Council Directive 91/414/EEC

Thiencarbazone-methyl

(BYH 18636)

Volume 3 / Annex B to the Report and Proposed Decision of the United Kingdom made to the European Commission under Article 8 of Council Directive 91/414/EEC

OECD Joint Review Project

Section B.6: Toxicology and metabolism
Part A: Evaluation and Assessment of the data submitted

December 2008

TXR # 0054800
DP Barcode D339801

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Background information

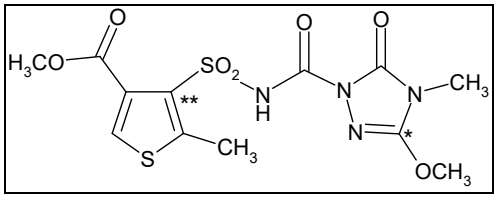
The evaluation of Annex II and III data is presented in Toxicology Part A Study evaluations. The risk assessment for the proposed uses of 'SC450' and 'SC 465' is presented in Part B.

In Part A a summary (green box) has been prepared by the Regulatory evaluator, indicating study reliability and identifying key points and conclusions. These summaries were peer reviewed by the US EPA and PMRA Canada.

B.6 Toxicological and Toxicokinetic Studies on the Active Substance

B.6.1 Absorption, distribution, metabolism and excretion (toxicokinetics) (IIA 5.1)

The toxicokinetics of thiencarbazone-methyl following oral gavage were investigated in four studies in the rat, using material ^{14}C-radiolabelled at the thiophene and dihydrotriazole positions.



* ^{14}C position of the [dihydrotriazole-3-^{14}C] label
** ^{14}C position of the [thiophene-4-^{14}C] label

B.6.1.1 Absorption, distribution and excretion (IIA 5.1.1)

Toxicokinetic studies - Single dose, oral route, in rats

Report:	**KIIA 5.1/01, Justus K.; 2006a (MRID #47070201)**
Title:	[Thiophene-4-^{14}C]BYH18636: Adsorption, Distribution, Excretion and Metabolism in the Rat
Report No &	MEF-05/176
Document No	M-268447-01-2
Guidelines:	EPA-Health Effects Guidelines, OPPTS 870.7485; EU Council Directive 91/414/EEC amended by Commission Directive 94/79/EC; PMRA Ref.: DACO 4.5.9 Metabolism/ Toxicokinetics in Mammals (Lab. Animal); OECD Guideline for Testing of Chemicals No. 417, Toxicokinetics, Japanese MAFF New Test Guidelines for Supporting Registration of Chemical Pesticides, 12 Nousan 8147
GLP	Yes

Executive Summary

The biokinetic behaviour and metabolism of BYH 18636 were investigated in the rat using [thiophene-4-^{14}C]BYH 18636.

In all five tests, between 92 and 100% of the administered dose were recovered in urine, bile, and faeces as well as in organs and tissues at sacrifice.

The absorption of radioactivity commenced immediately after the oral administration by gavage and was at least 48–50 % of the total recovered radioactivity at the low dose level (2 mg/kg bw).

In all tests, the excretion was almost complete (ca. 91 – 99 % of the administered dose) at the time of sacrifice, at 48 hours after dosing. Approximately one half of the radioactivity was excreted with the faeces and the other half with the urine. Only very minor amounts (1.4 % of the administered dose) were excreted with the bile. The plasma concentration courses and pharmacokinetic calculations demonstrated efficient elimination of the radioactivity from the systemic compartment.

The residues in the bodies at sacrifice were very low. Only ca. 0.5 – 0.7% of the administered dose was found in the total body, thereof ca. 0.04 – 0.1% in the skin, ca. 0.02 – 0.4% in the gastrointestinal tract and its contents, and ca. 0.2 - 0.4% in the organs and tissues. The highest dose normalised con-

centrations[i] were detected in the liver, showing low values of ca. 0.01 to 0.06. These were followed by values of ca. 0.007 to 0.02 in the lung, ca. 0.004 to 0.01 in the kidney, ca. 0.003 to 0.01 in the perirenal fat, < LOD to ca. 0.009 in the thyroid gland, and ca. 0.005 to 0.008 in the testis. All other organs and tissues were below 0.005.

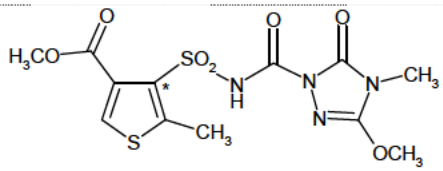
The biokinetic behaviour showed no dependence on the sex or pre-treatment. At the high dose level, the absorption was slightly lower, the course of the concentration in the plasma was less pronounced, relative residues and concentrations were lower in the liver and some other organs, and the excretion was slightly slower.

BYH 18636 was metabolised to only a low degree. Parent compound was the major component of the profiles and represented ca. 81 – 92% of the dose. One minor metabolite, BYH 18636-sulfonamide-carboxylic acid, was found with ca. 1 – 2% of the dose and one trace metabolite, BYH 18636-thienosaccharine, with ca. 0.1 – 0.2%. BYH 18636-sulfonamide was detected at ca. 0.7 – 1.5% of the dose, but this compound was only tentatively identified as metabolite. Cleavage of the urea group and hydrolysis of the methyl ester, followed by re-cyclisation to an intramolecular sulfonamide represent the metabolic pathway. The metabolism was not dependent on sex, dose level, or pre-treatment.

The biokinetic and metabolism of [thiophene-4-^{14}C]-BYH 18636 in rats is sufficiently understood.

I. Material and Methods

1. Test Material

Chemical structure		* position of the radiolabel
Radiolabeled test material	[Thiophene-4-^{14}C]BYH 18636	
Specific radioactivity	111.7 µCi/mg (= 4.13 MBq/mg)	
Chemical Purity	> 99% (certified, HPLC with UV detection at 210 nm)	
Radiochemical purity	>98% and >99% (certified, HPLC and TLC with radiodetection)	
Vehicle	0.5 % aqueous tragacanth solution	
Preparation of dosing solution	test 1, 2, 4, 5 (low dose): radiolabeled test compound suspended in aqueous tragacanth solution; test 3 (high dose): 1:50 mixture of radiolabeled and non-radio-labelled test compound suspended in aqueous tragacanth solution	

[i] The dose normalised concentration (CN) is defined as the radioactivity measured per gram of plasma or tissue, divided by the radioacitivity administered per gram of body weight. The dose normalised concentration is unitless and allows easy comparison of the concentrations obtained from experiments with different doses.

2. Test Animals

Species	Rat (*Rattus norvegicus*)
Strain	Wistar Hsd/Cpb: WU
Age/weight at study initiation	9 - 10 weeks (males), 12 – 13 weeks (females) / approx. 200 g
Source/breeder	Harlan & Winkelmann GmbH, 33178 Borchen, Germany
Sex and number of animals involved	test 1: 4 male rats; test 2: 4 female rats; test 3: 4 male rats ; test 4: 4 male rats; test 5: 6 male rats
Acclimation period	approx. one week in tests 1, 2, 3, 5 and three weeks in test 4
Identification	labelling by cage cards and with water-insoluble spots on the tail
Housing	in Makrolon® metabolism cages at 21 – 26°C; humidity: 37 – 57%; air changes: 10 – 15 times/h; photoperiod: 12 hrs dark / 12 hrs light
Diet	ad libitum with rat/mice maintenance long life diet (max. approx. 16 g per animal and day); last time fed ca. 16 hours prior to and again ca. 8 hours after dosing
Water	ad libitum tap water from the local mains supply in accordance with local drinking water regulation

3. Study Design

Table 5.1-1 Experiments conducted within ADME study of [thiophene-4-14C]-BYH 18636

Test Group	Dose of labelled material	Number/ sex	Sample collection schedule
test 1	2 mg/kg bw (low dose), single oral dose	4 males	plasma: 0.17, 0.33, 0.67, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 8.0, 24, 32, and 48 hours after administration urine: 4, 8, 12, 24, and 48 hours after administration faeces: 24 and 48 hours after administration blood, organs, and tissues: at sacrifice, 48 hours after administration
test 2	2 mg/kg bw (low dose), single oral dose	4 females	plasma: 0.17, 0.33, 0.67, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 8.0, 24, 32, and 48 hours after administration urine: 4, 8, 24, and 48 hours after adm. faeces: 24 and 48 hours after administration blood, organs, and tissues: at sacrifice, 48 hours after administration
test 3	100 mg/kg bw (high dose), single oral dose	4 males	plasma: 0.17, 0.33, 0.67, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 8.0, 24, 32, and 48 hours after administration urine: 4, 8, 24, and 48 hours after administration faeces: 24 and 48 hours after administration blood, organs, and tissues: at sacrifice, 48 hours after administration

test 4	2 mg/kg bw (low dose), single oral dose, (after pre-treatment with 14 daily doses of non-radiolabelled test material)	4 males	plasma: 0.17, 0.33, 0.67, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 8.0, 24, 32, and 48 hours after administration urine: 4, 8, 24, and 48 hours after administration faeces: 24 and 48 hours after administration blood, organs, and tissues: at sacrifice, 48 hours after administration
test 5	2 mg/kg bw (low dose), single oral dose (after bile-duct cannulation)	4 males	urine: 4, 8, 24, and 48 hours after administration bile: 4, 8, 24, 32 and 48 hours after administration faeces: 24 and 48 hours after administration skin, GIT, carcass: at sacrifice, 48 hours after administration

Dosing and sample collection

[Thiophene-4-^{14}C]BYH 18636 was administered by oral gavage to male rats at dose levels of 2 and 100 mg/kg bw and to female rats at 2 mg/kg bw. In finding the dose, the specific radioactivity, the toxicity of the test substance, and the stability in the administration suspension were taken into account. The low dose of 2 mg/kg bw is far below the effect level, whereas the high dose of 100 mg/kg bw is at the lower range of the effect level.

Application suspensions were prepared by suspension of radiolabeled test compound (low dose) or a mixture of radiolabeled and non-radiolabeled test compound at a ratio of 1:50 (high dose) in 0.5 % aqueous tragacanth solution. Each animal (4 animals per test) received 2 mL of the application suspension. The suspension was administered to the rats by oral gavage using a syringe attached to an animal-feeding knob cannula.

After administration of the radiolabeled test compound, the rats were kept individually in Makrolon® metabolism cages, which allowed for separate and quantitative collection of urine and faeces. Samples of plasma, urine, faeces and bile were collected separately for each animal at various times as given in Table 5.1a. Organs or tissues were dissected from the animals at sacrifice.

Collection of plasma

Blood samples were collected separately for each animal by pressing a capillary coated with heparin in a small cut in the tail vein. The capillaries were centrifuged using a hematocrit centrifuge to separate the plasma from the formed constituents (mainly erythrocytes). After centrifugation, the capillary was broken at the border between plasma and weight and radioactivity of formed constituents and the plasma (ca. 10 - 70 µL) were determined. By this method, it was possible to collect blood samples at the different time points from the same animals and thus creating plasma curves from single animals.

Collection of excreta

Immediately after the administration of the test compound, animals were transferred to metabolism cages. Urine and faeces were collected separately and quantitatively for each animal. Urine was collected in a cryogenic trap, and radioactivity was determined by LSC (liquid scintillation counter). Collected faeces were lyophilised (freeze-dried), weighed, homogenised and radioactivity was determined by combustion/LSC.

Collection of bile

A bile-duct cannulation experiment was performed with 6 male rats (test 5). Results of 4 animals were used for evaluation of study results, while 2 had to be regarded as outliers. Bile was collected at various times separately for each animal. The radioactivity was determined by LSC.

Sacrifice and organ/tissue sampling

All animals were sacrificed at 48 h after administration of radiolabeled test compound to obtain blood, organs and tissues, as well as GIT, skin and carcass. The blood collected at sacrifice was separated into plasma and erythrocytes by centrifugation. The organs and tissues were dissected, weighed immediately and again following lyophilisation. Finally, they were homogenised before aliquots were taken for the determination of radioactivity by combustion/LSC. The radioactivity in the small organs and tissues (renal fat, uterus, adrenal glands, thyroid, ovaries) was determined by LSC after solubilising in a tissue solubiliser. For these organs and tissues only the wet weight was recorded. The radioactivity remaining in the GIT, skin and residual carcass was determined by combustion/LSC in order to establish the radioactivity balance.

Metabolite analysis

For analysis and quantification of the parent compound and metabolites in urine, bile, and faeces, respective samples from all animals of a test were pooled, and samples from these pools were used for characterisation, identification and quantification. Urine and bile samples were analysed directly without preceded sample preparation. Pooled faeces samples were extracted with acetonitrile/water mixtures of increasing polarity (2 x acetonitrile, 2-3 x acetonitrile/ water 7:3, 2 x acetonitrile/water 1:1).

Characterisation, identification and quantification of parent compound and metabolites in pooled urine and bile samples and extracts from faeces was done using an HPLC method with RP18 HPLC column and detection via radioactivity and UV detector. Metabolites were identified by means of HPLC co-chromatography with non-radiolabeled reference compounds and by comparison of retention times and similar profiles. Chromatographic correspondence with the non-radiolabelled reference compounds was assessed by comparison of the UV-trace and the associated ^{14}C-trace. In addition, normal phase thin layer chromatography (TLC) with radioluminography and UV detection was used for analysis of samples and extracts and identification of components via co-chromatography. Finally, components were identified by LC/MS/MS.

II Results and Discussion

Recovery

Between 92 and 100 % of the administered dose were recovered from measurement of the total radioactivity in urine, bile, and faeces as well as in organs and tissues at sacrifice.

Table 5.1-2 Residues of [thiophene-4-^{14}C]-BYH 18636 in the rat

	Radioactivity in percent of dose administered				
	Test 1 2 mg/kg male	Test 2 2 mg/kg female	Test 3 100 mg/kg male	Test 4 2 mg/kg (14+1) pre-treatment male	Test 5 2 mg/kg bile test male
Urine	45.50	45.93	41.59	47.69	34.68
Bile	n.a.	n.a.	n.a.	n.a.	1.42
Faeces	49.17	45.39	57.68	48.09	60.18
Total excreted	94.68	91.32	99.27	95.78	96.29
Skin	0.071	0.081	0.043	0.048	0.114
Sum organs/tissues	0.381	0.405	0.155	0.399	0.303
Body without GIT	0.452	0.486	0.198	0.446	0.416
GIT	0.022	0.170	0.360	0.122	0.034
Total in body	0.474	0.656	0.558	0.569	0.451
Balance	95.15	91.98	99.83	96.34	96.74

	Radioactivity in percent of total radioactivity recovered				
	Test 1 2 mg/kg male	Test 2 2 mg/kg female	Test 3 100 mg/kg male	Test 4 2 mg/kg (14+1) pre-treatment male	Test 5 2 mg/kg bile test male
Urine	47.82	49.67	41.67	49.71	35.48
Bile	n.a.	n.a.	n.a.	n.a.	1.47
Faeces	51.68	49.61	57.77	49.70	62.58
Total excreted	99.50	99.28	99.44	99.41	99.53
Skin	0.075	0.089	0.043	0.050	0.117
Sum organs/tissues	0.401	0.445	0.156	0.416	0.313
Body without GIT	0.476	0.534	0.199	0.466	0.430
GIT	0.023	0.187	0.364	0.124	0.035
Total in body	0.499	0.720	0.563	0.590	0.466
Norm.-factor	1.05	1.09	1.00	1.04	1.04

Absorption

[Thiophene-4-^{14}C]BYH 18636 was very rapidly absorbed from the gastrointestinal tract of male and female rats in all test groups. The absorption was at least 48 – 50% of the total radioactivity recovered at the low dose level, calculated from the values of the urine and the body without the gastrointestinal tract. The elimination via the bile was negligible (ca. 1.5% of the recovered dose).

Distribution and Plasma Kinetics

The values from the pharmacokinetic model and from the plasma concentration courses demonstrated rapid absorption of the [thiophene-4-^{14}C]BYH 18636 related radioactivity followed by efficient elimination from the rats.

After very short absorption phases, maximum plasma concentrations of ca. 1.6 – 1.9 µg/g (denotes µg a.s. equiv./g) at the low dose level and 53 µg/g at the high dose level were already reached ca. 0.6 – 1 hours after administration. The absorption was followed by a fast initial elimination phase with half lives of 0.12 to 0.55 hours and a slower terminal elimination phase with half lives of ca. 8 to 29 hours. The mean residence times were approx. 7 to 15 hours. AUC values for the low dose tests ranged from 5.8 to 9.4 µg/g x h and AUC of the high dose test was 341 µg/g x h, showing a dose proportional behaviour. The dose normalised concentrations of the radioactivity in the plasma showed a roughly similar course for all tests. Steep increase of the plasma concentration reflected rapid absorption, which was immediately followed by a fast decline from continuous elimination.

Distinct dependence on the sex or pre-treatment was not observed, whereas the course of the concentration in the plasma of the high dosed rats was less pronounced: plasma maximum was lower and during the terminal elimination process the concentrations were higher than than at the low dose level.

Tissue distribution at sacrifice

The radiolabelled residues in the organs and tissues of the animals of all tests were determined at sacrifice, 48 h after the oral administration. The residues in the bodies were very low. Only ca. 0.5 – 0.7% of the administered dose was found in the total body, thereof ca. 0.04 – 0.1% in the skin, ca. 0.02 – 0.4% in the gastrointestinal tract and its contents (GIT), and ca. 0.2 - 0.4% in the organs and tissues. The values showed no distinct dependence on the sex or pre-treatment. The high dosed rats tended to show a lower value in the liver (0.06%) and a higher value in the GIT (0.4%).

Equivalent concentrations in the organs and tissues were rather low and values were measured between 0.0004 µg/g (denotes µg a.s. equiv./g) in the brain of the low dosed rats and ca. 1 µg/g in the liver of the high dosed rats. The highest dose normalised concentrations were detected in the liver, showing low values of ca. 0.01 to 0.06. These were followed by values of ca. 0.007 to 0.02 in the lung, ca. 0.004 to 0.01 in the kidney, ca. 0.003 to 0.01 in the perirenal fat, < LOD to ca. 0.009 in the thyroid gland, and ca. 0.005 to 0.008 in the testis. All other organs and tissues were below 0.005. The values showed no distinct dependence on the sex or pre-treatment, whereas the values of the high dosed rats were predominantly lower than the values of the low dosed rats.

Table 5.1-3 Dose-normalised concentration in ADME tests with [thiophene-4-^{14}C]-BYH 18636

Tissue/organ	Test 1 2 mg/kg male	Test 2 2 mg/kg female	Test 3 100 mg/kg male	Test 4 2 mg/kg (14+1) pre-treatment male
Erythrocytes	0.0012	0.0010	0.0013	0.0010
Plasma	0.0009	0.0026	0.0038	0.0025
Spleen	0.0007	0.0008	0.0008	0.0009
Liver	0.0522	0.0595	0.0111	0.0611
Kidney	0.0084	0.0095	0.0040	0.0066
Perirenal fat	0.0113	0.0073	0.0029	0.0091
Adrenal gland	0.0040	0.0033	0.0018	0.0026
Testis	0.0082	n.a.	0.0054	0.0056
Ovary	n.a.	0.0033	n.a.	n.a.
Uterus	n.a.	0.0023	n.a.	n.a.
Skeleton muscle	0.0012	0.0007	0.0006	0.0008
Bone femur	< LOD	0.0005	0.0006	< LOD
Heart	0.0014	0.0013	0.0011	0.0011
Lung	0.0177	0.0157	0.0068	0.0126
Brain	0.0003	0.0002	< LOD	< LOD
Thyroid gland	0.0092	0.0086	< LOD	< LOD
Skin	0.0033	0.0037	0.0018	0.0023
Carcass	0.0018	0.0025	0.0014	0.0018
GIT	0.0017	0.0196	0.0339	0.0095
Body without GIT	0.0052	0.0053	0.0022	0.0051
Total in body	0.0047	0.0066	0.0056	0.0057

n.a. = not applicable

Excretion

The excretion was fast. Already 24 hours after administration, ca. 73 – 94% of the administered dose had been excreted with the urine, bile, and faeces. At sacrifice, 48 hours after dosing, the excretion was almost complete (ca. 91 – 99% of the administered dose). Approximately one half of the radioactivity was excreted with the faeces (ca. 45 – 58% of the administered dose) and the other half with the urine (ca. 42 – 48%). The values and time course of excretion showed no distinct dependence on the sex or pre-treatment, whereas excretion of the high dosed rats was slightly slower. The male rats of the bile-duct cannulation experiment had excreted only very minor amounts of 1.4% of the administered dose with the bile at sacrifice.

Metabolism

The metabolic profiles of rat urine, bile, and faeces were recorded using reversed phase HPLC with radiodetection. Parent compound and all metabolites were first identified by LC-MS/MS in rat urine samples during preliminary metabolism investigations conducted in the quantitative whole body autoradiography study with [thiophene-4-^{14}C]-BYH 18636 (KIIA 5.1/03, Justus K., 2006, M-257644-01-2). In the current study, identification of parent compound and metabolites was achieved by co-chromatography with non-radiolabelled reference compounds using two chromatographic methods: reversed phase HPLC and normal phase TLC.

The identification rates were high, ca. 84 – 93% of the administered dose was identified. In the bile duct-cannulation test, the metabolite profiles of the bile and the urine were completely identified.

BYH 18636 was metabolised to only a low degree. Parent compound was the major component of the profiles and represented ca. 81 – 92% of the dose (tests 1 – 4). One minor metabolite, BYH 18636-sulfonamide-carboxylic acid, was found with ca. 1 – 2% of the dose and one trace metabolite, BYH 18636-thienosaccharine, with ca. 0.1 – 0.2%. BYH 18636-sulfonamide was detected at ca. 0.7 – 1.5% of the dose, but this compound was only tentatively identified as metabolite because trace amounts were present in the application suspensions and it might have formed from the parent compound by degradation in the faeces extract samples.

The metabolite profiles in excreta and the bile showed no dependence on sex, dose level, or pre-treatment.

Table 5.1-4 Metabolites in ADME tests with [thiophene-4-^{14}C]-BYH 18636

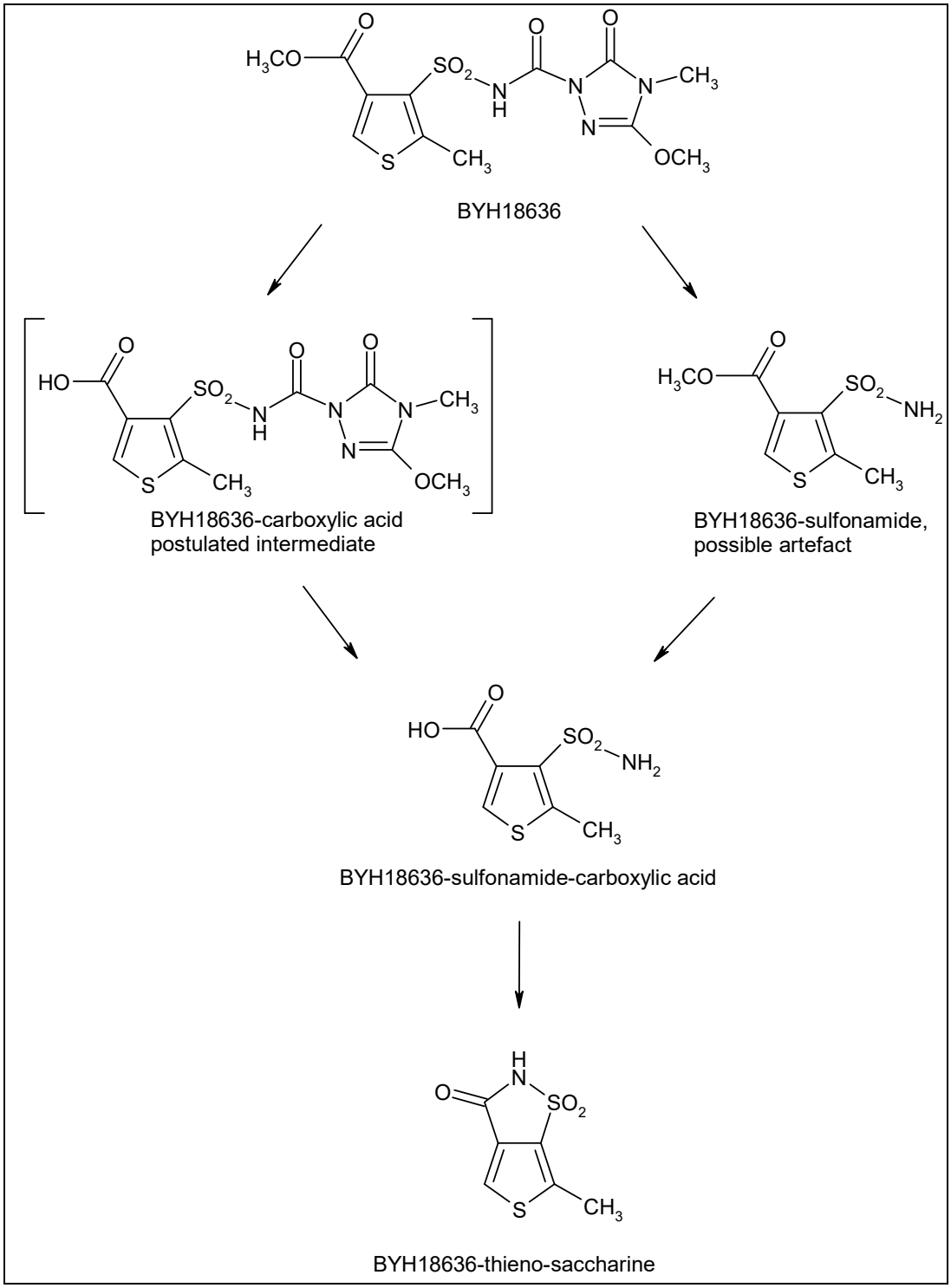
	Percent of administered dose				
	Test 1 2 mg/kg male	Test 2 2 mg/kg female	Test 3 100mg/kg male	Test 4 2 mg/kg pre-treat. male	Test 5 2 mg/kg bile test male
Excreted with urine, bile, and faeces	94.68	91.32	99.27	95.78	96.29
Compound (report name)					
BYH 18636-thienosaccharine	0.21	0.14	0.13	0.20	0.13
BYH 18636-sulfonamide-carboxylic acid	2.16	1.45	1.31	1.89	1.31
BYH 18636-sulfonamide*	0.69	1.52	n.d.	1.18	0.13
BYH 18636 (parent compound)	85.09	81.05	91.72	87.36	33.17
Total identified	88.15	84.16	93.16	90.63	34.74
unknown 1	0.10	0.00	0.00	0.00	0.00
unknown 2	0.03	0.00	0.00	0.00	0.00
Total unidentified	0.13	0.00	0.00	0.00	0.00
Faeces extracts not analysed	5.83	4.39	5.74	3.56	n.a.
Total characterised = total unidentified and faeces extracts not analysed	5.95	4.39	5.74	3.56	0.00
Solids after faeces extraction	0.56	0.41	0.36	0.55	n.a.
Urine samples not analysed (24 - 48 h)	n.a.	2.36	n.a.	1.04	1.33
Bile samples not analysed (24 - 48 h)	n.a.	n.a.	n.a.	n.a.	0.03
Faeces samples not analysed (0 - 48 h)	n.a.	n.a.	n.a.	n.a.	60.18
Total excreta samples not analysed	0.56	2.77	0.36	1.59	61.55
Total	94.67	91.32	99.27	95.78	96.28

n.a. = not applicable
n.d. = not detected
* tentatively identified as a metabolite, compound may be an artefact

Hydrolysis was the degradation reaction for the first metabolisation steps. The urea group was cleaved, releasing the thiophene-sulfonamide moiety, and the methyl ester was hydrolysed, yielding the metabolite BYH 18636-sulfonamide-carboxylic acid, which in turn was cyclised to BYH 18636-thienosaccharine, forming a new, intramolecular sulfonamide bond.

Based on these results, it is supposed that the biokinetic and metabolic behaviour of [thiophene-4-^{14}C]BYH 18636 in rats is sufficiently understood and the following biotransformation pathway is proposed (Figure 5.1-1).

Figure 5.1-1 Metabolic pathway of [thiophene-4-^{14}C]-BYH 18636 in rats



EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable The study (M41849131) is compliant with OECD 417: the four toxicokinetics studies reported in this section are considered to fully address the critical requirements of this guideline.
Conclusions	The study has adequately investigated the toxicokinetics of thiophene-labelled thiencarbazone-methyl following gavage administration. However limited cleavage of the molecule was shown, indicating that an additional study using a further radiolabel site may be required (see Justus & Spiegel, 2006, below). Thiencarbazone-methyl was found to be rapidly (predominantly within 24 hours; C_{max} attained within one hour) but moderately (~42-50%) absorbed following oral administration. Distribution at 48h post dose was extensive and relatively even, however higher levels of radioactivity were noted in the lungs, liver and fat. Metabolism of thiencarbazone-methyl was limited, with approximately 81-92% of the administered dose excreted as unchanged parent compound; three minor metabolites were identified. Excretion was relatively rapid and extensive, with little residual radioactivity (<1% of the administered dose) detected in tissues at 48 hours following administration; there is therefore little potential for bioaccumulation. Excretion was approximately equal in the urine (42-48%) and faeces (45-58%). Studies in cannulated male rats indicated little biliary excretion (1.4%). Toxicokinetic parameters were found to be largely independent of sex, dose level or dosing pattern.

Report:	**KIIA 5.1/02, Justus K., Spiegel K.; 2006 (MRID # 47070150)**
Title:	[Dihydrotriazole-3-^{14}C]BYH18636: Absorption, Distribution, Excretion and Metabolism in the Rat
Report No & Document No	MEF-06/049 M-275826-01-2
Guidelines:	EPA-Health Effects Guidelines, OPPTS 870.7485; EU Council Directive 91/414/EEC amended by Commission Directive 94/79/EC; PMRA Ref.: DACO 4.5.9 Metabolism/ Toxicokinetics in Mammals (Lab. Animal); OECD Guideline for Testing of Chemicals No. 417, Toxicokinetics, Japanese MAFF New Test Guidelines for Supporting Registration of Chemical Pesticides, 12 Nousan 8147
GLP	Yes

Executive Summary

The biokinetic behaviour and metabolism of BYH 18636 were investigated in the rat using [dihydro-triazole-3-^{14}C]-BYH 18636.

A group of 4 male rats was orally administered by gavage with a single dose of [dihydrotriazole-3-^{14}C]-BYH 18636 in 0.5% aqueous Tragacanth® at a target dose level of 2 mg/kg bw. The animals were sacrificed 2 days after dosing.

Approximately 99% of the administered dose was recovered in urine and faeces, as well as in organs and tissues at sacrifice.

The absorption of radioactivity commenced immediately after the oral administration and amounted to at least 55 % of the total recovered radioactivity.

The excretion was almost complete with approx. 98% of the administered dose detected in urine and faeces at the time of sacrifice, 48 hours after dosing. Approximately one half of the radioactivity was excreted with the faeces and the other half with the urine. The plasma concentration courses and toxicokinetic calculations demonstrated efficient elimination of the radioactivity from the systemic compartment.

Total radioactive residues (TRR) in the organs and tissues were rather low. The comparatively highest concentration was found in the thyroid gland with 0.0271 µg/g, which corresponded approximately to the blood plasma level (0.0248 µg/g). These values are followed by the levels found in liver (0.0165 µg/g), the adrenal gland 0.0161 µg/g) and the carcass (0.0141 µg/g). The equivalent concentrations in all other organs and tissues were below 0.01 µg/g.

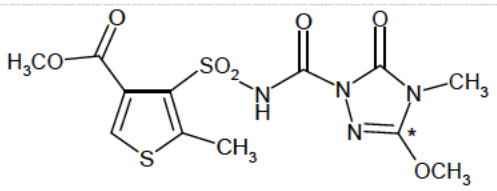
BYH 18636 was metabolised to a low extent. Parent compound was the major compound in the profiles and represented approx. 91% of the dose. Five trace metabolites were detected: BYH 18636-MMT, BYH 18636-OMT and methyl-carbamate, as well as two unknown metabolites, each representing less than 1% of the administered dose.

Metabolic degradation of BYH 18636 was started with hydrolysis resulting in BYH 18636-MMT. Desmethylation of BYH 18636-MMT led to BYH 18636-OMT and subsequent cleavage of the triazolinone moiety to methyl-carbamate.

The biokinetic and metabolic behaviour of [dihydrotriazole-3-^{14}C]BYH 18636 in rats is sufficiently understood.

I. Material and Methods

1. Test Material

Chemical structure	 * position of the radiolabel
Radiolabeled test material	[Dihydrotriazole-3-^{14}C]BYH 18636
Specific radioactivity	122 µCi/mg (= 4.5 MBq/mg)
Chemical Purity	> 98% (certified, HPLC with UV detection at 210 nm)
Radiochemical purity	>98% (certified, HPLC with radiodetection) >99% (certified, TLC with radiodetection)
Vehicle	0.5 % aqueous tragacanth solution
Preparation of dosing solution	radiolabeled test compound suspended in aqueous tragacanth solution

2. Test Animals

Species	Rat (*Rattus norvegicus*)
Strain	Wistar Hsd/Cpb: WU
Age/weight at study initiation	approx. 8 weeks / approx. 200 g
Source/breeder	Harlan & Winkelmann GmbH, 33178 Borchen, Germany
Sex and number of animals	4 male rats
Acclimation period	approximately one week
Identification	labelling by cage cards and with water-insoluble spots on the tail
Housing	in Makrolon® metabolism cages at approx. 24°C; humidity: 40 – 70%; air changes: 10 – 15 times/h; photoperiod: 12 h dark / 12 h light

Diet	ad libitum with rat/mice maintenance long life diet (max. approx. 16 g per animal and day); last time fed ca. 16 hours prior to and again ca. 6 hours after dosing
Water	ad libitum tap water from the local mains supply in accordance with local drinking water regulation

3. Study Design

Table 5.1-5 Experiments conducted within ADME study of [dihydrotriazole-3-^{14}C]-BYH 18636

Test Group	Dose of labelled material	Number/ sex	Sample collection schedule
test 1	2 mg/kg bw (low dose), single oral dose	4 males	plasma: 0.17, 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 24, 32, and 48 h after administration urine: 4, 8, 12, 24, and 48 hours after administration faeces: 24 and 48 hours after administration blood, organs, and tissues: at sacrifice, 48 hours after administration

[Dihydrotriazole-3-^{14}C]-BYH 18636 was administered by oral gavage to male rats at dose levels of 2 mg/kg bw. Samples of plasma, urine, faeces and bile were collected separately for each animal at various times.

The study was performed in parallel to study MEF-05/176 (KIIA 5.1/01, Justus K., 2006a). The study design (dose finding and administration, sampling and analytical methods) was the same as in that study and was described in detail in point KIIA 5.1/01.

II Results and Discussion

Recovery

Approximately 99% of the administered dose was recovered from measurements of the total radio-activity in urine, faeces and organs and tissues at sacrifice.

Table 5.1-6 Recovery of [dihydrotriazole-3-^{14}C]-BYH 18636 in the ADME study

	Radioactivity in percent of dose administered (mean values)	Radioactivity in percent of dose recovered (mean values)
Urine	54.16	54.77
Faeces	44.25	44.74
Total excreted	**98.41**	**99.51**
Skin	0.044	0.045
Sum organs/tissues	0.381	0.386
Body without GIT	0.425	0.431
GIT	0.067	0.068
Total in body	0.493	0.499
Balance	**98.90**	**–**
Normalisation factor	**–**	**1.01**

Absorption

The absorption rate was at least 55% of the total radioactivity recovered at the low dose level, calculated from the radioactivity detected in urine and the body without the gastrointestinal tract.

Distribution and plasma kinetics

The values from the pharmacokinetic model and from the plasma concentration courses demonstrated rapid absorption of the [dihydrotriazole-3-^{14}C]-BYH 18636 related radioactivity from the gastrointestinal tract followed by efficient elimination.

After a very short absorption phase, a maximum plasma concentration of approx. 3.02 µg/g (denotes µg a.s. equiv./g) was already reached 0.94 hours after administration. The absorption was followed by a fast initial elimination phase with a half live of 0.30 hours and a slower terminal elimination phase with a half live of 35.5 hours. The mean residence time was 15 hours. The AUC value was 13.6 µg/g x hours. The dose normalised concentrations of the radioactivity in the plasma showed the course already known from the [thiophene-4-^{14}C]BYH 18636 ADME study.

Tissue distribution at sacrifice

The radiolabelled residues in the organs and tissues of the animals were determined at sacrifice, 48 h after the oral administration. The residues in the bodies were very low. Approx. 0.49% of the administered dose was found in the total body, thereof 0.04% in the skin, 0.07% in the gastrointestinal tract and its contents (GIT), and approx. 0.38% in the organs and tissues. Among the organs and tissues, the highest residues were found in the carcass (0.32%), followed by the low residues in the liver (0.04%). All other analysed organs and tissues contained approx. 0.01% or less of the administered radioactivity.

Total radioactive residues (TRR) in the organs and tissues were low. The highest concentration was in the thyroid gland with 0.027 µg/g, which corresponded approximately to the blood plasma level (0.025 µg/g), followed by liver (0.017 µg/g), the adrenal gland (0.016 µg/g) and the carcass (0.014 µg/g). Residues in all other organs and tissues were below 0.01 µg/g.

Table 5.1-7 Equivalent concentration C and dose-normalised concentration CN (mean values) in the ADME study with [dihydrotriazole-3-^{14}C]-BYH 18636

Tissues/organs	Equivalent concentration C [µg/g]	Dose normalised concentration
Erythrocytes	0.0045	0.0020
Plasma	0.0248	0.0110
Spleen	0.0036	0.0016
Liver	0.0165	0.0073
Kidney	0.0061	0.0027
Perirenal fat	0.0046	0.0020
Adrenal gland	0.0161	0.0072
Testis	0.0028	0.0012
Skeleton muscle	0.0039	0.0017
Bone femur	0.0051	0.0023
Heart	0.0034	0.0015
Lung	0.0040	0.0018
Brain	0.0006	0.0003
Thyroid gland	0.0271	0.0121
Skin	0.0044	0.0020
Carcass	0.0141	0.0063
Body without GIT	0.0109	
GIT	0.0127	0.0056
Total in body	0.0111	

Excretion

The excretion of [dihydrotriazole-3-^{14}C]BYH 18636 was fast with approximately 97% of the administered dose excreted with urine and faeces within only 24 hours. At sacrifice, 48 hours after dosing, the excretion was almost complete (approximately 98% of the administered dose). About one half of the radioactivity was excreted with the faeces (approx. 44% of the administered dose) and the other half with the urine (approximately 54%).

