSO were determined using a Denver Basic pH meter (Denver Instrument Co., Arvada, Colorado) and an OSMETTE A freezing point osmometer (Precision Systems, Inc., Natick, Massachusetts). Alterations in the pH and osmolality of the culture medium have been shown to induce false positive responses in *in vitro* genotoxicity assays. There was no appreciable change in pH at this concentration as compared to the culture medium with solvent alone; however, there was an appreciable change in osmolality. When the osmolality was compared to culture medium alone, there was no appreciable change and therefore, the change in osmolality was deemed inconsequential to the assay (culture medium with the test material, pH = 7.61, osmolality = 371 mOsm/kgH_2O; culture medium

with 1% DMSO, pH = 7.70, osmolality = 452 mOsm/kgH_2O; culture medium alone, pH 7.69, osmolality = 324 mOsm/kgH_2O).

Assay A1 – Preliminary Toxicity Assay

In a preliminary toxicity assay, the test material was assayed at concentrations of 0 (solvent control), 13.5, 27, 54.1, 108.13, 216.25, 432.5, 865, 1730 and 3460 μg/ml in the absence and presence of an externally supplied metabolic activation system (S9). The highest concentration tested was based upon the initial solubility determination of the test material in the culture medium. In the absence of S9 activation, little to no toxicity was observed, with the relative cell survival (RCS) values ranging from 86.5 to 116.7%. However, in the highest concentration (*i.e.* 3460 μg/ml) the cells were inadvertently not plated due to a technical error. In the presence of S9 activation, all treated cultures showed little to no toxicity with RCS values ranging from 92.0 to 111.7%. Based upon the results of this assay, concentration levels of 0, 216.25, 432.5, 865, 1730, and 3460 μg/ml of the test material were selected for the initial gene mutation assay in the absence and presence of S9.

J. Mutagenicity assay

Assay B1 – Initial Mutagenicity Assay

In the initial mutagenicity assay (Assay B1), in the absence and presence of S9, little to no toxicity was observed based on RCS values. These values ranged from 82.7 to 118.7% in the absence of S9 and 88.2 to 104.1% in the presence of S9. The mutant frequencies observed in cultures treated with the test material in the absence and presence of S9 were not statistically different from the concurrent solvent control values and all mutant frequencies were within a reasonable range of historical background values.

Table 5.7.1.2-1: Results of Initial Mutagenicity Assay (assay B1)

	Initial Mutagenicity Assay (B1)			
Concentration	-S9		+S9	
μg/ml	%RCS	MF	%RCS	MF
Solvent Control	101.8	4.5	93.9	2.7
Solvent. Control	98.2	7.3	106.1	6.4
216.25	92.2	2.1	100.0	9.5
216.25	118.7	3.1	103.3	6.3
432.5	109.3	6.4	98.0	-[a]
432.5	100.5	12.4	100.6	4.4
865	82.7	9.1	88.2	5.7
865	90.2	14.2	90.0	8.3
1730	92.0	3.6	98.4	10.0
1730	90.0	19.0	95.7	3.4
3460	87.0	7.3	104.1	5.8
3460	90.6	4.2	89.2	11.3
Pos. Control[b]	36.9	428.8*	92.5	132.0*
Pos. Control[b]	36.2	395.5*	92.7	55.6*
Pos. Control[c]	ND	ND	101.2	92.2*
Pos. Control[c]	ND	ND	91.0	51.6*

RCS = Relative Cell Survival
MF = TG^r Mutants per 10^6 clonable cells
ND = Not done
[a]lost due to contamination
[b]Positive control = 621 μg/ml EMS (-S9); 4 μg/ml 20-MCA (+S9)
[c]Positive control = new lot of 20-MCA at 4 μg/ml (+S9)
*The frequency of TG^r mutants is significantly higher than the concurrent solvent control value.

Assay C1 – Confirmatory Mutagenicity Assay

In a confirmatory assay (Assay C1), the concentrations ranged from 1000 to 5175 μg/ml both in the absence and presence of S9. This increase in treatment concentration was due to a re-evaluation of the solubility of the test material, which indicated that the test material could be added to the treatment media at the limit concentration of 10 mM. There was little to no toxicity observed, as indicated by RCS values, in the absence of S9 activation (range from 86.6 to 108.6%) or the presence of S9 activation (range from 88.3 to 115.1%). The mutant frequencies observed in cultures treated with the test material in the absence of S9 and presence of S9 were not statistically different from the concurrent solvent control values and were within the range of the laboratory historical background.

Table 5.7.1.2-2: Results of Confirmatory Mutagenicity Assay (assay B1)

	Confirmatory Mutagenicity Assay (C1)			
Concentration	-S9		+S9	
µg/ml	%RCS	MF	%RCS	MF
Solvent Control	92.6	11.4	99.1	5.3
Solvent. Control	107.4	10.2	100.9	6.4
1000	95.1	8.3	88.3	7.5
1000	108.6	8.4	103.4	13.3
2000	92.9	3.6	107.2	10.6
2000	104.3	2.8	112.3	8.5
3000	86.6	8.0	102.1	10.2
3000	102.5	10.6	106.6	4.8
4000	89.0	10.7	115.1	5.4
4000	105.9	12.3	109.8	3.7
5175	89.8	14.4	101.5	12.6
5175	101.7	5.1	104.5	7.7
Pos. Control[a]	24.6	510.2*	89.1	208.8*
Pos. Control[a]	27.6	342.3*	104.5	238.4*
Pos. Control[b]	ND	ND	108.3	101.5*
Pos. Control[b]	ND	ND	102.1	130.3*
Pos. Control[c]	ND	ND	105.5	86.7*
Pos. Control[c]	ND	ND	114.0	119.1*

RCS = Relative Cell Survival
MF = TG^r Mutants per 10^6 clonable cells
ND = Not done
[a]Positive control = 621 µg/ml EMS (-S9); 4 µg/ml 20-MCA (+S9)
[b]Positive control = 6 µg/ml 20-MCA (+S9)
[c]Positive control = 8 µg/ml 20-MCA (+S9)
*The frequency of TG^r mutants is significantly higher than the concurrent solvent control value.

In both the initial and confirmatory mutagenicity assays, the positive control chemicals induced significant increases in mutant frequencies and these data confirmed the adequacy of the experimental conditions for detecting induced mutations.

The analytically observed concentrations of the test material in the stock solutions in Assay B1 ranged from 102.3 to 122.3% of target. In Assay C1, the observed concentration of the test material in the stock solutions ranged from 114.8 to 119.0% of target.

Based upon the frequency of TG^r mutants recovered in cultures treated with the test material, it was concluded that X768359 (BSTCA metabolite of penoxsulam) was not mutagenic in the CHO/HGPRT gene mutation assay.

B.6.7.1.2c *In Vitro* genotoxicity testing (IIA5.7.1.2c/01)

STUDY TYPE: *In vitro* mammalian cytogenetics OPPTS 870.5375; OECD 473

TEST MATERIAL (PURITY): X768359 (5-[2,2-difluoroethoxy)-6-trifluoromethyl-benzensulfonylamino]-1H-1,2,4-triazole-3-carboxylate) (99% (80% acid equivalent))

SYNONYMS: BSTCA metabolite of penoxsulam

CITATION: M. R. Schisler, and K. M. Kleinert (12 October 2006). EVALUATION OF X768359 (BSTCA METABOLITE OF PENOXSULAM) IN AN *IN VITRO* CHROMOSOMAL ABERRATION ASSAY UTILIZING RAT LYMPHOCYTES. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, 48674. Study ID: 061038, (12 October 2006). Unpublished

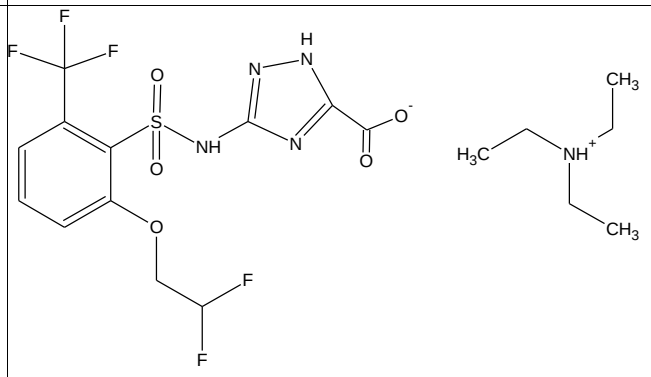
SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

SUMMARY: X768359 (5-[2,2-difluoroethoxy)-6-trifluoromethyl-benzensulfonylamino]-1H-1,2,4-triazole-3-carboxylate), the BSTCA metabolite of penoxsulam, was evaluated in an *in vitro* chromosomal aberration assay utilizing rat lymphocytes. Approximately 48 hours after the initiation of whole blood cultures, cells were treated in the absence and presence of S9 activation with concentrations ranging from 0 (solvent control) to 5175 μg/ml of X768359. The duration of treatment was 4 or 24 hours without S9 and 4 hours with S9. The highest concentration was based on the limit dose of 10 mM in this assay system. Based upon the mitotic indices, cultures treated for 4 hours with targeted concentrations of 0 (solvent control), 3000, 4000, and 5175 μg/ml in the absence and presence of S9 activation and cultures treated for 24 hours with 0 (solvent control), 865, 1730, and 3460 μg/ml were selected for determining the incidence of chromosomal aberrations. There were no significant increases in the frequencies of cells with aberrations in either the presence or absence of S9 activation. Cultures treated with the positive control chemicals (*i.e.*, mitomycin C without S9 and cyclophosphamide with S9) had significantly higher incidences of abnormal cells in all assays. Based upon these results, X768359, the BSTCA metabolite of penoxsulam, was considered to be non-genotoxic in this *in vitro* chromosomal aberration assay utilizing rat lymphocytes.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	X768359 (BSTCA metabolite of Penoxsulam)
	Description:	White solid
	Lot/Batch #:	lot # 035298-79, TSN105480
	Purity:	The BSTCA metabolite was isolated and provided as the triethylamine (TEA) salt. The purity of the BSTCA metabolite of penoxsulam TEA salt was determined to be 99% (80% acid equivalent) as measured by liquid chromatography with ultra violet detection. Structural identity was confirmed by infrared spectroscopy, mass spectroscopy, along with proton and carbon-13 nuclear magnetic resonance.
	CAS #:	Not Applicable
	Chemical Structure:	
	Solvent Used:	Dimethyl sulfoxide (DMSO)

2.	**Control Materials:**	
	Solvent Control (final concentration):	Dimethyl sulfoxide (DMSO) 1%
	Positive Control:	Nonactivation: Mitomycin C (MMC, Sigma, CAS No. 50-07-7), 0.5 µg/ml (4 hour) or 0.05 and 0.075 µg/ml (24 hour) Activation: Cyclophosphamide monohydrate (CP, Sigma, CAS No. 6055-19-2), 4 and 6 µg/ml

3.	**Activation**: S9 derived from							
	X	Induced	X	Aroclor 1254	X	Rat	X	Liver
		Not-induced		Phenobarbitol		Mouse		Lung
				None		Hamster		Other (name)
				Other (name)		Other (name)		

The S9 mix consisted of 10 mM $MgCl_2 \cdot 6H_2O$ (Sigma), 5 mM glucose-6-phosphate (Sigma), 4mM nicotinamide adenine dinucleotide phosphate (Sigma), 10mM $CaCl_2$ (Fisher, Fair Lawn,

New Jersey), 30 mM KCl (Sigma), and 50 mM sodium phosphate (pH 8.0, Sigma and Fisher). The reconstituted mix was added to the culture medium to obtain the desired final concentration of S9 in the culture, *i.e.*, 2% v/v. Hence, the final concentration of the co-factors in the culture medium was 1/5 of the concentrations stated above.