Metabolism

The metabolic profiles of rat urine and faeces extracts were recorded using reversed phase radio-HPLC. Parent compound and two metabolites were first identified by LC-MS/MS in rat urine during preliminary metabolism investigations conducted in the quantitative whole body autoradiography study with [dihydrotriazole-3-^{14}C]-BYH 18636 (MEF-05/179). In the current study, identification of parent compound and metabolites was achieved by co-chromatography with radiolabelled and non-radiolabelled reference items using predominantly two chromatographic methods: reversed phase HPLC and normal phase TLC.

The identification rate was high and approximately 93% of the administered dose was identified.

BYH 18636 was metabolised only to a low extent and represented approximately 91% of the dose. Five trace metabolites were detected (each of them < 1% of the administered dose): Methyl carbamate, BYH 18636-OMT, BYH 18636-MMT and two unknown metabolites. BYH 18636-MMT was found at approximately 0.2% of the administered dose in the application suspension. However, an additional amount corresponding to approximately 0.3% of the administered dose was produced in the rat and BYH 18636-MMT was therefore concluded to be a metabolite. Additionally, BYH 18636-triazolinone-carboxamide was detected at 0.1% of the administered dose and was presumably an artefact, since that amount was already present in the administration suspension.

Table 5.1-8 ADME study with [Dihydrotriazole-3-^{14}C]-BYH 18636

Test 1: male rats, 2 mg/kg bw	**Percent of administered dose**		
	Urine	Faeces	Sum
	0 – 48 h	0 – 48 h	0 – 48 h
Excreted with urine and faeces	54.16	44.25	98.41
Analysed extracts		39.10	
Compound (report name)			
methyl-carbamate	0.64	-	0.64
BYH 18636-OMT	0.36	-	0.36
BYH 18636-MMT*	0.47	-	0.47
BYH 18636-triazolinone-carboxamide**	0.11	-	0.11
BYH 18636 (parent compound)	52.40	38.67	91.07
Total identified	**53.97**	**38.67**	**92.64**
unknown 1	0.19	-	0.19
unknown 2	-	0.43	0.43
Total unknown	0.19	0.43	0.62
Extracts not analysed	-	4.17	4.17
Total characterised = Total unknown and extracts not analysed	0.19	4.17	4.79
Solids after extraction	-	0.98	0.98
Total	**54.16**	**44.25**	**98.41**

- = not detected

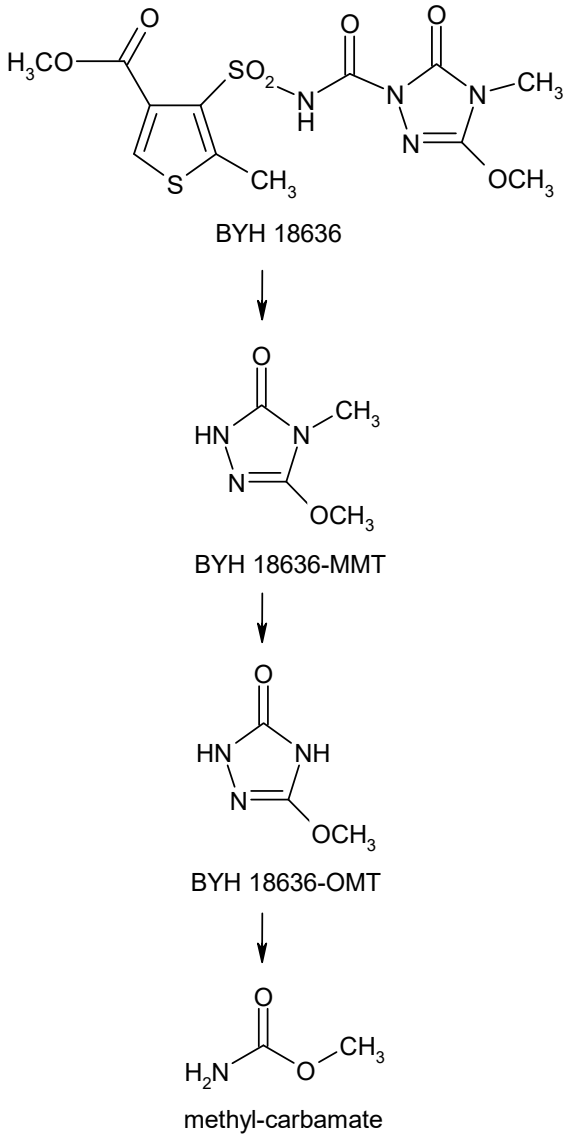
* The administration suspension contained 0.2% BYH 18636-MMT as impurity. Thus, only a part of the amount of BYH 18636-MMT detected in urine was considered to be a consequence of metabolism in the rat

** The administration suspension contained 0.1% BYH 18636-triazolinone-carboxamide as impurity. Thus, the presence of BYH 18636 triazolinone-carboxamide in urine was not considered to be a consequence of metabolism in the rat

Hydrolysis cleaved the urea group of BYH 18636 and resulted in BYH 18636-MMT. Desmethylation of BYH 18636-MMT led to BYH 18636-OMT and subsequent cleavage of the triazolinone moiety to methyl-carbamate.

Based on these results, it is concluded that the biokinetic and metabolic behaviour of [thiophene-4-^{14}C]BYH 18636 in rats is sufficiently understood and the biotransformation pathway presented on the following page is proposed:

Figure 5.1-2 Proposed metabolic pathway of [dihydrotriazole-3-^{14}C]BYH 18636 in rat



EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable The study (M21819148) is compliant with OECD 417. The four toxicokinetics studies reported in this section are considered to fully address the critical requirements of this guideline
Conclusions	The study has adequately investigated the toxicokinetics of dihydrotriazole-labelled thiencarbazone-methyl following repeated gavage administration at a low dose level to male rats. The use of a single sex and dose group is considered to be acceptable in light of the findings of the previous study (Justus, 2006a) in which the toxicokinetic parameters of thiophene-labelled thiencarbazone-methyl were found to be largely independent of sex, dose level or dosing pattern. Thiencarbazone-methyl was found to be rapidly (predominantly within 24 hours; C_{max} attained within one hour) but moderately (~55%) absorbed following oral administration. Distribution was extensive and relatively even, however slightly higher levels of radioactivity were noted in the adrenals and thyroids. In the absence of similar findings in the previous study using thiophem-labelled material (Justus, 2006a), it is assumed that this radioactivity is associated with metabolites formed following the cleavage of the thiencarbazone-methyl molecule and containing the triazole moiety, or from the breakdown products of these metabolites. Metabolism of thiencarbazone-methyl was limited, with 91% of the administered dose excreted as unchanged parent compound; five minor metabolites were detected, of which three were identified. Excretion was relatively rapid and extensive, with little residual radioactivity (<1% of the administered dose) detected in tissues at 48 hours following administration. The extent of excretion of radioactivity was comparable in the urine (44%) and faeces (54%).

Report:	**KIIA 5.1/03, Justus K.; 2006 (MRID 47727001).**
Title:	[Thiophene-4-^{14}C]BYH18636: Distribution of the total radioactivity in male rats determined by quantitative whole body autoradiography, determination of the exhaled $^{14}CO_2$
Report No & Document No	MEF-04/491 M-257644-01-2
Guidelines:	EPA-Health Effects Guidelines, OPPTS 870.7485; EU Council Directive 91/414/EEC amended by Commission Directive 94/79/EC; PMRA Ref.: DACO 4.5.9 Metabolism/ Toxicokinetics in Mammals (Lab. Animal); OECD Guideline for Testing of Chemicals No. 417, Toxicokinetics, Japanese MAFF New Test Guidelines for Supporting Registration of Chemical Pesticides, 12 Nousan 8147
GLP	Yes

Executive Summary

BYH 18636 was readily absorbed from the gastrointestinal tract, distributed among almost all organs and tissues, and excreted to approximately equal parts with the urine and faeces. Blackening by radioactivity originating from radiolabelled [thiophene-4-^{14}C]-BYH 18636 was most intense in the gastrointestinal tract, in the urinary bladder, and in the liver. Among the quantitatively analysed organs, tissues, and fluids, the highest equivalent concentrations were observed in the liver, blood, and kidney. Moderate peak concentrations were found in the lung, adrenal gland, myocardium, brown fat, skin,

salivary gland, pineal body, thyroid gland, and pituitary gland. Lower concentrations were reached in all other organs and tissues. The lowest peak concentrations were found in the spinal cord and brain.

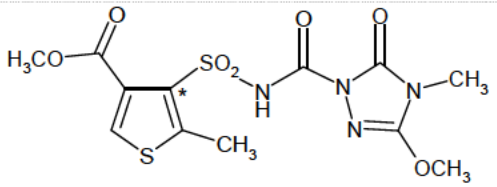
From peak values, a continuous decrease of radioactivity concentrations in the organs and tissues by several orders of magnitude, predominantly below the limit of detection or quantification was observed within the testing period of 7 days. Thus, continuous elimination of radioactivity from the body of the rats without any sign of retention was demonstrated.

Based on these results, the distribution of [thiophene-4-^{14}C]-BYH 18636 and corresponding metabolites in the organs and tissues of rats is supposed to be sufficiently understood.

Preliminary investigations on metabolism showed that BYH 18636 was metabolised only to a small degree. The parent compound was by far the major compound in the urine and faeces. The minor metabolite BYH 18636-sulfonamide-carboxylic acid and the trace metabolite BYH 18636-thienosaccharine were detected only in the urine. BYH 18636-sulfonamide, occurring as a trace component in the urine and faeces, was only tentatively identified as a metabolite, this compound was possibly an artefact.

I. Material and Methods

1. Test Material

Chemical structure	 * position of the radiolabel
Radiolabeled test material	[Thiophene-4-^{14}C]BYH 18636
Specific radioactivity	111.7 µCi/mg (= 4.13 MBq/mg)
Chemical Purity	> 99% (certified, HPLC with UV detection at 210 nm)
Radiochemical purity	>99% (certified, HPLC and TLC with radiodetection)
Vehicle	0.5 % aqueous tragacanth solution

2. Test Animals

Species	Rat (*Rattus norvegicus*)
Strain	Wistar Hsd/Cpb: WU
Age/weight at study initiation	approx. 9 weeks / approx. 206 g
Source/breeder	Harlan & Winkelmann GmbH, 33178 Borchen, Germany
Sex and number of animals	9 male rats
Acclimation period	approx. one week
Identification	labelling by cage cards and with water-insoluble spots on the tail
Housing	in Makrolon® metabolism cages at 23 - 25°C; humidity: 48 - 64%; air changes: 10 – 15 times/h; photoperiod: 12 hrs dark / 12 hrs light
Diet	ad libitum with rat/mice maintenance long life diet (max. approx. 18 g per animal and day); last time fed ca. 16 hours prior to and again ca. 8 hours after dosing
Water	ad libitum tap water from the local mains supply in accordance with local drinking water regulation

3. Study Design

Test Group	Dose of labelled material	Number/ sex	Sample collection schedule
-	3 mg/kg bw, single oral dose	8 males	autoradiography: 4, 8, 24, 48, 72, 96, 120, 144, and 168 h after administration urine: 4, 8, 24, 48, 72, 96, 120, 144, and 168 hours after administration faeces: 24, 48, 72, 96, 120, 144, and 168 hours after administration expired air: 24 and 48 hours after administration

Dosing and sample collection

Eight animals received each 2 mL of the application suspension of [thiophene-4-^{14}C]-BYH 18636 (resulting in a nominal dose of 3 mg/kg bw) and one control animal received 2 mL of the application suspension of non-radiolabelled test compound. The suspension was administered to the rats by oral gavage using a syringe attached to an animal-feeding knob cannula.

After administration of the radiolabelled test substance, the rats were kept individually in Makrolon® metabolism cages, which allowed for separate and quantitative collection of urine, faeces, and expired air.

Collection of excreta

Immediately after the administration of the test compound, animals were transferred to metabolism cages. Urine and faeces were collected separately and quantitatively for each animal. Urine was collected in a cryogenic trap, and radioactivity was determined by LSC (liquid scintillation counter). Collected faeces were lyophilised (freeze-dried), weighed, homogenised and radioactivity was determined by combustion/LSC.

Collection of air

Carbon dioxide and other volatiles from expired air were collected from four animals. The respective metabolism cages were attached to a high velocity air pump and ventilated with ca. 2 L of air per minute and cage. The out coming air was passed through a trapping system of two gas-washing bottles containing a 1:1-mixture of ethanolamine/ethanol. At sampling, the exact volume was determined, from which an aliquot was taken for the determination of radioactivity.

Analytical methods

Profiles from urine and faeces extract were recorded with reversed phase HPLC using radiodetection. Metabolites were identified with LC-MS/MS after isolation from urine. The identification was confirmed by HPLC co-chromatography with reference compounds.

Autoradiography

After sacrifice, the animals were fixed in a stretched position using a metal template and immediately frozen at ca. – 70°C in a dichloromethane/dry ice bath. After removal of the template, the animal body together with a series of blood standards was embedded in a slurry of carboxymethylcellulose (7 to 8%) on the platform of the microtome. The block was deep-frozen.

Sections with a thickness of 50 µm were cut from the block; the sections were attached to adhesive tape and freeze-dried overnight in the cooling cabinet of the microtome. From each animal, four sections showing the relevant organs and tissues were prepared. Imaging plates were exposed to the

sections, with two sections per plate, using exposure times between 48 and 120 hours. The exposed imaging plates were scanned using the Fuji BAS 5000® image analyser and the resulting autoradiography pictures were printed on a color printer. In addition, the sections were scanned using a color scanner. The sections were stored at –20°C at all times, except during exposure, which was performed at ambient temperature.

In order to correct for possible chemographic effects, one animal per test group was orally administered with the same dose of the non-radiolabelled test item and sacrificed after 4 hours. The sections from this control animal were exposed under identical conditions, using the longest exposure time which had been used for rat sections of animals treated with radiolabelled test item. No blackening of typical animal structures could be observed after an exposure time of 120 hours, so that the data obtained for the animals treated with radiolabelled test item were not corrected against the control animal.

The digital images of the autoradiograms obtained by the Fuji BAS 5000 system were used for the assessment of the distribution of radioactivity concentrations in different organs and tissues.

II Results and Discussion

The distribution of BYH 18636 in male rats was investigated by quantitative whole body autoradiography (QWBA) using the radioluminography (RLG) technique. The data were obtained over a testing period of 7 days following a single oral administration of radiolabelled [thiophene-4-^{14}C]-BYH 18636 at a dose level of 3 mg/kg body weight (bw). Eight rats were treated and sacrificed 1, 4, 8, 24, 48, 72, 120, and 168 hours after dosing.

BYH 18636 was readily absorbed from the gastrointestinal tract, distributed among almost all organs and tissues, and excreted to approximately equal parts with the urine and faeces. At 48 hours after dosage, the excretion was nearly complete. Only 0.01 % of the administered dose was expired, thus demonstrating the stability of the ^{14}C-thiophene labelling position.

Already at 1 hour after dosage, the radioactivity was distributed among almost all organs and tissues. Radioactivity related blackening was most intense in the gastrointestinal tract, the urinary bladder, and the liver. The pattern of blackening in the gastric mucosa of the stomach and also in the small intestine at about 4 h after administration indicated, that extrabiliary secretion into the gastrointestinal tract occurred to some extend.

Peak equivalent concentrations in the quantitatively analysed organs and tissues were reached already at 1 hour after dosage. The highest equivalent concentrations were found in the liver with up to 4.7 µg/g, followed by values of up to approx. 2.3 µg/g in the blood and 2.1 µg/g in the kidney. Moderate values of up to approx. 1.0 µg/g were found in the lung, adrenal gland and myocardium, 0.9 µg/g in the brown fat, 0.7 µg/g in skin and salivary gland as well as 0.6 µg/g in the pineal body, thyroid gland, and pituitary gland. All other organs and tissues showed peak values below 0.5 µg/g. Spinal cord and brain had the lowest peak concentrations with approx. 0.06 and 0.05 µg/g, respectively.

From peak values, the concentrations of radioactivity in the organs and tissues decreased continuously by several orders of magnitude during the test period of 7 days. At 168 hours after dosage, all values were below the limit of quantitation or detection, except for the nasal mucosa, liver and testes, which had a very low concentrations of 0.014, 0.009 and 0.005 µg/g, respectively. Thus, continuous elimination of radioactivity from the body of the rats without any sign of retention was demonstrated.

Based on these results, the distribution of [thiophene-4-^{14}C]-BYH 18636 and corresponding metabolites in the organs and tissues of rats is supposed to be sufficiently understood.

Table 5.1-9 TRR in organs and tissues

	TRR in organs and tissues [µg a.s. equivalents/g wet]							
	Animal no.							
	Time of sacrifice (hours after administration)							
Tissue/Organ	**744**	**745**	**746**	**747**	**748**	**749**	**750**	**751**
	1 h	**4 h**	**8 h**	**24 h**	**48 h**	**72 h**	**120 h**	**168 h**
Blood	2.252	0.352	0.082	0.013	0.006	< LOD	< LOD	---
Liver	4.700	1.138	0.441	0.137	0.121	0.056	0.027	0.009
Renal cortex	1.444	0.275	0.127	0.057	0.027	0.015	---	---
Renal medulla	2.079	0.688	0.172	0.033	0.022	0.013	---	---
Brown fat	0.860	0.173	0.098	0.060	0.038	0.022	0.009	---
Perirenal fat	0.247	0.057	0.052	0.046	0.034	0.012	0.008	---
Skeleton muscle	0.306	0.044	0.015	< LOQ	< LOD	< LOD	< LOD	---
Myocardium	0.983	0.149	0.041	0.007	< LOQ	< LOD	---	---
Lung	1.032	0.246	0.148	0.050	0.033	0.015	0.008	< LOQ
Spleen	0.365	0.054	0.015	< LOQ	< LOD	< LOD	< LOD	---
Pancreas	0.344	0.054	---	< LOD	< LOD	< LOD	< LOD	---
Bone marrow	0.404	0.071	---	---	---	---	---	---
Testes	0.343	0.155	0.079	0.046	0.027	0.019	0.012	0.005
Brain	0.050	0.011	< LOQ	< LOD	< LOD	< LOD	< LOD	---
Spinal cord	0.057	0.012	< LOQ	< LOD	---	---	---	---
Pituitary gland	0.561	0.094	0.031	---	---	---	---	---
Pineal body	0.631	0.079	0.024	---	---	---	---	---
Adrenal gland	1.013	0.158	0.046	0.007	< LOQ	< LOD	< LOD	< LOD
Thymus	0.352	0.055	0.015	< LOQ	< LOD	< LOD	---	---
Thyroid gland	0.584	0.107	0.039	0.019	0.016	---	---	---
Salivary gland	0.667	0.105	0.027	0.006	< LOQ	< LOD	---	---
Nasal mucosa	0.261	0.126	0.165	0.123	0.073	0.056	0.023	0.014
Skin	0.727	0.147	0.064	0.034	0.021	---	---	---
Vitreal body	0.162	0.056	0.022	< LOD	< LOD	< LOD	---	---

---: not discernable in the rat sections

Preliminary investigations on metabolism were conducted with selected excreta samples. A faeces extract was prepared and represented 98.4% of the recovered radioactivity in the faeces. Profiles from urine and faeces extract were recorded with reversed phase HPLC using radiodetection. Metabolites were identified with LC-MS/MS after isolation from urine. The identification was confirmed by HPLC co-chromatography with reference compounds. BYH 18636 was metabolised only to a small degree. The parent compound was by far the major compound in the urine and faeces. The minor metabolite BYH 18636-sulfonamide-carboxylic acid and the trace metabolite BYH 18636-thienosaccharine were detected only in the urine. Profiles of urine from different collection periods were very similar. BYH 18636-sulfonamide, occurring as a trace component in the urine and faeces, was only tentatively identified as a metabolite. The compound was possibly an artefact. BYH 18636 was shown to degrade considerably to BYH 18636-sulfonamide after several days at elevated temperature.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable The study (M1811322) complies with OECD 417. The four toxicokinetics studies reported in this section are considered to fully address the critical requirements of this guideline
Conclusions	The findings of this study are consistent with those of the previous study using thiophene-radiolabelled thiencarbazone-methyl (Justus, 2006a). Rapid absorption and excretion were apparent, with little tissue retention of radioactivity at 168 hours following administration. The highest tissue level at 168 hours was measured in the nasal mucosa. Findings indicate adequate exposure of the bone marrow. The only tissues with radioactivity levels exceeding blood at early time points are the liver and kidney; findings are consistent with ongoing excretion.

Report:	**KIIA 5.1/04, Schmeer K., Justus K.; 2006 (MRID 47727002).**
Title:	[Dihydrotriazole-3-^{14}C]BYH18636: Distribution of the total radioactivity in male rats determined by quantitative whole body autoradiography, determination of the exhaled $^{14}CO_2$
Report No & Document No	MEF-05/179 M-263383-01-2
Guidelines:	EPA-Health Effects Guidelines, OPPTS 870.7485; EU Council Directive 91/414/EEC amended by Commission Directive 94/79/EC; PMRA Ref.: DACO 4.5.9 Metabolism/ Toxicokinetics in Mammals (Lab. Animal); OECD Guideline for Testing of Chemicals No. 417, Toxicokinetics, Japanese MAFF New Test Guidelines for Supporting Registration of Chemical Pesticides, 12 Nousan 8147
GLP	Yes

Executive Summary

BYH 18636 was readily absorbed from the gastrointestinal tract, distributed among almost all organs and tissues, and excreted to approximately equal parts with the urine and faeces. Blackening by radioactivity originating from [dihydrotriazole-3-^{14}C]-BYH 18636 was most intense in the gastrointestinal tract, kidney, and in the liver. Among the quantitatively analyzed organs, tissues, and body fluids, the highest equivalent concentrations were observed in the liver, blood, and kidney. Moderate peak concentrations were found in the lung and myocardium, brown fat, adrenal gland, skin, salivary gland, thyroid gland, pituitary gland, and pineal body. Lower concentrations were reached in all other organs and tissues. The lowest peak concentrations were found in the spinal cord and brain.

From peak values, a continuous decrease of radioactivity concentrations in the organs and tissues by several orders of magnitude, predominantly below the limit of detection or quantification was observed already after 3 days. Thus, continuous elimination of radioactivity from the body of the rats without any sign of retention was demonstrated.

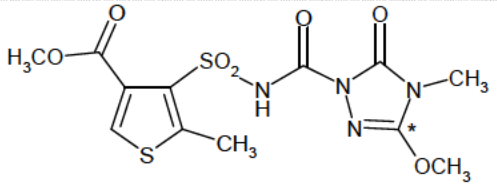
The distribution of [dihydrotriazole-3-^{14}C]-BYH 18636 and corresponding metabolites in the organs and tissues of rats is sufficiently understood.

Preliminary investigations on metabolism showed that BYH 18636 was metabolized only to a small degree. The test item was by far the major compound in the urine and faeces. Traces of BYH 18636-MMT and BYH 18636-triazolinone-carboxamide were identified in urine and traces of BYH 18636-

MMT were found in faeces as well. Both components can only tentatively be regarded as metabolites, since they were detected in the application solution as well.

I. Material and Methods

1. Test Material

Chemical structure	 * position of the radiolabel
Radiolabeled test material	[Dihydrotriazole-3-^{14}C]-BYH 18636
Specific radioactivity	117.83 µCi/mg (= 4.36 MBq/mg)
Chemical Purity	> 99% (certified, HPLC with UV detection at 210 nm)
Radiochemical purity	>99% (certified, HPLC and TLC with radiodetection)
Vehicle	0.5 % aqueous tragacanth solution

2. Test Animals

Species	Rat (*Rattus norvegicus*)
Strain	Wistar Hsd/Cpb: WU
Age/weight at study initiation	8 weeks / approx. 200 g
Source/breeder	Harlan & Winkelmann GmbH, 33178 Borchen, Germany
Sex and number of animals	9 male rats
Acclimation period	approximately one week
Identification	labelling by cage cards and with water-insoluble spots on the tail
Housing	in Makrolon® metabolism cages at 22 – 24°C; humidity: 53 – 66%; air changes: 10 – 15 times/h; photoperiod: 12 hrs dark / 12 hrs light
Diet	ad libitum with rat/mice maintenance long life diet (max. approx. 16 g per animal and day); last time fed ca. 16 hours prior to and again ca. 6 hours after dosing
Water	ad libitum tap water from the local mains supply in accordance with local drinking water regulation

3. Study Design

The study was performed in parallel to study MEF-04/491 (KIIA 5.1/03, Justus K., 2006). The study design (dose finding and administration, sampling and analytical methods) was the same as in that study and was described in detail in point KIIA 5.1/03.

II Results and Discussion

The distribution of BYH 18636 in male rats was investigated by quantitative whole body autoradiography (QWBA) using the radioluminography (RLG) technique. The data were obtained over a testing period of 7 days following a single oral administration of [dihydrotriazole-3-^{14}C]BYH 18636 at a dose level of 3 mg/kg body weight (bw). Eight rats were treated and sacrificed 1, 4, 8, 24, 48, 72, 120, and 168 hours after dosing.

BYH 18636 was readily absorbed from the gastrointestinal tract, distributed among almost all organs and tissues, and excreted to approximately equal parts with the urine and faeces. At 48 hours after dosage, the excretion was nearly complete. Only 0.8 % of the administered dose was expired, demonstrating a sufficient stability of the ^{14}C- dihydrotriazole labeling position.

Already at 1 hour after dosage, the radioactivity was distributed among almost all organs and tissues. Radioactivity related blackening was most intense in the gastrointestinal tract, the kidney, and the liver.

Peak equivalent concentrations in the quantitatively analyzed organs and tissues were reached already at 1 hour after dosage. The highest equivalent concentrations were found in the liver with up to 5.0 µg/g, followed by values of up to approx. 2.3 µg/g in the blood and 2.1 µg/g in the kidney. Moderate values of up to approx. 1.5 µg/g were found in the lung and 1.0 µg/g in the myocardium. Values between 0.9 µg/g and 0.5 µg/g were found in the adrenal gland, in the brown fat, in skin and salivary gland, in thyroid gland, pituitary gland as well as in the pineal body. All other organs and tissues showed peak values below 0.5 µg/g. Spinal cord and brain had the lowest peak concentrations with less than 0.1 µg/g.

From peak values, the concentrations of radioactivity in the organs and tissues decreased continuously by several orders of magnitude during the test period of 7 days. Already at 72 hours after dosage, almost all values except those for liver and nasal mucosa were below the limit of quantification or detection. Thus, continuous elimination of radioactivity from the body of the rats without any sign of retention was demonstrated.

Table 5.1-10 TRR in organs and tissues

Tissue/Organ	TRR in organs and tissues [µg/g]							
	Animal no.							
	753 1h	754 4h	755 8h	756 24h	757 48h	758 72h	759 120h	760 168h
Blood	2.254	0.363	0.086	0.011	0.005	---	---	---
Liver	5.038	1.257	0.210	0.031	0.015	0.007	0.007	< LOQ
Renal cortex	1.740	0.334	0.060	0.011	---	---	---	---
Renal medulla	2.149	0.483	0.097	0.012	---	---	---	---
Brown fat	0.847	0.111	0.031	---	---	---	---	---
Perirenal fat	0.246	0.072	---	---	---	---	---	---
Skeleton muscle	0.279	0.085	0.031	0.006	< LOQ	---	---	---
Myocardium	0.953	0.183	0.049	0.006	< LOD	---	---	---
Lung	1.470	0.250	0.056	---	---	---	---	---
Spleen	0.345	0.093	0.030	< LOQ	< LOQ	---	---	---
Pancreas	0.356	0.104	0.038	0.005	< LOQ	---	---	---
Bone marrow	0.463	0.091	0.034	---	---	---	---	---
Testes	0.338	0.125	0.034	< LOQ	< LOD	---	---	---
Brain	0.072	0.045	0.022	< LOD	< LOD	---	---	---
Spinal cord	0.080	0.044	0.020	< LOQ	< LOD	---	---	---
Pituitary gland	0.570	0.131	0.034	0.013	---	---	---	---
Pineal body	0.585	0.166	0.036	---	---	---	---	---
Adrenal gland	0.903	0.200	0.063	0.007	---	---	---	---
Thymus	0.405	0.092	0.033	< LOQ	---	---	---	---
Thyroid gland	0.621	0.160	---	---	---	---	---	---
Salivary gland	0.719	0.128	0.038	---	---	---	---	---
Nasal mucosa	0.222	0.071	0.035	0.021	0.012	0.007	0.007	0.007
Skin	0.741	0.169	0.050	---	---	---	---	---
Vitreal body	0.159	0.086	0.037	< LOQ	< LOQ	---	---	---

---: not discernable in the rat sections

Preliminary investigations on metabolism were conducted with selected excreta samples. A faeces extract was prepared and represented 98.9% of the recovered radioactivity in the faeces. Profiles from urine and faeces extract were recorded with reversed phase HPLC using radio detection. Metabolites were identified with LC-MS-MS after isolation from urine. The identification was confirmed by HPLC co-chromatography with reference items. BYH 18636 was metabolized only to a small degree. The test item was by far the major compound in the urine and faeces.

Traces of BYH 18636-MMT and BYH 18636-triazolinone-carboxamide were identified in urine and traces of BYH 18636-MMT were found in faeces as well. Both components can only tentatively be regarded as metabolites, since they were detected in the application solution as well.

Based on these results, the distribution of [dihydrotriazole-3-^{14}C]-BYH 18636 and corresponding metabolites in the organs and tissues of rats is supposed to be sufficiently understood.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable The study (M1811323) complies with OECD 417. The four toxicokinetics studies reported in this section fully are considered to fully address the critical requirements of this guideline.
Conclusions	The findings of this study are consistent with those of the previous study using dihydrotriazole-radiolabelled thiencarbazone-methyl (Justus & Spiegel, 2006). Rapid absorption and excretion of radioactivity were apparent, with little tissue retention of radioactivity at 168 hours following administration. Consistent with the previous study using thiophene-radiolabelled thiencarbazone-methyl (Justus, 2006), the highest (and only quantifiable) tissue levels of radioactivity at 168 hours were measured in the nasal mucosa.

B.6.2 Acute toxicity, irritancy and skin sensitisation studies (IIA 5.2)

B.6.2.1 Acute oral toxicity (IIA 5.2.1)

Report:	**KIIA 5.2.1/01, Schüngel M.; 2004, (MRID # 47070205)**
Title:	BYH 18636, Acute toxicity in the rat after oral administration
Report No &	AT01452
Document No	M-088833-01-2
Guidelines:	OECD 423 (2001); EEC Directive 67/548 Annex V – Method B.1.tris (2004); EPA Health Effects test Guidelines (OPPTS 870.1100) (1998)
GLP	Yes (certified laboratory)

Executive Summary
In an acute oral toxicity study using a stepwise procedure, two groups of three fasted, young adult female Wistar rats (HsdCpb:Wu) were given successively a single oral dose of BYH 18636 (batch number MIX-batch 702-73-06-0001, 96.2% purity) in 2% Cremophor EL of 2 000 mg/kg bw and were observed for 14 days.
The dose of 2 000 mg/kg bw was tolerated by both groups without mortalities, clinical signs, effects on weight gain or gross pathological findings.

According to OECD guideline 423, the LD_{50} cut-off of BYH 18636 was ≥ 5 000 mg /kg bw (category 5 / unclassified of the global harmonized classification system).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636
- **Description:** White powder
- **Lot/Batch:** MIX-batch 702-73-06-0001
- **Purity:** 96.2%
- **CAS:** 317815-83-1
- **Stability of test compound:** Stable at 1 and 200 mg/ml at room temperature for 4 hours

2. Vehicle and /or positive control: 2% Cremophor EL in demineralized water

3. Test animals:
- **Species**: Rat
- **Strain:** HsdCpb:Wu
- **Age:** 10 to 14 weeks approximately
- **Weight at dosing:** 167 to 183 g
- **Source:** Harlan/Winkelmann GmbH, 33178 Borchen, Germany
- **Acclimation period:** At least 5 days
- **Diet:** Provimi Kliba 3883.0.15 maus/Ratte Haltung, Kaiseraugst Switzerland, *ad libitum*
- **Water:** Tap water, *ad libitum*
- **Housing:** Animals were group caged conventionally in polycarbonate cages on low dust wood granulate bedding
- **Environmental conditions –**
  - **Temperature:** 22 ± 2°C
  - **Humidity:** 55 ± 5%
  - **Air changes:** Approximately 10 changes per hour
  - **Photoperiod:** Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS:

1. In life dates: 28 April to 18 May, 2004

2. Animal assignment and treatment

The substance was tested using a stepwise procedure, each step using three female rats. The animals were assigned to their groups by randomization. The random list was based on evenly distributed chance numbers by a sofware application. Following an overnight fast (16 to 24 hours), each group received a single dose of 2 000 mg/kg of BYH 18636 (96.2% purity) by gavage. The test substance was administered in demineralized water with 2% Cremophor EL at a volume of 10 ml/kg bw. Clinical signs and mortality rates were determined several times on the day of administration and subsequently at least once daily for an observation period of at least 14 days. Body weights were recorded on days 1, 8 and 15. On day 15, surviving animals were sacrificed and all animals were necropsied and examined for gross pathological changes.

Table IIA 5.2.1-1 Doses, mortality / animals treated

Dose (mg/kg bw)	Females
2 000 (1st)	0/3
2 000 (2nd)	0/3

3. Statistics

The data did not warrant statistical analysis.

II. RESULTS AND DISCUSSION

A. MORTALITY

Details are provided in table IIA 5.2.1-1. No mortalities occurred at 2 000 mg/kg bw, the only dose tested.

The oral LD_{50} cut-off was ≥ 5 000 mg /kg bw according to OECD guideline 423.

B. CLINICAL OBSERVATIONS

No clinical signs were observed.

C. BODY WEIGHT

There was no toxicological effect on body weight or body weight gain.

D. NECROPSY

No abnormalities were observed at gross necropsy.

III. CONCLUSION

The oral LD_{50} cut off of BYH 18636 was ≥ 5 000 mg/kg bw. BYH 18636 does not warrant classification as being toxic or harmful on the basis of its acute oral toxicity.

(Schüngel M. 2004)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable The study (T 5074266) is fully compliant with OECD 423 (2001) No treatment-related findings were apparent in this study following the administration of a single gavage (limit) dose of 2000 mg/kg bw.
Conclusions	The acute oral LD_{50} of thiencarbazone-methyl in female rats was found to be >2000 mg/kg bw under the conditions of this study. Thiencarbazone-methyl (96.2% purity) is therefore not classified for acute oral toxicity according to current EC criteria. EPA: For Toxicity Category/Classification EPA Toxicity Category III – LD_{50} > 2000 mg/kg - would be assigned.

Report:	**KIIA 5.2.1/02, Schüngel M.; 2006, [*MRID 47070205*]**
Title:	BYH 18636, Acute toxicity in the rat after oral administration
Report No &	AT03457
Document No	M-286151-01-2
Guidelines:	OECD 423 (2001); EEC Directive 67/548 Annex V – Method B.1.tris (2004); EPA Health Effects test Guidelines (OPPTS 870.1100) (1998)
GLP	Yes (certified laboratory)

Executive Summary

Based on the specification required for production of the technical BYH 18636, a new acute oral rat study was required. In a stepwise procedure, two groups of three fasted, young adult female Wistar rats (HsdCpb:Wu) were given successively a single oral dose of BYH 18636 (batch number GELL 420-151-1, 94.6% purity) in 2% Cremophor EL of 2 000 mg/kg bw and were observed for 14 days. The dose of 2 000 mg/kg bw was tolerated by both groups without mortalities, clinical signs, effects on weight gain or gross pathological findings.

According to OECD guideline 423, the LD_{50} cut-off of BYH 18636 was ≥ 5 000 mg /kg bw (category 5 / unclassified of the global harmonized classification system).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	BYH 18636
Description:	White powder
Lot/Batch:	GELL 420-151-1
Purity:	94.6%
CAS:	317815-83-1

Stability of test compound: Stable at 1 and 200 mg/ml at room temperature for 4 hours

2. Vehicle and /or positive control: 2% Cremophor EL in tap water

3. Test animals:

Species: Rat
Strain: HsdCpb:Wu
Age: 10 to 12 weeks approximately
Weight at dosing: 196 to 212 g
Source: Harlan/Winkelmann GmbH, 33178 Borchen, Germany
Acclimation period: At least 5 days
Diet: Provimi Kliba 3883.0.15 Maus/Ratte Haltung, Kaiseraugst Switzerland, *ad libitum*
Water: Tap water, *ad libitum*
Housing: Animals were group caged conventionally in polycarbonate cages on low dust wood granulate bedding
Environmental conditions –
Temperature: 22 ± 2°C
Humidity: 55 ± 5%
Air changes: Approximately 10 changes per hour
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS:

1. In life dates: July 19 to August 09, 2006

2. Animal assignment and treatment

The substance was tested using a stepwise procedure, each step using three female rats. The animals were assigned to their groups by randomization. The random list was based on evenly distributed chance numbers by a sofware application. Following an overnight fast (16 to 24 hours), each group received a single dose of 2 000 mg/kg of BYH 18636 (94.6% purity) by gavage. The test substance was administered in tap water with 2% Cremophor EL at a volume of 10 ml/kg bw. Clinical signs and mortality rates were determined several times on the day of administration and subsequently at least once daily for an observation period of at least 14 days. Body weights were recorded on days 1, 8 and 15. On day 15, surviving animals were sacrificed and all animals were necropsied and examined for gross pathological changes.

Table IIA 5.2.1-2 Doses, mortality / animals treated

Dose (mg/kg bw)	Females
2 000 (1st)	0/3
2 000 (2nd)	0/3

3. Statistics

The data did not warrant statistical analysis.

II. RESULTS AND DISCUSSION

A. MORTALITY

Details are provided in table IIA 5.2.1-2. No mortalities occurred at 2 000 mg/kg bw, the only dose tested.

The oral LD_{50} cut-off was ≥ 5 000 mg /kg bw according to OECD guideline 423.