4.	**Test cells:** Peripheral lymphocytes male CD ISG (Outbred Crl:CD (SD)IGSBR rats			
Media: RPMI 1640 medium (with 25 mM HEPES, GIBCO, Grand Island, New York) supplemented with 10% heat-inactivated dialyzed fetal bovine serum (GIBCO), antibiotics and antimycotics (Fungizone 0.25 µg/ml; penicillin G, 100 U/ml; and streptomycin sulfate, 0.1 mg/ml; GIBCO), 30 µg/ml PHA (HA16, Murex Diagnostics Ltd., Dartford, England), and an additional 2 mM L-glutamine (GIBCO).				
Properly maintained?	X	Yes		No
Periodically checked for *Mycoplasma* contamination?		Yes	X	No
Periodically checked for karyotype stability?	X	Yes		No

5.	**Test compound concentrations used:**	
	Nonactivated conditions:	Assay A1 – 4 hours 0, 54.1, 108.13, 216.25, 432.5, 865, 1730, and 3460 µg/ml Assay A1 – 24 hours 0, 27, 54.1, 108.13, 216.25, 432.5, 865, 1730, and 3460 µg/ml Assay B1 – 4 hours 0, 250, 500, 1000, 2000, 3000, 4000, and 5175 µg/ml
	Activated conditions:	Assay A1 – 4 hours 0, 54.1, 108.13, 216.25, 432.5, 865, 1730, and 3460 µg/ml Assay B1 – 4 hours 0, 250, 500, 1000, 2000, 3000, 4000, and 5175 µg/ml

F. TEST PERFORMANCE

1. Preliminary Cytotoxicity Assay: not performed

2. Cytogenetic Assay:

a.	**Cell exposure time:**	Test Material	Solvent Control	Positive Control
	Non-activated:	4 h & 24 h	4 h & 24 h	4 h & 24 h
	Activated:	4 h	4 h	4 h

b.	**Spindle inhibition**	
	Inhibition used/concentration:	Colcemid (1 µg/culture)
	Administration time:	2-3 hours (before cell harvest)

c.	**Cell harvest time after termination of treatment:**	Test Material	Solvent Control	Positive Control
	Non-activated:	0 and 20h	0 and 20h	0 and 20h
	Activated:	20 h	20 h	20 h

d. Details of slide preparation: The cells were swollen by hypotonic treatment (0.075 M

KCl), fixed with methanol:acetic acid (3:1), dropped on microscope slides, and stained in Giemsa.

e. Metaphase analysis:

No. of cells examined per dose: 200 cells/treatment, positive controls 100 cells				
Scored for structural?	X	Yes		No
Scored for numerical?	X	Yes If Y, list *(polyploidy),*		No
Coded prior to analysis?	X	Yes		No

f. Evaluation criteria: For a test to be acceptable, the chromosomal aberration frequency in the positive control cultures should be significantly higher than the solvent controls. The aberration frequency in the solvent control should be within reasonable limits of the laboratory historical values. A test chemical is considered positive in this assay if it induces a significant dose-related and reproducible increase in the frequency of cells with aberrations.

i. Statistical analysis: The proportions of cells with aberrations (excluding gaps) were compared by the following statistical methods. At each dose level, data from the replicates was pooled. A two-way contingency table was constructed to analyze the frequencies of aberrant cells. An overall Chi-square statistic, based on the table, was partitioned into components of interest. Specifically, statistics were generated to test the global hypotheses: 1) no difference in the average number of cells with aberrations among the dose groups, and 2) no linear trend of increasing number of cells with aberrations with increasing dose. An ordinal metric (0, 1, 2, etc.) was used for the doses in the statistical evaluation. If either statistic was found to be significant at alpha = 0.05, versus a one-sided increasing alternative, pairwise tests (*i.e.* control vs. treatment) were performed at each dose level and evaluated at alpha = 0.05, again versus a one-sided alternative.

Polyploid cells were analyzed by the Fisher Exact probability test. The number of polyploid cells were pooled across replicates for the analysis and evaluated at alpha = 0.05. The data was analyzed separately based on the presence or absence of S9 and based on the exposure time.

II. RESULTS

A. Preliminary cytotoxicity assay: Not performed

B. Cytogenetic assay:

pH and Osmolality

The pH and osmolality of treatment medium containing approximately 5150 µg/ml of the test material (approximately the limit dose of 10 mM limit) and medium containing 1% solvent (DMSO) was determined using a Denver Basic pH meter (Denver Instrument Co., Arvada, Colorado) and an OSMETTE A freezing point osmometer (Precision Systems, Inc., Natick, Massachusetts), respectively. There was no appreciable change in the pH or osmolality in the treatment medium containing the test material compared to culture medium alone (culture medium containing the test material, pH = 7.26, osmolality = 392 mOsm/kg H_20; culture medium with 1% DMSO, pH = 7.32, osmolality = 422 mOsm/kg H_20).

Assay A1

In the initial assay, cultures were treated with the test material in the absence and presence of S9 activation for 4 hours at concentrations of 0 (solvent control), 54.1, 108.13, 216.25, 432.5, 865, 1730, and 3460 µg/ml. Cultures were also treated continuously for 24 hours in the absence of S9 with the above concentrations plus an additional lower concentration of 27.0 µg/ml. The highest concentration tested was based upon the initial solubility determination of the test material in the culture medium. The analytically detected concentrations of the test material in the stock solutions (Assay A1) varied from 93.0 to 102.6% of the target.

With and without metabolic activation (4 hour treatment), the cultures did not display any toxicity as measured by relative mitotic indices. In the absence of S9, the mitotic indices for the treated cultures ranged from 71.4 to 102.0% relative to the solvent control values. In the presence of S9, the mitotic indices of the treated cultures ranged from 86.4 to 119.7% as compared to the solvent control values. Cultures treated continuously for 24 hours in the absence of S9 activation showed signs of toxicity at the highest concentration (*i.e.*, 3460 µg/ml) as measured by a relative mitotic index of 45.1%. The remaining concentrations had relative mitotic indices ranging from 73.6 to 104.4%.

A re-evaluation of the solubility of the test material indicated that the test material could be added to the treatment medium up to the limit concentration of 10 mM. Because the test material did not induce the desired level of toxicity in Assay A1 following the short-treatment, a repeat assay was conducted in the absence and presence of S9 with the limit concentration of 5175 µg/ml as the highest concentration (Assay B1). A repeat of the

continuous treatment in the absence of S9 was deemed unnecessary due to the desired level of toxicity seen at the highest concentration in Assay A1.

Assay B1

In Assay B1, cultures were treated with the test material in the absence and presence of S9 activation for 4 hours at concentrations of 0 (solvent control), 250, 500, 1000, 2000, 3000, 4000, and 5175 µg/ml. The highest concentration tested represented the 10 mM limit for this assay system. The analytically detected concentrations of the test material in the stock solutions (Assay B1) varied from 102.8 to 113.9% of the target.

With and without metabolic activation (4 hour treatment), the cultures did not display any toxicity as measured by relative mitotic indices. In the absence of S9, the mitotic indices for the treated cultures ranged from 79.8 to 109.6% relative to the solvent control values. In the presence of S9, the mitotic indices of the treated cultures ranged from 85.1 to 105.3% as compared to the solvent control values. Based upon these results, the three highest concentrations (*i.e.*, 3000, 4000, and 5175 µg/ml) were chosen for the determination of chromosomal aberration frequencies and incidence of polyploidy in the absence and presence of S9 with a 4 hour treatment.

Among the cultures treated with the positive control chemicals for 4 hour, 0.5 µg/ml of MMC and 4 µg/ml of CP were selected for evaluation of aberrations in the absence and presence of S9, respectively.

There were no significant increases in the incidence of polyploidy cells in any of the test material treated cultures as compared to the solvent control values.

In the 4 hour non-activation assay, the frequency of cells with aberrations in the solvent control was 0.5% and the corresponding values at treatment levels of 3000, 4000, and 5175 µg/ml were 0.5, 2.0, 2.5%, respectively. Although, the statistical analysis of the chromosomal frequency data identified an increasing linear trend among the treatment groups, the pairwise comparisons of the treatment groups to the solvent controls did not identify a significant response. The frequencies of aberrant cells observed in all of the test material treated cultures were within the range of the solvent control values reported in this study, as well as, the laboratory historical control values. Hence, this apparent linear trend was deemed to be a chance occurrence unrelated to the treatment and biologically insignificant.