B. CLINICAL OBSERVATIONS

No clinical signs were observed.

C. BODY WEIGHT

There was no toxicological effect on body weight or body weight gain.

D. NECROPSY

No abnormalities were observed at gross necropsy.

III. CONCLUSION

The oral LD_{50} cut off of BYH 18636 was ≥ 5 000 mg/kg bw. BYH 18636 does not warrant classification as being toxic or harmful on the basis of its acute oral toxicity.

(Schüngel M., 2006)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable The study (T 5077100) is fully compliant with OECD 423 (2001) No treatment-related findings were apparent in this study following the administration of a single gavage (limit) dose of 2000 mg/kg bw.
Conclusions	The acute oral LD_{50} of thiencarbazone-methyl in female rats was found to be >2000 mg/kg bw under the conditions of this study. Thiencarbazone-methyl (94.6% purity) is therefore not classified for acute oral toxicity according to current EC criteria. EPA: For Toxicity Category/Classification EPA Toxicity Category III – LD_{50} > 2000 mg/kg - would be assigned.

B.6.2.2 Acute dermal toxicity (IIA 5.2.2)

Report:	**KIIA 5.2.2/01, Schüngel M.; 2004 (MRID # 47070122)**
Title:	BYH 18636, Acute toxicity in the rat after dermal application
Report No &	AT 01445
Document No	M-088222-01-2
Guidelines:	OECD 402 (1987); EEC Directive 67/548 Annex V – Method B.3. (1992); EPA Health Effects Test Guidelines (OPPTS 870.1200; 1998)
GLP	Yes (certified laboratory)

Executive Summary:
In an acute dermal toxicity study, groups of young adult Wistar rats, 5/sex were exposed by the dermal route to BYH 18636 (batch number MIX-batch 702-73-06-0001, 96.2% purity). The test material was applied as received for 24 hours to 10% of each animal's body surface at a dose of 2 000 mg/kg. Animals were observed for the following 14 days.

The dermal LD_{50} for the males was > 2 000 mg/kg bw
for the females was > 2 000 mg/kg bw
for the combined sexes was > 2 000 mg/kg bw

BYH 18636 was regarded as of very low toxicity after dermal application. The only clinical sign observed was a partial reddening of the skin in one female from day 5 to day 7. Body weight and body weight gain of male rats were not affected by treatment. A decrease in body weight was observed on day 8 of the study in females. The females had recovered at the end of the study. On the basis of this study, BYH 18636 does not warrant classification for dermal toxicity.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636
- **Description:** White powder
- **Lot/Batch:** MIX-batch 702-73-06-0001
- **Purity:** 96.2%
- **CAS:** 317815-83-1
- **Stability of test compound:** Technical material specified for the duration of the study

2. Vehicle and /or positive control: test material dosed as received moistened with water

3. Test animals:
- **Species**: Rat
- **Strain:** HsdCpb:Wu
- **Age:** 9 to 13 weeks approximately
- **Weight at dosing:** 237 to 263 g for the males – 219 to 225 g for the females
- **Source:** Harlan/Winkelmann GmbH, 33178 Borchen, Germany
- **Acclimation period:** At least 5 days
- **Diet:** Provimi Kliba 3883.0.15 Maus/Ratte Haltung, Kaiseraugst Switzerland, *ad libitum*
- **Water:** Tap water, *ad libitum*
- **Housing:** Animals were caged individually in polycarbonate cages on low dust wood granulate bedding
- **Environmental conditions –**

Temperature: 22 ± 2°C
Humidity: 55 ± 5%
Air changes: Approximately 10 changes per hour
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS:

1. In life dates: 29 April to 13 May, 2004

2. Animal assignment and treatment

Animals were assigned by randomization to the test groups listed in Table IIA 5.2.2.-1. The random list was based on evenly distributed chance numbers especially generated for the study by a software application. On the day prior to dosing, the fur was clipped from the dorsal area of the trunk of each animal (approximately 10% of the body surface area). The test substance was administered as a single occluded dermal application and was applied moistened with distilled water. After an exposure period of 24 hours, the occlusion was removed and residual test material was removed with tepid water using soap and gently patting the area dry. Animals were observed for clinical signs and mortality several times on the day of dosing and subsequently at least once daily for an observation period of at least 14 days. Individual body weights were recorded on days 1, 8 and 15. On day 15, all animals were sacrificed by carbon dioxide and were necropsied and examined for gross pathological changes.

Table IIA 5.2.2.-1: Doses, mortality / animals treated

Dose (mg/kg bw)	Males	Females	Combined
2 000	0/5	0/5	0/10

3. Statistics

The data did not warrant statistical analysis.

II. RESULTS AND DISCUSSION

A. MORTALITY

Details are provided in table IIA 5.2.2.-1. No mortalities occurred at 2 000 mg/kg bw, the only dose level tested.

The dermal LD_{50} for males was > 2 000 mg/kg bw
for females was > 2 000 mg/kg bw
for the combined sexes was > 2 000 mg/kg bw

B. CLINICAL OBSERVATIONS

The only clinical sign observed was a partial reddening of the skin in one female from day 5 to day 7.

C. BODY WEIGHT

Body weight and body weight gain of male rats were not affected by treatment. A decrease in body weight was observed on day 8 for the females. The females had recovered at the end of the study.

D. NECROPSY

The necropsies performed at the end of the study revealed no particular findings.

III. CONCLUSION

The dermal LD_{50} of BYH 18636 was higher than 2 000 mg/kg bw in both sexes. BYH 18636 does not warrant classification as being toxic or harmful on the basis of its acute dermal toxicity.

(Schüngel M. 2004)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable The study (T 0074270)is fully compliant with OECD 402 (1987) Marginal weight loss was seen in females during Week 1; weight gain was seen in all animals during Week 2 however two females had not regained their initial weight by termination. No further treatment-related findings were apparent following a single dermal application at the limit dose.
Conclusions	The acute dermal LD_{50} of thiencarbazone-methyl in the rat was found to be >2000 mg/kg bw under the conditions of this study. Thiencarbazone-methyl is therefore not classified for acute dermal toxicity according to current EC criteria. EPA: For Toxicity Category/Classification EPA Toxicity Category III – LD_{50} > 2000 mg/kg - would be assigned.

B.6.2.3 Acute inhalation toxicity (IIA 5.2.3)

Report:	**KIIA 5.2.3/01, Pauluhn J.; 2004 (MRID # 47070123)**
Title:	BYH 18636, Acute inhalation toxicity in rats
Report No & Document No	AT01473 M-089443-01-2
Guidelines:	OECD 403 (1981); EEC Directive 92/69 Annex V – Method B.2. (1992); EPA Health Effects Test Guidelines (OPPTS 870.1300; 1998); Japan MAFF, Notification N° 12 Nousan-8147 (2000)
GLP	Yes (certified laboratory)

Executive Summary

In an acute inhalation study, groups of young adult Wistar rats (5/sex) were exposed by the inhalation route to BYH 18636 (batch number MIX-batch 702-73-06-0001, 96.2% purity) in air for 4 hours (nose only) at a concentration of 1060, 2018 and 5158 mg/m^3, respectively. A concurrent control group was exposed to an atmosphere using similar exposure conditions (15 L/min; conditioned dry air). Animals were observed for the following 14 days.

The inhalation LC_{50} for the males was > 5158 mg/m^3
for the females was > 5158 mg/m^3
for the combined sexes was > 5158 mg/m^3

BYH 18636 (solid aerosol) proved to be non-toxic via the inhalation route to rats. No mortality or

treatment-related clinical signs occurred up to the maximum technically attainable concentration. No changes in the reflex behaviour were observed. The rectal temperature was not affected by the treatment. No treatment-related significant effects were noted on body weight evolution. At necropsy no treatment-related findings were reported. On the basis of this study, BYH 18636 does not warrant classification as being harmful or toxic.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636
Description: White powder
Lot/Batch: MIX-batch 702-73-06-0001
Purity: 96.2%
CAS: 317815-83-1
Stability of test compound: certified for the duration of the study

2. Vehicle and /or positive control: the test substance was aerosolized as dry powder

3. Test animals:
Species: Rat
Strain: HsdCpb:Wu
Age: 2 months approximately
Weight at dosing: 174 to 234 g for the males and 161 to 194 g for the females
Source: Harlan/Winkelmann GmbH, 33178 Borchen, Germany
Acclimation period: At least 5 days
Diet: Provimi Kliba 3883 = NAFAG 9441 pellets maintenance diet for rats and mice, Kaiseraugst Switzerland, *ad libitum*
Water: Tap water, *ad libitum*
Housing: Animals were individually caged in conventional Makrolon® Type III$_H$ cages.
Environmental conditions –
Temperature: 22 ± 2°C
Humidity: 50 ± 10%
Air changes: Approximately 10 changes per hour
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS:

1. In life dates: 11 May to 09 June, 2004

2. Animal assignment and treatment

Animals were assigned to the test groups listed in Table IIA 5.2.3-1. The random list was based on evenly distributed chance numbers especially generated for the study by a software application. Animals were exposed to the aerosolized test substance in Plexiglas exposure tubes applying a directed-flow nose-only exposure principle. Animals were examined carefully several times on the day of exposure and at least once daily thereafter for 2 weeks. The following reflexes were tested: visual placing response, grip strength on wire mesh, abdominal muscle tone, corneal and pupillary reflexes, pinnal reflex, righting reflex, tail-pinch response, startle reflex with respect to behavioural changes stimulated by sounds (finger snapping) and touch (back). The rectal temperatures were measured shortly after cessation of exposure. Individual body weights were recorded before exposure and on days 3, 7 and 14. On day 15, all animals were sacrificed, necropsied and examined for gross pathological changes.

Table IIA 5.2.3-1 Doses, mortality / animals treated

Analytical concentration (mg/m^3)	Males	Females	Combined
0	0/5	0/5	0/10
1060	0/5	0/5	0/10
2018	0/5	0/5	0/10
5158	0/5	0/5	0/10

3. Generation of the test atmosphere / chamber description

Directed-flow nose-only inhalation chambers (TSE, 61348 Bad Homburg) were used. Two methods for dust generation were used: a Wright-Dust-Feeding system for intermediate concentrations and an Exactomat for high concentrations. The test substance concentration was determined by gravimetric analysis. Chamber samples were collected after the equilibrium concentration had been attained in hourly intervals. Two samples during each exposure were also taken for the analysis of the particle-size distribution using a Berner-type Aeras low-pressure critical orifice cascade impactor.

Gravimetric concentration (mg/m^3)	1060	2017.5	5157.5
Mass median aerodynamic diameter (µm)	6.28	2.35	17.56
Geometric standard deviation	3.04	1.88	2.73
Aerosol Mass < 3 µm (%)	25.5	65.2	4.1

The limit concentration of 5000 mg/m^3 was attained, however, at the expense of larger particles (no cyclone used). At 5158 mg/m^3, the Mass Median Aerodynamic Diameter was 17.56 µm and only 4.1% of particles had an aerosol mass < 3 µm. In order to achieve a particle size < 4 µm the test was repeated at 2000 mg/m^3 using the micronized test article and a cyclone. At 2017.5 mg/m3, the Mass Median Aerodynamic Diameter was 2.35 µm and 65.2% of particles had an aerosol mass < 3 µm. The study is nevertheless acceptable.

4. Statistics

A one-way ANOVA (vide infra) was used to analyse body weight gain data and rectal temperature measurements.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred up to 5158 mg/m^3, the maximum technically achievable concentration.

The 4 hour inhalation LC_{50} for the males was > 5158 mg/m^3
for the females was > 5158 mg/m^3
for the combined sexes was > 5158 mg/m^3

B. CLINICAL OBSERVATIONS

All rats tolerated the exposure without specific signs. A battery of reflex measurements was made on the first post-exposure day. In comparison to the rats of the control group, none of the rats of the treated groups exhibited changes in the reflex behaviour. The rectal temperature was not

affected by treatment.

C. BODY WEIGHT

Comparisons between the control and the exposure groups revealed no toxicologically significant changes in body weight in both sexes.

D. NECROPSY

Macroscopic changes causally related to the exposure of the test article were not observed.

III. CONCLUSION

The acute inhalation LC50 of BYH 18636 for the combined sexes was in excess of 5158 mg/m^3. BYH 18636 does not warrant classification as being toxic or harmful on the basis of its acute inhalation toxicity.

(Pauluhn J. 2004)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable The study (T2073309) is fully compliant with OECD 403 (1981). The absence of treatment-related findings at the higher concentration of 5158 mg/m^3 (5.158 mg/l) is reassuring, however the large particle size (MMAD 17.56 ±2.73 µm) at this concentration is noted. As the large majority of particles at this concentration were not of respirable size, this concentration (stated to be the maximum achievable) is not considered to be suitable for the derivation of the LC50 value.
Conclusions	The acute inhalation LC50 of thiencarbazone-methyl in the rat was therefore found to be >2018 mg/m^3 (2.018 mg/l; MMAD 2.35 ±1.88 µm) under the conditions of this study. However the absence of treatment-related findings at any concentration indicates that thiencarbazone-methyl should not be classified for acute inhalation toxicity according to current EC criteria: further testing is therefore not required. The absence of an effect on rectal temperature in this study indicates that thiencarbazone-methyl is not a significant respiratory irritant. EPA: For Toxicity Category/Classification EPA Toxicity Category IV would be assigned.

B.6.2.4 Skin irritancy (IIA 5.2.4)

Report:	**KIIA 5.2.4/01, Schüngel M.; 2004 (MRID # 47070203)**
Title:	BYH 18636, Acute skin irritation/corrosion on rabbits
Report No &	AT01648
Document No	M-129093-01-2
Guidelines:	OECD 404 (2002); EEC Directive 2004/73/EC Annex V – Method B.4. (2004); EPA Health Effects Test Guideline (OPPTS 870.2500; 1998)
GLP	Yes (certified laboratory)

Executive summary:
In a primary dermal irritation study, 3 young adult New Zealand female rabbits were exposed via the dermal route to 0.5 g of pulverized test substance (MIX-batch 702-73-06-0001, 96.3% purity) per animal. In the first step only one animal was used and three patches were applied successively to this animal. The first patch was removed after three minutes. As no serious skin reactions were observed, the second patch was removed after one hour and then the third patch applied and removed after four hours. The test was completed using two additional animals exposed for four hours. The test substance was applied as a powder moistened with water to the skin of the animal under a gauze patch. The animals were observed for 72 hours.

No erythema, eschar or œdema were observed at any time point. In this study, BYH 18636 was not a dermal irritant and does not warrant classification as being irritating to the skin.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636
- **Description:** White powder
- **Lot/Batch:** MIX-batch 702-73-06-0001
- **Purity:** 96.3%
- **CAS:** 317815-83-1
- **Stability of test compound:** Technical material specified for the duration of the study

2. Vehicle and /or positive control: test material dosed as received moistened with water.

3. Test animals:
- **Species**: Rabbit
- **Strain:** Crl:KBL(NZW)BR
- **Age:** Young adult animals
- **Weight at dosing:** 3.2 to 3.6 kg
- **Source:** Charles River, 88353 Kißlegg, Germany
- **Acclimation period:** At least 5 days
- **Diet:** Ssniff K-Z 4mm (Ssniff Spezialdiäten GmbH, 59494 Soest, Germany), approximately 100 g per animal per day
- **Water:** Tap water, *ad libitum*
- **Housing:** Animals were caged individually in cage units Metall/Noryl by EBECO
- **Environmental conditions –**
  - **Temperature:** 20 ± 3°C
  - **Humidity:** 55 ± 20%
  - **Air changes:** not mentioned
  - **Photoperiod:** Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS:

1. In life dates: October 26 to 29, 2004

2. Animal assignment and treatment

On the day prior to dosing, the fur was clipped on the right and left side from the dorsal-lateral area of the trunk of each rabbit. Care was taken to avoid abrading the skin. 0.5 g of the pulverized test substance moistened with water was applied to the skin of the animals under a gauze patch. The treated area was approximately 2.5 cm by 2.5 cm in size. The patch was placed on the dorso-lateral areas of the trunk of each rabbit and was held in place with non-irritating tape for the

duration of the exposure period. After the exposure period the dressing and the patch were removed and the exposed skin area was carefully washed with water without altering the existing response, or the integrity of the epidermis. The contralateral skin area not treated with the test substance served as control.

In the first step only one animal was used and three patches were applied successively to this animal. The first patch was removed after three minutes. As no serious skin reactions were observed, the second patch was removed after one hour and then the third patch applied and removed after four hours. The test was completed using two additional animals exposed for four hours. The responses were graded one hour later.

The dermal irritation was scored at 1, 24, 48 and 72 hours after patch removal. If no irritation indices were observed after 72 hours, the study was finished. If dermal irritation was observed, animals were monitored usually on days 7 and 14 after patch removal. The degree of erythema/eschar formation and oedema formation was recorded as specified by Draize and any serious lesion or toxic effects other than dermal irritation were also recorded. The body weight of each animal was recorded at the beginning of the study

II. RESULTS AND DISCUSSION

A. FINDINGS

No erythema, eschar or oedema was observed at any time point.

Table IIA. 5.2.4-1: Individual skin irritation scores according to the Draize scheme on the first animal

Observation (immediately after patch removal)	Duration of exposure	
	3 minutes	1 hour
Erythema (redness) And eschar formation	0	0
Oedema formation	0	0

Table IIA. 5.2.4-2: Individual and mean skin irritation scores after 4 hour exposure according the the Draize scheme

	Erythema and eschar			Oedema		
Animal number (body weight in kg)	1 (3.4)	2 (3.6)	3 (3.2)	1 (3.4)	2 (3.6)	3 (3.2)
1 hour	0	0	0	0	0	0
24 hours	0	0	0	0	0	0
48 hours	0	0	0	0	0	0
72 hours	0	0	0	0	0	0
Mean score 24-72 hours	0.0			0.0		
No positive response: mean scores < 2 = - Positive response : mean scores ≥ 2 = +						

III. CONCLUSIONS

BYH 18636 was non-irritant to the rabbit skin. On the basis of this study, BYH 18636 does not warrant classification as being irritating to the skin.

(Schüngel M. 2004)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable The study (T3073788) is fully compliant with OECD 404 (2002) Test animals were females. No dermal reactions were observed in any animal at any time point.
Conclusions	No evidence of skin irritation was seen under the conditions of this study. Thiencarbazone-methyl is therefore not classified as a skin irritant according to current EC criteria. EPA: For Toxicity Category/Classification EPA Toxicity Category IV would be assigned.

B.6.2.5 Eye irritancy (IIA 5.2.5)

Report:	**KIIA 5.2.5/01, Schüngel M.; 2005 (MRID # 47070124)**
Title:	BYH 18636, Acute eye irritation on rabbits
Report No &	AT02437
Document No	M-259012-01-2
Guidelines:	OECD 405 (2002); EEC Directive 2004/73/EC Annex V – Method B.4. (2004); EPA Health Effects Test Guideline (OPPTS 870.2400; 1998)
GLP	Yes (certified laboratory)

Executive summary:
In a primary eye irritation study, 0.1 g of pulverized test substance (MIX-batch 702-73-06-0001, 96.0% purity) was placed into the conjunctival sac of one eye of a rabbit after having gently pulled the lower lid away from the eyeball. The other eye, which remained untreated, served as control. Since severe irritation was not observed one hour after treatment, two further rabbits were treated as described. Eye irritation was scored and recorded at 1, 24, 48 and 72 hours after application. As no irritation indices were observed after 72 hours, the study was finished.
The degree of ocular lesions was recorded as specified by Draize and any serious lesion or toxic effects other than ocular lesions were also recorded. Body weight of each animal was recorded at the beginning of the study.
A slight redness of the conjunctivae was observed after 1 and 24 hours in all females (grade 2 for 2/3 females and grade 3 for 1/3 females after 1 hour, grade 1 for 3/3 females after 24 hours). According to the classification criteria BYH 18636 was not irritating to the eye and there were no systemic intolerance reactions.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636
- **Description:** White powder
- **Lot/Batch:** MIX-batch 702-73-06-0001
- **Purity:** 96.0%
- **CAS:** 317815-83-1
- **Stability of test compound:** Technical material specified for the duration of the study

2. Vehicle and /or positive control: test material dosed as received

3. Test animals:

Species:	Rabbit
Strain:	Crl:KBL(NZW)BR
Age:	Young adult animals
Weight at dosing:	2.6 to 3.2 kg
Source:	Charles River, 88353 Kißlegg, Germany
Acclimation period:	At least 5 days
Diet:	Ssniff K-Z 4mm (Ssniff Spezialdiäten GmbH, 59494 Soest, Germany), approximately 100 g per animal per day
Water:	Tap water, *ad libitum*
Housing:	Animals were caged individually in cage units Metall/Noryl by EBECO
Environmental conditions –	
Temperature:	20 ± 3°C
Humidity:	50 ± 25%
Air changes:	not mentioned
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS:

1. In life dates: June 21 to 24, 2005

2. Animal assignment and treatment

The testing strategy comprised a stepwise approach including the evaluation of existing data, the performance of a SAR evaluation for eye and skin corrosion/irritation, measurement of pH value, the evaluation of data on systemic toxicity via the dermal route, the performance of a validated *in vitro* test for skin corrosion (Human 3D Epidermal Skin Model) and *in vivo* testing for skin irritation/corrosion in rabbits before *in vivo* testing for eye irritation/corrosion in rabbits.

On the day before dosing, both eyes of each animal were examined including fluorescein examination. Only animals with healthy intact eyes were used. 0.1 g of pulverized test substance was placed into the conjunctival sac of one eye of the first animal after having gently pulled the lower lid away from the eyeball. The lids were gently held together for about one second in order. to prevent loss of the test substance. The other eye, which remained untreated, served as control. The eye was not rinsed for at least 24 hours following instillation. One hour after treatment a severe irritation was not observed, so two further rabbits were treated as described. The eye irritation was scored and recorded at 1, 24, 48 and 72 hours after application. If no irritation was observed after 72 hours, the study was finished. If eye irritation was observed, animals were monitored usually on days 7, 14 and 21 after application until the changes had completely subsided, however for not more than 21 days after application.
The degree of ocular lesions was recorded as specified by Draize and any serious lesion or toxic effects other than ocular lesions were also recorded. Body weight of each animal was recorded at the beginning of the study.

II. RESULTS

A. FINDINGS

Redness of the conjunctivae was observed after 1 and 24 hours in all females (grade 2 for 2/3 females and grade 3 for 1/3 females after 1 hour, grade 1 for 3/3 females after 24 hours).

Table IIA 5.2.5-1: Eye irritation scores according to the Draize scheme

	Cornea			Iris			Conjunctiva-redness			Conjunctiva-chemosis		
Animal number Time of observation	1	2	3	1	2	3	1	2	3	1	2	3
1 hour	0	0	0	0	0	0	2	2	3	0	0	0
24 hours	0	0	0	0	0	0	1	1	1	0	0	0
48 hours	0	0	0	0	0	0	0	0	0	0	0	0
72 hours	0	0	0	0	0	0	0	0	0	0	0	0
Mean scores 24-72 hours	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.3	0.0	0.0	0.0

Conjunctivae: Redness (refers to palpebral and bulbar conjunctivae; excluding cornea and iris)
Normal: 0 – Some blood vessels hyperaemic (injected): 1 – Diffuse, crimson colour; individual vessels not easily discernible: 2.
Chemosis: Swelling (refers to lids and/or nictating membranes)
Normal: 0 – Some swelling above normal: 1 – Obvious swelling, with partial eversion of lids: 2-Swelling, with lids about half closed: 3 – Swelling, with lids more than half closed: 4.

II. CONCLUSION

Slight to moderate ocular irritation was observed in all animals but had reversed by 48 hours. On the basis of this study, BYH 18636 does not warrant classification as being an eye irritant.

(Schüngel M. 2005)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable The study (T4075381) is fully compliant with OECD 405 (2002) Test animals were females. Signs of ocular irritation were limited to conjunctival erythema (up to Grade 3 according to the Draize scale); reactions declined in severity and were fully reversible within 48 hours. In order for classification as irritant the mean score for conjunctival redness needs to be ≥ 2.5 (mean score 24-72 hours individual animals).
Conclusions	The test material was found to be a mild eye irritant under the conditions of this study. The mean scores for ocular irritation are not sufficient to trigger classification of the test material as an eye irritant according to current EC criteria. EPA: Since there are no 'positive effects' at 24 hours, EPA Toxicity Category IV would be assigned.

B.6.2.6 Skin sensitisation (IIA 5.2.6)

Report:	**KIIA 5.2.6/01, Vohr H.-W.; 2004 (MRID # 47070204)**
Title:	BYH 18636, Study for the skin sensitization effect in guinea pigs (Guinea pig maximization test according to Magnusson and Kligman)
Report No & Document No	AT01388 M-083284-01-2
Guidelines:	OECD 406 (1992); EEC Directive 96/54/EC Annex V – Method B.6. (1996); EPA Health Effects Test Guideline (OPPTS 870.2600; 2003)
GLP	Yes (certified laboratory)

Executive summary:
In a dermal sensitisation study, BYH 18636 (MIX-batch 702-73-06-0001, 96.3% purity) in polyethylene glycol 400 was tested using young adult female SPF-bred guinea pigs of the strain Crl: HA. The treatment regime involved induction of sensitisation by intradermal injection of a suspension at 5% on day 1, induction of sensitisation by topical administration on day 8 and challenge by topical administration on day 22 of a suspension at 50%.

The challenge with the 50% BYH 18636 formulation led to no skin effects in the animals of the test substance group and the control group. Appropriate historical control data using alpha hexyl cinnamic aldehyde formulated in polyethylene glycol 400 demonstrated a positive response. On the basis of this study, BYH 18636 does not warrant classification as being a dermal sensitiser.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636
- **Description:** White powder
- **Lot/Batch:** MIX-batch 702-73-06-0001
- **Purity:** 96.3%
- **CAS:** 317815-83-1
- **Stability of test compound:** stable in vehicle at 1 and 50 % for at least 2 hours at room temperature

2. Vehicle and /or positive control: test material formulated in polyethylene glycol 400

3. Test animals:
- **Species**: Guinea pig
- **Strain:** Crl: HA
- **Age:** Young adult female animals
- **Weight at dosing:** 296 to 384 g
- **Source:** Charles River, 88353 Kißlegg, Germany
- **Acclimation period:** At least 5 days
- **Diet:** Provimi Kliba 3420 – maintenance Diet for Guinea pigs – (supplied by Provimi Kliba AG) *ad libitum*
- **Water:** Tap water, *ad libitum*
- **Housing:** Animals were caged by two or three in type IV Makrolon® Cages

Environmental conditions –

Temperature:	22 ± 3°C
Humidity:	55 ± 15%
Air changes:	at least 10 times per hour
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS:

1. In life dates: June 15 to July 09, 2004

2. Animal assignment and treatment

The treatment regime involved induction of sensitisation by intradermal injection on day 1, induction of sensitisation by topical administration on day 8 and challenge by topical administration on day 22. The doses for the induction and challenge treatments were selected on the basis of the results of the dose range-finding studies. Seven guinea pigs were used for those dose-range studies. The dorsal region and the flanks of the guinea pigs were shorn one day prior to the intradermal induction. Three injections were made on the left and the right side of the spinal column for each animal. Twenty animals were used for the test substance treated group and ten for the control group. All animals received at the first injection site complete Freund's adjuvant diluted with sterile physiological saline solution (1:1), at the second injection site 5% BYH 18636 formulated in polyethylene glycol 400 for the treated animals or vehicle alone for the control animals, and at the third injection site 5% BYH 18636 formulated at equal parts in polyethylene glycol 400 and complete Freund's adjuvant for treated animals or 1:1 mixture of polyethylene glycol 400 and complete Freund's adjuvant for control animals. The injection sites were visually assessed 2 and 7 days after the injection. The topical induction was performed one week after the intradermal induction. On the day prior to topical treatment, the test areas of the animals were shorn. Hypoallergenic patches were placed between and on the injection sites, covered with aluminium foil and held securely in place using an ORABAND® self-adhesive tape. In the treated group, 0.5 ml of 50% BYH 18636 formulation was applied to the skin and in the control group, 0.5 ml of polyethylene glycol 400 was applied. At the end of the 48 hours exposure period, the remaining test item was removed with sterile physiological saline solution. The challenge was performed three weeks after the intradermal induction. The dorsal region and the right flank of the animals were shorn one day prior to the challenge. A 50% formulation of BYH 18636 was placed on the right flank of the treated and control animals and vehicle was also placed and covered with a patch on the right flank of all animals as control. At the end of the exposure period, the remaining test item was removed with physiological saline solution, and 21 hours later the skin of the animals was shorn in the zone of the challenge area. The skin reactions were assessed 48 and 72 hours after the start of the application.

II. RESULTS AND DISCUSSION

Appearance, behaviour and mean body weight of the treated group were not different from the control group. After the intradermal induction the animals in the control group and in the treated group showed strong effects up to encrustation at the injection sites of the first induction. The challenge with the 50% BYH 18636 formulation led to no skin effects in the animals of the BYH 18636 treated group and the control group. Appropriate historical control data using alpha hexyl cinnamic aldehyde formulated in polyethylene glycol 400 demonstrated a positive response.

III. CONCLUSION

BYH 18636 did not exhibit dermal sensitisation potential under the test conditions. On the basis of this study, BYH 18636 does not warrant classification as being a skin sensitiser.

(Vohr H.-W. 2004)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable The study (T 4073040) is fully compliant with OECD 406 (1992) Dermal reactions following topical induction are not reported, however the concentration of the test material appears to be the highest achievable as a suspension in the vehicle. No dermal reactions were apparent following the challenge application. Although the Local Lymph Node Assay (LLNA) is PSD's preferred method for the assessment of skin sensitisation for reasons of animal welfare, the Maximisation method is scientifically valid and the results of this study are accepted.
Conclusions	No evidence of skin sensitisation was seen under the conditions of this study. The test material is therefore not classified as a skin sensitiser according to current EC criteria.

B.6.2.7 Potentiation/interaction of multiple active substances or products (IIA 5.2.7)

Potentiation/interaction of multiple active ingredients is currently not a requirement in the EU, the USA or Canada. Hence, no such studies were conducted and data/documents do not need to be submitted.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	The potential for combined (additive) toxicity is routinely considered in the UK for products containing two or more active substances. Given the common target of toxicity (urinary tract epithelium) and mode of action in the case of thiencarbazone-methyl and cyprosulfamide, the possibility of additive toxicity cannot be excluded.
Conclusions	A combined risk assessment is required for consumers and operators exposed to thiencarbazone-methyl and cyprosulfamide,

B.6.3 Short-term toxicity studies (IIA 5.3)

B.6.3.1 Oral short-term toxicity in the rat (IIA 5.3.1, 5.3.2)

Oral 28-day toxicity (IIA 5.3.1)

No GLP 28-day toxicity studies were performed with BYH 18636.

Oral 90-day toxicity (rat) (IIA 5.3.2)

Report:	**KIIA 5.3.2/01 Mc Elligott A. ; 2003 (MRID # 47070126)**
Title:	BYH 18636, 90-day toxicity study in the rat by dietary administration
Report No &	SA 02446;

Document No	M-104311-01-2
Guidelines:	OECD 408 (1998); EEC Directive 92/69/EC Annex V – Method B.26. (1992); EPA Health Effects Test Guideline (OPPTS 870.3100; 1998); M.A.F.F. in Japan notification 12 Nousan N°8147 (2000) guidelines.
GLP	Yes (certified laboratory)

Executive summary:
BYH 18636 (batch number NLL6954-10, 98% w/w purity) was administered continuously via the diet to separate groups of Wistar rats (10/sex/group) at dose levels of 0, 400, 2 000 and 7 000 ppm equivalent to 24.7, 123 and 439 mg/kg/day in males and 30.8, 154 and 543 mg/kg/day in females, respectively, for at least 90 days. An additional, 10 males and 10 females fed either 0 or 7 000 ppm of test diet for at least 90 days were maintained on control diet for a further 30 days to examine the reversibility of any effects seen.

At 7 000 ppm, one treatment-related mortality was recorded on Day 49 of the dosing phase. Clinical signs observed for this animal prior to death were a red soiled anogenital region and red coloured urine and macroscopic examination revealed that the probable cause of death was a urinary tract obstruction. At clinical chemistry evaluation, a tendency towards higher alkaline phosphatase activity was seen in male animals (+25%) at the end of the dosing phase only. At urinalysis, sulfonamide-like crystals were seen in the urine of 9/10 males and 10/10 females. The presence of these crystals in large amounts correlated with a cloudy appearance observed in the urine. Neither of these effects was observed at the end of the recovery phase of the study. At microscopic examination at the end of the dosing phase, treatment-related effects were observed in the kidney and urinary bladder in both male and female animals. Intrapelvic eosinophilic urolithiasis within the kidneys was found in 3/10 males and in 1/10 females. A similar eosinophilic urolithiasis was observed within the lumen of the urinary bladder in 2/10 males, this was correlated with gritty content (stones) observed macroscopically in 3/10 males. Within the urinary bladder, urothelial hyperplasia was found in 3/10 males and 1/10 females. Slight to mild collecting duct hyperplasia was found in 4/10 males and 2/10 females. Following the recovery period, treatment-related changes were again identified within the kidney and the urinary bladder in males and females at 7 000 ppm but at a lower frequency than at the end of the treatment phase. In the kidney, abnormal intrapelvic eosinophilic urolithiasis was found in 1/10 females. Hyperplasia in the collecting ducts was found in 1/8 males and 1/10 females, the affected male also had a mild simple diffuse urothelial hyperplasia in the urinary bladder.

At 2 000 ppm, there were no adverse treatment related findings observed in either sex but at the end of the treatment phase of the study, sulfonamide-like crystals were seen in the urine of 3/10 males and 4/10 females.

At 400 ppm, no treatment related findings were observed in either sex.

The No Observed Adverse Effect Level (NOAEL) in the Wistar rat when administered BYH 18636 in the diet over a 90-day period was 2 000 ppm (equivalent to 123 mg/kg/day for males and 154 mg/kg/day for females).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	BYH 18636
Description:	White crystals
Lot/Batch:	NLL6954-10
Purity:	98.0 %
CAS:	317815-83-1
Stability of test compound:	Stable in rodent diet at 50 and 15 000 ppm over a 102-day period at ambient temperature or over a 92-day freezing period followed by 10 days at ambient temperature

2. Vehicle and /or positive control: None

3. Test animals:

Species:	Rat
Strain:	Wistar Rj: WI (IOPS HAN)
Age:	7 weeks approximately
Weight at dosing:	259 to 302 g for the males – 181 to 220 g for the females
Source:	R. Janvier, Le Genest St Isle, France
Acclimation period:	16 days
Diet:	Certified rodent powdered and irradiated diet A04C-10 P1 from S.A.F.E. (Scientific Animal Food and Engineering, Epinay-sur-Orge, France), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were caged individually in suspended stainless steel wire mesh cages.
Environmental conditions –	
Temperature:	22 ± 2°C
Humidity:	55 ± 15%
Air changes:	Approximately 15 changes per hour
Photoperiod:	Alternating 12-hour light and dark cycles (7 am- 7 pm)

B. STUDY DESIGN:

1. In life dates: 31 December, 2002 to May 16, 2003

2. Animal assignment and treatment

There were 10 animals of each sex per dose group. Animals were assigned to dose groups using a randomization by weight. BYH 18636 was administered in the diet for at least 90 days to Wistar rats at the following doses – 0, 400, 2 000 and 7 000 ppm (equating approximately to 24.7, 123 and 439 mg/kg/day in males and 30.8, 154 and 543 mg/kg/day in females). A negative control group received plain diet. An additional, 10 males and 10 females fed either 0 or 7 000 ppm of test diet for at least 90 days were maintained on control diet for a further 30 days to examine the reversibility of any effects seen. Animal housing and husbandry were in accordance with the regulations of the Guide for the Care and Use of Laboratory Animals (Public Health Service, National Institute of Health, NIH publication N°86-23, revised 1985) and "Le Guide du Journal Officiel des Communautés Européennes L358, 18 Décembre 1986, N°86/609/CEE du 24 Novembre 1986".

3. Diet preparation and analysis

BYH 18636 was ground to a fine powder before being incorporated into the diet by dry mixing to provide the required concentrations. There were three preparations of each concentration for the whole study. The stability was demonstrated before the start of the study. Diet samples of 50 and 15 000 ppm were found to be stable over a 102-day period at ambient temperature or over a 92-day freezing period followed by 10 days at ambient temperature. The homogeneity of the diet was verified on the first and third preparations at 400 and 7 000 ppm and was within a range of 95 to 106 % of the nominal concentrations. Concentration was checked for each concentration of the three dietary preparations. The mean dietary concentrations throughout the study were 389, 1 945 and 6 952 ppm feed.

Table IIA 5.3.2-1 Study design

Test group	Concentration in diet (ppm)	Dose per animal (study averages)	Animals assigned

		Male (mg/kg bw/day)	Female (mg/kg bw/day)	Male	Female
1	0	0	0	10 + 10*	10 + 10*
2	400	24.7	30.8	10	10
3	2 000	123	154	10	10
4	7 000	439	543	10 + 10*	10 + 10*

* These additional 10 males and 10 females in control and high dosed groups were sacrificed following one month of recovery after termination of treatment.

Table IIA 5.3.2-2 Homogeneity and concentration of test material

	Nominal concentrations (ppm)	Analysed concentrations (ppm)	% of nominal concentration
Stability (pre-study)	50	H 51.4 M 51.5 B 50.5 S1 48.9 S2 49.9	103 103 101 98 100
	15 000	H 14 843 M 14 985 B 14 674 S1 14 962 S2 14 990	99 100 98 100 100
Homogeneity (first preparation)	400	H1 391 H3 398 M1 397 M3 391 B1 397 B3 387	98 100 99 98 99 97
	7 000	H1 7 017 H3 6 896 M1 6 858 M3 6 920 B1 6 928 B3 6 914	100 99 98 99 99 99
Homogeneity (third preparation)	400	H1 385 H3 384 M1 410 M3 401 B1 400 B3 380	96 96 103 100 100 95
	7 000	H1 7 420 H3 7 256 M1 7 118 M3 6 824 B1 6 795 B3 6 822	106 104 102 97 97 97
Concentration (first preparation)	400	393.5	98
	2 000	1 964	98
	7 000 (first load)	6 922	99
	7 000 (second load)	6 886	98
Concentration (second preparation)	400	380	95
	2 000	1 942	97
	7 000 (first load)	6 926	99
	7 000 (second load)	6 858	98
	400	393.3	98

Concentration (third preparation)	2 000	1 928	96
	7 000 (first load)	7 039	101
	7 000 (second load)	7 079	101

H = surface; M = middle; B = bottom of the preparation; S1 = stability after 102 days at ambient temperature, S2 = stability after 92-day frozen period and 10 days at ambient temperature. Samples 1, 2 and 3 for samples H, M or B: three different samples taken at the same level of the preparation, only two analyzed

4. Statistics

Data were analyzed by the Bartlett's test for homogeneity of variances. When the data were homogeneous, an ANOVA was performed followed by Dunnett's test on parameters showing a significant effect by ANOVA. When the data were not homogeneous even after transformation, a Kruskal-Wallis ANOVA was performed followed by the Dunn's test if the Kruskal-Wallis was significant. When one or more group variance(s) equaled 0, means were compared using non-parametric procedures. Group means were compared at the 5% and 1% levels of significance. Statistical analyses were carried out using Path/Tox System V4.2.2. (Module Enhanced Statistics).