Table 5.7.1.2-3. Mitotic Indices in Rat Lymphocyte Cultures Assay A1

	Mean mitotic index [a]		
	Assay A1		
Conc. µg/ml	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)
Solvent control	9.8 (100.0)	6.6 (100.0)	9.1 (100.0)
27	ND	ND	8.3 (91.2)
54.1	8.9 (90.8)	6.7 (101.5)	9.2 (101.1
108.13	10.0 (102.0)	5.9 (89.4)	8.2 (90.1)
216.25	8.0 (81.6)	5.7 (86.4)	9.5 (104.4)
432.5	7.0 (71.4)	7.2 (109.1)	8.0 (87.9)
865	9.5 (96.9)	7.7 (116.7)	9.4 (103.3)
1730	8.6 (87.8)	7.9 (119.7)	6.7 (73.6)
3460	7.2 (73.5)	7.2 (109.1)	4.1 (45.1)
Positive control [b]	2.9 (29.6)	2.5 (37.9)	5.4 (59.3)
Positive control [c]	ND	0.9 (13.6)	3.1 (34.1)

[a] values in parenthesis are % relative mitotic index

[b] Positive control = 0.5 µg/ml MMC (-S9, 4 hr); 4 µg/ml CP (+S9, 4 hr); 0.05 µg/ml MMS (-S9, 24 hr)

[c] Positive control = 6 µg/ml CP (+S9, 4hr); 0.075 µg/ml MMS (-S9, 24 hr)

ND = not done

Table 5.7.1.2-4. Mitotic Indices in Rat Lymphocyte Cultures Assay B1

	Mean mitotic index [a]	
	Assay B1	
Conc. µg/ml	-S9 (4 hr)	+S9 (4 hr)
Solvent control	10.4 (100.0)	9.4 (100.0)
250	11.3 (108.7)	9.9 (105.3)
500	11.4 (109.6)	8.7 (92.6)
1000	9.9 (95.2)	8.8 (93.6)
2000	9.7 (93.3)	9.4 (100.0)
3000	8.6 (82.7)	9.0 (95.7)
4000	8.3 (79.8)	8.9 (94.7)
5175	9.0 (86.5)	8.0 (85.1)
Positive control [b]	3.3 (31.7)	4.2 (44.7)
Positive control [c]	ND	1.9 (20.2)

[a] values in parenthesis are % relative mitotic index

[b] Positive control = 0.5 µg/ml MMC (-S9, 4 hr); 4 µg/ml CP (+S9, 4 hr)

[c] Positive control = 6 µg/ml CP (+S9, 4hr)

ND = not done

Table 5.7.1.2-5. Results of the Cytogentic Assay

	Total aberrations (excluding gaps)			No. of cells with aberrations (excluding gaps) [a]			Incidence of polyploidy (%)		
Conc. µg/ml	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)
Solvent control	1	5	4	0.5	2.5	2.0	0.0	0.5	0.5
865	ND	ND	3	ND	ND	1.0	ND	ND	0.0
1730	ND	ND	4	ND	ND	2.0	ND	ND	0.5
3000	0	0	ND	0.5	0.0	ND	0.5	0.0	ND
3460	ND	ND	2	ND	ND	1.0	ND	ND	0.0
4000	4	5	ND	2.0	2.5	ND	0.0	0.0	ND
5175	5	4	ND	2.5	2.0	ND	0.5	0.5	ND
Positive control [b]	23	36	28	34.0*	36.0*	16.6*	0.0	0.0	0.0

[a] Values are percentages

[b] Positive control = 0.5 µg/ml MMC (-S9, 4 hr); 4 µg/ml CP (+S9, 4 hr); 0.05 µg/ml MMC (-S9, 24 hr)

*Significantly different from solvent controls, $\alpha = 0.05$.

ND = not done

It was concluded that under the experimental conditions used, X768359, the BSTCA metabolite of penoxsulam, was non-genotoxic in this *in vitro* chromosomal aberration test.

B.6.7.1.3. Mutagenicity studies on BST

B.6.7.1.3a *In Vitro* genotoxicity testing (Reference IIA5.7.1.3a/01)

STUDY TYPE: *(Bacterial system, Salmonella typhimurium; Escherichia coli)*/ mammalian activation gene mutation assay; OPPTS 870.5100[6]; OECD 471 (formerly OECD 471 & 472).

TEST MATERIAL (PURITY): X697134 (3-([{2,2-difluoroethyoxy)-6-(trifluormethyl)phenyl]sulfonyl}-amino-1H-1,2,4-triazole) (99%)

SYNONYMS: BST metabolite of penoxsulam

CITATION: M. S. Mecchi (21 July 2006). *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay Preincubation Method with a Confirmatory Assay with X697134 (BST Metabolite of Penoxsulam). Covance Laboratories Inc., Vienna, Virginia. Study ID 6736-157, 13 February 2006 – 21 July 2006. Unpublished.

SPONSOR: The Dow Chemical Company

SUMMARY:

In a reverse gene mutation assay in bacteria, tester strains TA98, TA100, TA1535, and TA1537 of *S. typhimurium* and tester strain WP2*uvr*A of *E. coli* were exposed to X697134 (BST Metabolite of Penoxsulam) (99% a.i.), in dimethylsulfoxide using the preincubation method at concentrations of 33.3, 100, 333, 1000, 3330, and 5000 µg per plate in the presence and absence of mammalian metabolic activation.

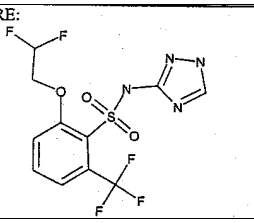
X697134 (BST Metabolite of Penoxsulam) was tested up to the limit concentration (5000 µg per plate) with all strains. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as acceptable. This study satisfies the requirement for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	X697134 (BST Metabolite of Penoxsulam)
	Description:	White powder
	Lot #:	035434-5
	Purity:	99% a.i.
	CAS #:	not provided
		STRUCTURE: 

[6]870.5100 - Reverse mutation *E. coli* WP2 and WP2uvrA; *S. typhimurium* TA 97, TA98, TA100, TA1535, TA1537
870.5140 - Gene mutation *Aspergillus nidulans*
870.5250 - Gene mutation *Neurospora crassa*

	Solvent Used:	Dimethylsulfoxide

2.	**Control Materials:**	
	Negative:	NA
	Solvent (final conc'n):	Dimethylsulfoxide at 50 µL per plate
	Positive:	Nonactivation: sodium azide 2.0 µg/plate TA100, TA1535 2-nitrofluorene 1.0 µg/plate TA98 ICR-191 2.0 µg/plate TA1537 4-nitroquinoline-N-oxide 0.4 µg/plate WP2*uvr*A
		Activation: benzo[a]pyrene 2.5 µg/plate TA98 2-aminoanthracene 2.5 µg/plate TA100, TA1535, and TA1537 2-aminoanthracene 25.0 µg/plate WP2*uvr*A

3.	**Activation**: S9 derived from									
	x	induced		x	Aroclor 1254	x	Rat	x	Liver
		non-induced			Phenobarbitol		Mouse		Lung
					None		Hamster		Other (name)
					Other (name)		Other (name)		

S9 mix composition: H_2O (70%), 1M NaH_2PO_4/Na_2HPO_4, pH 7.4 (10%), 0.25M Glucose-6-phosphate (2%), 0.10M NADP (4%), 0.825M KCl/0.2M $MgCl_2$ (4%), and S9 Homogenate (10%).

4.	**Test organisms:** *S. typhimurium* strains									
		TA97	x	TA98	x	TA100		TA102		TA104
	x	TA1535	x	TA1537		TA1538	x	WP2*uvr*A (*E. coli*)		
Properly maintained?							x	Yes		No
Checked for appropriate genetic markers (*rfa* mutation, R factor)?							x	Yes		No

5. Test compound concentrations used:

Nonactivated conditions:
Cytotoxicity test: TA100 and WP2*uvr*A at 10.0, 33.3, 100, 333, 1000, 3330, and 5000 µg per plate; two replicates.
Mutagenicity assay: TA98, TA100, TA1535, TA1537, and WP2*uvr*A at 33.3, 100, 333, 1000, 3330, and 5000 µg per plate; three replicates.

Activated conditions:
Cytotoxicity test: TA100 and WP2*uvr*A at 10.0, 33.3, 100, 333, 1000, 3330, and 5000 µg per plate; two replicates.
Mutagenicity assay: TA98, TA100, TA1535, TA1537, and WP2*uvr*A at 33.3, 100, 333, 1000, 3330, and 5000 µg per plate; three replicates.

B. TEST PERFORMANCE:
1. Type of *Salmonella* assay: pre-incubation (20 minutes)

2. <u>Protocol</u>:

Tester strains were exposed to the test article via the preincubation modification of the Ames Test originally described by Yahagi *et al.* (1975) and Maron and Ames (1983). This methodology has been shown to detect a wide range of classes of chemical mutagens. In the preincubation methodology, S9 mix (or phosphate buffer, where appropriate), tester strain, and test article were preincubated for approximately 20 minutes prior to addition of molten agar. The agar and preincubation reaction mixture were mixed and then overlaid onto a minimal agar plate. Following incubation, revertant colonies were counted. Test article, vehicle controls, and positive controls were plated in triplicate. The condition of the bacterial background lawn was evaluated both macroscopically and microscopically (using a dissecting microscope) for indications of cytotoxicity and test article precipitate. Evidence of cytotoxicity was scored relative to the vehicle control plate and was recorded along with the revertant counts for all plates at that concentration. Revertant colonies were counted either by automated colony counter or by hand.

3. <u>Statistical Analysis</u>: Mean and standard deviation were calculated for each replicate.

4. <u>Evaluation Criteria</u>: Before assay data were evaluated, criteria for a valid assay had to be met. The following criteria were used to determine a valid assay:

Salmonella typhimurium tester strain cultures exhibited sensitivity to crystal violet to demonstrate the presence of the *rfa* wall mutation. Cultures of tester strains TA98 and TA100 exhibited resistance to ampicillin to demonstrate the presence of the pKM101 plasmid.

Demonstrating the requirement for histidine (*Salmonella typhimurium*) or tryptophan (*Escherichia coli*), tester strain cultures exhibited a characteristic number of spontaneous revertants per plate when plated along with the vehicle under selective conditions. Acceptable ranges for mean vehicle controls were as follows:

TA98	8	-	60
TA100	60	-	240
TA1535	4	-	45
TA1537	2	-	25
WP2*uvr*A	5	-	40

Demonstrating that appropriate numbers of bacteria were plated, density of tester strain cultures were greater than or equal to 1.0×10^9 bacteria per mL and/or had reached a target density demonstrated to produce cultures with at least 1.0×10^9 bacteria per mL (see Study Deficiencies).

Demonstrating that tester strains were capable of identifying a mutagen, the mean value of a positive control for a respective tester strain exhibited at least a 3-fold increase over the mean value of the vehicle control for that strain. An acceptable positive control in the presence of S9 mix for a specific strain was evaluated as having demonstrated both integrity of the S9 mix and ability of the tester strain to detect a mutagen.

A minimum of three non-toxic concentrations was required to evaluate assay data. Cytotoxicity was detectable as a decrease in the number of revertant colonies per plate and/or by a thinning or disappearance of the bacterial background lawn. Thinning of the bacterial background lawn not accompanied by a reduction in the number of revertants per plate was not evaluated as an indication of cytotoxicity.

Once criteria for a valid assay had been met, responses observed in the assay were evaluated.

For a test article to be considered positive, it had to produce at least a 2-fold (TA100) or 3-fold (TA98, TA1535, TA1537, and WP2*uvr*A) concentration-related and reproducible increase in the mean revertants per plate over the mean revertants per plate of the appropriate vehicle control. A response that did not meet all three of the above criteria (magnitude,

concentration-responsiveness, reproducibility) was not evaluated as positive.

II. RESULTS

A. Preliminary cytotoxicity assay

Seven concentrations of test article, from 10.0 to 5000 □ g per plate, were tested in Trials 28097-A1 with tester strains TA100 and WP2*uvr*A. No cytotoxicity was observed with either tester strain in the presence or absence of S9 mix as evidenced by no decreases in the number of revertants per plate and normal bacterial background lawns.

B. Mutagenicity assay

The data for the mutagenicity assay were generated in Trials 28097-B1 and 28097-C1.

The tester strains used in the preincubation mutagenicity assay were *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* tester strain WP2*uvr*A.

The assay was conducted with six concentrations of the test article (33.3, 100, 333, 1000, 3330, and 5000 □ g per plate) in the presence and absence of S9 mix along with concurrent vehicle and positive controls using three plates per concentration.

In the initial mutagenicity assay (Trial 28096-B1), all data were acceptable and no positive increases in the mean number of the revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix.

In the confirmatory mutagenicity assay (Trial 28096-C1), all data were acceptable and no positive increases in the mean number of the revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix.

III. CONCLUSION

Under the conditions of this study, X697134 (BST metabolite of penoxsulam) did not show evidence of mutagenic potential.

B.6.7.1.3b *In Vitro* genotoxicity testing (Reference IIA5.7.1.3b/01)

STUDY TYPE: Mammalian cells in culture gene assay in CHO-K_1-BH_4 cells; USEPA OPPTS 870.5300; OECD Guideline 476; EC, B.17.

TEST MATERIAL (PURITY): X697134; (3-([{2,2-difluoroethyoxy)-6-(trifluormethyl)phenyl]sulfonyl}-amino-1H-1,2,4-triazole) (99%)

SYNONYMS: BST metabolite of penoxsulam

CITATION: S. D. Seidel, M. R.., Schisler, and. K. M. Kleinert. (August 28, 2006). EVALUATION OF X697134 (BST METABOLITE OF PENOXSULAM) IN THE CHINESE HAMSTER OVARY CELL/HYPOXANTHINE-GUANINE-PHOSPHORIBOSYL TRANSFERASE (CHO/HGPRT) FORWARD MUTATION ASSAY. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, 48674. Study ID: 061019, (August 28, 2006). Unpublished

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

SUMMARY:

X697134 (3-([{2,2-difluoroethyoxy)-6-(trifluormethyl)phenyl]sulfonyl}-amino-1H-1,2,4-triazole), the BST metabolite of penoxsulam, was evaluated in the *in vitro* Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward gene mutation assay. The genotoxic potential of the test material was assessed in at least two independent assays in the absence and presence of an externally supplied metabolic activation (S9) system at concentrations ranging from 153.13 to 2450 µg/ml. The highest concentration was based on limit of solubility of the test material in the treatment medium. The adequacy of the experimental conditions for detection of induced mutation was confirmed by employing positive control chemicals, ethyl methanesulfonate for assays without S9 and 20-methylcholanthrene for assays with S9. Solvent control cultures were treated with the solvent used to dissolve the test material (*i.e.* dimethyl sulfoxide). Based upon the frequency of TG^r mutants recovered in cultures treated with the test material, it was concluded that X697134, the BST metabolite of penoxsulam, was non-mutagenic in the assay system employed.

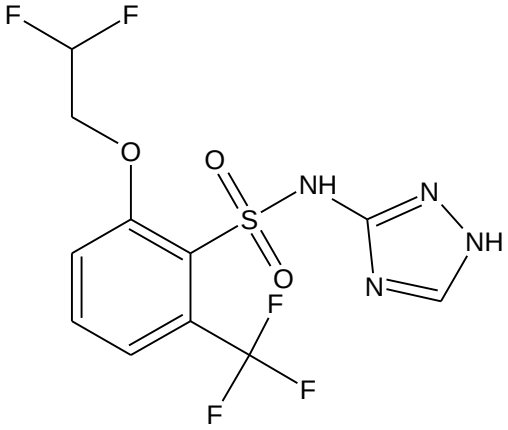
This study is acceptable and satisfies the guideline requirement for an Mammalian cells in culture gene assay in CHO-K_1-BH_4 cells; USEPA OPPTS 870.5300; OECD Guideline 476; EC, B.17.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	X697134 (BST metabolite of penoxsulam)
	Description:	White solid
	Lot/Batch #:	# 035434-5, TSN105514
	Purity:	99%
	CAS #:	Not applicable

	Chemical Structure:	
	Solvent Used:	Dimethyl sulfoxide (DMSO, Sigma, St. Louis, Missouri)

2.	**Control Materials:**	
	Solvent control (final conc'n):	Dimethyl sulfoxide (DMSO) 1%
	Positive control:	Nonactivation: Ethyl methanesulfonate (EMS, CAS No. 62-50-0), 621 µg/ml
		Activation: 20-methylcholanthrene (20-MCA, CAS No. 56-49-5), 4, 6, and 8µg/ml

3.	**Activation**: S9 derived from								
	X	induced		X	Aroclor 1254	X	Rat	X	Liver
		non-induced			Phenobarbitol		Mouse		Lung
					None		Hamster		Other
					Other		Other		

The S9 mix consisted of the following co-factors: 10 mM $MgCl_2 \cdot 6H_2O$, 5 mM glucose-6-phosphate, 4 mM nicotinamide adenine dinucleotide phosphate, 10 mM $CaCl_2$, 30 mM KCl, and 50 mM sodium phosphate (pH 8.0). The reconstituted mix was added to the culture medium to obtain the desired final concentration of S9 in the culture, *i.e.*, 2% v/v. Hence, the final concentration of the co-factors in the culture medium was 1/5th of the concentrations stated above.

4.	**Test cells:** mammalian cells in culture			
		mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
	X	Chinese hamster ovary (CHO) cells		list any others

Media: The cells were routinely maintained in Ham's F-12 nutrient mix (GIBCO, Grand Island, New York) supplemented with 5% (v/v) heat-inactivated (56°C, 30 minutes), dialyzed fetal bovine serum (GIBCO), antibiotics and antimycotics (penicillin G, 100 units/ml; streptomycin sulfate, 0.1 mg/ml; fungizone, 25 µg/ml; GIBCO) and an additional 2 mM L-glutamine (GIBCO). The selection medium used for the detection of $HGPRT^-$ mutants was Ham's F-12 nutrient mix without hypoxanthine, supplemented with 10 µM 6-thioguanine (GIBCO) and 5% serum and the above-mentioned antibiotics.

Properly maintained?	X	Yes		No
Periodically checked for Mycoplasma contamination?	X	Yes		No
Periodically checked for karyotype stability?	X	Yes		No
Periodically "cleansed" against high spontaneous background?	X	Yes		No

5.	**Locus Examined:**		Thymidine kinase (TK)	X	Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)		Na^+/K^+ ATPase
	Selection agent:		bromodeoxyuridine (BrdU		8-azaguanine (8-AG)		ouabain
			fluorodeoxyuridine (FdU)	X	6-thioguanine (6-TG)		
			trifluorothymidine (TFT)				

6.	**Test compound concentrations used:**	
	Nonactivated conditions:	Assay A1 0, 9.6, 19.1, 38.3, 76.6, 153.13, 306.25, 612.5, 1225, and 2450 μg/mL Assay B1 0, 153.13, 306.25, 612.5, 1225, and 2450 μg/mL Assay C1 0, 153.13, 306.25, 612.5, 1225, and 2450 μg/mL
	Activated conditions:	Assay A1 0, 9.6, 19.1, 38.3, 76.6, 153.13, 306.25, 612.5, 1225, and 2450 μg/mL Assay B1 0, 153.13, 306.25, 612.5, 1225, and 2450 μg/mL Assay C1 0, 153.13, 306.25, 612.5, 1225, and 2450 μg/mL

B. TEST PERFORMANCE

6. Cell treatment:

a. Cells were exposed to test compound, negative/solvent or positive controls for 4 hours (nonactivated) and 4 hours (activated).

b. After washing, cells were cultured for 8 days (expression period) before cell selection.

c. After expression,2 x 10^5cells/dish (10 dishes/ group) were cultured for 9 days in selection medium to determine numbers of mutants and 200 cells/dish (3 dishes/group) were cultured for 7 days without selective agent to determine cloning efficiency.

2. Statistical Methods: The frequency of mutants per 10^6 clonable cells was statistically evaluated using a weighted analysis of variance; weights were derived from the inverse of the mutation frequency variance. The actual plate counts are assumed to follow a Poisson distribution therefore the mean plate count was used as an estimate of variance.

If the analysis of variance was significant at alpha = 0.05, a Dunnett's t-test was conducted, comparing each treated group and the positive control to the negative control (alpha = 0.05, one-sided). An additional comparison of the positive control to the negative control (alpha = 0.05) was conducted using a linear contrast statement. Linear dose-related trend tests were performed if any of the pairwise comparisons of test material with the negative control yielded significant differences.