C. METHODS:

1. Observations

The animals were observed twice daily for moribundity and mortality (once daily on weekends or public holidays). Observed clinical signs were recorded at least once daily for all animals and detailed physical examinations were performed once during the acclimatization phase and at least weekly during the treatment period. On study weeks 11 to 12, a neurotoxicity assessment was performed for all animals (except for animals of the recovery groups) by observers who were blind with respect to the dose level. Animals were tested individually for motor activity using an automated photocell recording apparatus designed to measure quantitatively spontaneous exploratory motor activity in a novel environment. Motor activity was recorded for 90 minutes with data being collected at 15-minute intervals throughout the session. For sensor reactivity, the following reflexes and responses were recorded: pupillary reflex, surface righting reflex, corneal reflex, flexor reflex, auditory startle response and tail pinch response. The fore- and hindlimb grip strength of all animals were measured quantitatively using a grip strength apparatus equipped with one pull and one push strain gauge. The mean of three successive measurements was determined for both fore- and hindlimb grip strength.

2. Body weight

Body weights were recorded three times during the acclimatization phase, on the first day of test substance administration, then at weekly intervals throughout the treatment and recovery periods and before necropsy.

3. Food consumption and compound intake

Food consumption was recorded weekly; the weekly mean achieved dosage intake for each week and for weeks 1 to 13 was calculated for each sex.

4. Ophthalmic examination

During the acclimatization period all animals were subjected to an ophthalmological examination. After instillation of an atropinic agent (Mydriaticum, Merk Sharp and Dohme) each eye was

examined by means of an indirect ophthalmoscope. During Week 12, all surviving animals from control and high dose groups were re-examined as no significant findings were observed at the end of the dosing phase (Week 12), no further ophthalmological examination was performed at the end of the recovery phase.

5. Clinical chemistry

On study days 91, 92 or 93 and on recovery phase days 31 and 32, prior to necropsy, blood samples were taken from all surviving animals in all groups by puncture of the retro-orbital venous plexus. Animals were diet fasted overnight prior to bleeding and anesthetized by inhalation of isoflurane. Blood was collected on EDTA for haematology, on lithium heparin for plasma and clot activator for serum for clinical chemistry and on sodium citrate for coagulation parameters.
The following haematology parameters were assayed using a Advia 120 (Bayer Diagnostics, Puteaux, France): red blood cell count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, reticulocyte count, white blood cell count and differential count evaluation and platelet count. A blood smear was prepared and stained with Wright stain. It was examined when results of Advia 120 determinations were abnormal. Prothrombin time was assayed on an ACL 3000 (Instrumentation Laboratory, Paris, France).
Any significant change in the general appearance of the plasma and the serum was recorded. Total bilirubin, glucose, urea, creatinine, total cholesterol, triglycerides, chloride, sodium, potassium, calcium and inorganic phosphorus concentrations and aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma-glutamyltransferase activities were assayed on plasma samples, total protein and albumin concentrations were assayed on serum samples using an Hitachi 911 (Roche Diagnostics, Meylan, France). Globulin and albumin/globulin ratio values were calculated.

6. Urinalysis

In the morning, overnight urine samples were collected on study Days 85 or 86 from the exposure phase animals and on recovery Day 29 from the surviving extra animals in the control and high dose groups allocated to the recovery phase. Food and water were not accessible during urine collection.
Any significant change in the general appearance of the urine was recorded. The urine volume was measured. pH was assayed using a Clinitek 200+ and Ames Multistix dipsticks (Bayer Diagnostics, Puteaux, France). Urinary refractive index was measured using a RFM320 refractometer (Bioblock Scientific, Illkirch, France).
The following semi-quantitative parameters were assayed using a Clinitek 200+ and Ames Multistix dipsticks: glucose, bilirubin, ketone bodies, occult blood, protein and urobilinogen. Microscopic examination of the urinary sediment was performed after centrifugation of the urine. The presence of red blood cells, white blood cells, epithelial cells, bacteria, casts and crystals was graded.

7. Sacrifice and pathology

On study days 91, 92 or 93 and on recovery days 31 or 32, a complete necropsy was performed on all surviving animals. Animals were deeply anaesthetized by intraperitoneal injection of pentobarbital, then exsanguinated before necropsy. All animals were fasted prior to scheduled sacrifice. All animals, either found dead or killed for humane reasons, were necropsied. The necropsy included the examination of all major organs, tissues and body cavities. Macroscopic abnormalities were recorded, sampled and examined microscopically. Adrenal gland, brain, epididymides, heart, kidney, liver, ovary, pituitary gland, prostate gland, spleen, testis, thymus, thyroid gland (with parathyroid gland) and uterus (including cervix) were weighed fresh at scheduled sacrifice only. Paired organs were weighed together.
The following organs or tissues were sampled: adrenal gland, aorta, articular surface (femoro-tibial), bone (sternum), bone marrow (sternum), brain, epididymis, oesophagus, exorbital

(lachrymal) gland, eye and optic nerve, Harderian gland, heart, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidney, larynx/pharynx, liver, lung, lymph nodes (submaxillary, mesenteric), mammary gland, nasal cavities, ovary, pancreas, pituitary gland, prostate gland, sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, submaxillary (salivary) gland, testis, thymus, thyroid gland (with parathyroid), tongue, trachea, urinary bladder, uterus (with cervix), vagina.
A bone marrow smear was prepared from femur, stained with May-Grünwald Giemsa, but not examined. Tissues samples were fixed by immersion in neutral buffered 10% formalin with the exception of the eye, optic nerve, Harderian gland, epididymis and testis that were fixed in Davidson's fixative. Histopathological examinations were performed on all tissues from all the animals in the control and high dose groups and all decedents in all groups. The liver, kidney, lung, spleen, thyroid gland, thymus and urinary bladder were examined in all animals in the study.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS:

1. Clinical signs of toxicity and neurotoxicity assessment

Clinical signs considered to be treatment related were confined to the decedent male at 7 000 ppm. where red anogenital soiling was observed on Day 21 and red coloured urine was observed on Days 22, 42 and 43. All other clinical signs observed during the course of the study were considered to be incidental and not related to treatment with the test substance.

At 7 000, 2 000 and 400 ppm in both sexes, overall mean exploratory locomotor activity was comparable to control values. In addition, the pattern of the locomotor activity over time was similar to the control. All reflexes and responses evaluated were unaffected by the treatment. The minor differences observed in the auditory startle and tail pinch responses were considered to reflect inter-individual variation rather than any treatment-related effects. The fore- and hindlimb grip strength were comparable to control values. The statistically significant differences noted in males at 400 and 7 000 ppm for the hindlimb grip strength were considered not to be neurotoxicologically relevant in view of their low amplitude and the absence of a dose-effect.

2. Mortality

There were four mortalities during the dosing phase of the study. One male animal at 7 000 ppm was found dead on Day 49 of the study. Another male animal from this group was sacrificed on Day 30 following an accidental trauma on Day 29. This animal had pilo-erection and reduced motor activity on Day 29. One male animal from the control group was found dead on Day 67. A number of clinical signs were observed for this animal prior to death including; soiled fur around the anogenital region (on Day 57 and 64), reduced motor activity, absence of grasping reflex, half closed eyes and coldness to touch (Day 66). One female in the control group was sacrificed for humane reasons on Day 86, this animal had a left thoracic mass from Day 50 onwards. All of the mortalities during the dosing phase were animals allocated to the recovery groups. There were no further mortalities during the recovery phase of the study.

B. BODY WEIGHT AND BODY WEIGHT GAIN:

There were no modifications to mean body weight or mean body weight change in the treated groups compared to the control animals during the dosing or recovery phase of the study.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

Food consumption was unaffected by treatment at any dose level compared to the control group during the dosing phase or the subsequent recovery phase of the study. The test material intake at 400, 2 000 and 7 000 ppm corresponded to 24.7, 123 and 439 mg/kg bw/day in males and 30.8,

154 and 543 mg/kg bw/day in females respectively.

D. OPHTHALMOSCOPIC EXAMINATION:

No treatment-related ocular abnormalities were observed at ophthalmoscopic examination.

E. BLOOD ANALYSIS:

1. Haematological findings

At the end of the dosing and the recovery phases, no toxicologically significant change was observed for the parameters assayed in the study. The few statistically significant differences which were observed were not considered to be relevant in view of their occurrence at low dose and/or the variation of the individual values.

2. Clinical chemistry findings

At the end of the dosing phase, a tendency towards higher alkaline phosphatase activity was seen in males at 7000 ppm (+25%, $p<0.05$). This effect was not observed at the end the recovery period. No other toxicologically significant change was noted.

F. URINALYSIS:

At the end of the dosing phase, sulfonamide-like crystals were seen in the urine from rats treated at 7 000 ppm (9/10 males and 10/10 females) and at 2 000 ppm (3/10 males and 4/10 females). The presence of these crystals in large amounts correlated with cloudy appearance observed in the urine. At the end of the recovery period, no such effects were observed.

G. SACRIFICE AND PATHOLOGY:

1. Organ weight

At final sacrifice, statistically significant changes for organ weights were observed for pituitary gland but were considered as incidental as they were not associated with any histopathological changes. At recovery phase sacrifice, mean liver weights were found to be statistically significantly higher in females at 7 000 ppm, but this change was considered not to be toxicologically relevant since it was not associated with histological changes and liver weight change after the period of treatment.

2. Gross and histopathology

Four rats died prematurely, two males at 7 000 ppm, one control male and one control female animal. One male dosed at 7 000 ppm was sacrificed on Day 30 following an accidental trauma on Day 29. Gross observations (included white or red foci on the liver, one testis, the thymus and on the glandular region of the stomach), and histological findings (haemorrhages or necrosis within the liver, one testis, the thymus, the heart, the brain and meninges of the spinal cord, erosions of the stomach and intraalveolar blood in the lung), were consistent with an accidental trauma and post trauma agonal changes. One male dosed at 7 000 ppm was found dead on Day 49. This animal had a distended and ruptured urinary bladder associated with a gritty content and a red focus around the torn mucosa. Yellow liquid content was found in the abdominal cavity. Pelvic dilatation in the kidneys was associated with a gritty content in the pyelic cavity, this animal also had a small thymus. These changes were found at microscopic level with a marked bilateral pelvic dilatation on kidneys, slight hyperplasia of the collecting ducts, marked obstructive nephropathy and a mild diffuse simple urothelial hyperplasia on the urinary bladder associated with a serosal inflammation. Lymphoid depletion was seen in spleen and lymph nodes (mesenteric). A urinary tract obstruction was the probable cause of death. Although one animal in the control group had similar findings, the cause of this obstruction was likely to be induced by treatment since similar changes were observed at final sacrifice especially in the urinary bladder.

One control male was found dead at Day 67. At necropsy, enlarged kidneys with bilateral pelvic dilatation were associated with a distended urinary bladder characterized by multiple red foci. These changes were found at the microscopic level with a marked bilateral pelvic dilatation on the kidney, mild hyperplasia of the collecting ducts, marked obstructive nephropathy and a mild diffuse simple urothelial hyperplasia on the urinary bladder. A moderate myocardial necrosis, mild myocardial haemorrhages associated with slight valvular inflammation were observed within the heart. Lymphoid depletion was seen in spleen and lymph nodes (mesenteric and submaxillary). A urinary tract obstruction was the probable cause of death. One control female was killed for humane reasons on Day 86. This animal had a left thoracic mass identified as a fibrosarcoma.

At final sacrifice, a treatment related effect was seen in the urinary bladder, of animals dosed at 7 000 ppm, a gritty content (with stones) was observed in 3/10 males. All other gross pathology changes were considered as incidental and not treatment-related.
At recovery phase sacrifice, all gross pathology changes were considered as incidental and not treatment related.

Treatment-related histological changes were identified within the kidneys and the urinary bladder in males and females at 7 000 ppm only. Within the kidneys, intrapelvic eosinophilic urolithiasis was found in 3/10 males and in 1/10 female. A similar eosinophilic urolithiasis was observed within the lumen of the urinary bladder in 2/10 males at 7 000 ppm. This abnormal content within the urinary tract was associated with lesions of the urothelial epithelium with a higher incidence and severity than those observed in controls, mainly in males. Within the urinary bladder, mild to moderate simple diffuse urothelial hyperplasia was found in 3/10 males and 1/10 females at 7 000 ppm. Slight to mild collecting duct hyperplasia was found in 4/10 males and 2/10 females at 7 000 ppm. It must be stressed that some of these hyperplastic changes are incidentally encountered in control Wistar rats, but this abnormal eosinophilic urolithiasis can induce the same urinary tract changes.

Table IIA 5.3.2-3 Incidence of Selected Pathological Findings

Incidence of treatment related lesions of the kidneys at final sacrifice								
Sex	MALES				FEMALES			
Dose (ppm)	0	400	2 000	7 000	0	400	2 000	7 000
Number examined	10	10	10	10	10	10	10	10
Intrapelvic urolithiasis, eosinophilic								
Total	0	0	0	3	0	0	0	1
Collecting duct hyperplasia								
Total	0	0	0	4	2	1	0	2
Incidence of treatment related lesions of the urinary bladder at final sacrifice								
Urolithiasis, eosinophilic								
Total	0	0	0	2	0	0	0	0
Urothelial hyperplasia, simple, diffuse								
Total	0	0	0	3	1	1	0	1

At recovery phase sacrifice, treatment-related changes were again identified within the kidneys and the urinary bladder in males and females at 7 000 ppm. Within the kidneys, abnormal intrapelvic eosinophilic urolithiasis was found in 1/10 females. Collecting ducts hyperplasia was found in 1/8 males and 1/10 females. A mild simple diffuse urothelial hyperplasia was found in 1/8 males in the urinary bladder.

III. CONCLUSION

At 7 000 ppm several lesions were seen within the kidney and the urinary bladder which were in conjunction with urinary calculi. Following the recovery period, treatment-related changes were again identified within the kidney and the urinary bladder in males and females at 7 000 ppm but at a lower frequency than at the end of the treatment phase. At 2 000 ppm, sulfonamide-like crystals were seen in the urine of 3/10 males and 4/10 females. They were considered not to be adverse findings, as no histopathological findings were observed at this dose level. No treatment related findings were observed at 400 ppm in either sex.

The No Observed Adverse Effect Level (NOAEL) in the Wistar rat when administered BYH 18636 in the diet over a 90-day period was 2 000 ppm (equivalent to 123 mg/kg/day for males and 154 mg/kg/day for females).

(McElligott A., 2003)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY

Name of authority: Pesticides Safety Directorate, UK

Reviewer's Comments

Reliability rating: Totally reliable

The study (SA02446) is compliant with OECD 408 (1998).

It is noted that the test material used in this study (98.0%) is of higher purity than that currently manufactured (minimum specified purity 94.6%).

The significantly increased plasma alkaline phosphatase activity seen in 7000 ppm males of the main group was not seen in main group females or in the recovery groups. The finding is considered to be treatment-related and, although of small magnitude, is also considered to be of toxicological significance. Similar findings were not apparent in the chronic rat study (Schladt *et al*, 2007), at the (slightly lower) top dose level of 5000 ppm.

Statistically significant effects on mean pituitary weight seen in all of the treated main groups of males are not consistent and do not indicate a treatment-related effect. Similar effects were not apparent in the recovery groups.

A slight (but statistically significant) reduction in mean absolute testes weight seen in 7000 ppm males of the main group is not considered to be of clear toxicological significance in the absence of histopathological correlates. Similar effects were not apparent in the recovery groups.

Additional findings in the 90-day rat study

Parameter		Dose level (ppm)			
		0	400	2000	7000
AP (IU/l)	**M**	60	62	64	75*
		62	-	-	66
	F	39	45	43	48
		45	-	-	39
Testes weight (g)	**M**	3.96	3.82	4.13	3.74*
Testes weight (% body weight)		0.781	0.730	0.807	0.737
Testes weight (% brain weight)		185.5	180.5	188.8	175.8
Pituitary weight (g)	**M**	0.013	0.012	0.011*	0.011**
Pituitary weight (% body weight)		0.0026	0.0022**	0.0022*	0.0022**
Pituitary weight (% brain weight)		0.63	0.55	0.50*	0.53**

Conclusions

The results of this study indicate that thiencarbazone-methyl is of relatively low toxicity in the rat. Findings show that the kidney and bladder (and to a lesser extent the liver) are the target organs of thiencarbazone-methyl toxicity; observations in these organs and correlating clinical signs and urinalysis findings indicate that precipitation of crystals of thiencarbazone-methyl (and/or its metabolites) in the urine may be the initial effect. The subsequent formation of urinary calculi is postulated to cause local irritation (manifested as urothelial hyperplasia) of the renal collecting ducts and urinary bladder; dysuria, urinary tract obstruction and obstructive nephropathy.

	A NOAEL of 2000 ppm (equivalent to mean achieved dietary intakes of 123 and 154 mg/kg bw/d in males and females respectively) can be determined for this study, based on the treatment-related mortality, clinical chemistry (increased alkaline phosphatase activity) and histopathological (renal and urinary bladder) findings at the top dose level of 7000 ppm (equivalent to mean intakes of 439 and 543 mg/kg bw/d in males and females respectively). The presence of crystals in the urine seen in both sexes at 2000 ppm is not considered to constitute an adverse effect in the absence of associated histopathological findings. The NOAELs for this study are therefore the same as proposed by the Notifier.

B.6.3.2 Oral short-term toxicity in the mouse

- **Oral 90-day toxicity in the mouse (IIA 5.3.2)**

Report:	**KIIA 5.3.2/02 Langrand-Lerche C.;2004 (MRID # 47070127)**
Title:	BYH 18636, 90-day toxicity study in the mouse by dietary administration
Report No &	SA 03086;
Document No	M-000411-01-2
Guidelines:	OECD 408 (1998); EEC Directive 92/69/EC Annex V – Method B.26. (1992); EPA Health Effects Test Guideline (OPPTS 870.3100; 1998); M.A.F.F. in Japan notification 12 Nousan N°8147 (2000) guidelines.
GLP	Yes (certified laboratory)

Executive summary:
BYH 18636 (batch number NLL6954-10, 98.0 to 99.7 % w/w purity), was administered continuously via the diet to groups of C57BL/6 mice (10/sex/group) at concentrations of 0, 500, 2 000 and 4 000 ppm for at least 90 days (equating approximately to 0, 76, 315 and 637 mg/kg/day in males and 0, 103, 409 and 789 mg/kg/day in females).

At 4 000 ppm, the only treatment-related finding was a urinary bladder calculus observed at autopsy in one male. This finding was accompanied by marked submucosal inflammatory cell infiltration, minimal diffuse urothelial inflammation and moderate diffuse urothelial hyperplasia of urinary bladder.
At 2 000 and 500 ppm, no treatment-related findings were observed in either sex.

The NOEL of BYH 18636 in this study was 2 000 ppm in males (equating to 315 mg/kg/day) and 4 000 ppm in the females (equating to 789 mg/kg/day).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	BYH 18636
Description:	White crystals
Lot/Batch:	NLL6954-10
Purity:	98.0 (December 03, 2002), 99.7 % (June 06, 2003)
CAS:	317815-83-1
Stability of test compound:	Stable in rodent diet at 50 and 15 000 ppm over a 102-day period at ambient temperature or over a 92-day freezing period followed by 10 days at ambient temperature

2. Vehicle and /or positive control: None

3. Test animals:

Species:	Mouse
Strain:	Ico: C57BL/6 J @ Ico
Age:	6 to 7 weeks approximately
Weight at dosing:	20.8 to 23.2 g for the males – 16.4 to 19.3 g for the females
Source:	Charles River Laboratories, L'Arbresle, France
Acclimation period:	7 days
Diet:	Certified rodent powdered and irradiated diet A04C-10 P1 from S.A.F.E. (Scientific Animal Food and Engineering, Epinay-sur-Orge, France), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were caged individually in suspended stainless steel wire mesh cages.
Environmental conditions –	
Temperature:	22 ± 2°C
Humidity:	55 ± 15%
Air changes:	Approximately 15 changes per hour
Photoperiod:	Alternating 12-hour light and dark cycles (7 am- 7 pm)

B. STUDY DESIGN:

1. In life dates: 07 May to 14 August, 2003

2. Animal assignment and treatment

There were 10 animals of each sex per dose group. Animals were assigned to dose groups using a randomization by weight. BYH 18636 was administered in the diet for at least 90 days to C57BL/6 mice at the following doses – 0, 500, 2 000 and 4 000 ppm (equating approximately to 0, 76, 315 and 637 mg/kg/day in males and 0, 103, 409 and 789 mg/kg/day in females). A negative control group received plain diet. Animal housing and husbandry were in accordance with the regulations of the Guide for the Care and Use of Laboratory Animals (Public Health Service, National Institute of Health, NIH publication N°86-23, revised 1985) and "Le Guide du Journal Officiel des Communautés Européennes L358, 18 Décembre 1986, N°86/609/CEE du 24 Novembre 1986".

3. Diet preparation and analysis

BYH 18636 was ground to a fine powder before being incorporated into the diet by dry mixing to provide the required concentrations. There was one preparation of each concentration for the whole study. The stability had been demonstrated in a previous study where diet samples of 50 and 15 000 ppm were found to be stable over a 102-day period at ambient temperature or over a 92-day freezing period followed by 10 days at ambient temperature. The homogeneity of the diet was verified at 500 and 4 000 ppm and was within a range of 92 to 102% of the nominal concentrations. Concentration was checked for the intermediate dose level. The mean dietary concentrations throughout the study were 501, 1 984 and 3 894 ppm feed.

Table IIA 5.3.2-4 Study design

Test group	Concentration in diet (ppm)	Dose per animal (study averages)		Animals assigned	
		Male (mg/kg bw/day)	Female (mg/kg bw/day)	Male	Female
1	0	0	0	10	10
2	500	76	103	10	10
3	2 000	315	409	10	10

4	4 000	637	789	10	10

Table IIA 5.3.2-5 Homogeneity and concentration of test material

	Nominal concentrations (ppm)	Analysed concentrations (ppm)	% of nominal concentration
Homogeneity (first load)	500	H1 505 H3 462 B1 494 B3 508	101 92 99 102
	4 000	H1 3 929 H3 3 934 B1 3 861 B3 3 860	98 98 97 97
Concentration (first load)	500	492	98
	2 000	2 011	101
	4 000	3 896	97
Concentration (second load)	500	509	102
	2 000	1 957	98
	4 000	3 891	97

H = surface; B = bottom of the preparation. Samples 1, 2 and 3 for samples H or B: three different samples taken at the same level of the preparation, only two analyzed.

Homogeneity and concentration results were within the target ranges of 85 to 115% of the nominal concentration. The stability of the test substance in the diet had been demonstrated in the rat 90-day study (M-104311-01-2), where BYH 18636 was found to be stable in the diet over a 102-day period at ambient temperature or a 92-day freezing period followed by 10 days at ambient temperature.

4. Statistics

Data were analyzed by the Bartlett's test for homogeneity of variances. When the data were homogeneous, an ANOVA was performed followed by Dunnett's test on parameters showing a significant effect by ANOVA. When the data were not homogeneous even after transformation, a Kruskal-Wallis ANOVA was performed followed by the Dunn's test if the Kruskal-Wallis was significant. When one or more group variance(s) equaled 0, means were compared using non-parametric procedures. Group means were compared at the 5% and 1% levels of significance. Statistical analyses were carried out using Path/Tox System V4.2.2. (Module Enhanced Statistics).

C. METHODS:

1. Observations

The animals were observed twice daily for moribundity and mortality (once daily on weekends or public holidays). Observed clinical signs were recorded at least once daily for all animals and detailed physical examinations were performed once during the acclimatization phase and at least weekly during the treatment period.

2. Body weight

Body weights were recorded twice during the acclimatization phase, on the first day of test substance administration, then at weekly intervals throughout the treatment period and before necropsy.

3. Food consumption and compound intake

Food consumption was recorded weekly; the weekly mean achieved dosage intake for each week

and for weeks 1 to 13 was calculated for each sex.

4. Ophthalmic examination
Not performed.

5. Clinical chemistry
On study days 91, 92 or 93, in the morning, prior to necropsy, blood samples were taken from all animals in all groups by puncture of the retro-orbital venous plexus. Animals were diet fasted overnight prior to bleeding and anaesthetized by inhalation of isoflurane. Blood was collected on lithium heparin for plasma chemistry determinations. Any significant change in the appearance of the plasma was recorded and the following clinical chemistry parameters were measured: total bilirubin, total protein, albumin, total cholesterol and urea concentrations and aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities.

6. Urinalysis
Urinalysis was not performed.

7. Sacrifice and pathology
On study days 91, 92 or 93, a complete necropsy was performed on all animals. Animals were deeply anaesthetized by intraperitoneal injection of pentobarbital, then exsanguinated before necropsy. All animals were fasted prior to scheduled sacrifice. The following organs were weighed fresh at scheduled sacrifice only: adrenal gland, brain, heart, kidney, liver, spleen, testis, thymus and uterus (including cervix). The following organs or tissues were sampled: adrenal gland, aorta, articular surface (femoro-tibial), bone (sternum), bone marrow (sternum), brain, epididymis, oesophagus, exorbital (lachrymal) gland, eye and optic nerve, gall bladder, Harderian gland, heart, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidney, larynx/pharynx, liver, lung, lymph nodes (submaxillary, mesenteric), mammary gland, nasal cavities, ovary, pancreas, pituitary gland, prostate gland, sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, submaxillary (salivary) gland, testis, thymus, thyroid gland (with parathyroid), tongue, trachea, urinary bladder, uterus (with cervix), vagina. A bone marrow smear was prepared from femur, stained with May-Grünwald Giemsa, but not examined. Tissues samples were fixed by immersion in neutral buffered 10% formalin with the exception of the eye, optic nerve, Harderian gland, epididymis and testis that were fixed in Davidson's fixative. Histopathological examinations were performed on all tissues from all the animals in the control and high dose groups. The liver, kidney, lung, thyroid gland and urinary bladder were examined in all animals in the study.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS:

1. Clinical signs of toxicity
There were no treatment-related clinical signs of toxicity observed in animals at any dose.

2. Mortality
No mortality occurred in the test animals at any dose level.

B. BODY WEIGHT AND BODY WEIGHT GAIN:

The body weight evolution of animals was unaffected by treatment in any dose group.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

Food consumption was unaffected by treatment in any dose group. Overall averages for consumption of test material at 500, 2 000 and 4 000 ppm in feed corresponded to 76, 315 and 637 mg/kg bw/day in males and 103, 409 and 789 mg/kg bw/day in females.

D. BLOOD ANALYSIS:

No toxicological significant change was noted for the parameters assayed.

E. SACRIFICE AND PATHOLOGY:

1. Organ weight

There was no relevant change in terminal body weights and organ weights of treated males and females when compared to control animals.

2. Gross and histopathology

All gross pathology findings were considered to be incidental in origin and not treatment-related. At terminal sacrifice, changes considered to be associated with the administration of BYH 18636 were present in the urinary bladder of one male given 4 000 ppm. No treatment-related findings were present in females given 4 000 ppm or in either sex given 2 000 or 500 ppm.
A urinary bladder calculus was present in 1/10 males given 4 000 ppm. This finding was accompanied by marked, submucosal inflammatory cell infiltration, minimal, diffuse urothelial inflammation and moderate, diffuse urothelial hyperplasia of the urinary bladder. There were no other treatment-related histopathological findings. All of the other pathological findings encountered were considered to have arisen spontaneously.

III. CONCLUSION

The No Observed Effect Level (NOEL) of BYH 18636 in this study was 2 000 ppm in the male (equivalent to 315 mg/kg/day) and 4 000 ppm in the female mouse (equivalent to 789 mg/kg/day).

(Langrand-Lerche C., 2004)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Reliable with restrictions The study (SA03086) is compliant with OECD 408 (1998) with the following deviations: The guideline specifies that histopathology of the brain should include investigation of 'representative regions including cerebrum, cerebellum and medulla pons'. The study report states only that the brain was investigated histopathologically, however the applicant has clarified that the brain was sectioned at the level of the optic chiasm (including the basal ganglia, septum, cortex and anterior hypothalamus); at the hippocampus (containing the cortex and brain stem at the transition of diencephalon to mesencephalon) and at the third section containing the cerebellum/ brain stem (medulla oblongata) The epididymides and ovaries were not weighed, as specified in OECD 408, however this is not considered to affect the integrity of the study in the absence of effects on these organs in other studies in the mouse or in other species. It is noted that the test material used in this study (98.0-99.7%) is of higher purity than that currently manufactured (minimum specified purity 94.6%).

	Thiencarbazone-methyl was found to be of relatively low toxicity in the mouse. Treatment-related findings in this study were limited to a single incidence of urinary bladder calculus with associated histopathology in one top dose male. Findings are therefore consistent with the mode of toxicity in the rat and indicate that the urinary bladder is also a target of thiencarbazone-methyl toxicity in the mouse.
Conclusions	A NOAEL of 2000 ppm for males (equivalent to a mean achieved dietary intake of 315 mg/kg bw/d) can be determined for this study, based on the urinary bladder calculus and associated histopathology seen at 4000 ppm (equivalent to 637 mg/kg bw/d). A NOAEL of 4000 ppm (equivalent to 789 mg/kg bw/d) can be determined for females in the absence of any treatment-related findings at the top dose level. The NOAELs for this study are therefore the same as proposed by the Notifier.

B.6.3.3 Oral short-term toxicity in the dog (IIA 5.3.3, IIA 5.3.4)

Oral 90-day toxicity in the dog (IIA 5.3.3)

Report:	**KIIA 5.3.3/01 Eigenberg D.A., 2005 (47070129)**
Title:	90-day toxicity feeding study in the Beagle dog with Technical Grade BYH 18636
Report No & Document No	201290-1 M-261742-02-1
Guidelines:	OECD 409 (1998); EPA Health Effects Test Guideline (OPPTS 870.3150; 1998); M.A.F.F. in Japan notification 12 Nousan N°8147 (2000) guidelines.
GLP	Yes (certified laboratory)

Executive summary:

Technical grade BYH 18636 (Mix Batch 702-73-06-0001, 95.5 to 96.1% purity) was administered in the diet to Beagle dogs (4/sex/dose) at dose levels of 0 (concurrent vehicle control) 1 000, 5 000 and 10 000 ppm for a period of at least 100 days (equivalent to 34, 149 and 335 mg/kg/day for the males and 32, 159 and 351 for the females, respectively). Clinical observations were conducted twice daily (except once on weekends and holidays), food consumption was measured daily and body weights were taken weekly. Clinical chemistry, haematology and urinalysis were performed on all animals once prior to administration of the test substance and during study weeks five, nine and fourteen. Ophthalmic examinations were performed pre-exposure and pre-sacrifice. A gross necropsy was performed, organ weights were taken and tissues were examined microscopically.

At 10 000 ppm: Findings were limited to the urinary bladder of males and females, with calculi observed grossly and haemorrhage, inflammatory changes and epithelial hyperplasia observed microscopically.

At 5 000 ppm: No treatment-related effects were observed in either males or females.

At 1 000 ppm: No treatment-related effects were observed in either males or females.

The No Observed Effect Level for this study was 5 000 ppm (equivalent to 149 and 159 mg/kg/day for males and females, respectively).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636

Description: white powder
Lot/Batch: Mix-batch 702-73-06-0001
Purity: 95.5 % (June 16, 2004), 96.1 % (January 26, 2005)
CAS: 317815-83-1
Stability of test compound: Stable in diet over 7 days at ambient temperature and 56 days at freezer temperature

2. Vehicle and /or positive control: corn oil and acetone

3. Test animals:
Species: Dog
Strain: Beagle
Age: 7 months approximately
Weight at dosing: 6.1 to 9.6 kg for the males – 5.0 to 9.0 kg for the females
Source: Marshal Farms USA, Inc, North Rose New York
Acclimation period: 7 days
Diet: Purina certified canine diet Etts 5006-3; presented to the animals for 3-6 hours/day, beginning 9 days prior to study initiation and continuing throughout the study; *ad libitum* prior to this time.
Water: Tap water, *ad libitum*
Housing: Animals were caged individually in stainless steel runs.
Environmental conditions –
Temperature: 23.5 ± 5.5°C
Humidity: 50 ± 20 %
Air changes: Approximately 14 to 15 changes per hour
Photoperiod: Alternating 12-hour light and dark cycles (6 am- 6 pm)

B. STUDY DESIGN:

1. In life dates: August 30 to December 10, 2004 at Bayer CropScience LP, Stilwell, Kansas, USA

2. Animal assignment and treatment

Prior to initiation of test compound administration, the dogs were randomly assigned to dose groups, based on weight. Weight variation of animals used was targeted not to exceed ± 20%. Four animals per sex were treated at the following concentrations with BYH 18636 whereas controls received untreated diet.

Table IIA 5.3.3-1 Study design

Test group	Concentration in diet (ppm)	Dose per animal (average)		Animals assigned	
		Male (mg/kg bw/day)	Female (mg/kg bw/day)	Male	Female
1	0	0	0	4	4
2	1 000	34	32	4	4
3	5 000	149	159	4	4
4	10 000	335	351	4	4

3. Diet preparation and analysis

BYH 18636 was mixed in the feed at the designed nominal concentrations without correcting for the active ingredient purity. All feed mixtures were prepared weekly and stored under freezer conditions until presented to the animals. Corn oil, at 1% by weight of the diet, along with acetone, was used as a vehicle to suspend the test substance prior to being mixed in the diet. The control diet was prepared the same way as the treated diet, excluding only the test substance. The homogeneity of the test substance in the diet and the stability of the active ingredient of the test substance in feed, stored at room temperature for 7 days and freezer temperature for 56 days, were determined. The concentration of the active ingredient in the feed was verified for study weeks 1, 2, 3, 7, 11 and 15 for all dose groups. The analytically determined mean concentrations ranged from 96 to 99% of the nominal concentrations. The test substance was found to be homogenously distributed in the feed and was stable at both room temperature and freezer conditions.

4. Statistics

Statistical significance was determined at $p \leq 0.05$ for all tests with the exception of Bartlett's test, in which a probability value of $p \leq 0.001$ was used. All tests were two-tailed, except for gross and histopathologic lesion evaluations that were one-tailed.

Continuous data were analyzed by the Bartlett's test for homogeneity of variances. When the data were homogeneous, an ANOVA was performed followed by Dunnett's test on parameters showing a significant effect by ANOVA. When the data were not homogeneous, a Kruskal-Wallis ANOVA was performed followed by the Mann-Whitney U-test to identify statistical significance between groups.

Frequency data, that were examined statistically, were initially analyzed by a Chi-Square procedure. If there was statistical significance using the Chi-Square test, each treatment group was compared to the control group using a Fisher's Exact test.

C. METHODS:

1. Observations

The animals were observed twice daily for clinical signs of toxicity except on weekends and holidays when observations were performed once a day. Detailed clinical examinations were performed at initiation of dosing and weekly thereafter, except for study day 98, when observations for clinical signs of toxicity were not performed.

2. Body weight

Body weights were recorded weekly throughout the treatment, except on study day 98, and before necropsy.

3. Food consumption and compound intake

Food consumption was recorded daily; the weekly mean achieved dosage intake for each week was calculated for each sex. The mean achieved dosage intake was also calculated from days 0 to 91.

4. Ophthalmic examination

Following the acclimation period and prior to intitiation of dosing, ophthlamic examinations were conducted on all animals. Ophthalmic examinations were also conducted on all animals just prior to termination of the study.

5. Clinical chemistry

Clinical chemistry and complete blood count, including differentials, were performed on all animals once prior to administration of the test substance. Following initiation of dosing, haematology and clinical chemistry were assayed on all animals in all groups during study weeks

five, nine and forteen. Animals were diet fasted overnight prior to blood collection, which was drawn via jugular venipuncture.
The following haematology parameters were assayed: red blood cell count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, reticulocyte count, blood cell morphology, red blood cell distribution width, haemoglobin distribution width, white blood cell count and differential count evaluation and platelet count. Activated partial thromboplastin time and prothrombin time were measured.
Total bilirubin, glucose, albumin, globulins, albumin/globulin ratio, total protein, urea, uric acid, creatinine, total cholesterol, triglycerides, chloride, sodium, potassium, calcium and phosphorus concentrations and aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactic acid dehydrogenase, creatine phosphokinase and gamma-glutamyltransferase activities were assayed.

6. Urinalysis

Urinalysis was performed on all animals once prior to administration of the test substance and on all animals during study weeks five, nine and fourteen. Urine volume was collected over a 24 hour period.
Any significant change in the general appearance of the urine was recorded. The urine volume was measured. pH and specific gravity were assayed.
The following parameters were assayed: glucose, bilirubin, ketones, blood, protein, nitrites, leukocytes and urobilinogen. Microscopic examination of the urinary sediment was performed.

7. Sacrifice and pathology

On study days 100, 101 or 102, a complete necropsy was performed on all animals. Animals were deeply anaesthetized by intravenous injection of Fatal-Plus, then exsanguinated before necropsy. The necropsy consisted of a systematic gross examination of each animal's general physical condition, body orifices, external and internal organs and tissues. Adrenal gland, brain, heart, kidney, liver (with gall bladder), lung, ovary, pituitary gland, prostate gland, spleen, testis, thymus, thyroid gland (with parathyroid gland) and uterus (including cervix) were weighed fresh.
The following organs or tissues were sampled: adrenal gland, aorta, bone (sternum, rib), bone marrow, brain, epididymis, oesophagus, eye and optic nerve, Fallopian tube, heart, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidney, larynx/pharynx, liver with gall bladder, lung, lymph nodes, mammary gland, nasal cavities, ovary, pancreas, pituitary gland, prostate gland, salivary gland, sciatic nerve, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, testis, thymus, thyroid gland (with parathyroid), trachea, urinary bladder, uterus (with cervix), ureter and vagina.
Tissues samples were processed routinely and stained with haematoxylin and eosin. Histopathological examinations were performed on all tissues (except vagina) from all the animals in the control and high dose groups. Target organs and gross lesions from all dose groups were examined histologically.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS:

1. Mortality

No animals were found dead or sacrificed *in-extremis* in this study.

2. Clinical signs of toxicity

There were no treatment-related clinical signs.

B. BODY WEIGHT AND BODY WEIGHT GAIN:

There were no treatment-related effects on body weight.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

There were no treatment-related effects on food consumption.

Table IIA 5.3.3-2 Mean food consumption

Test group	Concentration in diet (ppm)	Mean food consumption Days 1 -102 (g/animal/day)	
		Male	Female
1	0	300	251
2	1 000	323	257
3	5 000	279	245
4	10 000	280	257

The mean achieved dosage intake per group was: 31, 147 and 328 mg/kg/day in males and 29, 161 and 343 mg/kg/day in females.

D. OPHTHALMIC EXAMINATION:

No treatment-related ocular abnormalities were observed at ophthalmic examination.

E. BLOOD ANALYSIS:

1. Haematological findings

There were no treatment-related changes in male or female dogs in any treatment group.

2. Clinical chemistry findings

There were no clinical chemistry treatment-related changes.

The few scattered statistically significant changes in clinical chemistry parameters (chloride, triglycerides and total bilirubin in males and albumin /globulin ratio, phosporus, potassium and glucose in females) were considered not to be treatment-related, because the changes were small and not dose-dependent.

F. URINALYSIS:

There were a few scattered statistical differences among urine values during the study. No changes were considered to be treatment-related.

G. SACRIFICE AND PATHOLOGY:

1. Organ weight

The terminal body weights were similar in control and treated dogs.

No statistically significant differences were obtained in absolute organ weights in both male and female dogs. The statistically significant increase in relative thyroid weight in the 10 000 ppm males and liver and ovary weights in 10 000 ppm females and the statistically significant decreased uterine weight in 5 000 ppm females were considered not to be treatment-related since the changes were small, not dose-dependent and did not correlate with any histopathological changes.

2. Gross and histopathology

The changes noted that were considered treatment-related were limited to the urinary bladder of 10 000 ppm treated males and females. Calculi in the urinary bladder were observed in 3/4 males and 2/4 females.

Numerous incidental microscopic observations were noted in control and treated dogs. Minimal mineralization of the renal papilla of the kidney was noted in most control and treated dogs. The only microscopic observations that were considered to be treatment-related were the haemorrhage, inflammatory changes and epithelial hyperplasia associated changes in the urinary bladder of both male and female 10 000 ppm treated dogs.

Table IIA 5.3.3-3 Incidence of Selected Pathological Findings

Sex	MALES				FEMALES			
Dose (ppm)	0	1 000	5 000	10 000	0	1 000	5 000	10 000
Number examined	4	4	4	4	4	4	4	4
Urinary bladder								
Epithelium hyperplasia	0	0	0	3	0	0	0	2
Inflammation	0	0	0	2	0	0	0	0
Haemorrhage	0	0	0	2	0	0	0	1

IV. CONCLUSION

The No Observed Adverse Effect Level for this study was 5 000 ppm (149 and 159 mg/kg/day for males and females, respectively).

(Eigenberg D.A., 2005)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable The study (04-S76-WH) is fully compliant with OECD 409 (1998). Weights of the epididymides were recorded at necropsy (this measurement is not specified in the Notifier's summary). Food consumption by 10000 ppm males was consistently (but not statistically significantly) lower than controls during the later part of the study: this finding is not considered to be of toxicological relevance in the absence of any significant effect of treatment on bodyweight or weight gain. A statistically significant increase in plasma potassium concentration (shown below) seen in treated groups of females at later time points is not considered to be treatment-related. A clear dose-response relationship is not apparent for this finding, which is associated with low concurrent control values. A statistically significant increase in relative liver weight seen in 10000 ppm females is associated with a (non-significant) increase in the absolute weight of this organ. The finding is not considered to be of toxicological significance in the absence of any clinical chemistry or histopathological correlates.

A slight (but significant) increase in mean relative ovary weight is not considered to be of toxicological significance in the absence of any histopathological correlates or similar findings in the 12-month dog study (Eigenberg, 2007).

Additional findings in the 90-day dog study

Parameter / time point		Dose level (ppm)			
		0	1000	5000	100000
		Plasma potassium concentration (mmol/l)			
M	D-7	4.9	5.0	4.9	5.1
	D29	4.7	5.3	5.1	5.2
	D58	4.9	4.9	5.0	4.9
	D93	4.6	4.6	4.7	4.7
F	D-7	5.1	4.7	4.8	4.5
	D29	4.7	5.1	5.1	5.2
	D58	4.0	4.6*	4.3	4.5*
	D93	4.1	4.5*	4.5*	4.8*
Liver weight (g)	M	313.4	302.7	304.6	316.2
	F	255.3	247.8	262.6	315.0
Liver weight (%)	M	3.595	2.982	3.232	3.575
	F	3.284	2.895	3.355	4.105*
Ovary weight (g)	F	0.988	0.664	0.674	1.561
Ovary weight (%)		0.012	0.008	0.009	0.020*

Conclusions

Thiencarbazone-methyl was found to be of relatively low toxicity in the dog. The urinary bladder was found to be the target of toxicity, as is also the case for the short-term toxicity studies in the rat and mouse. Treatment-related findings in this study were limited to the presence of calculi in the urinary bladder at the top dose level of 10000 ppm, with associated histopathological changes (hyperplasia, inflammation and haemorrhage) seen in the transitional epithelium

A NOAEL of 5000 ppm (equivalent to mean achieved dietary intakes of 149 and 159 mg/kg bw/d in males and females respectively) can be determined for this study, based on the urinary bladder calculi and associated findings seen in both sexes at the top dose level of 10000 ppm (equivalent to 335 and 351 mg/kg bw/d in males and females respectively.

The NOAELs for this study are therefore the same as proposed by the Notifier.

B.6.3.4 Oral 1 year toxicity (dog) (IIA 5.3.4)

Report:	**KIIA 5.3.4/01 Eigenberg D.A., 2007 (MRID # 47070133).**
Title:	A chronic toxicity feeding study in the Beagle dog with Technical Grade BYH 18636
Report No & Document No	201497-1 M-284899-02-1
Guidelines:	OECD 452 (1981); EPA Health Effects Test Guideline (OPPTS 870.4100; 1998); M.A.F.F. in Japan notification 12 Nousan N°8147 (2000) guidelines.
GLP	Yes (certified laboratory)

Executive summary:

Technical grade BYH 18636 was administered in the diet to Beagle dogs (4/sex/dose) at dose levels of 0, 1000, and 4000 ppm for at least 370 days, and 8000 ppm for 21 days which was then reduced to 7000 ppm for males and females on study day 21, due to the presence of urinary calculi in males. Since urinary calculi persisted in males treated at 7000 ppm, a washout period of 4 days was conducted for this group between study days 52 and 55, before continuation of the treatment at 6000

ppm starting on study day 56 for the remainder of the 12-month treatment period. Cageside observations were conducted daily, detailed clinical observations were conducted weekly, food consumption was measured daily, and body weights were taken weekly. Ophthalmic examinations were performed once pre-exposure and just prior to necropsy. Clinical chemistry, haematology, and urinalysis measurements were taken once pre-exposure and during study weeks 14, 27, 40 and 53. A gross necropsy was performed, organ weights were taken, and tissues were examined microscopically.

At 6000 ppm in males/7000 ppm in females:
In males, calculi were observed in the urinary bladder and were associated with micropathology findings in the bladder of slight to moderate transitional cell hyperplasia, slight congestion, slight haemorrhage, slight inflammation, minimal calculus, and/or moderate ulceration.
At 4000 ppm and 1000 ppm:
No treatment-related effects were observed.

The NOAEL for this study is 4000 ppm for males, equivalent to 117 mg/kg bw/day, and 7000 ppm for females, equivalent to 200 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636
- **Description:** white powder
- **Lot/Batch:** Mix-batch 702-73-06-0001
- **Purity:** 96.4% (analysis on 01/12/2003), 96.4% (analysis on 03/06/2005), 95.7% (analysis on 17/11/2005)
- **CAS:** 317815-83-1
- **Stability of test compound:** Stable in diet at 100 and 13 000 ppm over 7 days at ambient temperature and 56 days at freezer temperature

2. Vehicle and /or positive control: corn oil (1%) and acetone

3. Test animals:
- **Species**: Dog
- **Strain:** Beagle
- **Age:** 5 to 6 months approximately
- **Weight at dosing:** 6.4 to 8.7 kg for the males – 5.0 to 6.4 kg for the females
- **Source:** Marshal BioResources, North Rose, New York
- **Acclimation period:** 7 days
- **Diet:** Purina certified canine diet Etts 5006-3; presented to the animals for 3-6 hours/day, beginning 6 days prior to study initiation and continuing throughout the study; *ad libitum* prior to this time.
- **Water:** Tap water, *ad libitum*
- **Housing:** Animals were caged individually in stainless steel runs.
- **Environmental conditions –**
  - **Temperature:** 23.5 ± 5.5°C
  - **Humidity:** 50 ± 20 %
  - **Air changes:** At least 10.5 changes per hour
  - **Photoperiod:** Alternating 12-hour light and dark cycles (6 am- 6 pm)

B. STUDY DESIGN:

1. In life dates: February 9, 2005 to February 22, 2006 at Bayer CropScience LP, Stilwell, Kansas, USA

2. Animal assignment and treatment

Prior to initiation of test compound administration, the dogs were randomly assigned to dose groups, based on weight. Weight variation of animals used was targeted not to exceed ± 20% of the mean weight for each sex. Four animals per sex were treated at the following concentrations with BYH 18636 whereas controls received untreated diet.
The nominal concentrations chosen for this study were 0 (concurrent vehicle control), 1000, 4000, and 8000 ppm (4/sex/dose) of technical grade BYH 18636 mixed with dog ration. Due to the presence of urinary calculi in high-dose group males, the dose level of 8000 ppm was reduced to 7000 ppm for both sexes in the high-dose group on study day 21. Since urinary calculi persisted in males treated at 7000 ppm, a washout period of 4 days was conducted for this group between study days 52 and 55, before continuation of the treatment at 6000 ppm from study day 56.

Table IIA 5.3.4-1 Study design

Test group	Sex	Concentrations in the diet (ppm)				Doses in mg/kg/day
		Days 0 to 20	Days 21 to 51	Days 52 to 55	Days 56 to termination	
1	Male	0				0
	Female	0				0
2	Male	1000				29
	Female	1000				27
3	Male	4000				117
	Female	4000				127
4	Male	8000	7000	Washout	6000	179
	Female	8000	7000			200

Selection of the dose levels for this study was based on a 90-day feeding study in the Beagle dog, which used doses of 0 (concurrent vehicle control), 1000, 5000, and 10000 ppm. In this study, there were urinary calculi in 3 of 4 males and 2 of 4 females in the 10000 ppm treated dogs. Microscopically, compound-related changes were epithelial hyperplasia, inflammation, and haemorrhage in the urinary bladder of the 10000 ppm males and females, which were probably due to the irritation by the calculi.

3. Diet preparation and analysis

All feed mixtures were prepared weekly by mixing appropriate amounts of the test substance with Purina Certified Canine Diet 5006-3 and then storing the mixture under freezer conditions until presented to the animals. Corn oil, at 1% by weight of the diet, along with acetone were used as vehicles to suspend the test substance prior to being mixed in the diet. The control diet was prepared in the same manner as the treated diet, excluding only the test substance. The test substance was homogeneously distributed in the diet and was stable in the feed stored at room temperature for 7 days and freezer temperature for 56 days. For the homogenenity analysis, the mean concentration was 103 ppm (103% of the nominal concentration) for the 100 ppm test level and 13081 ppm (101% of the nominal concentration) for the 13000 ppm test level. After 56 days of freezer storage, a 1% decline in concentration was found at both 100 and 13000 ppm. After 7 days at room temperature, no decline was observed at 100 ppm and a 6% decline in concentration was found at 13000 ppm.

The concentration of the active ingredient in the feed was verified during the study for the first three weeks in which a dose was used in the study, except the 6000 and 7000 ppm dose groups which were verified for the first four weeks of use, and then once every 4 or 5 weeks until the end of the study. The concentration of the active ingredient in the feed for the 8000 ppm dose was verified for study weeks 1, 2 and 3. The mean concentrations for the study were 98% and 99% of the nominal levels; %RSD values ranged from 2.60 to 4.54.

4. Statistics

Statistical significance was determined at $p \leq 0.05$ for all tests with the exception of Bartlett's test, in which a probability value of $p \leq 0.001$ was used. All tests were two-tailed, except for gross and histopathologic lesion evaluations that were one-tailed.

Continuous data were analyzed by the Bartlett's test for homogeneity of variances. When the data were homogeneous, an ANOVA was performed followed by Student's t-test on parameters showing a significant effect by ANOVA. When the data were not homogeneous, a Kruskal-Wallis ANOVA was performed followed by the Mann-Whitney U-test to identify statistical significance between groups.

Frequency data, that were examined statistically, were initially analyzed by a Chi-Square procedure. If there was statistical significance using the Chi-Square test, each treatment group was compared to the control group using a Fisher's Exact test.

C. METHODS:

1. Observations

a. Cageside observations

All study animals were observed at least twice daily for clinical signs of toxicity (except once daily on weekends and holidays).

b. Clinical examinations

Detailed clinical observations for clinical signs of toxicity were performed on all animals at treatment initiation (study day 0) and on a weekly basis thereafter.

2. Body weight

Body weights were recorded weekly throughout the treatment. Body weights were also taken immediately prior to necropsy to allow for calculation of organ-to-body weight ratios.

3. Food consumption and compound intake

Each animal's food consumption was measured daily throughout the study. The intake of test compound in mg/kg/day was calculated for males and females using the analytical concentration of BYH 18636 in the feed and the following equation: (average food consumption per week/average body weight per week) x ppm in the feed/1000. The calculation for the high-dose group was calculated using the number of weeks that the various dose concentrations were administered to the dogs.

4. Ophthalmic examination

Following the acclimation period and prior to intitiation of dosing, ophthlamic examinations were conducted on all animals. Ophthalmic examinations were also conducted on all animals just prior to termination of the study.

5. Clinical chemistry

Clinical chemistry and complete blood count, including differentials, were performed on all animals once prior to administration of the test substance and during weeks 14, 27, 40 and 53. Animals were diet fasted overnight prior to blood collection, which was drawn via jugular venipuncture.

The following haematology parameters were assayed: red blood cell count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, reticulocyte count, blood cell morphology, red blood cell distribution width, haemoglobin distribution width, white blood cell count and differential count evaluation and platelet count. Activate partial thromboplastin time and prothrombin time were measured.

Total bilirubin, glucose, albumin, globulins, albumin/globulin ratio, total protein, urea, uric acid,

creatinine, total cholesterol, triglycerides, chloride, sodium, potassium, calcium and phosphorus concentrations and aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactic acid dehydrogenase, creatine phosphokinase and gamma-glutamyltransferase activities were assayed.

6. Urinalysis

Urinalysis was performed on all animals once prior to administration of the test substance and on all animals during study weeks 14, 27, 40 and 53. Urine volume was collected over a 24 hour period.

Any significant change in the general appearance of the urine was recorded. The urine volume was measured. pH and specific gravity were assayed.

The following parameters were assayed: glucose, bilirubin, ketones, blood, protein, nitrites, leukocytes and urobilinogen. Microscopic examination of the urinary sediment was performed.

7. Sacrifice and pathology

Animals were deeply anaesthetized by intravenous injection of Fatal-Plus, then exsanguinated before necropsy. The necropsy consisted of a systematic gross examination of each animal's general physical condition, body orifices, external and internal organs and tissues. Adrenal gland, brain, heart, kidney, liver, lung, ovary, pituitary gland, prostate gland, spleen, testis, thymus, thyroid gland (with parathyroid gland) and uterus (including cervix) were weighed fresh.

The following organs or tissues were sampled: adrenal gland, aorta, bone (sternum, rib), bone marrow, brain, epididymis, oesophagus, eye and optic nerve, Fallopian tube, heart, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidney, larynx/pharynx, liver, lung, lymph nodes, mammary gland, ovary, pancreas, pituitary gland, prostate gland, salivary gland, sciatic nerve, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, testis, thymus, thyroid gland (with parathyroid), trachea, urinary bladder, uterus (with cervix), ureter and vagina.

Tissues samples were processed routinely and stained with haematoxylin and eosin. Histopathological examinations were performed on all tissues (except vagina) from all the animals in the control and high dose groups. Target organs and gross lesions from all dose groups were examined histologically.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS:

1. Clinical signs of toxicity

The only compound-related clinical observation was the presence of fine sand-like yellow granular particles in the urine of 3 out of 4 high-dose group males. This sign was observed starting on study day 13 and persisted throughout the study: up to day 357 in one male, day 118 in the second male, and day 292 in the last male.

2. Mortality

No animals were found dead or sacrificed *in-extremis* in this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN:

There were no treatment-related effects on body weight or body weight gain.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

There were no treatment-related effects on food consumption.

Table IIA 5.3.4-2 Mean BYH 18636 intake

Test group	Concentration in diet (ppm)	Mean intake Days 1 -364 (mg/kg/day)	
		Male	Female
1	0	0	0
2	1 000	29	27
3	4 000	117	127
4	8 000/7 000/6 000	179	200

The mean achieved dosage intake per group was: 29, 117 and 179 mg/kg/day in males and 27, 127 and 200 mg/kg/day in females.

D. OPHTHALMIC EXAMINATION:

No treatment-related ocular abnormalities were observed at ophthalmic examination.

E. BLOOD ANALYSIS:

1. Haematological findings

There were no treatment-related changes in male and female dogs at any treatment group.

2. Clinical chemistry findings

There were no clinical chemistry treatment-related changes.

F. URINALYSIS:

There was a non-statistical increase of mean urinary protein in high-dose group males at days 93, 184, 275, and 366. This increase in mean urinary protein values was attributed primarily to increases in two high-dose group males – YO3001 (100 mg/dl on days 93, 184, 275, and 366) and YO3002 (100 mg/dl on day 93 and 300 mg/dl on days 184, 275 and 366). These two males had urinary bladder calculi present at necropsy along with micropathology changes which could account for increased urinary protein. However, one animal (YO003) in the control group had urinary protein values of 100 mg/dl at day 275 and 300 mg/dl at day 366 which would question whether BYH 18636 had an effect on urinary protein.
No other changes were considered treatment-related.

G. SACRIFICE AND PATHOLOGY:

1. Organ weight

Terminal body weights and organ weights were not affected by compound-administration.

Variability in mean organ weights occurred in the high-dose group. Decreases in mean male kidney weights; decreases in mean male spleen weights; increases in mean male testis weights; decreases in female liver weights; increases in mean female pituitary weights; and increases in mean female thyroid weights were considered not to be treatment-related. These changes were minimal and considered not to be biologically significant. None of these tissues had associated micropathology considered to be compound-related. None of these mean changes were statistically significantly different from the control group. All of these mean changes were within historical control values from this lab.

The only statistically significantly changed organ weight occurred as an increase in the absolute thyroid weight for mid-dose group females. This change was not dose-dependent and not considered to be compound-related.

3. Gross and histopathology

Calculus and abnormal consistency noted in the urinary bladders of two high-dose group males were considered to be compound-related. All other gross findings were considered to be incidental and/or background and not related to compound-administration.

As per protocol, micropathology evaluation was conducted on all tissues from the control and high-dose groups. Based primarily on gross (calculus) and micropathology (transitional cell hyperplasia) changes noted in the urinary bladders of high-dose group males, the male low- and mid-dose group urinary bladders were also processed and evaluated microscopically.

Compound-related micropathology lesions in the urinary bladder were limited to two males in the high-dose group. Both had calculi present in the urinary bladder at necropsy. The micropathology lesions consisted of the following: a slight to moderate transitional cell hyperplasia; a slight congestion; a slight haemorrhage; a slight inflammation; a minimal calculus; and/or moderate ulceration. All other micropathology lesions were considered to be incidental and not related to compound-administration.

III. CONCLUSION

The NOAEL for this study was 4000 ppm for males, equivalent to 117 mg/kg bw/day, and 7000 ppm for females, equivalent to 200 mg/kg bw/day.

(Eigenberg D.A., 2007)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY

Name of authority: Pesticides Safety Directorate, UK

Reviewer's Comments

Reliability rating: Totally reliable

The study (201497-1) is fully compliant with OECD 452. Minor and isolated deviations from the protocol regarding the light/dark cycle, temperature range, airflow and feeding time are not considered to have affected the integrity of the study.

In contrast to the Notifier's statement that there no treatment-related findings on bodyweight or bodyweight gain, it is considered that weight gain by top dose males was adversely affected by administration of the test material. Mean bodyweights in this group were consistently slightly (but not significantly) lower than controls from Day 7 to Day 56, but were comparable from Day 70 following the 'washout' period and treatment at the reduced dose level of 6000 ppm. Similar findings were not apparent in females and food consumption was comparable in all groups.

A small number of statistically significant clinical chemistry findings (increased plasma creatinine kinase activity, increased plasma phosphorus concentration) were seen during the treatment phase, but are consistent with results seen pre-test and are therefore not considered to be treatment-related. A statistically significant increase in lactate dehydrogenase activity seen in top dose males at 6 months (group values: 94, 113, 65, 223* U/l) is not considered to be treatment-related in the absence of similar findings at other time points.

Slightly (but not statistically significantly) lower absolute and relative kidney weights in top dose males are not considered to be of clear toxicological significance in the absence of clinical chemistry or histopathological correlates.

Additional findings in the one-year dog study

Observation / time point		Dose level (ppm) 0	1000	4000	6000
Weight (g) (males)	D0	7615.0 -	7417.8 97.4%	7528.8 98.9%	7656.5 100.5%
	D7	7935.5 -	7724.8 97.3%	7773.5 98.0%	7911.3 99.7%
	D28	8554.5 -	8439.8 98.7%	8632.5 100.9%	8558.0 100.0%
	D42	8785.0 -	8908.5 101.4%	8967.8 102.1%	8773.3 99.9%
	D49	9055.5 -	8989.3 99.3%	9134.5 100.9%	8741.5 96.5%
	D56	9004.0 -	8954.0 99.4%	9172.0 101.9%	8681.8 96.4%
	D63	9311.8 -	9224.0 99.1%	9375.5 100.7%	8970.3 96.3%
	D70	9369.8 -	9385.0 100.2%	9665.0 103%	9221.5 98.4%
Kidney weights					
M	(g) (% bw)	62.863 0.569	55.382 0.509	56.379 0.515	52.670 0.464
F	(g) (% bw)	36.274 0.431	35.019 0.413	40.793 0.458	36.346 0.424

Conclusions	The results of this study are consistent with the 90-day dog study (Eigenberg, 2005) and confirm the urinary bladder as the target of thiencarbazone-methyl toxicity in this species. A NOAEL for males of 4000 ppm (117 mg/kg bw/d) can be determined for this study, based on the presence of urinary bladder calculi and associated urinalysis observations and histopathological findings in the transitional epithelium at the top dose level of 8000/7000/6000 ppm (equivalent to a mean achieved dietary intake of 179 mg/kg bw/d). A NOAEL of females of 7000 ppm (equivalent to a mean intake of 200 mg/kg bw/d) can be determined for this study in the absence of any clearly treatment-related findings at the top dose level. The NOAELs for this study are therefore the same as proposed by the Notifier.

B.6.3.5 28-day inhalation toxicity (rodents) (IIA 5.3.5)

Based on the results of the acute inhalation toxicity study and the physical properties of BYH 18636 (compound not volatile with an extrapolated vapour pression of 8.8 x 10^{-14} Pa at 20°C), no repeated inhalation study is triggered.

B.6.3.6 90-day inhalation toxicity (rodents) (IIA 5.3.6)

Based on the results of the acute inhalation toxicity study and the physical properties of BYH 18636 (compound not volatile with an extrapolated vapour pression of 8.8 x 10^{-14} Pa at 20°C),, no repeated inhalation study is triggered.

B.6.3.7 Percutaneous 28-day toxicity (rodents) (IIA 5.3.7) (MRID # 47070213)

Based on the results of the acute dermal toxicity study and the physical properties of BYH 18636, no repeated dermal study is triggered in the EU or in the USA. For Canada, the use of animals for this study is not justified, based on the overall toxicology profile for this compound and dermal penetration data indicating that its potential for dermal absorption is low (See waiver request from **Zhou J., 2007, M-285873-01-1**).

B.6.3.8 Percutaneous 90-day toxicity (rodents) (IIA 5.3.8)

Based on the results of the acute dermal toxicity study and the physical properties of BYH 18636, no repeated dermal study is triggered in the EU or in the USA. For Canada, the use of animals for this study is not justified, based on the overall toxicology profile for this compound and dermal penetration data indicating that its potential for dermal absorption is low (See waiver request from Zhou J., 2007, M-285873-01-1).

B.6.4 Genotoxicity studies (IIA 5.4)

B.6.4.1 Genotoxicity *in vitro* (IIA 5.4.1, IIA 5.4.2, IIA 5.4.3)

In vitro genotoxicity - Bacterial assay for gene mutation (IIA 5.4.1)

Report:	**KIIA 5.4.1/01, Wirnitzer U., 2005 (MRID # 47070137)**
Title:	BYH 18636, Salmonella/microsome test, plate incorporation and preincubation method
Report No & Document No	AT02274 M-257354-01-2
Guidelines:	OECD 471 (1997); EEC Directive 2000/32/EC Method B13/14 (2000); EPA Health Effects Test Guideline (OPPTS 870.5100; 1998)
GLP	Yes (certified laboratory)

Executive summary:
In this *in vitro* assessment of the mutagenic potential of BYH 18636 (Mix-batch 702-73-06-0001, 96.4% of purity), histidine dependent auxotrophic mutants of Salmonella typhimurium, strains TA 1535, TA 1537, TA 98, TA 100 and TA 102 were exposed to BYH 18636 up to 5000 µg/plate, diluted in dimethyl sulphoxide (DMSO). For each bacterial strain and dose level, triplicate plates were used in both the presence and absence of an Aroclor 1254-induced rat liver metabolic activation system (S9 mix). DMSO was also used as a negative control. Specific positive controls were used for each strain. After 48 hours of incubation at 37°C, the numbers of revertant colonies were scored using an automated colony counter. Due to strain-specific bacteriotoxic effect, one independent repeat of the plate incorporation test was performed at doses from 20 to 1200 µg/plate depending on the strain. Another assay testing a pre-incubation for 20 minutes at 37°C was also performed at doses from 10 to 400 µg/plate.

Doses up to 20 µg/plate did not cause any bacteriotoxic effects. At higher doses, BYH 18636 had a strong, strain-specific bacteriotoxic effect, so that the range could only to be used to a limited extent up to 400 µg/plate for assessment purposes. Substance precipitation occurred at the dose of 1581 µg/plate and above.

BYH 18636 did not cause any significant increase in the number of revertant colonies in either the presence or absence of metabolic activation.

All the positive control compounds produced expected increases in the number of revertant colonies, thereby demonstrating the sensitivity of the assay and the efficacy of the S9 mix.

Therefore, BYH 18636 was non-mutagenic with or without S9 mix in the plate incorporation as well as in the pre-incubation modification of the Salmonella/microsome test.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636
- **Description:** Solid, fine, white powder
- **Lot/Batch:** MIX-batch 702-73-06-0001
- **Purity:** 96.4%
- **CAS:** 317815-83-1
- **Stability of test compound:** Stable for the duration of the study

2. Control materials:
- **Negative:** Culture medium
- **Solvent:** DMSO

Positive: Non activated.
Sodium azide (Serva) for TA 1535 at 10 µg/plate,
Nitrofurantoin (Sigma) for TA 100 at 0.2 µg/plate,
4-Nitro-1,2-phenylene diamine (Merck-Schuchardt) for TA 1537 at 10 µg/plate and TA 98 at 0.5 µg/plate,
Mitomycin C (Fluka) for TA 102 at 0.2 µg/plate only in plate incorporation trials,
Cumene hydroperoxide (Sigma) for TA 102 in pre-incubation trials only at 50 µg/plate,
2-Aminoanthracene (Aldrich) for the activating effect of the S9 mix in all strains at 3 µg/plate.

3. Test organisms:

Species: Salmonella typhimurium LT2 mutants

Strain: Histidine-auxotrophic strains TA 1535, TA 100, TA 1537, TA 98 and TA 102

Source: Strains obtained from Prof. Bruce Ames in 1997 and stored in the laboratory since then.

4. Test compound concentrations:

Plate incorporation assay: First assay for all strains with or without S9 mix: 16, 50, 158, 500, 1581 and 5000 µg/plate
Second assay for TA 1535, TA 1537 and TA 102 with or without S9 mix: 20, 40, 60, 80 and 100 µg/plate
Second assay for TA 100 with or without S9 mix: 400, 600, 800, 1000 and 1200 µg/plate
Second assay for TA 98 with or without S9 mix: 100, 200, 300 and 400 µg/plate,
Third assay for TA 98 with S9 mix: 50, 100, 200, 300 and 400 µg/plate

Pre-incubation assay: For TA 1535, TA 1537 and TA 102 with or without S9 mix: 10, 20, 30, 40, 50 and 100 µg/plate,
For TA 100 and TA 98 with or without S9 mix: 30, 40, 50, 100, 200 and 400 µg/plate.

B. STUDY DESIGN AND METHODS:

The in life phase of the study was performed between February 22 to March 17, 2005 at Bayer Healthcare AG (PH-R&D Toxicology International).
The Salmonella/microsome test is a screening method which detects point mutations caused by chemical agents *in vitro*. Auxotrophic mutants of Salmonella typhimurium are used to demonstrate this effect. For this purpose, the rate of reversion to prototrophy is evaluated in negative control and treated groups.

1. Plate incorporation assay:

Due to the low solubility of BYH 18636, BYH 18636 or the control material were dissolved in 0.2 ml of DMSO. DMSO (0.2 ml) containing BYH 18636 or controls were added to glass vessels with 0.1 ml of bacterial cultures grown overnight, 0.5 ml of S9 mix or buffer and 2 ml of soft agar. The mixture was placed in a waterbath at 45°C for 30 seconds, shaken and overlaid onto Petri dishes containing solid agar. After 48 hours of incubation at 37°C, the numbers of revertant colonies were scored using an automated colony counter. Three plates were used, both with and without S9 mix, for each strain and dose. The doses for the first trial were routinely determined on the basis of a standard protocol with a maximum dose of 5000 µg/plate and at least 5 additional doses. If less than three doses were used for assessment, at least two repeats were performed.

2. Pre-incubation assay:
An independent repeat was performed as pre-incubation of the previously described mixture in a water bath at 37°C for 20 minutes. At the end of the preincubation period, 2 ml of molten soft agar were added to the tubes, the content mixed and plated onto Petri dishes with solid agar. After 48 hours of incubation at 37°C, the numbers of revertant colonies were also scored using an automated colony counter.

3. Assessment criteria:
A reproducible and dose-related increase in mutant colonies of at least one strain was considered to be positive. For TA 1535, TA 100 and TA 98, this increase should be about twice that of negative controls, whereas for TA 1537, at least a threefold increase should be reached. For TA 102 an increase of about 100 mutants should be reached. Otherwise, the result was considered as negative.

II. RESULTS AND DISCUSSION

There was no indication of a bacteriotoxic effect of BYH 18636 at doses of up to 20 µg/plate. The total bacterial counts consistently produced results comparable to the negative controls, or differed only insignificantly. No inhibition of growth was noted as well. Higher doses had a strong, strain-specific bacteriotoxic effect. Therefore only results obtained up to 400 µg/plate were used for assessment purposes. At 1581 µg/plate, the substance started to precipitate.
None of the five strains concerned showed in the plate incorporation test a dose-related and biologically relevant increase in mutant colonies over those of the negative controls. This applied both to the tests with and without S9 mix and was confirmed by the results of the pre-incubation trials.
The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, mitomycin C, cumene hydroperoxide and 2-aminoanthracene increased mutant colonies to well over those of the negative controls, and thus demonstrated the system's sensitivity and the activity of the S9 mix.

III. CONCLUSION

No indication of mutagenic effects of BYH 18636 could be found at assessable doses of up to 400 µg/plate in any of the Salmonella typhimurium strains used in the assay.

(Wirnitzer U., 2005)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY

Name of authority

Pesticides Safety Directorate, UK

Reviewer's Comments

Reliability rating: Reliable with restrictions

The study (T0073262) was fully compliant with OECD 471 (1997)

The study was performed with test material of higher purity (96.4%) than the minimum currently proposed (950 g/kg) for the specification of the manufactured material.

The highest concentrations of the test material used in this study were limited by cytotoxicity and solubility. The efficacy of the S9 batch (from male Sprague-Dawley rat liver) was additionally demonstrated using reference mutagens in addition to the single positive control chemical (2-aminoanthracene) used in the study.

The laboratory's criteria for a positive response is stated to be an increased mutant count of twice the negative control value (strains TA1535, TA100, TA98); three times the negative control value (strain TA1537) or 100 more than the negative control value (TA102). The responses for TA100 with the positive control compound (nitrofurantoin) in the absence of metabolic activation in the first and second (plate incorporation) assays do not therefore meet the laboratory's criteria for a positive response; however the response in the third (pre-incubation) assay does meet the laboratory's criteria. While the response of strain TA102 to the positive control compounds does meet the laboratory's criteria for a positive response, the magnitude of the response in some assays (less than twice the negative control) is not great. The laboratory's criterion for a positive response in this strain is therefore questioned. The Notifier has stated that '*a positive control is not used to demonstrate that high doses of known mutagens induce strong mutagenic effects. Positive controls should be used in a way which demonstrates that the test system used detects reproducibly relatively weak effects of low doses of known mutagens thus demonstrating the sensitivity of the used test system.*' This argument is not accepted. Although the assay is shown to be capable of detecting a weak response to a low concentration of a strong mutagen, in the absence of the demonstration of a marked response, it is unclear whether the assay is capable of detecting a weak mutagen.

Performance of positive control compounds in the Ames test

Conditions		Revertant colonies/plate									
		TA1535		TA100		TA1537		TA98		TA102	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
1st assay	-control	17	10	164	209	11	7	21	45	219	316
	+control	728	150	309	1384	114	136	159	1146	553	683
	Acceptable*	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓
2nd assay	-control	23	29	150	178	6	13	20	86	235	354
	+control	685	188	256	1419	87	161	92	1231	412	735
	Acceptable*	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓
3rd assay	-control	21	18	131	155	8	6	21	31	243	329
	+control	651	184	495	1626	131	187	181	1532	461	777
	Acceptable*	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

**according the laboratory's criteria*

Conclusions

No evidence of genotoxicity was seen under the conditions of this study. However the sensitivity of this study is considered to be limited in the absence of a consistent response to the positive control compounds in strain TA100 and the magnitude of the response to the positive control compounds in strain TA102.

Report:	**KIIA 5.4.1/02, Herbold B., 2007 (MRID # 47070136)**
Title:	BYH 18636, Salmonella/microsome test, plate incorporation and preincubation method
Report No & Document No	AT03630 M-283701-01-2
Guidelines:	OECD 471 (1997); EEC Directive 2000/32/EC Method B13/14 (2000); EPA Health Effects Test Guideline (OPPTS 870.5100; 1998)
GLP	Yes (certified laboratory)

Executive summary:
Based on the specification required for production of the technical BYH 18636, a new Ames test was required. In this *in vitro* assessment of the mutagenic potential of BYH 18636 (batch GELL 420-151-1, 94.6% of purity), histidine dependent auxotrophic mutants of Salmonella typhimurium, strains TA 1535, TA 1537, TA 98, TA 100 and TA 102 were exposed to BYH 18636 up to 480 µg/plate, diluted in dimethyl sulphoxide (DMSO). For each bacterial strain and dose level, triplicate plates were used in both the presence and absence of an Aroclor 1254-induced rat liver metabolic activation system (S9 mix). DMSO was also used as a negative control. Specific positive controls were used for each strain. After 48 hours of incubation at 37°C, the numbers of revertant colonies were scored using an automated colony counter. The independent repeat of the plate incorporation test was performed at doses of up to and including 512 µg/tube.

Doses up to and including 30 µg/plate did not cause any bacteriotoxic effects. At higher doses, BYH 18636 had a strong, strain-specific bacteriotoxic effect, so that the range could only be used to a limited extent up to and including 256 µg/plate for assessment purposes.

BYH 18636 did not cause any significant increase in the number of revertant colonies in either the presence or absence of metabolic activation.

All the positive control compounds produced expected increases in the number of revertant colonies, thereby demonstrating the sensitivity of the assay and the efficacy of the S9 mix.

Therefore, BYH 18636 was non-mutagenic with or without S9 mix in the plate incorporation as well as in the pre-incubation modification of the Salmonella/microsome test.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636
Description: fine white powder
Lot/Batch: GELL 420-151-1
Purity: 94.6% (June 21, 2006)
CAS: 317815-83-1
Stability of test compound: Stable for the duration of the study

2. Control materials:
Negative: Culture medium
Solvent: DMSO
Positive: Non activated.
Sodium azide (Serva) for TA 1535 at 10 µg/plate,
Nitrofurantoin (Sigma) for TA 100 at 0.2 µg/plate,
4-Nitro-1,2-phenylene diamine (Merck-Schuchardt) for TA 1537 at 10 µg/plate and TA 98 at 0.5 µg/plate,
Mitomycin C (Fluka) for TA 102 at 0.2 µg/plate only in plate

	incorporation trials, Cumene hydroperoxide (Sigma) for TA 102 in pre-incubation trials only at 50 µg/plate, 2-Aminoanthracene (Aldrich) for the activating effect of the S9 mix in all strains at 3 µg/plate.