3.Evaluation Criteria: For an assay to be acceptable, the mutant frequency in positive controls should have been significantly higher than the solvent controls. An additional criteria, was that the mutant frequency in the solvent controls should have been within reasonable limits of the laboratory historical control values and literature values. The test chemical was considered positive if it induced a statistically significant, dose related, reproducible increase in mutant frequency. The final interpretation of the data took into consideration such factors as the mutant frequency and cloning efficiencies in the solvent controls.

II. RESULTS

K. Preliminary cytotoxicity

pH and Osmolality

The pH and osmolality of treatment medium containing approximately 2450 µg/ml of the test material (limit of solubility in culture medium) and medium containing 1% DMSO were determined using a Denver Basic pH meter (Denver Instrument Co., Arvada, Colorado) and an OSMETTE A™ freezing point osmometer (Precision Systems, Inc., Natick, Massachusetts). Alterations in the pH and osmolality of the culture medium have been shown to induce false positive responses in *in vitro* genotoxicity assays. The changes in both the pH and osmolality at this concentration as compared to the culture medium with solvent alone (culture medium with the test material, pH = 7.08, osmolality= 432 mOsm/kg H_20; culture medium with 1% DMSO, pH = 7.34, osmolality = 467 mOsm/kg H_20) were deemed to be inconsequential to the assay conduct.

™ Trademark of Precision Systems Inc.

Assay A1 – Preliminary Toxicity Assay

In a preliminary toxicity assay, the test material was assayed at concentrations of 0, 9.6, 19.1, 38.3, 76.6, 153.13, 306.25, 612.5, 1225 and 2450 µg/ml in the absence and presence of an externally supplied metabolic activation system (S9). The highest concentration tested was based upon the limited solubility of the test material in the culture medium. The cultures treated without S9 activation showed no toxicity, as determined by, relative cell survival (RCS) values ranging from 100.9 to 127.0%. In the presence of S9 activation, little to no toxicity was observed with the RCS values ranging from 63.8 to 115.1%. Based upon the results of this assay, concentration levels of 0, 153.13, 306.25, 612.5, 1225, and 2450 µg/ml of the test material were selected for the initial gene mutation assay in the absence and presence of S9.

L. Mutagenicity assay

Assay B1 – Initial Mutagenicity Assay

In the initial mutagenicity assay (Assay B1), in the absence and presence of S9, little to no toxicity was observed. The RCS values ranged from 87.8 to 118.1% in the absence of S9 and 72.8 to 93.7% in the presence of S9. The mutant frequencies in the test material treated cultures were not statistically different from the concurrent solvent control values and all mutant frequencies were within a reasonable range of historical background values. The positive control chemicals induced significant increases in mutant frequencies in both the absence and presence of S9 and these data confirmed the adequacy of the experimental conditions for detecting induced mutations.

Table 5.7.1.3-1: Results of Initial Mutagenicity Assay (Assay B1)

	Initial Mutagenicity Assay (B1)			
Concentration	-S9		+S9	
µg/ml	%RCS	MF	%RCS	MF
Solvent Control	104.8	3.4	106.2	7.1
Solvent. Control	95.2	6.5	93.8	6.0
153.13	88.6	1.5	76.6	10.8
153.13	87.8	7.0	93.7	1.6
306.25	106.0	10.9	90.2	10.4
306.25	97.3	5.8	86.9	6.2
612.5	118.1	7.0	81.6	6.3
612.5	109.0	7.1	93.4	3.1
1225	104.3	1.8	83.2	2.6
1225	111.0	5.1	76.9	11.1
2450[a]	111.8	7.5	84.0	2.0
2450 [a]	101.5	9.7	72.8	10.0
Pos. Control[b]	38.4	572.0*	92.2	40.9*
Pos. Control[b]	39.0	450.0*	97.6	31.8*

RCS = Relative Cell Survival
MF = TG^r Mutants per 10^6 clonable cells
ND = Not done
[a]Precipitation in treatment medium
[b]Positive control = 621 µg/ml EMS (-S9); 4 µg/ml 20-MCA (+S9)
*The frequency of TG^r mutants is significantly higher than the concurrent solvent control value.

Assay C1 – Confirmatory Mutagenicity Assay

In a confirmatory assay (Assay C1), the concentrations tested ranged from 153.13 to 2450 µg/ml without and with S9. In the absence and presence of S9, no toxicity was observed as indicated by RCS values, ranging from 100.1 to 120.8% (-S9) and 91.9 to 101.2% (+S9). The mutant frequencies observed in cultures treated with the test material in the absence of S9 and presence of S9 were not significantly different from the concurrent solvent control values and were within the range of the laboratory historical background. The positive control chemical in the absence of metabolic activation (*i.e.*, EMS) induced statistically significant increases in mutant frequency compared to the concurrent solvent control values. However, the positive control in the presence of metabolic activation (*i.e.*, 20-MCA) failed to induce a statistically significant response compared to the concurrent solvent control values. Therefore, the assay was repeated in the presence of metabolic activation with higher concentrations of 20-MCA (Assay D1).

Table 5.7.1.3-2: Results of Confirmatory Mutagenicity Assay (assay C1)

	Confirmatory Mutagenicity Assay (C1)			
Concentration	-S9		+S9	
µg/ml	%RCS	MF	%RCS	MF
Solvent Control	105.1	10.9	97.3	20.7
Solvent. Control	94.9	12.5	102.7	13.2
153.13	108.7	12.7	97.7	5.7
153.13	111.5	8.4	101.2	11.5
306.25	110.2	4.3	99.0	9.5
306.25	120.8	11.0	92.3	12.0
612.5	113.7	15.1	95.8	10.9
612.5	100.1	8.0	97.5	15.5
1225	113.7	10.6	91.9	11.9
1225	102.5	3.4	96.3	11.9
2450[a]	102.0	4.7	95.4	13.4
2450 [a]	103.8	18.4	93.5	16.5
Pos. Control[b]	25.6	292.6*	100.2	41.1
Pos. Control[b]	35.5	352.5*	92.3	22.5

RCS = Relative Cell Survival
MF = TG^r Mutants per 10^6 clonable cells
ND = Not done
[a]Precipitation in treatment medium
[b]Positive control = 621 µg/ml EMS (-S9); 4 µg/ml 20-MCA (+S9)
*The frequency of TG^r mutants is significantly higher than the concurrent solvent control value.

Assay D1 – Repeat Confirmatory Mutagenicity Assay in the Presence of S9

In the repeat confirmatory assay (Assay D1), in the presence of S9, the concentrations tested ranged from 153.13 to 2450 µg/ml. There was little to no toxicity observed, as indicated by RCS values ranging from 65.3 to 102.2%. The mutant frequencies observed in cultures treated with the test material in the presence of S9 were not significantly different from the concurrent solvent control values and were within the range of the laboratory historical background. The positive control chemical, 20-MCA, induced statistically significant increases in mutant frequency and this data confirmed the adequacy of the experimental conditions for detecting induced mutations.

Table 5.7.1.3-3: Repeat Confirmatory Mutagenicity Assay in the Presence of S9 (Assay D1)

Confirmatory Mutagenicity Assay (D1)		
Concentration	+S9	
µg/ml	%RCS	MF
Solvent Control	102.2	5.5
Solvent. Control	97.8	5.5
153.13	89.7	4.8
153.13	96.8	-[a]
306.25	87.1	1.5
306.25	102.2	3.0
612.5	91.7	1.3
612.5	89.1	0.8
1225	89.6	12.5
1225	79.8	7.9
2450[b]	70.7	7.0
2450 [b]	65.3	3.4
Pos. Control[c]	85.7	155.0*
Pos. Control[c]	83.0	231.3*
Pos. Control[d]	67.4	131.4*
Pos. Control[d]	91.2	157.9*
Pos. Control[e]	72.7	86.7*
Pos. Control[e]	69.7	115.0*

RCS = Relative Cell Survival
MF = TG^r Mutants per 10^6 clonable cells
ND = Not done
[a]Lost due to contamination
[b]Precipitation in treatment medium
[c]Positive control = 4 µg/ml 20-MCA (+S9)
[d]Positive control = 6 µg/ml 20-MCA (+S9)
[e]Positive control = 8 µg/ml 20-MCA (+S9)
*The frequency of TG^r mutants is significantly higher than the concurrent solvent control value.

The analytically observed concentrations of the test material in the stock solutions in Assay B1 ranged from 109.6 to 113.1% of target. In Assay C1, the observed concentration of the test material in the stock solutions ranged from 104.9 to 109.6% of target. In Assay D1, the observed concentration of the test material in the stock solutions ranged from 109.8 to 112.7% of target.

Conclusions

Based upon the frequency of TG^r mutants recovered in cultures treated with the test material, it was concluded that XDE-638 BST Metabolite was not mutagenic in the CHO/HGPRT gene mutation assay.