3. Test organisms:

Species:	Salmonella typhimurium LT2 mutants
Strain:	Histidine-auxotrophic strains TA 1535, TA 100, TA 1537, TA 98 and TA 102
Source:	Strains obtained from Prof. Bruce Ames in 1997 and stored in the laboratory since then.

4. Test compound concentrations:

Plate incorporation assay:	For all strains with or without S9 mix: 15, 30, 60, 120, 240 and 480 µg/plate
Pre-incubation assay:	For TA 1535, TA 1537 and TA 98 with or without S9 mix: 8, 16, 32, 64, 128 and 256 µg/plate For TA 100 with or without S9 mix: 16, 32, 64, 128, 256 and 512 µg/plate For TA 102 with or without S9 mix: 4, 8, 16, 32, 64 and 128 µg/plate.

B. STUDY DESIGN AND METHODS:

The *in life* phase of the study was performed between December 1 to 18, 2006 at Bayer HealthCare AG (PH-GDD Health Care Toxicology).
The Salmonella/microsome test is a screening method which detects point mutations caused by chemical agents *in vitro*. Auxotrophic mutants of Salmonella typhimurium are used to demonstrate this effect. For this purpose, the rate of reversion to prototrophy is evaluated in negative control and treated groups.

1. Plate incorporation assay:
DMSO (0.1 ml) containing BYH 18636 or controls were added to glass vessels with 0.1 ml of bacterial cultures grown overnight, 0.5 ml of S9 mix or buffer and 2 ml of soft agar. The mixture was placed in a waterbath at 45°C for 30 seconds, shaken and overlaid onto Petri dishes containing solid agar. After 48 hours of incubation at 37°C, the numbers of revertant colonies were scored using an automated colony counter. Three plates were used, both with and without S9 mix, for each strain and dose. The doses for the first trial were determined on the basis of a previous conducted study with the same active ingredient (AT02274). At least 5 additional doses were routinely used. If less than three doses were used for assessment, at least two repeats were performed.

2. Pre-incubation assay:
An independent repeat was performed as pre-incubation of the previously described mixture in a water bath at 37°C for 20 minutes. At the end of the pre-incubation period, 2 ml of molten soft agar were added to the tubes, the content mixed and plated onto Petri dishes with solid agar. After 48 hours of incubation at 37°C, the numbers of revertant colonies were also scored using an automated colony counter.

3. Assessment criteria:
A reproducible and dose-related increase in mutant colonies of at least one strain was considered to be positive. For TA 1535, TA 100 and TA 98, this increase should be about twice that of negative controls, whereas for TA 1537, at least a threefold increase should be reached. For TA 102 an increase of about 100 mutants should be reached. Otherwise, the result was considered as negative.

II. RESULTS AND DISCUSSION

There was no indication of a bacteriotoxic effect of BYH 18636 at doses of up to and including 30 µg/plate. The total bacterial counts consistently produced results comparable to the negative controls, or differed only insignificantly. No inhibition of growth was noted as well. Higher doses had a strong, strain-specific bacteriotoxic effect. Therefore only results obtained up to and including 256 µg/plate were used for assessment purposes.
None of the five strains concerned showed in the plate incorporation test a dose-related and biologically relevant increase in mutant colonies over those of the negative controls. This applied both to the tests with and without S9 mix and was confirmed by the results of the pre-incubation trials.
The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, mitomycin C, cumene hydroperoxide and 2-aminoanthracene increased mutant colonies to well over those of the negative controls, and thus demonstrated the system's sensitivity and the activity of the S9 mix.

III. CONCLUSION

No indication of mutagenic effects of BYH 18636 could be found in any of the Salmonella typhimurium strains used in the assay.

(Herbold B., 2007)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY

Name of authority: Pesticides Safety Directorate, UK

Reviewer's Comments:

Reliability rating: Reliable with restrictions

The study (T4076830) is fully compliant with OECD 471 (1997)

The study is essentially identical to the previous study (Wirnitzer, 2005), but was performed using material of lower purity (94.6% compared to 96.4% in the previous study), and stated to be representative of the proposed specification.

The highest concentrations of the test material used were limited by cytotoxicity. In contrast to the previous study (Wirnitzer, 2005), the positive control compounds demonstrated the sensitivity of the assay in the absence and presence of metabolic activation for all strains (according to the laboratory's criteria). However the response of the positive control compounds was less marked in strain TA102 (mean revertant colony counts less than twice the solvent control). The efficacy of the S9 batch (from male Sprague-Dawley rats) was additionally demonstrated using reference mutagens in addition to the single positive control chemical (2-aminoanthracene) used in the study. While the response of strain TA102 to the positive control compounds does meet the laboratory's criteria for a positive response, the magnitude of the response in some assays (less than twice the negative control) is not great. The laboratory's criterion for a positive response in this strain is therefore questioned.

Performance of positive control compounds in the Ames test

Compound	Revertant colonies/plate: strain TA102			
	1st assay		2nd assay	
	-S9	+S9	-S9	+S9
-ve control	211	263	214	309
+ve control	534	512	415	522

Conclusions: No evidence of genotoxicity was seen under the conditions of this study, however the response to the positive control compounds of TA102 shows a limited sensitivity of this strain.

In vitro genotoxicity - Test for clastogenicity in mammalian cells (IIA 5.4.2)

Report:	**KIIA 5.4.2/01, Thum M., 2005 (MRID # 47070145)**
Title:	BYH 18636, *In vitro* chromosome aberration test with Chinese Hamster V79 cells
Report No &	AT02499
Document No	M-259268-01-2
Guidelines:	OECD 473 (1997); EEC Directive 2000/32/EC Method B10 (2000); EPA Health Effects Test Guideline (OPPTS 870.5375; 1998)
GLP	Yes (certified laboratory)

Executive summary:
In this *in vitro* assessment of the clastogenic potential of BYH 18636 (Mix-batch 702-73-06-0001, 96.3 to 96.4% of purity), Chinese Hamster V79 cells were exposed to BYH 18636 at 100, 200 and 400 µg/ml, diluted in dimethyl sulphoxide (DMSO). For each dose level, duplicate cultures were used in both the presence and absence of an Aroclor 1254-induced rat liver metabolic activation system (S9 mix). DMSO was also used as a negative control. Mitomycin C, which produces crosslinks in the DNA, and cyclophosphamide, which induces chromosomal damage after metabolic activation, were used as positive controls, diluted in Hanks' balanced salt solution. After 4 hours treatment, the medium was changed and the cells were harvested 14 hours later. A second harvest was performed 30 hours after the start of the study. In a second experiment, the cells were exposed to BYH 18636 at 400 µg/plate or controls without S9 mix for 18 hours and harvested at the end of the treatment period. Colcemid was added to each flask two hours prior to harvest to arrest the cells in a metaphase-like stage of mitosis.

Cytotoxic effects were observed at 400 µg/plate with and without S9 mix after 4 hours treatment whereas no cytotoxic effects were observed after 18 hours treatment without S9 mix. Precipitation in the medium did not occur.

None of the cultures treated with BYH 18636 in the presence and in the absence of S9 mix showed biologically relevant or statistically significant increased numbers of aberrant metaphases.

The positive controls mitomycin C and cyclophosphamide induced clastogenic effects and demonstrated the sensitivity of the test system and the activity of the used S9 mix.

BYH 18636 was considered not to be clastogenic for mammalian cells *in vitro*.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:		BYH 18636
Description:		fine, white powder
Lot/Batch:		MIX-batch 702-73-06-0001
Purity:		96.3 to 96.4%
CAS:		317815-83-1
Stability of test compound:		Stable for the duration of the study
2. Control materials:	**Negative:**	Culture medium (Eagle's minimal essential medium)
	Solvent:	DMSO for BYH 18636 and Hanks's balanced salt solution for positive controls (Seromed)
	Positive:	Mitomycin C (Fluka, batch 454188/2 44903116) without S9 mix at 0.1 µg/ml (4-hr treatment); 0.03 µg/ml (18-hrs treatment). Cyclophosphamide (Endoxan 100 mg injection vials of dry substance, Baxter Oncology GmbH) with S9 mix at 2 µg/ml.

3. Test organisms:

Cell line: Chinese hamster V79 lung cells

Source: Cells obtained from Dr Utesch, Merk AG, Darmstadt in 1993, stored in the laboratory since then.

4. Test compound concentrations:

BYH 18636 was used in each trial at 100, 200 and 400 µg/ml (4hrs -/+S9; 18 hrs –S9).

B. STUDY DESIGN AND METHODS:

The experimental phase of the study was performed from November 23, 2004 to July 8, 2005 at Bayer Healthcare AG (PH-R&D Toxicology International).
The *in vitro* cytogenetic test is a mutagenicity test system for the detection of chromosome aberrations in cultured mammalian cells. The test is designed to detect structural aberrations (chromatid and chromosome aberrations) in cells at their first post-treatment mitosis.

1. Determination of cytotoxicity:

Duplicate cultures were exposed in a pre-test to BYH 18636 at concentrations ranging from 1 to 400 µg/ml with or without S9 mix for 4 hours. In addition, cells were exposed without S9 mix for 18 hours to concentrations ranging from 10 to 400 µg/ml. The mitotic index was determined for all cultures. The number of mitotic cells among a total of 1000 cells per culture was determined using a light microscope at a magnification of about 630. All cells which were not in interphase were defined as mitotic.
In the main study, cultures with a total incubation period of 8 hours were additionally and exclusively used to determine the cytotoxicity of BYH 18636 at concentrations ranging from 100 to 400 µg/ml.

Concentrations of up to 400 µg/ml BYH 18636 did not change the pH or the osmolality in the medium of the pre-test.

2. Treatment protocol:

Chinese hamster V79 cells were passaged on the day prior to treatment. Approximately $1x10^6$ cells were seeded in 20 ml of medium per 75 m^2 flasks and incubated at 37°C in a CO_2-incubator (5% CO_2). Unless reported otherwise, the cells were grown in Eagle's minimal essential medium containing 10% foetal calf serum. Immediately before treatment, the medium was removed from the cultures. For the trials without S9 mix, 20 ml of medium containing 2% foetal calf serum and 0.2 ml of test substance solution were added to each flask. For the trials with S9 mix, 19 ml of medium containing 2% foetal calf serum, 1 ml of S9 mix and 0.2 ml of test substance solution were added to each flask. The cells were incubated for 4 hours at 37°C. After 4 hours of treatment, the medium was removed, the cells were washed with PBS and 20 ml of fresh medium containing 10% foetal calf serum was added to the flasks. The flasks were placed in a CO_2-incubator for the remaining incubation time. In the cultures treated for 18 hours, the medium was not removed.

0.2 ml of Colcemid-solution (40 µg/ml) was added to each flask 2 hours prior to the end of the incubation period to arrest the cells in a metaphase-like stage of mitosis(c-metaphase).

Positive controls and solvent controls (0.2 ml of solvent per culture) were set up in parallel and handled as described for BYH 18636-treated cultures. Untreated controls and solvent controls were used as negative controls.

4. Chromosome preparations:

After the removal of the medium from each flask, the cells were trypsinized, suspended in medium and centrifuged for approximately 5 minutes at 700 rpm. The supernatant was removed and 1 to 2 ml of a hypotonic solution (0.4% KCl; 37°C) was added to each tube. Within 4 minutes, the

volume was brought to 6 ml with additional hypotonic solution and cells were resuspended. The cells were centrifuged again and fixed with cold (4°C) fixative (ethanol/acetic acid 3:1) for 20 minutes at room temperature. Cells were pelleted and resuspended in fixative as before and centrifuged again. The pelleted cells were resuspended in a small volume of fixative and the suspension was dropped onto clean slides. The slides were allowed to dry for at least 2 hours. Thereafter, they were submerged in pure methanol for 3 minutes and stained for 15-20 minutes in 3% Giemsa solution. Slides were rinsed twice in water and once in acetone and were then kept in xylene for about 30 minutes. The slides were allowed to dry completely and covered. At least two slides were generated per culture.

5. Evaluation criteria:

Coded slides were evaluated using a light microscope at a magnification of about 1000. Chromosomes of approximately 200 metaphases per concentration, 100 metaphases from each of two parallel cultures, were examined. Only metaphases containing the modal chromosome number (22) were analyzed unless exchanges were detected. The following aberrations were recorded: gaps (an achromatic lesion within a chromatid arm without dislocation of the chromatid end), break (a discontinuity of one chromatid with dislocation of the chromatid end), fragment (part of chromosome without centromere), deletion (result of a break with the terminal chromatid part of the chromosome missing within the metaphase under assessment), exchange (exchange of chromatid-parts between different chromosomes or within the same chromosome), multiple aberration (when five or more structural changes occur within one metaphase). Observed polyploidy metaphases were recorded but not used for assessment.

6. Assessment criteria:

An assay was acceptable, if there was a biologically relevant increase in chromosome aberrations induced by positive controls and if the numbers of aberrations for the negative controls were in the expected range.
An increased incidence of gaps of both types without a concomitant increase of other aberration types was considered not to be an indication of a clastogenic effect.
A test was considered positive, if there was a relevant and statistically significant increase in the aberration rate.
A test was considered negative, if there was no such increase at any time interval or if there were statistically significant values, which were, however, within the range of historical negative controls.
A test was considered equivocal, if there was an increase above the range of historical negative controls which was statistically significant but not considered relevant, or if an increase occurred, which was considered relevant, but which was not statistically significant.

7. Statistics:

The statistical analysis was performed by pair-wise comparison of BYH 18636-treated and positive control groups to the respective solvent control group.
The mitotic index was statistically analyzed (provided that it was reduced compared to the mean of the corresponding solvent control) using the one-sided Chi^2-test.
The numbers of metaphases with aberrations (including and excluding gaps) and of metaphases with exchanges were compared (provided that these data superceded the respective solvent control). The one-side Chi^2-test was used for the statistical evaluation.
A difference was considered to be significant, if the probability of error was below 5%.

II. RESULTS AND DISCUSSION

A. Mitotic and survival indices:

In experiments with or without S9 mix, the mitotic indices in the treated cultures when compared to the solvent control, were not reduced by 23%. The cultures treated with positive controls (mitomycin C without S9 mix and cyclophosphamide with S9 mix) also showed no reduction in mitosis rate.
In experiments without S9 mix, the survival indices in the treated cultures were relevantly reduced

compared to solvent controls at 400 µg/ml after 4 hours of treatment but no relevant reduction was observed after 18 hours of treatment. The cultures treated with mitomycin C showed no reduction in survival rate.
In experiments with S9 mix, the treated cultures showed a 24% reduction of the survival rate at 400 µg/ml when compared to solvent control. The positive control cyclophosphamide also reduced the survival rate.

B. Chromosome aberrations:

In experiments without S9 mix, no biologically relevant and statistically significant increases in the numbers of metaphases with aberrations were detected after 4 hours of treatment and total culture times of 18 or 30 hours. The same was true for a treatment period and a culture time of 18 hours. The treatment with the positive control mitomycin C resulted in a clear and statistically significant increase of metaphases with aberrations and demonstrated the sensitivity of the test system.

In experiments with S9 mix, no biologically relevant and statistically significant increase in numbers of metaphases with aberrations was detected after 4 hours of treatment and total culture times of 18 or 30 hours. The positive control cyclophosphamide induced statistically significant and biologically relevant increases in metaphases with aberrations and demonstrated the sensitivity of the test system and the activity of the used S9 mix.

III. CONCLUSION

None of the cultures treated with BYH 18636 up to 400 µg/ml in the absence or presence of S9 mix showed statistically significant or biologically relevant increases in numbers of metaphases with aberrations. The positive controls mitomycin C and cyclophosphamide induced clear clastogenic effects and demonstrated the sensitivity of the test system.

Based on the results of this test, BYH 18636 was considered not to be clastogenic for mammalian cells *in vitro*.

(Thum M., 2005)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable The study (T1074271) is fully compliant with OECD 473 (1997) The study was performed with test material of higher purity (96.4%) than the minimum currently proposed (950 g/kg) for the specification of the manufactured material. Only limited cytotoxicity (~25% reduction in the survival index) was seen at the highest concentration of the test material use in the initial assay, however the highest concentrations of the test material were limited by its solubility in the vehicle (40 mg/ml in DMSO). Appropriate positive control compounds confirmed the sensitivity of the assay. The test material was not found to induce structural or numerical chromosomal aberrations under the conditions of this study.
Conclusions	No evidence of clastogenicity or aneugenicity was seen under the conditions of this study.

Report: **KIIA 5.4.2/02, Thum M., 2007 (MRID # 47070144)**
Title: BYH 18636, *In vitro* chromosome aberration test with Chinese Hamster V79 cells

Report No &	AT03625
Document No	M-283558-01-2
Guidelines:	OECD 473 (1997); EEC Directive 2000/32/EC Method B10 (2000); EPA Health Effects Test Guideline (OPPTS 870.5375; 1998)
GLP	Yes (certified laboratory)

Executive summary:
Based on the specification required for production of the technical BYH 18636, a new study on the *in vitro* assessment of the clastogenic potential of BYH 18636 (batch GELL 420-151-1, 94.6% of purity) was performed. Chinese Hamster V79 cells were exposed to BYH 18636 at 100, 200 and 400 µg/ml, diluted in dimethyl sulphoxide (DMSO). For each dose level, duplicate cultures were used in both the presence and absence of an Aroclor 1254-induced rat liver metabolic activation system (S9 mix). DMSO was also used as a negative control. Mitomycin C, which produces crosslinks in the DNA, and cyclophosphamide, which induces chromosomal damage after metabolic activation, were used as positive controls, diluted in Hanks' balanced salt solution. After 4 hours treatment, the medium was changed and the cells were harvested 14 hours later. A second harvest was performed 30 hours after the start of the study. In a second experiment, the cells were exposed to BYH 18636 at 400 µg/plate or controls without S9 mix for 18 hours and harvested at the end of the treatment period. Colcemid was added to each flask two hours prior to harvest to arrest the cells in a metaphase-like stage of mitosis.

No cytotoxic effects were observed at 400 µg/plate without S9 mix after 4 hours and 18 hours treatment. Cytotoxic effects were observed at 200 µg/plate and above. Precipitation in the medium did not occur.

None of the cultures treated with BYH 18636 in the presence and in the absence of S9 mix showed biologically relevant or statistically significant increased numbers of aberrant metaphases.

The positive controls mitomycin C and cyclophosphamide induced clastogenic effects and demonstrated the sensitivity of the test system and the activity of the used S9 mix.

BYH 18636 was considered not to be clastogenic for mammalian cells *in vitro*.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636
- **Description:** fine, white powder
- **Lot/Batch:** GELL 420-151-1
- **Purity:** 94.6% (June 21, 2006)
- **CAS:** 317815-83-1
- **Stability of test compound:** Stable for the duration of the study

2. Control materials:
- **Negative:** Culture medium (Eagle's minimal essential medium)
- **Solvent:** DMSO for BYH 18636 and Hanks's balanced salt solution for positive controls (Seromed)
- **Positive:** Mitomycin C (Fluka, batch 1149026 10605077) without S9 mix at 0.1 µg/ml (4-gr treatment); 0.03 µg/ml (18-hrs treatment).
  Cyclophosphamide (Endoxan 100 mg injection vials of dry substance, Baxter Oncology GmbH) with S9 mix at 2 µg/ml.

3. Test organisms:
- **Cell line**: Chinese hamster V79 lung cells

Source: Cells obtained from Dr Utesch, Merk AG, Darmstadt in 1993, stored in the laboratory since then.

4. Test compound concentrations:

BYH 18636 was used in each trial at 100, 200 and 400 µg/ml (4 hrs -/+S9; 18 hrs –S9).

B. STUDY DESIGN AND METHODS:

The experimental phase of the study was performed from July 18 to November 16, 2006 at Bayer Healthcare AG (PH-GDD Toxicology).
The *in vitro* cytogenetic test is a mutagenicity test system for the detection of chromosome aberrations in cultured mammalian cells. The test is designed to detect structural aberrations (chromatid and chromosome aberrations) in cells at their first post-treatment mitosis.

1. Determination of cytotoxicity:

Duplicate cultures were exposed in a pre-test to BYH 18636 at concentrations ranging from 100 to 400 µg/ml with or without S9 mix for 4 hours. In addition, cells were exposed without S9 mix for 18 hours to concentrations ranging from 100 to 400 µg/ml. The mitotic index was determined for all cultures. The number of mitotic cells among a total of 1000 cells per culture was determined using a light microscope at a magnification of about 630. All cells which were not in interphase were defined as mitotic.
In the main study, cultures with a total incubation period of 8 hours were additionally and exclusively used to determine the cytotoxicity of BYH 18636 at concentrations ranging from 100 to 400 µg/ml.

Concentrations of up to 400 µg/ml BYH 18636 did not change the pH or the osmolality in the medium of the pre-test.

2. Treatment protocol:

Chinese hamster V79 cells were passaged on the day prior to treatment. Approximately $1x10^6$ cells were seeded in 20 ml of medium per 75 m^2 flasks and incubated at 37°C in a CO_2-incubator (5% CO_2). Unless reported otherwise, the cells were grown in Eagle's minimal essential medium containing 10% foetal calf serum. Immediately before treatment, the medium was removed from the cultures. For the trials without S9 mix, 20 ml of medium containing 2% foetal calf serum and 0.2 ml of test substance solution were added to each flask. For the trials with S9 mix, 19 ml of medium containing 2% foetal calf serum, 1 ml of S9 mix and 0.2 ml of test substance solution were added to each flask. The cells were incubated for 4 hours at 37°C. After 4 hours of treatment, the medium was removed, the cells were washed with PBS and 20 ml of fresh medium containing 10% foetal calf serum was added to the flasks. The flasks were placed in a CO_2-incubator for the remaining incubation time. In the cultures treated for 18 hours, the medium was not removed.

Colcemid-solution (0.2 ml of 40 µg/ml) was added to each flask 2 hours prior to the end of the incubation period to arrest the cells in a metaphase-like stage of mitosis(c-metaphase).

Positive controls and solvent controls (0.2 ml of solvent per culture) were set up in parallel and handled as described for BYH 18636-treated cultures. Untreated controls and solvent controls were used as negative controls.

3. Chromosome preparations:

After the removal of the medium from each flask, the cells were trypsinized, suspended in medium and centrifuged for approximately 5 minutes at 700 rpm. The supernatant was removed and 1 to 2 ml of a hypotonic solution (0.4% KCl; 37°C) was added to each tube. Within 4 minutes, the volume was brought to 6 ml with additional hypotonic solution and cells were resuspended. The cells were centrifuged again and fixed with cold (4°C) fixative (ethanol/acetic acid 3:1) for 20 minutes at room temperature. Cells were pelleted and resuspended in fixative as before and

centrifuged again. The pelleted cells were resuspended in a small volume of fixative and the suspension was dropped onto clean slides. The slides were allowed to dry for at least 2 hours. Thereafter, they were submerged in pure methanol for 3 minutes and stained for 15-20 minutes in 3% Giemsa solution. Slides were rinsed twice in water and once in acetone and were then kept in xylene for about 30 minutes. The slides were allowed to dry completely and covered. At least two slides were generated per culture.

4. Evaluation criteria:

Coded slides were evaluated using a light microscope at a magnification of about 1000. Chromosomes of approximately 200 metaphases per concentration, 100 metaphases from each of two parallel cultures, were examined. Only metaphases containing the modal chromosome number (22) were analyzed unless exchanges were detected. The following aberrations were recorded: gaps (an achromatic lesion within a chromatid arm without dislocation of the chromatid end), break (a discontinuity of one chromatid with dislocation of the chromatid end), fragment (part of chromosome without centromer), deletion (result of a break with the terminal chromatid part of the chromosome missing within the metaphase under assessment), exchange (exchange of chromatid-parts between different chromosomes or within the same chromosome), multiple aberration (when five or more structural changes occur within one metaphase). Observed polyploidy metaphases were recorded but not used for assessment.

5. Assessment criteria:

An assay was acceptable, if there was a biologically relevant increase in chromosome aberrations induced by positive controls and if the numbers of aberrations for the negative controls were in the expected range.

An increased incidence of gaps of both types without a concomitant increase of other aberration types was considered not to be an indication of a clastogenic effect.

A test was considered positive, if there was a relevant and statistically significant increase in the aberration rate.

A test was considered negative, if there was no such increase at any time interval or if there were statistically significant values, which were, however, within the range of historical negative controls.

A test was considered equivocal, if there was an increase above the range of historical negative controls which was statistically significant but not considered relevant, or if an increase occurred, which was considered relevant, but which was not statistically significant.

6. Statistics:

The statistical analysis was performed by pair-wise comparison of BYH 18636-treated and positive control groups to the respective solvent control group.

The mitotic index was statistically analyzed (provided that it was reduced compared to the mean of the corresponding solvent control) using the one-sided Chi^2-test.

The numbers of metaphases with aberrations (including and excluding gaps) and of metaphases with exchanges were compared (provided that these data superceded the respective solvent control). The one-side Chi^2-test was used for the statistical evaluation.

A difference was considered to be significant, if the probability of error was below 5%.

II. RESULTS AND DISCUSSION

A. Mitotic and survival indices:

In experiments with or without S9 mix, the mitotic indices in the treated cultures when compared to the solvent control, were not relevantly reduced. The cultures treated with mitomycin C without S9 mix also showed no reduction in mitosis rate. The cultures treated with cyclophosphamide and S9 mix showed reduction in mitosis rate.

In experiments without S9 mix, the survival indices in the treated cultures were not relevantly reduced. The cultures treated with mitomycin C showed a reduction in survival rate.

In experiments with S9 mix, the treated cultures showed a relevant reduction of the survival rate at 200

µg/ml and above. The positive control cyclophosphamide also reduced the survival rate.

B. Chromosome aberrations:

In experiments without S9 mix, no biologically relevant and statistically significant increases in the numbers of metaphases with aberrations were detected after 4 hours of treatment and total culture times of 18 or 30 hours. The same was true for a treatment period and a culture time of 18 hours. The treatment with the positive control mitomycin C resulted in a clear and statistically significant increase of metaphases with aberrations and demonstrated the sensitivity of the test system.

In experiments with S9 mix, no biologically relevant and statistically significant increase in numbers of metaphases with aberrations was detected after 4 hours of treatment and total culture times of 18 or 30 hours. The positive control cyclophosphamide induced statistically significant and biologically relevant increases in metaphases with aberrations and demonstrated the sensitivity of the test system and the activity of the used S9 mix.

III. CONCLUSION

None of the cultures treated with BYH 18636 up to 400 µg/ml in the presence or absence of S9 mix showed statistically significant or biologically relevant increases in numbers of metaphases with aberrations. The positive controls mitomycin C and cyclophosphamide induced clear clastogenic effects and demonstrated the sensitivity of the test system.

Based on the results of this test, BYH 18636 was considered not to be clastogenic for mammalian cells *in vitro*.

(Thum M., 2007)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable The study (T5076831) is fully compliant with OECD 473 (1997) The study is essentially a repeat of the previous study (Thum, 2005) using material of lower purity (94.6% compared to 96.3-96.4% in the previous study), and representative of the proposed specification. Only limited cytotoxicity (10-25% reduction in the survival index) was seen at the highest concentration of the test material use in the initial assay, however the highest concentrations of the test material were limited by its solubility in the vehicle (40 mg/ml in DMSO). Appropriate positive control compounds confirmed the sensitivity of the assay.
	The test material was not found to induce structural or numerical chromosomal aberrations.
Conclusions	No evidence of clastogenicity or aneugenicity was seen under the conditions of this study.

In vitro genotoxicity - Test for gene mutation in mammalian cells (IIA 5.4.3)

Report: **KIIA 5.4.3/01, Herbold B., 2005 (MRID # 47070141)**

Title: BYH 18636, V79/HPRT-test *in vitro* for the detection of induced forward mutations

Report No & Document No	AT02752 M-263744-01-2
Guidelines:	OECD 476 (1997); EEC Directive 2000/32/EC Method B17 (2000); EPA Health Effects Test Guideline (OPPTS 870.5300; 1998)
GLP	Yes (certified laboratory)

Executive summary:
The purpose of the study was to assess the point mutagenic potential of BYH 18636 (Mix batch 702-73-06-0001, 96.0 to 96.4 % of purity) at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in V79 cells.
For dose selection, a preliminary cytotoxicity test was conducted with and without an Aroclor 1254-induced rat liver metabolic activation system (S9 mix) using concentrations of BYH 18636 ranging from 25 to 600 µg/ml. Although BYH 18636 was tested up to and above the solubility of BYH 18636 in the vehicle (20 mg/ml corresponding to 200 µg/ml in the test culture), no cytotoxic effects were observed. BYH 18636 was tested in the first mutation experiment and the following independent repeats at concentrations ranging from 60 to 600 µg/ml (-/+S9).
Exponentially growing V79 cells were plated in 20 ml culture medium in two 75 cm^2 flasks. After attachment, cells were exposed with or without S9 mix to vehicle alone or to a range of concentrations of the test substance for 5 hours. Thereafter, each cell monolayer was washed with PBS, trypsinized and replated into 3 Petri dishes. Per culture one flask and 3 Petri dishes were used. These dishes were incubated for 6 to 8 days to allow colony development and to determine the cytotoxicity associated with each test substance directly after treatment (survival to treatment).
Cells ($3x10^5$ cells/ml) in 75 cm^2 flasks were incubated to permit growth and expression of induced mutations. Cells were subcultured (= count 1, normally after 3 days) by reseeding cells in 75 cm^2 flasks. At the end of the expression period (=count 2, normally a total of 6 days), cultures were reseeded in Petri dishes without hypoxanthine but containing 6-TG for selection of mutants. In addition, 200 cells per dish were seeded in 5 ml culture medium to determine the absolute cloning efficiency for each concentration. After incubation for 6 to 8 days, the colonies were fixed, stained with Giemsa and counted to determine the number of 6-TG resistant colonies in the mutation assay dishes and the number of colonies in the cloning efficiency dishes.

With and without S9 mix BYH 18636 induced no biologically relevant decreases in survival to treatment and no decreases in relative population growth were observed. Precipitation of BYH 18636 in the culture medium was not observed. With and without S9 mix there was no biologically relevant increase in mutant frequency above that of the vehicle controls. Ethyl methanesulfonate and dimethyl benzanthracene induced clear mutagenic effects demonstrating the sensitivity of the test system and the activity of the S9 mix.

Based on these results, BYH 18636 was considered to be non-mutagenic in the V79/HPRT forward mutation assay, both with and without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	BYH 18636
Description:	white powder
Lot/Batch:	MIX-batch 702-73-06-0001
Purity:	96.4% (December 6, 2004), 96.0% (May 19, 2005)
CAS:	317815-83-1
Stability of test compound:	Stable for the duration of the study
2. Control materials: Negative:	Culture medium [Eagle's minimal essential medium supplemented with 1% L-glutamine, 1% MEM-vitamins, 1% MEM NEAA, 1% penicillin/streptomycin and 10% foetal calf serum (FCS)]

Solvent: DMSO for BYH 18636 and Dimethylbenzanthracene not exceeding 1% (v/v) in the culture medium. No solvent needed for ethyl methanesulfonate as it is a liquid.

Positive: Ethyl methanesulfonate (EMS), a directly alkylating agent, used at a final concentration of 900 µg/ml in non-activation trials.
Dimethylbenzanthracene (DMBA), promutagen requiring a metabolic activation, used at a final concentration of 20 µg/ml for trials with S9 mix.

3. Test organisms:

Cell line: Chinese hamster V79 lung cells

Source: Cells obtained from Prof. G. Speit, University of Ulm, Germany. These cells have since been recloned to maintain karyotypic stability. They have a modal chromosome number of 22 and a rapid population doubling time (10 to 14 hours)

Culture condition: Incubation performed at 37°C in a humidified atmosphere with about 5% CO_2.

4. Test compound concentrations:

BYH 18636 was used at 25, 50, 100, 200, 400 and 600 µg/ml -/+ in the clonal cytotoxicity assay and at 60, 120, 240, 360, 480 and 600 µg/ml -/+ in the mutagenic assays (2 Trials –S9; 3 Trials +S9).

5. Metabolic activation:

The S9 fraction was isolated from the livers of Aroclor 1254 induced male Sprague Dawley rats. The preparation dated from April 6, 2004 (protein content from 22.1 to 22.2 mg/ml) and was kept frozen at -80°C. The batch was tested for contamination and cytotoxicity prior to use in the first study. Cofactors were freshly dissolved in sodium phosphate buffer (150 mM, pH 7.4)

B. STUDY DESIGN AND METHODS:

The experimental phase of the study was performed April 25 to July 11, 2005 at Bayer Healthcare AG (PH-R&D Toxicology International).

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

1. Determination of cytotoxicity:

Exponentially growing V79 cells were plated in 20 ml culture medium in a 75 cm^2 flask with a total volume of 275 ml ($4x10^6$ cells per flasks). For each concentration, one culture was available. After attachment (16 to 24 hours later), cells were exposed without S9 mix to vehicle alone or to a range of concentrations of the test substance for 5 hours in 20 ml medium containing 2% FCS. In experiments with metabolic activation 1 ml of medium was replaced by 1 ml of S9 mix. Thereafter, each cell monolayer was washed with PBS, trypsinized and replated in 5 ml culture medium at a density of 200 cells into 3 Petri dishes (diameter of □60 mm). These dishes were incubated for 6 to 8 days to allow colony development. Thereafter, colonies were fixed with 95% methanol, stained with Giemsa (Merk; stock solution diluted 1:5 with deionized water) and counted automatically using an Artek counter,

when there was no interference by precipitation on the plates or colouration of the plates. Cytotoxicity was expressed by comparison of colonies in treated cultures versus vehicle control cultures (relative cloning efficiency).

2. Treatment protocol without metabolic activation:

Exponentially growing V79 cells were plated in 20 ml culture medium in two 75 cm^2 flasks per concentration ($4x10^6$ cells per flask) including all control groups. After attachment (16 to 24 hours later), cells were exposed to vehicle alone or to a range of concentrations of the test substance for 5 hours in 20 ml culture medium with reduced serum content (2%). Thereafter, cell monolayers were washed with PBS, trypsinized and replated in 20 ml culture medium using $1.5x10^6$ cells per 75 cm^2 flask and in 5 ml culture medium using 200 cells per Petri dish (diameter of 60 mm). One flask and 3 Petri dishes were used per culture. These dishes were incubated for 6 to 8 days to allow colony development and to determine the cytotoxicity associated with each test substance directly after treatment (survival to treatment).

Cells in 75 cm^2 flasks were incubated to permit growth and expression of induced mutations. Cells were subcultured (= count 1, normally after 3 days) by reseeding $1.5x10^6$ cells into 20 ml of medium in 75 cm^2 flasks. At the end of the expression period (=count 2, normally a total of 6 days), cultures were reseeded in Petri dishes (diameter of 100 mm) at $3x10^5$ cells per dish (8 dishes per culture) in 20 ml culture medium without hypoxanthine but containing 10 µg/ml 6-TG for selection of mutants. In addition, 200 cells per dish (diameter of 60 mm, 3 dishes per culture) were seeded in 5 ml culture medium to determine the absolute cloning efficiency for each concentration. After incubation for 6 to 8 days, the colonies were fixed, stained with Giemsa and counted to determine the number of 6-TG resistant colonies in the mutation assay dishes and the number of colonies in the cloning efficiency dishes.

Two trials were performed.

3. Treatment protocol with metabolic activation:

The activation assay was performed independantly. The procedure was identical to the non-activation assay except for the addition of S9 mix. In these experiments 19 ml instead of 20 ml culture medium and additonally 1 ml of S9 mix were added to the flasks for the treatment period, resulting in a concentration of 5% S9 mix in the cultures. The number of 6-TG resistant mutants and viability were determined as in the non-activation assay.

Three trials were performed.

4. Parameters assessed:

The parameter "survival to treatment" in % was determined on the basis of the following calculation:

$$\frac{\text{Mean number of colonies (treated cultures) x 100}}{\text{Mean number of colonies (vehicle control cultures)}}$$

The "absolute population growth" was calculated using the following formula:

Absolute population growth (for each culture) = cell count 1 x cell count 2

The parameter "relative population growth" shows the cumulative growth of the treated cell populations, relative to the vehicle control.

$$\frac{\text{Absolute population growth treated culture x 100}}{\text{Absolute population growth of corresponding vehicle control culture}}$$

The ability of cells to form colonies at the time of mutant selection is measured by the parameter "absolute cloning efficiency". It is expressed in %.

$$\frac{\text{Mean number of colonies per dish} \times 100}{200}$$

- The "mutant frequency" is calculated for each group by dividing the total number of mutant colonies by the number of cells seeded (usually 8-10 plates at $3x10^5$ cells per plate), corrected for the absolute cloning efficiency. The mutant frequency is expressed as 6-TG resistant mutants par 10^6 clonable cells.

$$\frac{\text{Total number of mutant colonies} \times 100}{\text{Number of evaluated dishes} \times 3 \times 10^5 \times \text{C.E.}}$$

5. Acceptance criteria:

- The average cloning efficiency of the negative and vehicle controls should be at least 50%.
- The average of mutant frequency of the vehicle controls should not exceed 25×10^{-6} cells.
- The mutant frequency of the two cultures of the vehicle and /or the negative control should differ only to an acceptable extent. As a rule of thumb, the difference of mutant frequencies should not be greater than 5×10^{-6}.
- The positive control should induce an average mutant frequency of at least three times that of the vehicle control.
- If not limited by the solubility of the test substance in the vehicle the highest concentration should induce cytotoxicity of about 80 to 90% or should be a concentration where precipitation occurs in the medium. The survival at the lowest concentration should be in the range of the negative control.
- For the calculation of an acceptable mutant frequency at least 5 dishes per culture should be available and relative survival to treatment, relative population growth and absolute cloning efficiency should be 10% or greater.

However, these criteria may be overruled by good scientific judgement.

6. Assessment criteria:

- Mutant frequencies was only used for assessment, if at least 5 dishes per culture were available and relative survival to treatment, relative population growth and absolute cloning efficiency were 10% or greater.
- A trial was considered positive if a concentration-related and in parallel cultures reproducible increase in mutant frequencies was observed. To be relevant, the increase in mutant frequencies should be at least two to three times that of the highest negative or vehicle control value observed in the respective trial. If this result was repoduced in a second trial, the test substance was considered to be mutagenic.
- Despite these criteria, a positive result was only considered relevant, if no significant change in osmolality compared to the vehicle control was observed. Otherwise, unphysiological culture conditions may be the reason for the positive result.
- A test substance was judged as equivocal if there was no strictly concentration related increase in mutation frequencies but if one or more concentrations induced a reproducible and biologically relevant increase in mutant frequencies in all trials.
- An assay was considered negative if no reproducible and relevant increases of mutant frequencies were observed.

However, these criteria may be overruled by good scientific judgement.

7. Statistical analysis:

The statistical analysis relied on the mutant frequencies which were submitted to a weighted analysis of variance as well as to a weighted recursive regression, both with Poisson derived weights.

The two mutant frequency values obtained per group were, although somewhat related, considered as independent measurements thus increasing the power of the statistical tests applied. Since the protocol of the HPRT assay required at least two independent trials, the overall analysis without respectively with activation was the most important one for classifying substances into mutagens and non-mutagens. However, separate analyses were run for each trial in order to examine the consistency of the results.

All acceptable groups were included in the weighted analysis of variance followed by pairwise comparisons to the vehicle control on a nominal significance level of $\alpha = 0.05$ using the Dunnett test. The regression analysis part was performed on the basis of the actual concentrations thereby omitting the positive, negative and vehicle controls. If there was a significant concentration related increase of the mutant frequency ($\alpha = 0.05$) in the main analysis the highest concentration was dropped and the analysis repeated. This procedure was repeated until $p > 0.05$. In that way eliminated concentrations were flagged correspondingly.

II. RESULTS AND DISCUSSION

1. General remarks:

In the absence and in the presence of S9 mix Chinese hamster V79 cells were exposed to BYH 18636 at concentrations of up to and including 600 µg/ml. With and without S9 mix neither biologically relevant cytotoxicity nor substance precipitation in the medium were observed. However, at 240 µg/ml and above, the formulation in the vehicle was a white suspension.

The means of the absolute cloning efficiency for the vehicle controls in the mutation experiments were 82.9% and 84.7% in the experiments without activation, and 72.5%, 84.0% and 97.9% in the experiemnts with activation.These results demonstrated good cloning conditions for the experiments.

2. Mutation assay without metabolic activation:

Two trials were performed. The mutant frequencies of the negative controls (means of 1.3×10^{-6} and 2.6×10^{-6}, respectively) and of the vehicle controls (means of 0.75×10^{-6} and 3×10^{-6}, respectively) were all within the normal range. The positive control EMS induced clear mutagenic and statistically significant effects in all trials (means of 236.7×10^{-6} and 630.1×10^{-6}, respectively).

For BYH 18636 treated cultures concentration-related decreases were observed neither in relative survival to treatment nor in relative population growth. Relevant BYH 18636 induced increases in mutant frequencies could not be found (ranging from 0.5×10^{-6} to 1.6×10^{-6} in the first trial, and from 2.4×10^{-6} to 8.8×10^{-6} in the second trial). In addition, the overall statistical analysis revealed no statistically significant increases. Therefore, BYH 18636 was evaluated as non-mutagenic in the non activation trial.

3. Mutation assay with metabolic activation:

Three trials were performed with S9 mix. The mutant frequencies of the negative controls (means of 7.5×10^{-6} , 7.1×10^{-6} and 2.8×10^{-6}, respectively) and of the vehicle controls (means of 7.9×10^{-6} , 5.2×10^{-6} and 2.4×10^{-6}, respectively) were all within the normal range. The positive control DMBA induced clear mutagenic and statistically significant effects in all trials (means of 33.4×10^{-6} , 31.3×10^{-6} and 60.0×10^{-6}, respectively).

The BYH 18636 treated cultures showed no concentration-related decreases neither in relative survival to treatment nor in relative population growth. Relevant BYH 18636 induced increases in mutant frequencies could not be found (ranging from 7.1×10^{-6} to 24.3×10^{-6} in the first trial, from 2.6×10^{-6}

to 12.7 x 10^{-6} in the second trial and from 0.5 x 10^{-6} to 4.5 x 10^{-6} in the third trial). In addition, the overall statistical analysis revealed no statistically significant increases. Therefore, BYH 18636 was evaluated as non-mutagenic in the activation trial.

III. CONCLUSION

No cytotoxic effects were induced by BYH 18636 tested in the V79/HPRT- test in concentrations ranging from 60 to 600 µg/ml with and without S9. No precipitation of BYH 18636 in the medium was observed. However, BYH 18636 was tested up to and over its limit of solubility in the vehicle. BYH 18636 induced no biologically relevant increases in mutant frequencies.

The positive controls EMS and DMBA had a marked mutagenic effect.

Based on these results, BYH 18636 was considered not to be mutagenic in the V79/HPRT forward mutation assay.

(Herbold B., 2005)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Reliable with restrictions The study (T1073263) was performed broadly in compliance with OECD 476 (1997), but see the detailed comments below. The study was performed with test material of higher purity (96.4%) than the minimum currently proposed (950 g/kg) for the specification of the manufactured material. PSD has general concerns regarding the sensitivity of the HPRT assay following advice from the UK independent advisory committee, the COM (Committee on Mutagenicity). The COM advises that the mouse lymphoma (TK) assay is the preferred mammalian cell mutation assay. This preference is due to the ability of the mouse lymphoma assay to detect clastogenic endpoints in addition to mutagenic endpoints, and also for reasons of statistical power. OECD guideline 476 (1997) states that the number of cells used should be at least ten times the inverse of the spontaneous mutation frequency. This is considered to present practical difficulties with regard to the use of surface cultures of cells used in the HPRT assay compared to the suspension cultures used in the mouse lymphoma assay. However it is considerer that the number of cells used for each replicate in this assay ($4x10^6$) is adequate, based on the laboratory's background range. No evidence of cytotoxicity was seen in this study: the highest tested concentration of thiencarbazone-methyl was limited by solubility in the vehicle. No evidence of mutagenicity was seen in the absence of metabolic activation in Experiment 1. In the presence of metabolic activation, slight (but not statistically significant) increases in mutation frequency were seen in some replicates at a number of concentrations, but with no apparent relationship to concentration. Findings do not meet the laboratory's criteria for a positive response, however the sensitivity of the experiment has to be questioned given the magnitude of response for the positive control.

No evidence of mutagenicity was seen in the absence of metabolic activation in Experiment 2. Mutation frequencies in the presence of metabolic activation were comparable at all concentrations and were less variable than in Experiment 1, however the magnitude of response seen with the positive control was again low (and comparable to those in Experiment 1).

Mutation frequencies in the presence of metabolic activation in Experiment 3 were comparable at all concentrations and were lower than seen in the previous two Experiments. The response to the positive control was also of greater magnitude in this Experiment.

Summary of results in the mammalian cell mutagenicity assay

[µg/ml]	Mutation frequency ($x10^{-6}$ cells)				
	Experiment 1		Experiment 2		Experiment 3
	-S9	+S9	-S9	+S9	+S9
0	0.5	7.6	3.7	5.8	3.4
	2.1	7.4	1.4	8.4	2.2
DMSO	0.5	8.0	1.5	3.9	2.4
	1.0	7.8	4.5	6.7	2.4
60	0.6	8.9	6.1	5.6	1.3
	0.5	10.6	5.5	7.6	2.9
120	0.5	19.3	2.0	8.8	1.1
	0.5	9.5	3.0	8.4	0.6
240	0.5	11.2	4.9	2.6	4.5
	0.5	24.3	5.2	6.7	1.3
360	0.6	16.1	4.5	10.4	2.8
	1.1	17.1	3.8	4.5	1.7
480	0.5	7.1	8.8	7.5	0.5
	1.0	9.5	3.8	4.1	1.5
600	0.5	21.7	2.4	12.7	1.0
	1.6	14.3	-	4.1	0.7
EMS 900	256.6	-	617.1	-	-
	216.8	-	643.1	-	-
DMBA 20	-	36.0	-	27.2	62.4
	-	30.7	-	35.3	57.6

Conclusions: No evidence of mutagenicity was seen under the conditions of this study, however there are some concerns about the performance of the positive control compound (in the presence of metabolic activation) in two of the three experiments.

Report: **KIIA 5.4.3/02, Herbold B., 2007 (MRID # 47070140)**
Title: BYH 18636, V79/HPRT-test *in vitro* for the detection of induced forward mutations
Report No & Document No: AT03686 / M-284851-01-2
Guidelines: OECD 476 (1997); EEC Directive 2000/32/EC Method B17 (2000); EPA Health Effects Test Guideline (OPPTS 870.5300; 1998)
GLP Yes (certified laboratory)

Executive summary:
Based on the specification required for production of the technical BYH 18636, a new study to assess the point mutagenic potential of BYH 18636 (GELL 420-151-1, 94.6 % of purity) at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in V79 cells was performed.
Dose selection was based on the results from a preliminary cytotoxicity test conducted in the previous study (M-263744-01-2), where concentrations of BYH 18636 ranging from 25 to 600 µg/ml were used with and without an Aroclor 1254-induced rat liver metabolic activation system (S9 mix). Although

BYH 18636 was tested up to and above the solubility of BYH 18636 in the vehicle (20 mg/ml corresponding to 200 µg/ml in the test culture), no cytotoxic effects were observed. BYH 18636 was tested in the first mutation experiment and the following independent repeats at concentrations ranging from 30 to 600 µg/ml -/+S9.
Exponentially growing V79 cells were plated in 20 ml culture medium in two 75 cm^2 flasks. After attachment, cells were exposed with or without S9 mix to vehicle alone or to a range of concentrations of the test substance for 5 hours. Thereafter, each cell monolayer was washed with PBS, trypsinized and replated into 3 Petri dishes. Per culture one flask and 3 Petri dishes were used. These dishes were incubated for 6 to 8 days to allow colony development and to determine the cytotoxicity associated with each test substance directly after treatment (survival to treatment).
Cells ($3x10^5$ cells/ml) in 75 cm^2 flasks were incubated to permit growth and expression of induced mutations. Cells were subcultured (= count 1, normally after 3 days) by reseeding cells in 75 cm^2 flasks. At the end of the expression period (=count 2, normally a total of 6 days), cultures were reseeded in Petri dishes without hypoxanthine but containing 6-TG for selection of mutants. In addition, 200 cells per dish were seeded in 5 ml culture medium to determine the absolute cloning efficiency for each concentration. After incubation for 6 to 8 days, the colonies were fixed, stained with Giemsa and counted to determine the number of 6-TG resistant colonies in the mutation assay dishes and the number of colonies in the cloning efficiency dishes.

With and without S9 mix BYH 18636 induced no biologically relevant decreases in survival to treatment and no decreases in relative population growth were observed. Precipitation of BYH 18636 in the culture medium was not observed. However, BYH 18636 was tested up to its limit of solubility in the vehicle. With and without S9 mix there was no biologically relevant increase in mutant frequency above that of the vehicle controls. Ethyl methanesulfonate and dimethyl benzanthracene induced clear mutagenic effects demonstrating the sensitivity of the test system and the activity of the S9 mix.

Based on these results, BYH 18636 was considered to be non-mutagenic in the V79/HPRT forward mutation assay, both with and without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636
Description: white crystalline powder
Lot/Batch: GELL 420-151-1
Purity: 94.6%
CAS: 317815-83-1
Stability of test compound: Stable for at least 24 hours from 0.01 to 60 mg/ml at room temperature

2. Control materials:
Negative: Culture medium [Eagle's minimal essential medium supplemented with 1% L-glutamine, 1% MEM-vitamins, 1% MEM NEAA, 1% penicillin/streptomycin and 10% foetal calf serum (FCS)]
Solvent: DMSO for BYH 18636 and Dimethylbenzanthracene not exceeding 1% (v/v) in the culture medium. No solvent needed for ethyl methanesulfonate as it is a liquid.
Positive: Ethyl methanesulfonate (EMS), a directly alkylating agent, used at a final concentration of 900 µg/ml in non-activation trials.
Dimethylbenzanthracene (DMBA), promutagen requiring a metabolic activation, used at a final concentration of 20 µg/ml for trials with S9 mix.

3. Test organisms :

Cell line: Chinese hamster V79 lung cells

Source: Cells obtained from Prof. G. Speit, University of Ulm, Germany. These cells have since been recloned to maintain karyotypic stability. They have a modal chromosome number of 22 and a rapid population doubling time (10 to 14 hours)

Culture condition: Incubation performed at 37°C in a humidified atmosphere with about 5% CO_2.

4. Test compound concentrations:

BYH 18636 was used at 30, 60, 120, 240, 360, 480 and 600 µg/ml -/+ in the mutagenic assays (2 Trials).

5. Metabolic activation:

The S9 fraction was isolated from the livers of Aroclor 1254 induced male Sprague Dawley rats. The preparation dated from September 13, 2005 (protein content from 24.0 mg/ml) and was kept frozen at -80°C. The batch was tested for contamination and cytotoxicity prior to use in the first study. Cofactors were freshly dissolved in sodium phosphate buffer (150 mM, pH 7.4)

B. STUDY DESIGN AND METHODS:

The experimental phase of the study was performed from August 1 to October 10, 2006 at Bayer Healthcare AG (PH-GDD Toxicology).

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

1. Determination of cytotoxicity:

Exponentially growing V79 cells were plated in 20 ml culture medium in a 75 cm^2 flask with a total volume of 275 ml ($4x10^6$ cells per flasks). For each concentration, one culture was available. After attachment (16 to 24 hours later), cells were exposed without S9 mix to vehicle alone or to a range of concentrations of the test substance for 5 hours in 20 ml medium containing 2% FCS. In experiments with metabolic activation 1 ml of medium was replaced by 1 ml of S9 mix. Thereafter, each cell monolayer was washed with PBS, trypsinized and replated in 5 ml culture medium at a density of 200 cells into 3 Petri dishes (diameter of 60 mm). These dishes were incubated for 6 to 8 days to allow colony development. Thereafter, colonies were fixed with 95% methanol, stained with Giemsa (Merk; stock solution diluted 1:5 with deionized water) and counted automatically using an Artek counter, when there was no interference by precipitation on the plates or colouration of the plates. Cytotoxicity was expressed by comparison of colonies in treated cultures versus vehicle control cultures (relative cloning efficiency).

2. Treatment protocol without metabolic activation:

Exponentially growing V79 cells were plated in 20 ml culture medium in two 75 cm^2 flasks per concentration ($4x10^6$ cells per flask) including all control groups. After attachment (16 to 24 hours later), cells were exposed to vehicle alone or to a range of concentrations of the test substance for 5 hours in 20 ml culture medium with reduced serum content (2%). Thereafter, cell monolayers were washed with PBS, trypsinized and replated in 20 ml culture medium using $1.5x10^6$ cells per 75 cm^2

flask and in 5 ml culture medium using 200 cells per Petri dish (diameter of 60 mm). One flask and 3 Petri dishes were used per culture. These dishes were incubated for 6 to 8 days to allow colony development and to determine the cytotoxicity associated with each test substance directly after treatment (survival to treatment).

Cells in 75 cm^2 flasks were incubated to permit growth and expression of induced mutations. Cells were subcultured (= count 1, normally after 3 days) by reseeding $1.5x10^6$ cells into 20 ml of medium in 75 cm^2 flasks. At the end of the expression period (=count 2, normally a total of 6 days), cultures were reseeded in Petri dishes (diameter of 100 mm) at $3x10^5$ cells per dish (8 dishes per culture) in 20 ml culture medium without hypoxanthine but containing 10 µg/ml 6-TG for selection of mutants. In addition, 200 cells per dish (diameter of 60 mm, 3 dishes per culture) were seeded in 5 ml culture medium to determine the absolute cloning efficiency for each concentration. After incubation for 6 to 8 days, the colonies were fixed, stained with Giemsa and counted to determine the number of 6-TG resistant colonies in the mutation assay dishes and the number of colonies in the cloning efficiency dishes.

Two trials were performed.

3. Treatment protocol with metabolic activation:

The activation assay was performed independantly. The procedure was identical to the non-activation assay except for the addition of S9 mix. In these experiments 19 ml instead of 20 ml culture medium and additonally 1 ml of S9 mix were added to the flasks for the treatment period, resulting in a concentration of 5% S9 mix in the cultures. The number of 6-TG resistant mutants and viability were determined as in the non-activation assay.

Two trials were performed.

4. Parameters assessed:

The parameter "survival to treatment" in % was determined on the basis of the following calculation :

$$\frac{\text{Mean number of colonies (treated cultures) x 100}}{\text{Mean number of colonies (vehicle control cultures)}}$$

The "absolute population growth" was calculated using the following formula:

Absolute population growth (for each culture) = cell count 1 x cell count 2

The parameter "relative population growth" shows the cumulative growth of the treated cell populations, relative to the vehicle control.

$$\frac{\text{Absolute population growth treated culture x 100}}{\text{Absolute population growth of corresponding vehicle control culture}}$$

The ability of cells to form colonies at the time of mutant selection is measured by the parameter "absolute cloning efficiency". It is expressed in %.

$$\frac{\text{Mean number of colonies per dish x 100}}{200}$$

The "mutant frequency" is calculated for each group by dividing the total number of mutant colonies by the number of cells seeded (usually 8-10 plates at $3x10^5$ cells per plate), corrected for the absolute

cloning efficiency. The mutant frequency is expressed as 6-TG resistant mutants par 10^6 clonable cells.

$$\frac{\text{Total number of mutant colonies x 100}}{\text{Number of evaluated dishes x 3 x } 10^5 \text{ x C.E.}}$$

5. Acceptance criteria:

- The average cloning efficiency of the negative and vehicle controls should be at least 50%.
- The average of mutant frequency of the vehicle controls should not exceed 25 x 10^{-6} cells.
- The mutant frequency of the two cultures of the vehicle and /or the negative control should differ only to an acceptable extend. As a rule of thumb, the difference of mutant frequencies should not be greater than 5 x 10^{-6}.
- The positive control should induce an average mutant frequency of at least three times that of the vehicle control.
- If not limited by the solubility of the test substance in the vehicle the highest concentration should induce cytotoxicity of about 80 to 90% or should be a concentration where precipitation occurs in the medium. The survival at the lowest concentration should be in the range of the negative control.
- For the calculation of an acceptable mutant frequency at least 5 dishes per culture should be available and relative survival to treatment, relative population growth and absolute cloning efficiency should be 10% or greater.

However, these criteria may be overruled by good scientific judgement.

6. Assessment criteria:

- Mutant frequencies was only used for assessment, if at least 5 dishes per culture were available and relative survival to treatment, relative population growth and absolute cloning efficiency were 10% or greater.
- A trial was considered positive if a concentration-related and in parallel cultures reproducible increase in mutant frequencies was observed. To be relevant, the increase in mutant frequencies should be at least two to three times that of the highest negative or vehicle control value observed in the respective trial. If this result was repoduced in a second trial, the test substance was considered to be mutagenic.
- Despite these criteria, a positive result was only considered relevant, if no significant change in osmolality compared to the vehicle control was observed. Otherwise, unphysiological culture conditions may be the reason for the positive result.
- A test substance was judged as equivocal if there was no strictly concentration related increase in mutation frequencies but if one or more concentrations induced a reproducible and biologically relevant increase in mutant frequencies in all trials.
- An assay was considered negative if no reproducible and relevant increases of mutant frequencies were observed.

However, these criteria may be overruled by good scientific judgement.

7. Statistical analysis:

The statistical analysis relied on the mutant frequencies which were submitted to a weighted analysis of variance as well as to a weighted recursive regression, both with Poisson derived weights.

The two mutant frequency values obtained per group were, although somewhat related, considered as independent measurements thus increasing the power of the statistical tests applied. Since the protocol of the HPRT assay required at least two independent trials, the overall analysis without respectively

with activation was the most important one for classifying substances into mutagens and non-mutagens. However, separate analyses were run for each trial in order to examine the consistency of the results.

All acceptable groups were included in the weighted analysis of variance followed by pairwise comparisons to the vehicle control on a nominal significance level of $\alpha = 0.05$ using the Dunnett test. The regression analysis part was performed on the basis of the actual concentrations thereby omitting the positive, negative and vehicle controls. If there was a significant concentration related increase of the mutant frequency ($\alpha = 0.05$) in the main analysis the highest concentration was dropped and the analysis repeated. This procedure was repeated until $p > 0.05$. In that way eliminated concentrations were flagged correspondingly.

II. RESULTS AND DISCUSSION

1. General remarks:

In the absence and in the presence of S9 mix Chinese hamster V79 cells were exposed to BYH 18636 at concentrations of up to and including 600 µg/ml. With and without S9 mix no substance precipitation occurred in the medium.
The means of the absolute cloning efficiency for the vehicle controls in the mutation experiments were 64.7% and 76.5% in the experiments without activation, and 62.7% and 72.9% in the experiments with activation. These results demonstrated good cloning conditions for the experiments.

2. Mutation assay without metabolic activation:

Two trials were performed. The mutant frequencies of the negative controls (means of 3.7×10^{-6} and 5.8×10^{-6}, respectively) and of the vehicle controls (means of 2.2×10^{-6} and 10.7×10^{-6}, respectively) were all within the normal range. The positive control EMS induced clear mutagenic and statistically significant effects in all trials (means of 571.4×10^{-6} and 879.7×10^{-6}, respectively).

For BYH 18636 treated cultures no cytotoxic effects of 80 to 90% were induced. However, BYH 18636 was tested up to its limits of solubility in the vehicle. Relevant BYH 18636 induced increases in mutant frequencies could not be found (ranging from 0.0×10^{-6} to 5.2×10^{-6} in the first trial, and from 2.6×10^{-6} to 15.7×10^{-6} in the second trial). In addition, the overall statistical analysis revealed no statistically significant increases. Therefore, BYH 18636 was evaluated as non-mutagenic in the non-activation trial.

3. Mutation assay with metabolic activation:

Two trials were performed with S9 mix. The mutant frequencies of the negative controls (means of 0.8×10^{-6} and 3.2×10^{-6}, respectively) and of the vehicle controls (means of 1.3×10^{-6} and 5.2×10^{-6}, respectively) were all within the normal range. The positive control DMBA induced clear mutagenic and statistically significant effects in all trials (means of 105.9×10^{-6} and 56.5×10^{-6}, respectively).

For BYH 18636 treated cultures no cytotoxic effects of 80 to 90% were induced. However, BYH 18636 were tested up to its limit of solubility in the vehicle. Relevant BYH 18636 induced increases in mutant frequencies could not be found (ranging from 0.0×10^{-6} to 4.0×10^{-6} in the first trial and from 1.2×10^{-6} to 6.3×10^{-6} in the second trial). In addition, the overall statistical analysis revealed no statistically significant increases. Therefore, BYH 18636 was evaluated as non-mutagenic in the activation trial.

III. CONCLUSION

No cytotoxic effects of 80 to 90% were induced by BYH 18636 tested in the V79/HPRT- test in concentrations ranging from 30 to 600 µg/ml with and without S9. However, BYH 18636 was tested up to its limit of solubility in the vehicle. BYH 18636 induced no biologically relevant increases in mutant frequencies.
The positive controls EMS and DMBA had a marked mutagenic effect.
Based on these results, BYH 18636 was considered not to be mutagenic in the V79/HPRT forward mutation assay.

(Herbold B., 2007)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable This study (T6076832) is essentially a repeat of the previous study (Herbold, 2005) but performed with test material of lower purity (94.6% compared to 96.0-96.4% used in the previous assay), and representative of the proposed specification. The study is fully compliant with OECD 476 (1997): see comments regarding the general sensitivity of HPRT assays in the previous study (Herbold, 2005). The number of cells used for each replicate in this assay ($4x10^6$) is considered to be adequate, based on the laboratory's background range of mutation frequency. No evidence of cytotoxicity was seen in this study: the highest tested concentration of thiencarbazone-methyl was limited by solubility in the vehicle. Mutation frequencies were unaffected by treatment with thiencarbazone-methyl and were less variable than seen in the previous study. The performance of the positive control compound in the presence of metabolic activation was satisfactory in both experiments.
Conclusions	No evidence of mutagenicity was seen under the conditions of this study.

B.6.4.2 Genotoxicity *in vivo* (IIA 5.4.4, IIA 5.4.5, IIA 5.4.6)

In vivo genotoxicity testing (somatic cells) (metaphase analysis in rodent bone marrow, or micronucleus test in rodent (IIA 5.4.4)

Report:	**KIIA 5.4.4/01, Herbold B.; 2004 (MRID # 47070214)**
Title:	BYH 18636, Micronucleus test on the male mouse
Report No &	AT01568
Document No	M-123179-01-2
Guidelines:	OECD 474 (1997); EEC Directive 2000/32/EC Method B12 (2000); EPA Health Effects Test Guideline (OPPTS 870.5395; 1998)
GLP	Yes (certified laboratory)

Executive summary:
The selection of BYH 18636 doses was based on a pilot study, where groups of 3 males and 3 females received two intraperitoneal injections separated by 24 hours. A dose of 1000 mg/kg of BYH 18636 was used. Clinical signs were recorded for up to at least 24 hours after the second injection and 1 of 3 animals of each sex died. Based on these results, as no substantial differences between sexes in toxicity were observed, males only were used in this study and 500 mg/kg of BYH 18636 was chosen as MTD. The dose-range of BYH 18636 tested in the study was 125, 250 and 500 mg/kg.

Each group comprised 5 male mice. The negative control group was treated with two intraperitoneal injections of 0.5% aqueous Cremophor emulsion and the positive control group received only one intraperitoneal injection of cyclophosphamide at 20 mg/kg. BYH 18636 treated groups also received two intraperitoneal injections separated by 24 hours. Five additional animals were treated at 500 mg/kg of BYH 18636 in case of mortality in the initial group or need of replacement of slides due to

pathological altered ratio of polychromatic to normochromatic erythrocytes.

Bone marrow from at least one femur from each animal was sampled 24 hours after the last intraperitoneal injection. Slides of bone marrow cells were prepared and scored for the occurrence of micronucleated polychromatic erythrocytes (micronucleated PCEs), of micronucleated normochromatic erythrocytes and PCE/total erythrocytes ratios. 2000 polychromatic erythrocytes were counted per animal.
Three of 10 treated mice died during the test period, due to the acute intraperitoneal toxicity of 500 mg/kg of BYH 18636. Animals treated at 125, 250 and 500 mg/kg showed the following symptoms until their sacrifice: apathy, loss of weight, spasm, periodically stretching of body, difficulty in breathing and slitted eyes. There were no statistically significant changes in mean PCE/total erythrocyte ratio observed for any of the BYH 18636 treated groups or the control group.

There were no biologically significant variations in the incidence of micronucleated polychromatic erythrocytes between the control and the BYH 18636 treated groups. The statistical significance observed at 500 mg/kg was considered to be of no biological relevance, since the absolute value of 4.2/2000 was clearly within the range of the historical negative controls. No relevant variations were observed between the negative control and BYH 18636 treated groups in the number of micronucleated normochromatic erythrocytes.

The positive control, cyclophosphamide, caused a clear increase in the number of polychromatic erythrocytes with micronuclei but did not alter the ratio of polychromatic to normochromatic erythrocytes.

In conclusion, there was no indication of a clastogenic effect of intraperitoneally administered BYH 18636 in the micronucleus test on the male mouse.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636
Description: Fine white powder
Lot/Batch: MIX-batch 702-73-06-0001
Purity: 96.3%
CAS: 317815-83-1
Stability of test compound: Stable for the duration of the study

2. Control materials:
Negative: None
Solvent: 0.5% aqueous Cremophor emulsion
Positive: cyclophosphamide used in form of Endoxan 100 mg injection vials of dry substance (Baxter Oncology GmbH)

3. Test animals:
Species: Mouse
Strain: Hsd/Win: NMRI
Age: 6 to 12 weeks approximately
Weight at dosing: 37 to 44 g (males only)
Source: Harlan/Winkelmann GmbH, 33178 Borchen, Germany
Number of animals per dose: Range-finding test: 3 animals/sex
Micronucleus assay: 5 males/group
Animal husbandry: The animals were properly maintained.

4. Test compound concentrations: **Range-finding test:** 2 intraperitoneal injections of 1000 mg/kg separated by 24 hours
Micronucleus assay: 0, 125, 250 and 500 mg/kg

B. TEST PERFORMANCE

Bayer Health Care AG (PH-PD P Health Care Toxicology) conducted the study during the period July 20 to September 09, 2004.

1. Treatment and sampling times

Sampling took place 24 hours after the last intraperitoneal injection; the positive control was sampled at 24 hours after the only one intraperitoneal injection.

2. Tissues and cells examined

Bone marrow; 2 000 polychromatic erythrocytes (PCEs) examined per animal; the number of normochromatic eryhtrocytes (NCEs, more mature RBCs) per 2 000 PCEs was noted.

3. Details of slide preparation

At 24 hours after the second intraperitoneal injection of BYH 18636 or vehicle control, or 24 hours after the only one intraperitoneal injection of positive control, the appropriate groups of animals were sacrificed. Bone marrow smears were prepared from at least one intact femur for each animal. Cell smears were prepared and stained according to conventional cytological procedures.

Coded slides were scored for the presence of micronuclei in 2000 PCEs per animal. The ratio of PCEs to NCEs was also recorded for each animal, as an indication of cytotoxicity to the target tissue. The number of normochromatic erythrocytes showing micronuclei was also established.

4. Evaluation Criteria

To determine whether a statistically significant response in MN PCE frequency was treatment related, the following criteria were applied:

- Whether or not there were dose-dependent effects that were consistent with a treatment-induced response and
- The degree of the response in relation to both concurrent and historical vehicle and positive control data

5. Statistical methods

The BYH 18636 group(s) with the highest mean (provided this superceded the negative control mean) and the positive control were checked by Wilcoxon's non-parametric rank sum test with respect to the number of micronucleated polychromatic erythrocytes and the number of normochromatic erythrocytes.

The rate of normochromatic erythrocytes containing micronuclei was examined if the micronuclear rate for polychromatic erythrocytes was already relevantly increased. In this case, the group with the highest mean was compared with the negative control using the one-sided Chi^2-test.

II. RESULTS AND DISCUSSION

A. RANGE-FINDING TEST

Three males and three females received two intraperitoneal injections at 24 hours interval of BYH 18636 at 1000 mg/kg. The animals were sacrificed 24 hours after the last injection. One male and one female died. Clinicals signs were apathy, roughened fur, loss of weight, spasm, periodically stretching of body, difficulty in breathing, slitted eyes and closed eyes.

B. MICRONUCLEUS ASSAY:

1. Toxicity

At 500 mg/kg, 3/10 males died. The treated males showed the following compound-related symptoms until sacrifice: apathy, roughened fur, loss of weight, spasm, periodically stretching of body, difficulty in breathing and slitted eyes.

2. PCE ratio

There was no evidence of target cell cytotoxicity (a significant decrease in the PCE/total erythrocyte ratio) at any dose level (see Table IIA 5.4.4-1)

3. Micronucleated polychromatic erythrocytes

There were no biologically significant variations in the incidence of micronucleated polychromatic erythrocytes between the control and the BYH 18636 treated groups. The statistical significance observed at 500 mg/kg was considered to be of no biological relevance, since the absolute value of 4.2/2000 was clearly within the range of the historical negative controls. No relevant variations were observed between the negative control and BYH 18636 treated groups in the number of micronucleated normochromatic erythrocytes.

The positive control, cyclophosphamide, caused a clear increase in the number of polychromatic erythrocytes with micronuclei but did not alter the ratio of polychromatic to normochromatic erythrocytes.

III. CONCLUSION

BYH 18636 did not induce micronucleated polychromatic erythrocytes in male mice at doses up to 500 mg/kg.

Table IIA 5.4.4-1: Summary of micronucleus results in male mice 24 hours after the last injection (PCE ratio data)

Number of animals	Vehicle control	BYH 18636 125 mg/kg	BYH 18636 250 mg/kg	BYH 18636 500 mg/kg	Positive control
5	1.6 (1.1)	2.4 (1.5)	2.8 (2.0)	4.2 (1.5) *	31.0 (5.8) **

* $p < 0.05$; ** $p < 0.01$ in non-parametric Wilcoxon ranking test

(Herbold B., 2004)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK

Reviewer's Comments	Reliability rating: Totally reliable The study is compliant with OECD 474 (1997). The study was performed with test material of higher purity (96.4%) than the minimum currently proposed (950 g/kg) for the specification of the manufactured material. The use of mice of a single sex in this study is considered to be acceptable in the absence of any marked sex differences in the toxicity of thiencarbazone-methyl and based on the results of the range-finding study. The use of intraperitoneal dosing in this study has not been justified but is considered to be acceptable and is likely to result in adequate exposure of the bone marrow. The top dose level used in this study is considered to be sufficiently high, based on the mortality and signs of toxicity seen at this dose level in the main study and at 1000 mg/kg bw in the range-finding study. The slight (but statistically significant) increase in the proportion of micronucleated polychromatic erythrocytes seen in the 500 mg/kg bw dose group is within the laboratory's background range of 2.0-5.6 (figures from 27 studies performed in 2002-2003) and is associated with an unusually low concurrent control value of 1.6. This finding is therefore not considered to be of toxicological significance. Mouse micronucleus assay: summary of findings

Micronucleated PCEs per 2000 [range]				
-control	125 mg/kg bw	250 mg/kg bw	500 mg/kg bw	+control
1.6 ± 1.1 [0-3]	2.4 ± 1.5 [1-4]	2.8 ± 2.0 [1-6]	4.2* ± 1.5 [2-6]	31.0** ± 5.8 [21-36]

Conclusions	No evidence of genotoxicity was seen under the conditions of this study.

In vivo genotoxicity (somatic cells) - DNA repair or mouse spot tests (IIA 5.4.5)

Based on the results of the *in vitro* and *in vivo* studies as reported under points 5.4.1 to 5.4.4, no further studies in germ cells were triggered.

In vivo studies in germ cells (IIA 5.4.6)

Based on the results of the *in vitro* and *in vivo* studies as reported under points 5.4.1 to 5.4.4, no further studies in germ cells were triggered.

B.6.5 Chronic toxicity and carcinogenicity studies (IIA 5.5)

B.6.5.1 Chronic toxicity and carcinogenicity in the rat

Long-term (2 years) oral toxicity in the rat (IIA 5.5.1)

The long term (2 years) oral toxicity of BYH 18636 in rats was investigated in a combined chronic/carcinogenicity study reported under point IIA 5.5.2.

Carcinogenicity study in the rat (IIA 5.5.2)

Report: **KIIA 5.5.2/01 Schladt L., Rühl-Fehlert C., Hartmann E., 2007 (MRID # 47070134)**

Title:	BYH 18636, Combined Chronic Toxicity and Carcinogenicity Study in Wistar Rats (Dietary Administration for 2 Years)
Report No & Document No	AT03629 M-284355-01-2
Guidelines:	OECD 453 (1981); EEC Directive 88/302/EEC – Annex V - Method B.33. (1988); EPA Health Effects Test Guideline (OPPTS 870.4300; 1998); M.A.F.F. in Japan notification 12 Nousan N°8147 (2000) guidelines.
GLP	Yes (certified laboratory)

Executive summary:
BYH 18636 (Mix-batch 702-73-06-0001, 96.0 - 96.4% of purity) was administered in the diet to 60 male and 60 female Wistar rats per dose group, in doses of 0, 500, 2500 or 5000 ppm (main groups) for a scheduled treatment period of 2 years (carcinogenicity part of the study). Furthermore, BYH 18636 was administered in the diet to 10 male and 10 female rats per dose group, in doses of 0, 200, 500, 2500 or 5000 ppm (satellite groups) for a treatment period of 1 year (chronic part of the study). Treatment resulted in the following mean daily test substance intake for main groups (averaged over the study period of two years and given in ascending dosages): 22.8, 115.2 and 234.0 mg/kg body weight for males and 29.9, 152.9 and 313.4 mg/kg for females. During their treatment period of one year mean daily test substance intake of satellite group animals was for the males: 10.6, 27.2, 136.4 and 268.60 mg/kg body weight and for the females: 13.2, 35.8, 176.7 and 366.6 mg/kg.

The animals were regularly observed and weighed and food intake was determined. Furthermore, ophthalmological investigations (before the study start: all animals; after one year and at the end of the treatment period: all main group animals of the control and high dose groups), a functional observational battery and motor activity assessment (once in week 50 on animals of the satellite groups) as well as clinical laboratory investigations of blood and urine samples (after 3 months, 6 months and near the end of the first year of treatment from animals of the satellite groups and after 3 months, 6 months, 12 months, 18 months and near the end of the second year of treatment from animals of the main groups) were performed. At necropsy, selected organs were weighed and tissues were subjected to gross and histopathological investigations.

Microscopy of urinary sediment showed the presence of crystals in nearly all animals of the 5000 ppm group as well as in two males and two females of the 2500 ppm group after 78 weeks of treatment. However, at the end of the treatment period no such crystals were observed in the sediment. Furthermore, histopathological investigation confirmed no evidence of treatment-related effects in the kidneys/urinary tract.

The investigations gave no evidence for treatment-related adverse effects. Furthermore, under the conditions described the administration of BYH 18636 to male and female rats did not produce any evidence of an oncogenic effect of the test substance.