B.6.7.1.3c *In Vitro* genotoxicity testing (Reference IIA5.7.1.3c/01)

STUDY TYPE: *In vitro* mammalian cytogenetics OPPTS 870.5375; OECD 473

TEST MATERIAL (PURITY): X697134; (3-([{2,2-difluoroethyoxy)-6-(trifluormethyl)phenyl]sulfonyl}-amino-1H-1,2,4-triazole) (99%)

SYNONYMS: BST metabolite of penoxsulam

CITATION: G. D. Charles, M. R. Schisler, and K. M. Kleinert. (2006). EVALUATION OF X697134 (BST METABOLITE OF PENOXSULAM) IN AN *IN VITRO* CHROMOSOMAL ABERRATION ASSAY UTILIZING RAT LYMPHOCYTES. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, 48674. Study ID: 061018, (June 28, 2006). . Unpublished

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

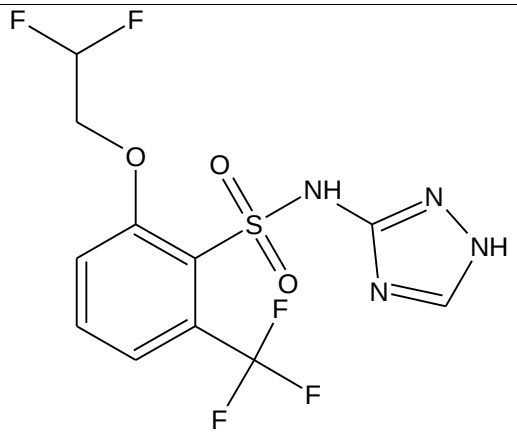
SUMMARY: X697134, the BST metabolite of penoxsulam (3-([{2,2-difluoroethyoxy)-6-(trifluormethyl)phenyl]sulfonyl}-amino-1H-1,2,4-triazole) was evaluated in an *in vitro* chromosomal aberration assay utilizing rat lymphocytes. Approximately 48 hours after the initiation of whole blood cultures, cells were treated either in the absence or presence of S9 activation with concentrations ranging from 0 (solvent control) to 2450 μg of X697134, the BST metabolite of penoxsulam, per ml of culture medium. The duration of treatment was 4 or 24 hours without S9 and 4 hours with S9. The highest concentration was based on the solubility of the test material in the vehicle. Based upon the mitotic indices, cultures treated for 4 hours with targeted concentrations of 0 (solvent control), 612.5, 1225, and 2450 μg/ml in the absence and presence of S9 activation and cultures treated for 24 hours with 0 (solvent control), 76.6, 153.13, and 306.25 μg/ml were selected for determining the incidence of chromosomal aberrations. There were no significant increases in the frequencies of cells with aberrations in either the absence or presence of S9 activation. Cultures treated with the positive control chemicals (*i.e.*, mitomycin C without S9 and cyclophosphamide with S9) had significantly higher incidences of abnormal cells in all assays. Based upon these results, X697134, the BST metabolite of penoxsulam, was considered to be non-genotoxic in this *in vitro* chromosomal aberration assay utilizing rat lymphocytes.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	X697134 (BST METABOLITE OF PENOXSULAM)
	Description:	White solid
	Lot/Batch #:	035434-5, TSN105514
	Purity:	The purity of the test material was determined to be 99% by liquid chromatography with ultra violet detection. Structural identity was confirmed by infrared spectroscopy, mass spectroscopy, along with proton and carbon-13 nuclear magnetic resonance.
	CAS #:	Not applicable

	Chemical Structure:	
	Solvent Used:	Dimethyl sulfoxide, DMSO

2.	**Control Materials:**	
	Solvent Control (final concentration):	Dimethyl sulfoxide (DMSO) 1%
	Positive Control:	Nonactivation: Mitomycin C (MMC, Sigma, CAS No. 50-07-7), 0.5 µg/ml (4 hour) or 0.05 and 0.075 µg/ml (24 hour) Activation: Cyclophosphamide monohydrate (CP, Sigma, CAS No. 6055-19-2), 4 and 6 µg/ml

3.	**Activation**: S9 derived from							
	X	Induced	X	Aroclor 1254	X	Rat	X	Liver
		Not-induced		Phenobarbitol		Mouse		Lung
				None		Hamster		Other (name)
				Other (name)		Other (name)		

The S9 mix consisted of 10 mM $MgCl_2 \cdot 6H_2O$ (Sigma), 5 mM glucose-6-phosphate (Sigma), 4mM nicotinamide adenine dinucleotide phosphate (Sigma), 10mM $CaCl_2$ (Fisher, Fair Lawn, New Jersey), 30 mM KCl (Sigma), and 50 mM sodium phosphate (pH 8.0, Sigma and Fisher). The reconstituted mix was added to the culture medium to obtain the desired final concentration of S9 in the culture, *i.e.*, 2% v/v. Hence, the final concentration of the co-factors in the culture medium was 1/5 of the concentrations stated above.

4.	**Test cells:** Peripheral lymphocytes from male CD ISG (Outbred Crl:CD (SD)) rats			
Media: RPMI 1640 medium (with 25 mM HEPES, GIBCO, Grand Island, New York) supplemented with 10% heat-inactivated dialyzed fetal bovine serum (GIBCO), antibiotics and antimycotics (Fungizone 0.25 µg/ml; penicillin G, 100 U/ml; and streptomycin sulfate, 0.1 mg/ml; GIBCO), 30 µg/ml PHA (HA16, Murex Diagnostics Ltd., Dartford, England), and an additional 2 mM L-glutamine (GIBCO).				
Properly maintained?	X	Yes		No
Periodically checked for *Mycoplasma* contamination?		Yes	X	No
Periodically checked for karyotype stability?	X	Yes		No

5.	**Test compound concentrations used:**	
	Nonactivated conditions:	Assay A1 – 4 hours 0, 38.3, 76.6, 153.13, 306.25, 612.5, 1225, and 2450 μg/ml Assay A1 – 24 hours 0, 19.1, 38.3, 76.6, 153.13, 306.25, 612.5, 1225 and 2450 μg/ml
	Activated conditions:	Assay A1 – 4 hours 0, 38.3, 76.6, 153.13, 306.25, 612.5, 1225, and 2450 μg/ml

G. TEST PERFORMANCE

1. Preliminary Cytotoxicity Assay: Not performed

2. Cytogenetic Assay:

a.	**Cell exposure time:**	Test Material	Solvent Control	Positive Control
	Non-activated: Activated:	4 h & 24 h 4 h	4 h & 24 h 4 h	4 h & 24 h 4 h

b.	**Spindle inhibition**	
	Inhibition used/concentration:	Colcemid (1 μg/culture)
	Administration time:	2-3 hours (before cell harvest)

c.	**Cell harvest time after termination of treatment:**	Test Material	Solvent Control	Positive Control
	Non-activated: Activated:	0 and 20h 20 h	0 and 20h 20 h	0 and 20h 20 h

d. Details of slide preparation: The cells were swollen by hypotonic treatment (0.075 M KCl), fixed with methanol:acetic acid (3:1), dropped on microscope slides, and stained in Giemsa.

e. Metaphase analysis:

No. of cells examined per dose: 200 cells/treatment, positive controls 100 cells				
Scored for structural?	X	Yes		No
Scored for numerical?	X	Yes If Y, list *(polyploidy),*		No
Coded prior to analysis?	X	Yes		No

f. Evaluation criteria: For a test to be acceptable, the chromosomal aberration frequency in the positive control cultures should be significantly higher than the solvent controls. The aberration frequency in the solvent control should be within reasonable limits of the laboratory historical values. A test chemical is considered positive in this assay if it induces a significant dose-related and reproducible increase in the frequency of cells with aberrations.

j. Statistical analysis: The proportions of cells with aberrations (excluding gaps) were compared by the following statistical methods. At each dose level, data from the replicates was pooled. A two-way contingency table was constructed to analyze the frequencies of aberrant cells. An overall Chi-square statistic, based on the table, was partitioned into components of interest. Specifically, statistics were generated to test the global hypotheses: 1) no difference in the average number of cells with aberrations among the dose groups, and 2) no linear trend of increasing number of cells with aberrations with increasing dose. An ordinal metric (0, 1, 2, etc.) was used for the doses in the statistical evaluation. If either statistic was found to be significant at alpha = 0.05, versus a one-sided increasing alternative, pairwise tests (*i.e.* control vs. treatment) were performed at each dose level and evaluated at alpha = 0.05, again versus a one-sided alternative.

Polyploid cells were analyzed by the Fisher Exact probability test. The number of polyploid cells were pooled across replicates for the analysis and evaluated at alpha = 0.05. The data was analyzed separately based on the presence or absence of S-9 and based on the exposure time.

II. RESULTS

pH and Osmolality:

The pH and osmolality of treatment medium containing approximately 2450 µg/ml of the test material (solubility limitations in the solvent) and medium containing 1% solvent (DMSO) was determined using a Denver Basic pH meter (Denver Instrument Co., Arvada, Colorado) and an OSMETTE A freezing point osmometer (Precision Systems, Inc., Natick, Massachusetts), respectively. The changes in both the pH or osmolality at this concentration as compared to the culture medium with solvent alone (culture medium with the test material, pH = 7.18, osmolality = 394 mOsm/kg H_20; culture medium with 1% DMSO, pH = 7.41, osmolality = 424 mOsm/kg H_20) were deemed to be inconsequential to the assay conduct.

Assay A1

In the initial assay, cultures were treated with the test material in the absence and presence of S9 activation for 4 hours at concentrations of 0 (solvent control), 38.3, 76.6, 153.13, 306.25, 612.5, 1225, and 2450 µg/ml. Cultures were also treated continuously for 24 hours in the absence of S9 with the above concentrations plus an additional lower concentration of 19.1 µg/ml. The highest concentration evaluated was based on solubility limitations of the test material in the solvent. Furthermore, there was also a slight precipitate in the treatment medium at the highest concentration level (2450 µg/ml). The analytically detected concentrations of the test material in the stock solutions (Assay A1) varied from 107.5 to 111.5% of the target.

Short Treatment

Without and with metabolic activation (4 hour treatment), the cultures displayed varying levels of toxicity as measured by relative mitotic indices. In the absence of S9, the mitotic indices for the treated cultures ranged from 47.7 to 111.0% relative to the solvent control values. In the presence of S9, the relative mitotic indices of the treated cultures ranged from 58.4 to 106.7% as compared to the solvent control values. Based upon these results, cultures treated with the top three concentrations (*i.e.*, 612.5, 1225, and 2450 µg/ml) were chosen for the determination of chromosomal aberration frequencies and incidence of polyploidy both in the absence and presence of S9 activation for the 4 hour treatment

Among the cultures treated with the positive control chemicals for 4 hour, 0.5 µg/ml of MMC and 4 µg/ml of CP were selected for evaluation of aberrations in the absence and presence of S9, respectively.

There were no significant increases in the incidence of polyploidy cells in any of the test material treated cultures as compared to the solvent control values.

In the 4 hour non-activation assay, the frequency of cells with aberrations in the solvent control was 0.5% and the corresponding values at treatment levels of 612.5, 1225 and 2450 µg/ml were 2.0, 1.5, and 2.5%, respectively. In the activation assay, cultures treated with the test material at concentrations of 612.5, 1225 and 2450 µg/ml had aberrant cell frequencies of 2.0, 1.5, and 1.0%, respectively as compared to the solvent control value of 1.0%. Statistical analyses of these data did not identify significant differences between the solvent control and any of the treated cultures without or with S9 activation. The frequencies of aberrant cells observed in the test material treated cultures were within the laboratory historical background range.

Significant increases in the frequency of cells with aberrations were observed in cultures treated with the positive control chemicals. Aberrant cell frequencies in MMC (- S9, 4 hour treatment), and CP (+ S9, 4 hour treatment) cultures were 48%, and 30%, respectively.

Continuous Treatment

Based upon the clearly negative findings in the 4 hour treatment in the absence of metabolic activation, slides from the continuous 24 hour treatment were evaluated. Excessive toxicity was evident at the highest treatment level (*i.e.*, 2450 µg/ml) as determined by a relative mitotic index of 5%. The remaining treatment levels had mitotic indices ranging from 17.5 to 100.0% relative to the solvent control values. Based on these results, cultures treated with 76.6, 153.13, and 306.25 µg/ml of the test material were chosen for the determination of chromosomal aberration frequencies and incidence of polyploidy in the 24 hour continuous treatment in the absence of metabolic activation. Cultures treated with 0.05 µg/ml MMC were selected for evaluation to serve as the positive control for the 24 hour assay in the absence of metabolic activation.

There were no significant increases in the incidence of polyploidy cells in any of the test material treated cultures as compared to the solvent control values.

The frequency of aberrant cells in the solvent control was 4.0% and this value was within the range of the laboratory historical negative controls. The corresponding values at treatment levels of 76.6, 153.13, and 306.25 μg/ml were 3.0, 3.0, and 5.0%, respectively. There were no statistically significant differences between the test material treated cultures and the solvent control values, and all values were within the laboratory historical background range.

Significant increases in the frequency of cells with aberrations were observed in cultures treated with the positive control chemical. Aberrant cell frequency in MMC treated cultures was 32%.

A second assay with treatment of cultures in the presence of S9 was not considered necessary in this study since the results of the initial test yielded clearly negative results.

Table 5.7.1.3-4. Mitotic Indices and Polyploidy Incidence in Rat Lymphocyte Cultures

	Mean mitotic index [a]			Incidence of polyploidy (%)		
	Assay A1			Assay A1		
Conc. µg/ml	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)	-S9 (4 hr)	+S9 (4 hr)	-S9 (24hr)
Solvent control	10.9 (100.0)	8.9 (100.0)	8.0 (100.0)	0.5	0.5	0.0
19.1	ND	ND	7.6 (95.0)	ND	ND	ND
38.3	9.1 (83.5)	9.5 (106.7)	8.0 (100.0)	ND	ND	ND
76.6	12.1 (111.0)	6.4 (71.9)	6.4 (80.0)	ND	ND	0.5
153.13	9.7 (89.0)	6.5 (73.0)	5.4 (67.5)	ND	ND	0.0
306.25	10.0 (91.7)	6.8 (76.4)	3.4 (42.5)	ND	ND	0.0
612.5	7.0 (64.2)	5.2 (58.4)	1.7 (21.3)	0.0	0.0	ND
1225	6.2 (56.9)	5.9 (66.3)	1.4 (17.5)	0.0	0.0	ND
2450	5.2 (47.7)	5.8(65.2)	0.4 (5.0)	0.0	0.5	ND
Positive control [b]	2.7 (24.8)	3.5 (39.3)	2.8 (35.0)	0.0	0.0	0.5
Positive control [c]	ND	1.5 (16.9)	2.4 (30.0)	ND	ND	ND

[a] values in parenthesis are % relative mitotic index

[b] Positive control = 0.5 µg/ml MMC (-S9, 4 hr); 4 µg/ml CP (+S9, 4 hr); 0.05 µg/ml MMS (-S9, 24 hr)

[c] Positive control = 6 µg/ml CP (+S9, 4hr); 0.075 µg/ml MMS (-S9, 24 hr)

ND = not done

Table 5.7.1.3-5. Results of the Cytogentic Assay

	Total aberrations (excluding gaps)			No. of cells with aberrations (excluding gaps) [a]		
	Assay A1			Assay A1		
Conc. µg/ml	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)
Solvent control	1	2	8	0.5	1.0	4.0
76.6	ND	ND	6	ND	ND	3.0
153.13	ND	ND	5	ND	ND	3.0
306.25	ND	ND	11	ND	ND	5.0
612.5	4	4	ND	2.0	2.0	ND
1225	3	3	ND	1.5	1.5	ND
2450	5	2	ND	2.5	1.0	ND
Positive control [b]	33	35	37	48.0*	30.0*	32.0*

[a] Values are percentages
[b] Positive control = 0.5 µg/ml MMC (-S9, 4 hr); 4 µg/ml CP (+S9, 4 hr); 0.05 µg/ml MMC (-S9, 24 hr)
*Significantly different from solvent controls, $\alpha = 0.05$.
ND = not done

Based on the results of mutagenicity testing with 5-OH-638, BSTCA and BST, there was no evidence of a genotoxic potential for penoxsulam metabolites.

B.8.5.2 Predicted Environmental Concentrations in surface water (PECsw) and sediment (PECsed) (Annex IIIA 9.2.3)

An additional refined risk assessment for aquatic plants was required following review by EFSA of a previously presented refined risk assessment. Additional refinement to the aquatic risk assessment was provided by higher-tier modelling of rice paddy systems using the RICEWQ and RIVWQ modelling systems. This is described in the following report:

Report: Karpouzas, D.G. and Capri, E. (2007): Report on Higher Tier Risk Assessment of the Herbicide Penoxsulam Applied in Rice Crop for Calculating Exposure in Surface Water Systems (Ref. K23)

Guidelines: Not applicable

GLP: No

Methodology: A higher tier aquatic exposure assessment for penoxsulam was conducted using the rice model RICEWQ in conjunction with the surface water model RIVWQ. Basin-scale scenarios representative of rice cultivation in three of the main rice producing countries of Europe (Greece, Italy and Spain) were developed. A single scenario for Greece (Axios river basin), five scenarios for Italy (Vercelli, Lomellina settedrionale, Lomellina meridionale, Ticino, Mantova) and four scenarios for Spain (Sevilla, Extremadura, Taragona-Ebro Delta, Valencia) were developed. All scenarios comprised a simulated rice cultivated basin of varying area consisting of rice paddy blocks associated with ditches or small drainage canals receiving water from the paddies, which in turn discharged their waters into secondary larger canals and subsequently into larger river systems. The main characteristics of the different scenarios are summarized in Table B.8.5.2-1.

The fate of penoxsulam in the paddy fields was simulated with the RICEWQ model which was parameterized based on the soil properties and agronomic and water management practices for each scenario, and the good agricultural practice for penoxsulam. The key pesticide input parameters are shown in Table B.8.5.2-2. For all simulations, the rice paddies were assumed to be drained when treated with penoxsulam (40 g a.s./ha), then flooded the next day. Water was held in the paddy for five days after which water was released from the paddy to the immediately adjacent drainage ditches. Daily losses of penoxsulam due to overflow or controlled drainage (provided by RICEWQ) and spray drift (calculated with the FOCUS Drift Calculator tool) were used as incremental mass loadings for the RIVWQ model which calculated the predicted environmental concentrations in the water and sediment of the receiving surface water bodies (based on a daily time step). The simulations were run for 20 years for Greece (using weather data from the FOCUS Thiva scenario) and 5 or 6 years for Italy and Spain (using local weather data).

Table B.8.5.2-1 Description of the Scenarios for the RICEWQ/RIVWQ Simulations

	Greece	**Italy**	**Spain**
No. of scenarios:	1	5	4
Area of catchment (ha):	2000	20	600 - 1000
Area of fields (ha):	4	10	1.5 - 10
Percent of area treated:	30	30 or 100	25
No. of years simulated:	20	5	5 or 6
Dimensions of ditches (width x depth, m):	NA	1 x 1	1.5 x 0.5
Dimensions of canals (width x depth,	4 x 1.5	2 x 1	4 x 1

m):			
Dimensions of river (width x depth, m):	60 x 3	25 x 2	50 x 2
Water flow velocity in ditches (m^3/s):	NA	0.01	0.01
Water flow velocity in canals (m^3/s):	0.1	0.06	0.06
Water flow velocity in river (m^3/s):	0.3	0.1	0.1

NA = not applicable (fields drained directly to canals)

Table B.8.5.2-2 Key Input Parameters for Penoxsulam in the RICEWQ Model

Parameter	Value
Application rate (g/ha):	40
Water solubility (mg/L):	410
Koc (L/kg):	94
DT50 in paddy water (days):	5.9
DT50 in paddy soil (days):	1
DT50 on foliage (days):	10
DT50 in ditch/canal/river water (days):	15
DT50 in ditch/canal/river sediment (days):	23

Findings: The maximum, the 95th percentile and the 90th percentile PECsw values are shown in Tables B.8.5.2-3 to B.8.5.2-6. The maximum PECsed values are shown in Table B.8.5.2-7.

In the Greek scenario, maximum and 95th percentile PECsw values in the drainage canals were 0.11 and 0.08 µg/L respectively. The corresponding PECsw values in the river of the Greek scenario were low (0.003 and < 0.001 µg/L, respectively)

In the five Italian scenarios, maximum PECsw values for penoxsulam in the ditches were 0.17 – 0.48 µg/L for the simulations where 30% of the area was treated on the same day and 0.31 – 0.43 µg/L for the simulations where the whole area was treated over 14 days. Maximum PECsw values were 0.01 - 0.11 µg/L in drainage canals and ≤ 0.001 µg/L in the rivers of the Italian scenarios.

In the four Spanish scenarios, the maximum PECsw values for penoxsulam were 0.09 - 0.49 µg/L in the ditches, 0.01 – 0.03 µg/L in the canals and ≤ 0.002 µg/L in the rivers.

In all scenarios, the sediment PEC values for penoxsulam were markedly lower than its corresponding surface water due to the low affinity of this herbicide to sediment and soil.

Table B.8.5.2-3 RICEWQ/RIVWQ PECsw Values (µg/L) for Penoxsulam in the Greek Scenario

Water Body	PECsw		
	Max	95th Perc.	90th Perc.
Canal	0.114	0.078	0.072
River	0.003	<0.001	<0.001

Table B.8.5.2-4 RICEWQ/RIVWQ PECsw Values (µg/L) for Penoxsulam in the Italian Scenario with Treatment of 30% of the Area on the Same Day

Scenario	Ditch			Canal			River		
	Max	95th Perc.	90th Perc.	Max	95th Perc.	90th Perc.	Max	95th Perc.	90th Perc.
Lomellina meridionale	0.214	0.159	0.130	0.014	0013	0.012	<0.001	<0.001	<0.001
Lomellina settedrionale	0.475	0.311	0.248	0.021	0.019	0.017	<0.001	<0.001	<0.001
Mantova	0.168	0.148	0.141	0.012	0.012	0.012	<0.001	<0.001	<0.001
Ticino	0.285	0.231	0.192	0.070	0.058	0.058	0.001	0.001	0.001
Vercelli	0.375	0.292	0.231	0.026	0.023	0.021	<0.001	<0.001	<0.001

Table B.8.5.2-5 RICEWQ/RIVWQ PECsw Values (µg/L) for Penoxsulam in the Italian Scenario with Treatment of the Whole Area over 14 Days

Scenario	Ditch			Canal			River		
	Max	95th Perc.	90th Perc.	Max	95th Perc.	90th Perc.	Max	95th Perc.	90th Perc.
Lomellina meridionale	0.259	0.231	0.221	0.017	0.017	0.017	<0.001	<0.001	<0.001
Lomellina settedrionale	0.377	0.348	0.303	0.033	0.030	0.027	<0.001	<0.001	<0.001
Mantova	0.313	0.283	0.257	0.016	0.016	0.016	<0.001	<0.001	<0.001
Ticino	0.425	0.373	0.360	0.106	0.090	0.074	0.001	0.001	0.001
Vercelli	0.350	0.327	0.316	0.063	0.059	0.055	<0.001	<0.001	<0.001

Table B.8.5.2-6 RICEWQ/RIVWQ PECsw Values (µg/L) for Penoxsulam in the Spanish Scenarios

Scenario	Ditch			Canal			River		
	Max	95th Perc.	90th Perc.	Max	95th Perc.	90th Perc.	Max	95th Perc.	90th Perc.
Sevilla	0.493	0.304	0.234	0.031	0.021	0.019	0.001	<0.001	<0.001
Extremadura	0.268	0.177	0.142	0.016	0.011	0.009	<0.001	<0.001	<0.001
Taragona	0.087	0.057	0.049	0.011	0.006	0.005	<0.001	<0.001	<0.001
Valencia	0.253	0.137	0.111	0.021	0.019	0.018	0.002	0.001	<0.001

Table B.8.5.2-7 Maximum PECsed Values (µg/kg) for Penoxsulam in the RICEWQ/RIVWQ Scenarios

Country	Scenario	Ditch	Canal	River
Greece	-	NA	0.009	<0.001
Italy	Lomellina meridionale	0.023	0.002	<0.001
	Lomellina settedrionale	0.026	0.002	<0.001
	Mantova	0.026	0.002	<0.001
	Ticino	0.034	0.004	<0.001
	Vercelli	0.028	0.003	<0.001
Spain	Sevilla	0.023	0.002	<0.001
	Extremadura	0.014	0.001	<0.001
	Taragona	0.005	0.001	<0.001
	Valencia	0.012	0.001	<0.001

An additional refined risk assessment was provided, based on surface water modelling, in order to demonstrate acceptable risk to aquatic plants.

Report: Karpouzas D. G., E. Capri (2007): Report on higher tier risk assesment of the herbicide Penoxsulam applied in rice crop for calculating exposure in surface water systems

An additional refinement risk assessment for aquatic plants was provided by a higher-tier exposure assessment, using the RICEWQ and RIVWQ modelling systems, in order to evaluate the realistic PECsw after application of Penoxsulam to rice crops in european countries. Rice paddies were assumed to be drained when treated with penoxsulam (40 g a.s./ha), then flooded the next day. Water was held in the paddy for 5 days after which water was released from the paddy to the immediately adjacent drainage ditches. Water in drainage ditches flow from each paddy into larger canals and then into rivers. Since rice is cultivated in large river basin a more realistic exposure assessment was performed at the basin-scale level using scenarios representative of rice cultivation at member-state level. Thus, basin-scale scenarios representative of rice cultivation in three main rice producing countries of EU, Greece, Italy and Spain were developed. More specifically, a single scenario for Greece (Axios river basin), five scenarios for Italy (Vercelli, Lomellina settedrionale, Lomellina meridionale, Ticino, Mantova) and four scenarios for Spain (Sevilla, Extremadura, Taragona-Ebro Delta, Valencia) were developed. All scenarios comprised a simulated rice cultivated basin of varying area consisting of rice paddy blocks associated with ditches or drainage canals receiving water from the paddies, which in turn, discharged their waters into secondary larger canals and subsequently into larger river systems. Concentrations of Penoxsulam were calculated in each segment of the water flow regime (paddy, ditch, canal and river) over several years of weather simulations. A detailed summary of the refined modelling report provided as Reference K23. The TER values for all modelled scenarios were calculated using the 95th percentile PEC_{sw} values of ditchs, canals and rivers and the standard laboratory E_bC_{50} of *Lemna gibba*, 14 day EC50 = 3.29 μg a.s./L (Tab. 10.2.10)

Tab. 10.2.10 (M3S6 final version up date oct. 2007) ***Refined Toxicity Exposure Ratios for the higher aquatic plant Lemna in surface water: RICEWQ/RIVWQ modelling***

Refined Exposure Scenario[(a)]	**rice area treated %**	**E_bC_{50} (µg a.s./L)**	**95th Percentile PEC_{sw} [(b)] (µg a.s./L)**	**TER**	**Annex VI Trigger**
Greece- Drainage canals	30		0.078	42	10
Greece-River			<0.001	>3000	
Italy-Lomellina meridionale-Ditches					
Italy-Lomellina meridionale-Ditches	30		0.159	20.7	
Italy-Lomellina meridionale-Drainage	100		0.231	14.2	
canals	30		0.013	253	10
Italy-Lomellina meridionale-Drainage	100		0.017	194	
canals	30 – 100		<0.001	>3000	
Italy-Lomellina meridionale-River					
Italy-Lomellina settentrionale-Ditches					
Italy-Lomellina settentrionale-Ditches	30		0.311	10.6	
Italy-Lomellina settentrionale-Drainage	100		0.348	**9.5**	
canals	30		0.019	170	10
Italy-Lomellina settentrionale-Drainage	100		0.030	109	
canals	30 – 100		<0.001	>3000	
Italy-Lomellina settentrionale-River					
Italy-Mantova-Ditches	30		0.148	22.2	
Italy-Mantova-Ditches	100		0.283	11.6	
Italy-Mantova -Drainage canals	30		0.012	274	10
Italy-Mantova -Drainage canals	100		0.016	200	
Italy-Mantova-River	30 – 100		<0.001	>3000	
Italy-Ticino-Ditches	30	3.29	0.231	14.2	
Italy-Ticino-Ditches	100		0.373	**8.8**	
Italy-Ticino-Drainage canals	30		0.058	56	10
Italy-Ticino-Drainage canals	100		0.090	36	
Italy-Ticino-River	30 – 100		0.001	3200	
Italy-Vercelli-Ditches	30		0.292	11.3	
Italy-Vercelli-Ditches	100		0.327	10.1	
Italy-Vercelli -Drainage canals	30		0.023	140	10
Italy-Vercelli -Drainage canals	100		0.059	55	
Italy-Vercelli-River	30 – 100		<0.001	>3000	
Spain-Sevilla-Ditches			0.304	10.8	
Spain-Sevilla-Drainage canals	25		0.021	156	10
Spain-Sevilla-River			<0.001	>3000	
Spain-Extremadura-Ditches			0.177	18.6	
Spain-Extremadura-Drainage canals	25		0.011	299	10
Spain-Extremadura-River			<0.001	>3000	
Spain-Taragona-Ditches			0.057	57.7	
Spain-Taragona-Drainage canals	25		0.006	548	10
Spain-Taragona-River			<0.001	>3000	
Spain-Valencia-Ditches			0.137	24	
Spain-Valencia-Drainage canals	25		0.019	173	10
Spain-Valencia-River			0.001	3000	

(a) **Greece**: Axios river basin, 30% of the total rice cultivated area treated with Penoxsulam during a 20 day period, PECsw based on 20 years simulation

Italy: 30% of the total rice cultivated area in each scenario treated with Penoxsulam on the same day, or 100% of the total rice cultivated area in each scenario treated with Penoxsulam during two week period, PECsw based on 5 years simulation.

Spain: 25% of the total rice cultivated area in each scenario treated with Penoxsulam during a week period, PECsw
based on 5 years simulation (Sevilla, Valencia) and 6 years simulation (Extremadura, Taragona)
(b) Number of simulation years depends from meteorological data availability.

Findings

The TER values for ditches, for all modelled scenarios, are above or very close to the trigger value. All TER values for canals and rivers result above the trigger value. Ditches immediately adjacent to rice paddies are intimately associated with rice production, and are not independent water body that can sustain viable aquatic plant communities over the long term. Furthermore, proper functioning of drainage ditches requires that they be periodically managed to allow unimpeded water flow from the rice paddies. Considered that drainage canals and rivers should be considered the ecologically relevant aquatic habitats requiring protection, the risk for aquatic macrophytes can be considered acceptable in all modelled scenarios.

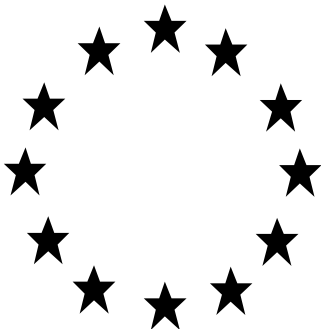
Conclusion

An adequate risk assessment for penoxsulam in all modelled scenarios was achieved using the 95th percentile PEC_{sw} values derived from higher tier modelling in association with the standard laboratory-derived E_bC_{50} in Lemna gibba (14 day E_bC_{50} = 3.29 µg a.s./L) and evaluated against the TER trigger value of 10.

A possible safe use of penoxsulam was demonstrated for aquatic macrophytes. Realistic national scenarios should be considered at member state level.

ANNEX C
Volume 4

Council Directive 91/414/EEC



DE-638
PENOXSULAM

Addendum to Volume 4
(August 2008)

Annex C to the Report and Proposed Decision of Italy made to the European Commission under 91/414/EEC

Confidential Information

Confidential information available at RMS