Therefore, the present study established a No-Observed-Effect Level (NOEL) of 5000 ppm (corresponding to 234.0 or 313.4 mg/kg body weight/day) in males or females, respectively.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	BYH 18636
Description:	White powder
Lot/Batch:	Mix-batch 702-73-06-0001
Purity:	96.0% (analyzed in July, 2003), 96.2% (analyzed in December 2003), 96.3% (analyzed in May 2004), 96.4% (analyzed in December 2004), 96.0% (analyzed in May 2005)
CAS:	317815-83-1
Stability of test compound:	Stable in rodent diet at 10 and 7 000 ppm over a 15-day period at ambient temperature or over a 15-day freezing

period

2. Vehicle and /or positive control: None

3. Test animals:

Species: Rat
Strain: Wistar (Hsd Cpb:WU)
Age: 6 to 7 weeks approximately
Weight at dosing: 161 g(136 to 190 g) for the males –
129 g (110 to 150 g) for the females
Source: Harlan Winkelmann GmbH, Borchen, Germany
Acclimation period: about one week
Diet: *ad libitum*: Provimi Kliba Maus/Ratte-Haltung-GLP, (Provimi Kliba SA 25 G4 (3883.9.25) by Provimi Kliba SA, 4303-Kaiseraugst, Switzerland
Water: Tap water, *ad libitum*
Housing: Individually in Makrolon® cages Type III h, Bedding material: Low-dust wood shavings supplied by Ssniff GmbH in 59494-Soest, Germany.
Environmental conditions –
Temperature: 22 ± 2°C
Humidity: Approximately 55 %
Air changes: At least 10 changes per hour
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN:

1. In life dates: November 03, 2003 to November 18, 2005

2. Animal assignment and treatment

The animals were allocated to their final cage number specified by random list, on the basis of evenly distributed random numbers especially generated for this study using a computer program. The dosing schedule and the distribution of the animals to the study groups are noted in the following table:

Table IIA 5.5.2-1 Study design

Test group	Concentration in diet (ppm)	Dose per animal (week period 1 to 104)		Animals assigned/sex	
		Male (mg/kg bw/day)	Female (mg/kg bw/day)	Main study 104 weeks	Interim sacrifice 52 weeks
1	0	0	0	60	10
2	200	11 *	13 *	-	10
3	500	23	30	60	10
5	2500	115	153	60	10
4	5000	234	313	60	10

*: week period 1 to 52

Dose selection: The dose levels were selected based on the results from a previous 90-day dietary study in the rat. In this study, Wistar rats were administered BYH 18636 via the diet at dose levels of 0, 400, 2500, and 7000 ppm for at least 90 days. Dietary administration of up to 7000 ppm resulted in the mortality of 1/10 males treated at 7000 ppm with a urinary tract obstruction as the probable cause of death. One animal found dead in the control group presented a similar condition but due to the nature of the treatment-related findings observed in other animals from the 7000 ppm group at final sacrifice, it was considered highly likely that the obstruction in the animal from

the 7000 ppm group was induced by treatment with BYH 18636. For 7000 ppm rats at urinalysis, sulfonamide-like crystals were seen in the urine of 9/10 males and 10/10 females at the end of the dosing phase. At microscopic examination at the end of the dosing phase, treatment-related effects were observed in the kidney and the urinary bladder in both males and females. Intrapelvic eosinophilic urolithiasis within the kidneys was found in 3/10 males and 1/10 females. A similar eosinophilic urolithiasis within the lumen of the urinary bladder was seen in 2/10 males; this was correlated with gritty content observed macroscopically in 3/10 males. There were no adverse treatment-related findings observed at 2000 ppm in either sex but at the end of the treatment period, sulfonamide-like crystals were seen in the urine of 3/10 males and 4/10 females.

Based on these results, the dose levels for the carcinogenicity study were 200 (interim sacrifice only), 500, 2500 and 5000 ppm.

3. Diet preparation and anlysis

The test compound was mixed in the diet at the appropriate concentrations at room temperature and maximally used over the stability period determined by analytical investigations. The test compound was blended with the diet Provimi Kliba SA 25 G4 (3883.9.25).
Chemical analysis of the diet formulations prior to the start of the study ensured a homogeneous distribution of the compound in the food / test substance mixtures for the concentrations 10 and 7000 ppm in 15 kg mixtures. The compound was shown to be stable in these mixtures for at least 15 days after storing under animal room conditions. Analyses during in-life phase of the study included check of compound content (all doses including 0 ppm) and homogeneity (low and high dose only) at beginning and termination of the study as well as once during every quarter of the study.

Homogeneity Analysis: The investigations revealed that the test compound was homogenously distributed in the mixtures within the Company's defined limits.

Stability Analysis: The stability of the compound in these mixtures was given for at least 15 days after storing under animal room conditions.

Concentration Analysis: The investigations revealed that the concentration of the test compound in the mixtures agreed with the target concentrations within the Company's defined limits.

4. Statistics

The statistical evaluation of clinical chemistry, haematology, urinalysis, body and organ weights as well as food intake data was performed using SAS routines.
Statistical evaluations on body weight and organ weight data were done using the Dunnett-Test in connection with a variance analysis. A Kruskal-Wallis-Test with a Steel-Test was performed for analyzing data of food intake.
All variables that were not dichotomous are described by sex, dose group and date using appropriate measures of central tendency (mean, median) and general variability (standard deviation, minimum, maximum).
For the statistical evaluation of samples drawn from continuously distributed random variables three types of statistical tests were used, the choice of the test being a function of prior knowledge obtained in former studies. Provided that the variables in question were approximately normally distributed with equal variances across treatments, the Dunnett´s-Test was used, if heteroscedasticity appeared more likely, a p-value adjusted Welch test was applied. If the evidence based on experience with historical data indicated that the assumptions for a parametric analysis of variance could not be maintained, distribution-free tests in lieu of ANOVA was carried out, i.e. the Kruskal-Wallis test followed by adjusted Mann-Whitney-Wilcoxon tests (U tests) when appropriate.

With respect to data collected in the functional observational battery categorical variables were analyzed with a repeated measures analysis of variance followed by a one-way analysis of variance using the SAS procedure PROC CATMOD. As part of the one-way analysis, contrasts

were performed to compare the results of each treated group with those of the control group. The logic of the analysis plan for continuous variables was analogous to that of the categorical variables, but using the SAS procedure PROC GLM.

C. METHODS:

1. Observations

a. Cageside Observations

All animals were inspected twice daily (once daily on weekends and public holidays) for signs of toxicity and mortality. In addition, general condition and behavior of the animals of satellite groups were checked and recorded daily ("in cage observation").

b. Clinical Examinations

A careful clinical examination ("outside cage observation") was made once prior to treatment and weekly up to necropsy in all animals of all groups. Any clinical signs and abnormalities including changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions, autonomic activity, changes in gait, posture, and response to handling, clonic/tonic movements, stereotypes and bizarre behaviour were recorded with time of onset, location and grading. For satellite animals this included an observation in a standard arena outside the home cage ("open field observation").

c. Neurological Evaluations

Functional Observational Battery:

The functional observational battery was performed during the treatment period in week 50 on animals of the satellite groups.

Near the end of the first year of treatment the following examinations were conducted individually on all animals of satellite groups (not blind):

- home cage observation: posture, piloerection, gait abnormalities, involuntary motor movements, vocalization, others;
- observations during handling: ease of removing, reaction to being handled, muscle tone, palpebral closure, lacrimation, nasal discharge, salivation, stains, others;
- open field observations: piloerection, respiratory abnormalities, posture, involuntary motor movements, stereotypy, bizarre behaviour, gait abnormalities, vocalization, arousal, rearing, defecation, urination, others
- reflex/physiologic observations: approach response, touch response, auditory response, tail pinch response, grip strength, pupil size, body temperature, body weight.

Motor Activity Measurement:

Motor activity measurements were performed during the treatment period in week 50 on all animals of the satellite groups.

Motor activity (MA) and locomotor activity (LMA) were examined as activity for the entire 60-minute session and activity during each 10-minute interval. Motor activity was measured as the number of beam interruptions that occurred during the test session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Thus, for locomotor activity, only one interruption of a given beam was counted until the animal relocated in the maze and interrupted one of the other beams. Habituation was evaluated as a decrement in activity during the test session.

2. Body weight

The individual body weights of animals of all groups were determined just prior to the administration of the test substance, once a week during the first 13 weeks of the study and every 4 weeks thereafter. Furthermore, body weights were recorded immediately before scheduled necropsies, for calculation of relative organ weights.

3. Food Consumption and test compound intake

The individual food consumption of all animals was determined once a week during the first 13 weeks of the study and every 4 weeks thereafter.
The test compound intake was calculated from the food intake.
The following data were calculated:
- for each interval: daily food intake per animal, mean daily food intake per animal, mean daily food intake per kg body weight, mean daily test substance intake per kg body weight;
- for the total feeding period: mean food intake per animal and day, mean food intake per kg body weight and day, mean test substance intake per animal and day, mean test substance intake per kg body weight and day.
- Furthermore, cumulative food intake per animal, cumulative food intake per kg body weight, cumulative test substance intake per animal and cumulative test substance intake per kg body weight were calculated.

4. Ophthalmological Examination

All animals were subjected to ophthalmological inspection before the study start. After one year and at the end of the treatment period all main group animals of the control and high dose groups were investigated.
The pupillary reflex of both eyes was first tested in a darkened room and the anterior regions of the eye were inspected. After dilating the pupils with Mydriaticum Roche® or Mydriaticum Stulln® drops, the refractive elements of the eye as well as iris and fundus were examined using an indirect ophthalmoscope. In addition, the optical media was examined with a ZEISS photo-slit lamp.

5. Haematology and Clinical Chemistry

Blood samples were taken from fasted animals after 3 months, 6 months and near the end of the first year of treatment from all animals of the satellite groups and after 3 months, 6 months, 12 months, 18 months and near the end of the second year of treatment from the first 10 surviving animals of the main groups.
Occasionally, sample quantity may have been insufficient to permit determination of all intended parameters, or no determination was possible due to technical faults. The planned number of measurements per group may, therefore, not necessarily be obtained in each case.

The blood samples for determination of glucose concentration were taken in the morning from the caudal vein of fasted non-anaesthetized animals. The blood samples used for determining the other parameters in peripheral blood were collected in the morning from the retro-orbital venous plexus of fasted (for about 16 hrs) animals anaesthetized with diethyl ether. The blood obtained was treated as follows:
The samples for the haematological determinations were collected in tubes coated with EDTA (anticoagulant). The samples for the determinations of the thromboplastin time (HQUICK) were collected in tubes with sodium-citrate. The samples for the determinations of electrolyte concentrations remained untreated. The samples for other biochemical tests were heparinized. The blood samples for glucose determinations were mixed with perchloric acid (1+10) to precipitate proteins.

The following haematological parameters were measured: haematocrit, haemoglobin, leukocyte count, erythrocyte count, leukocyte differential count, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular volume, reticulocyte count, prothrombin time, thrombocytes.

Blood smears: from all animals of the carcinogenicity part of the study (main groups), which were killed in moribund condition as well as from all surviving animals after 12 months, after 18 months and near the end of the study, blood was collected from one of the caudal veins to prepare blood smears.
A differential blood count was performed on the smears obtained from animals killed in moribund condition and from the blood smears obtained from the surviving animals of the

high dose and control group near the end of the study. Differential blood count was performed for the next lower dose groups if there was a major discrepancy between the highest dose group and the controls.

The following clinical chemistry parameters were determined:
Any significant change in the general appearance of the plasma and the serum was recorded. Total bilirubin, glucose, urea, creatinine, total cholesterol, triglycerides, chloride, sodium, potassium, calcium and inorganic phosphorus concentrations and aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, glutamate dehydrogenase, creatine kinase and lactate dehydrogenase activities were assayed on plasma samples, total protein and albumin concentrations were assayed on serum samples. Globulin and albumin/globulin ratio values were calculated.

6. Urinalysis

Urine was collected after 3 months, 6 months and near the end of the first year of treatment from all animals of the satellite groups. Furthermore, urine was collected after 18 months and near the end of the second year of treatment from the first living 10 animals of the main groups (collection period was about 16 hours; urine was sampled at room temperature; during the urine collection periods water was offered *ad libitum*, but food was not supplied). The following parameters were examined:
Any significant change in the general appearance of the urine was recorded. The urine volume and urinary refractive index were measured. pH was assayed. The following semi-quantitative parameters were assayed using dipsticks: glucose, bilirubin, ketone bodies, occult blood, protein and urobilinogen. Microscopic examination of the urinary sediment was performed after centrifugation of the urine.

7. Sacrifice and Pathology

All animals scheduled for necropsy were sacrificed by exsanguination under deep diethyl ether anaesthesia, necropsied and their organs and tissues subjected to thorough gross pathological examination. Changes were described in terms of localization, size, colour and consistency whenever appropriate.
Animals that died spontaneously or were sacrificed in a moribund state during the study were necropsied at the earliest opportunity. From these animals the organs and tissues were handled as described above.
The following organs were weighed: adrenal gland, brain, epididymides, heart, kidney, liver, ovary, spleen, testis and uterus.
The following organs or tissues were sampled: adrenal gland, aorta, bone (sternum/femur), bone, brain, epididymis, oesophagus, lachrymal gland, eye and optic nerve, harderian gland, heart, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidney, larynx/pharynx, liver, lung, lymph nodes, mammary gland, nasal cavities, ovary, pancreas, parathyroids, Peyer's patches, pituitary gland, prostate gland, sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, testis, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus (with cervix),ureters, vagina. These organs were histopathologically examined in all animals of the main groups and in control and high dose group animals of the satellite groups (with the exception of abnormalities, kidneys, adrenals, liver, lungs, which were examined in all animals of these groups).
From all sacrificed animals (from deceased animals as far as possible) the tissues mentioned above as well as all other tissues showing abnormalities were preserved in 10% formalin. Urinary bladder and lungs were fixed by instillation prior to fixation.

II. RESULTS

A. OBSERVATIONS

1. Mortality

From the animals scheduled for a one-year treatment one male from the control group and one

female from the 200 ppm group had to be sacrificed in moribund condition. One male rat from the 200 ppm group was found dead.

The table IIA 5.5.2-2 shows the cumulative mortality of the animals scheduled for the two-year treatment at quarterly intervals from the start of the study until week 107. Related to ascending dosages at the end of week 104 the number of intercurrently died animals/group were (given in ascending dosages): males: 26 / 21 / 34 / 25; females: 25 / 14 / 18 / 14. A few further animals died or had to be killed in moribund condition during the following weeks, in which necropsy was performed. There was no treatment-related effect on the survival.
The cause of death in these animals was frequently local or disseminated tumour growth or degenerative changes, in males often chronic progressive nephropathy. There was no evidence of any dose-correlated changes contributing to the death of the animals. The death of one male from the 2500 ppm group and one female from the 500 ppm group was due to the blood collection procedure.

Table IIA 5.5.2-2: Intercurrent Deaths of Animals Scheduled for a 2 Year Treatment

Sex	Males				Females			
Dose (ppm)	0	500	2500	5000	0	500	2500	5000
N	60	60	60	60	60	60	60	60
Week								
1-13	0	0	1	1	0	0	0	0
1-26	0	0	1	1	0	0	0	0
1-39	0	0	3/1§	2	0	0	0	0
1-52	2	0	3/1§	3	1	2/1§	0	0
1-65	3	2	6/1§	5	2	4/1§	4	2
1-78	6	7	12/1§	11	8	5/1§	5	6
1-91	17	15	19/1§	17	16	9/1§	9	8
1-104	26	21	34/1§	25	25	14/1§	18	14
1-107	26	22	34/1§	25	26	15/1§	18	15

§ = animals that died during/after blood sampling

2. Clinical signs

The incidences for palpable masses were 5/4/7/4 for males and 22/16/14/10 for females and gave therefore no evidence of a treatment-related increase for this finding. Also the observation of animals for other clinical signs of toxicity revealed no treatment-related changes.

3. Neurological Evaluations

Neurological evaluations were performed on all animals of the satellite groups. In summary, the investigations/observations during FOB in the home cage, during handling and in the open field, and the manipulative tests (e.g. grip strength, auditory response) showed no relevant signs or symptoms indicating evidence for a treatment-related effect in rats exposed to the test substance.
The activity determinations over the entire 60 minutes observation period did not reveal a relevant effect on motor (MA) and locomotor activity (LMA).
The results of the 10-minute intervals indicate that treated and control groups reacted in a similar way and that there were no relevant differences in the first 10-minute intervals, which are regarded as best indicator of increases or decreases in activity before the animals habituate to a lower level of activity.

B. BODY WEIGHT

Body weights of treated animals were not significantly decreased compared to the respective control groups.

Table IIA 5.5.2-3: Mean Body Weights (BW) and Body Weight Gains (BWG) - Main Groups

Dietary levels	0	500 ppm	2500 ppm	5000 ppm
MALES Initial BW (g)	160	158	157	157
Final BW (g)	530	521	515	532
BWG Wk 1 (g) (% C)	47.0	46.4 (-1.28)	46.7 (-0.64)	46.5 (-1.06)
BWG Wk 1-13 (g) (%C)	272	271 (-0.37)	272 (0)	274 (0.74)
BWG Wk 13-26 (g) (% C)	70.2	64.4 (-8.26)	63.4 (-9.69)+	67.1 (-4.42)
BWG Wk 26-54 (g) (% C)	44.2	46.7 (5.66)	41.7 (-5.66)	44.1 (-0.23)
BWG Wk 54-74 (g) (% C)	8.99	11.2 (24.58)	6.28 (-30.14)	7.24 (-19.47)
BWG Wk 74-105 (g) (% C)	-40	-33 (-17.50)	-36 (-10.00)	-24 (-40.00)
Overall BWG Wk -1-105 (g)	370	366 (-1.08)	360 (-2.70)	374 (1.08)
FEMALES Initial BW (g)	130	128	127	127
Final BW (g)	330	343	330	342
BWG Wk 1 (g) (% C)	19.3	19.6 (1.55)	19.6 (1.55)	19.9 (3.11)
BWG Wk 1-13 (g) (%C)	116	117 (0.86)	116 (0)	117 (0.86)
BWG Wk 13-26 (g) (% C)	32.4	31.0 (-4.32)	29.3 (-9.57)	29.1 (-10.19)
BWG Wk 26-54 (g) (% C)	39.2	36.5 (-6.89)	37.4 (-4.59)	38.4 (-2.04)
BWG Wk 54-74 (g) (% C)	22.4	25.4 (13.39)	25.8 (15.18)	27.4 (22.32)
BWG Wk 74-105 (g) (% C)	-5.5	0.21 (103.82)	-3.5 (36.36)	3.10 (156.36)
Overall BWG Wk -1-105 (g)	201	216 (7.46)	203 (1.00)	214 (6.47)

C = control; Wk = week + Statistically different ($p \leq 0.05$) from the control.
% C = % of change difference controls
Remark: the statistical difference is based on the actual data and not on the % of change.

Table IIA 5.5.2-4: Mean Body Weights (BW) and Body Weight Gains (BWG) - Satellite Groups

Dietary levels	0 ppm	200 ppm	500 ppm	2500 ppm	5000 ppm
MALES Initial BW (g)	165	163	164	162	160
Final BW (g)	546	527	533	539	541
BWG Wk 1 (g) (% C)	46.9	46.4 (-1.07)	48.3 (2.99)	46.4 (-1.07)	50.9 (8.53)
BWG Wk 1-13 (g) (%C)	273	253 (-7.33)	262 (-4.03)	278 (1.83)	275 (0.73)
BWG Wk 13-26 (g) (% C)	87.7	72.2 (-17.67)	74.0 (-15.62)	71.8 (-18.13)	55.8 (-36.37)++
BWG Wk 26-53 (g) (% C)	29.0	33.8 (16.55)	33.4 (15.17)	27.9 (-3.79)	50.4 (73.79)
Overall BWG Wk -1-53 (g)	383	365 (-4.70)	369 (-3.66)	378 (-1.31)	381 (0.52)
FEMALES Initial BW (g)	130	132	128	128	130
Final BW (g)	284	313	291	297	299
BWG Wk 1 (g) (% C)	17.8	24.0 (34.83)+	16.3 (-8.43)	18.5 (3.93)	17.8 (0.0)

BWG Wk 1-13 (g) (%C)	91.9	113 (22.96)+	98.0 (6.64)	102 (10.99)	99.8 (8.60)
BWG Wk 13-26 (g) (% C)	34.5	38.1 (10.43)	33.7 (-2.32)	37.9 (9.86)	33.4 (-3.19)
BWG Wk 26-53 (g) (% C)	27.7	28.1 (1.44)	31.5 (13.72)	29.3 (5.78)	35.9 (29.60)
Overall BWG Wk -1-53 (g)	154	182 (18.18)	163 (5.84)	169 (9.74)	169 (9.74)

C = control; Wk = week
+ Statistically different ($p \leq 0.05$) from the control. ++ Statistically different ($p \leq 0.01$) from the control.
% C = % of diffenrece to controls

Remark: the statistical difference is based on the actual data and not on the % change.

C. FOOD CONSUMPTION AND TEST COMPOUND INTAKE

1. Food Consumption

There were no relevant differences in food intake in treated groups compared to the respective control groups.

The only exception was the lower food intake of females of the 200 ppm satellite group (related to body weight about 11%), which had, on the other hand, the highest body weights of the female satellite groups. A toxicological relevance is not inferred from these data.

Table IIA 5.5.2-5: Cumulative and Mean Daily Food Intake (Main Groups)

Group Means for Main Groups					
Dose ppm	Days	g/animal total	g/animal per day	g/kg body weight total	g/kg body weight per day
Male					
0	707	15752	22.28	32084	45.38
500	707	15618	22.09	32225	45.58
2500	707	15752	22.28	32579	46.08
5000	707	16226	22.95	33095	46.81
Female					
0	708	12510	17.67	43068	60.83
500	708	12333	17.42	42353	59.82
2500	708	12312	17.39	43294	61.15
5000	708	12801	18.08	44377	62.68

Table IIA 5.5.2-6: Cumulative and Mean Daily Food Intake (Satellite Groups)

Group Means for Satellite Groups					
Dose ppm	Days	g/animal total	g/animal per day	g/kg body weight total	g/kg body weight per day
Male					
0	364	8769	24.09	19008	52.22
200	364	8408	23.10	19288	52.99
500	364	8867	24.36	19816	54.44
2500	364	9005	24.74	19860	54.56
5000	364	8831	24.26	19554	53.72
Female					
0	364	6395	17.57	27002	74.18
200	364	6272	17.23	24006	65.95
500	364	6304	17.32	26066	71.61
2500	364	6326	17.38	25728	70.68
5000	364	6479	17.80	26692	73.33

2. Test compound Intake

Treatment resulted in the following mean daily test compound intake (averaged over the study period and given in ascending dosages) of the main groups: 22.8, 115.2 and 234.0 mg/kg body weight for males and 29.9, 152.9 and 313.4 mg/kg for females.

During their treatment period of one year animals of satellite groups ingested per day for the males: 10.6, 27.2, 136.4 and 268.6 mg/kg body weight and for females: 13.2, 35.8, 176.7 and 366.6 mg/kg.

Table IIA 5.5.2-7: Cumulative and Mean Daily Test Compound Intake (Main Groups)

Group Means for Main Groups					
Dose ppm	Days	mg/animal total	mg/animal per day	mg/kg body weight total	mg/kg body weight per day
Male					
500	707	7812	11.05	16113	22.8
2500	707	39380	55.70	81446	115.2
5000	707	81142	114.77	165466	234.0
Female					
500	708	6167	8.71	21176	29.9
2500	708	30777	43.47	108232	152.9
5000	708	63996	90.39	221894	313.4

Table IIA 5.5.2-8: Cumulative and Mean Daily Test Compound Intake (Satellite Groups)

Group Means for Satellite Groups					
Dose ppm	Days	mg/animal total	mg/animal per day	mg/kg body weight total	mg/kg body weight per day
Male					
200	364	1682	4.62	3858	10.6
500	364	4434	12.18	9908	27.2
2500	364	22513	61.85	49653	136.4
5000	364	44146	121.28	97770	268.6
Female					
200	364	1256	3.45	4801	13.2
500	364	3152	8.66	13031	35.8
2500	364	15816	43.45	64319	176.7
5000	364	32392	88.99	133457	366.6

D. OPHTHALMOLIGICAL EXAMINATION

Ophthalmologic investigations after one year of treatment and near the end of the study revealed no treatment-related effects on the eyes.

E. BLOOD ANALYSES

1. Haematology

Haematological investigations produced no evidence for relevant treatment-related effects on red or white blood cells or blood coagulation. Differential blood count performed on smears prepared from blood of the caudal vein of main group animals of the high dose and control group near the end of the treatment period revealed no evidence of treatment-related effects. Histopathological investigation indicated no need for the other smears (with the exception of those of animals killed in moribund condition) to be investigated.

The differential blood counts performed in animals sacrificed in a moribund condition resulted in a considerable variation of the relative amounts of the elements of the leucocyte population. However, there was no evidence for a specific test substance-related effect.

2. Clinical Chemistry

The triglyceride concentration in peripheral blood of main group females was lowest at 5000 ppm (mostly statistically significantly). A significant difference was also observed after 78 weeks at 2500 ppm. It should be mentioned that the differences of the last two determinations were partly caused by the relatively high value of one control females resulting in a correspondingly higher mean for the control group.

The investigations gave no evidence for further relevant treatment-related effects on enzyme activities, substrates or electrolytes in peripheral blood.

Table IIA 5.5.2-9: Triglyceride concentration (mmol/l) in main female groups

	Days 93/94	Days 184/186	Days 359/360	Days 549/551	Days 725/726
0 ppm	**0.66**	**0.84**	**1.18**	**2.74**	**3.22**
500 ppm	**0.57**	**0.74**	**0.86**	**1.62**	**2.57**
2500 ppm	**0.62**	**0.55**	**0.97**	**1.41 ***	**3.24**
5000 ppm	**0.53**	**0.51 ***	**0.70***	**1.12 ****	**1.83**

*: significantly different at $p \leq 0.05$ **: significantly different at $p \leq 0.01$

F. URINALYSIS

Quantitative and semiquantitative urinalysis revealed no evidence of treatment-related effects. At microscopy of sediment, crystals were observed in nearly all the investigated animals of the 5000 ppm group as well as in two males and two females of the 2500 ppm group after 78 weeks of treatment. However, at the end of the treatment period no such crystals were observed in the sediment.

Table IIA 5.5.2-10: Urinalysis – Sediment - Main Groups

Dose ppm	No. of animals with Finding: / No. of animals investigated	A	B
Males	Day 543/ 544		
0	10	0	0
500	10	0	0
2500	10	2	0
5000	10	9	0
Females	Day 543/ 544		
0	10	0	0
500	10	0	0
2500	10	2	0
5000	10	7	3

A = round, yellow, birefractive crystalloid structures;
most similar in shape to Leucin or Biurate crystals;
soluble in: acetic acid, hydrochloride acid, soda lye, ethanol;
partly soluble in: heat

B = yellow, birefractive bushy aggregates of crystals;
soluble in: hydrochloride acid;
partly soluble in: acetic acid, soda lye
not soluble in: ethanol, heat

G. SACRIFICE AND PATHOLOGY

1. Organ weight

Determination of organ weights of animals of the satellite groups at necropsy after one year of treatment revealed no significant differences between treated groups and the respective control groups.

Determination of organ weights of animals of the main groups at the end of the study revealed a few differences between treated and control animals. As the differences to control were slight with no correlated findings (e.g. at histopathological evaluation) they were regarded to be of no toxicological relevance.

Table IIA 5.5.2-11: Absolute Organ Weights – Main Groups

Dose ppm	Body W. G	Brain mg	Adrenals mg	Heart mg	Liver mg	Spleen mg	Kidneys mg	Testes mg	Epididym. mg
m	Terminal Sacrifice								
0	529	2226	71	2132	19554	1228	3966	3656	1507
500	527	2236	61	2041	18730	1141	3869	3770	1484
2500	519	2190	65	1954	18300	1098	3790	3612	1423
5000	537	2237	68	2032	19266	1170	3842	3809	1516
f	Terminal Sacrifice							Ovaries mg	Uterus mg
0	328	1951	70	1476	12335	688	2502	191	1387
500	345	2007 +	73	1464	11993	678	2534	174	1366
2500	332	1981	77	1420	11404	667	2496	167	1440
5000	344	2000	75	1393	11824	658	2429	180	1476

+ significantly different at $p \leq 0.05$

Table IIA 5.5.2-12: Relative Organ Weights – Main Groups

Dose ppm	Body W. G	Brain --	Adrenals --	Heart --	Liver mg/100g	Spleen --	Kidneys --	Testes --	Epididym. --
m	Terminal Sacrifice								
0	529	424	14	408	3722	233	761	693	285
500	527	427	12	391	3574	218	739	720	283
2500	519	426	13	381	3534	212	733	695	275
5000	537	419	13	379	3606	219	720	714	283
f	Terminal Sacrifice							Ovaries mg/100g	Uterus mg/100g
0	328	602	22	456	3801	213	770	58	430
500	345	587	21	427	3495 +	199	739	51	402
2500	332	606	23	433	3460 +	202	764	51	443
5000	344	590	22	408 +	3451 +	192	711 +	53	439

+ significantly different at $p \leq 0.05$

2. Gross Pathology

The interim sacrifice after 12 months, no treatment-related gross pathological findings were observed.

Amongst the animals attributed to the 2-year treatment period (terminal sacrifice and decedents), the most frequent findings were nodules of the pituitary gland in females (incidence: 22 -13 -17 -16), which were often combined with depressions of the cerebrum in this sex (incidence: 15 -5 -8 -7), surface changes of the kidneys in males (incidence: 35 -34 -30 -30), nodules of the uterus in females (incidence: 8 -13 -13 -15) and of the skin of females (incidence: 12 -10 -10 -6).
An unusual distribution was observed in the incidence of nodules in the thyroid gland of males which was 1 -2 -2 -8. In females this finding was observed only in one animal of the 2500 ppm group (incidence 0 - 0 -1 -0).

3. Microscopic Pathology

a. Non-neoplastic changes in Animals scheduled for 12-month treatment

In the kidneys, the severity score of chronic progressive nephropathy was reduced in males of the high dose group when compared to males of the other groups including controls. End-stage lesions of chronic progressive nephropathy usually scored with

grade 4 or 5 were absent in high dose males. Harderian metaplasia of the lacrimal gland ("Harderization") was also found with reduced severity scores in males of the high dose group. Besides these observations, no increase or decrease of non - neoplastic changes influenced by the treatment was detectable.

b. Non-neoplastic changes in animals scheduled for 24-month treatment

Most of the non-neoplastic findings observed at microscopic evaluation, were associated with the age of the animals and were degenerative, inflammatory or proliferative in nature.

As statistically significant decreased incidence in males (all animals including decedents) at the highest concentration level was obtained for some non-neoplastic findings. In females (all animals including decedents) no finding was significantly decreased or increased at the highest concentration level except a decrease of Harderian gland atrophy. These differences were considered not to be toxicologically relevant

None of the non-neoplastic changes with statistical significance were considered treatment-related.

Table IIA 5.5.2-13: Non-Neoplastic Changes Exhibiting Statistical Significance

INCIDENCE OF FINDINGS Animals scheduled for terminal sacrifice				
Dose (ppm)	**0**	**500**	**2500**	**5000**
Organ/Sex / Finding				
Nose / male				
inflammatory cells, luminal	7	2	2	0 ++
Lung / male				
vascular mineralization	16	13	19	7 +
Liver / male				
hyperplastic bile duct	54	51	47	46 +
Testes / male				
focal atrophy	28	17+	15 +	16 +
Harderian glands / female				
Focal atrophy	13	7	10	5 +

+ = $p < 0.05$; ++ = $p < 0.01$;

c. Neoplastic changes in Animals scheduled for 12-month treatment

No treatment-related tumours were found after 12 months. However, a few tumours were seen in rats of the chronic part of the toxicity study. These were one adenoma of the adrenal cortex (male, 500 ppm), three stromal polyps of the uterus (one female at 200, 2500 and 5000 ppm, each), one c-cell adenoma of the thyroid gland (female, control), and one mammary gland adenocarcinoma (female, 200 ppm). These few tumours were found irrespective to the treatment with the test substance.

d. Neoplastic changes in animals scheduled for 24-month treatment

The incidence of fibroadenomas in the mammary gland of females at 5000 ppm was statistically significant lower compared to the control incidence and that a few tumours showed a negative trend (granular cell tumour, brain, males; malignant pheochromocytoma, adrenal medulla, males; fibroadenoma, mammary gland, females) in the trend test statistics according to Peto.

At necropsy an increased incidence of thyroid gland nodules was observed in males (incidence: 1 - 2 - 2 - 8). The correlating histopathological changes were diverse and included c-cell tumours, follicular cell tumours and hyperplasias of the adjacent parathyroid gland. C-cell carcinomas were observed in males of the high concentration group only (incidence in males including decedents: 0 - 0 - 0 - 2 or in %: 0 – 0 – 0 – 3.3) and showed a significant positive trend in the Peto-test.
The focal c-cell hyperplasia (incidence in %: 13.3-6.9-15.0-6.7) and adenoma (incidence in %: 6.7-6.9-3.3-10.0) as precursor lesions of the c-cell carcinoma were not elevated accordingly. This observation and the historical data from 16 own studies (range: for focal c-cell hyperplasia: 3.4 -34.0%, mean: 14.7%; for c-cell adenoma: 2.1-24.0%, mean: 11.1%; for c-cell carcinoma: 0.0 -6.0%, mean 0.9%) from our laboratory with the same rat strain demonstrates that the incidence of c-cell carcinomas was within the historical control incidence and was a chance finding.

Nodules in the uterus of females were present at necropsy at an incidence of 8/13/13/15. The vast majority of them correlated with stromal polyps which are a frequent finding in aged Wistar rats. Uterine adenocarcinomas were slightly higher in incidence at the high dose level (incidences were 2/ 1/ 4/ 5), however without statistical significance and well within the incidence of historical data. No influence of treatment can be inferred from these data.

A few neoplastic findings were significantly reduced compared to the control group and/or showed a negative trend of the incidences. These were brain granular cell tumors and malignant pheochromocytomas of the adrenal medullas in males and mammary gland fibroadenoma in females. These tumors are either generally infrequent (granular cell tumors, malignant pheochromocytomas) or known to occur at relatively high and varying incidences (mammary gland fibroadenoma) in carcinogenicity studies in Wistar rats from our laboratory.

III. CONCLUSION

Under the conditions described the administration of BYH 18636 to male and female rats did not produce any evidence of an oncogenic effect of the test substance.
The No-Observed-Adverse-Effect Level (NOAEL) was 5000 ppm (equating to 234.0 or 313.4 mg/kg body weight/day in males or females, respectively).

(Schladt L., 2007)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK

Reviewer's Comments

Reliability rating: Totally reliable

The study (T3073715) is fully compliant with OECD 453.

The survival of animals in this study was unaffected by treatment and is considered to be adequate for the assessment of carcinogenicity. With the exception of 2500 ppm males, survival to 104 weeks exceeded 50%. Survival in 2500 ppm males reached 50% at Week 99; however a higher rate of survival was seen in males at the high dose level of 5000 ppm.

It is questionable whether the highest dose (5000 ppm) used in this study was adequate given the apparent absence of treatment-related findings in either sex. However the choice of top dose level is considered to be justified based on the results of the 90-day rat study, in which mortality (secondary to the critical effect of urinary tract toxicity) was observed at a dose level of 7000 ppm.

Treatment-related effects on the urinary tract were limited to the presence of crystals (assumed to be thiencarbazone-methyl or a metabolite) in the urine of the majority of animals at the top dose level of 5000 ppm and in two animals/sex at 2500 ppm. Findings are not considered to be of toxicological significance in the absence of macroscopic or histopathological correlates.

Lower plasma triglyceride concentrations were observed in females at a number of time points at 5000 ppm and at one time point at 2500 ppm. The Notifier does not consider these findings to represent a treatment-related adverse effect but has not included any further reasoning to support this view. The Study Director indicates that these findings are considered to be related to treatment. A review of findings in both sexes (including the satellite groups) indicates an association with treatment in both sexes at 5000 ppm. Findings are lower at the majority of time points, most notably at later time points, even when excluding outlying values in the controls (as shown below). Findings are therefore considered to be treatment-related and potentially adverse. The finding at 18 months in 2500 ppm females is not considered to be clearly related to treatment due to a lack of consistency between time points and the association with a high concurrent control value, attributable at least in part to an outlier. Similar findings were not observed in the 90-day rat study (McElligott, 2003) at dose levels of up to 7000 ppm, however it is notable that, in the chronic toxicity study, effects were only apparent after exposure for six months.

Effects on plasma triglyceride concentration in the rat chronic toxicity study

Time point	**Mean triglyceride concentration** (mmol/l) [mean excluding outlier value]							
	M				**F**			
	0	**500**	**2500**	**5000**	**0**	**500**	**2500**	**5000**
3 months (satellite)	0.94 1.50	0.95 1.05	1.44 1.22	0.93 1.06	0.66 0.56	0.57 0.49	0.62 0.49	0.53 0.43
6 months (satellite)	0.97 2.14	0.97 1.34	1.54 1.51	1.35 0.98	0.84 0.65	0.74 0.53	0.55 0.53	0.51* 0.55
12 months (satellite)	1.95 4.61 [3.33]	2.12 2.75	2.52 3.05	2.43 1.94*	1.18 1.27	0.86 0.94	0.97 0.98	0.70* 0.86
18 months	2.56	3.04	2.65	2.19	2.74 [2.27]	1.62	1.41*	1.12**
24 months	4.15 [3.47]	3.17	2.72	2.25*	3.22 [2.33]	2.57	3.24	1.83

Conclusions	No evidence of carcinogenicity was seen under the conditions of this study. A NOAEL of 2500 ppm (equivalent to mean achieved dietary intakes of 115 and 153 mg/kg bw/d in males and females respectively) can be determined based on the clinical chemistry findings (reduced plasma triglyceride concentration) at the top dose level of 5000 ppm (equivalent to 234 and 313 mg/kg bw/d respectively). It is acknowledged that this is a conservative interpretation and that findings at 5000 ppm represent a minimal NOAEL. The Notifiers proposed NOAEL of 5000 ppm is therefore not agreed.

B.6.5.2 Chronic toxicity and carcinogenicity in the mouse

Carcinogenicity study in the mouse (IIA 5.5.3)

Report:	**KIIA 5.5.3/01 Wason S., 2006 (MRID # 47070135)**
Title:	BYH 18636, Carcinogenicity study of BYH 18636 in the C57BL/6J mouse by dietary administration
Report No & Document No	SA 04062 M-280301-01-2
Guidelines:	OECD 451 (1981); EEC Directive 88/302/EEC – Annex V - Method B.32. (1987); EPA Health Effects Test Guideline (OPPTS 870.4200; 1998); M.A.F.F. in Japan notification 12 Nousan N°8147 (2000) guidelines.
GLP	Yes (certified laboratory)

Executive summary:

Groups of 60 male and 60 female C57BL/6J mice were fed diet containing 0, 200, 1000 or 4000 ppm of BYH 18636 (mix-batch 702-73-06-0001) for at least 28 weeks. After 28 weeks, 10 males and 10 females from each group allocated to the 28-week phase of the study were necropsied at the scheduled interim sacrifice. The remaining 50 animals/sex/group, allocated to the carcinogenicity phase of the study, continued treatment until the scheduled final sacrifice of the study after at least 78 weeks of treatment. The mean intake of BYH 18636 over 18 months was calculated to be 0, 29.2, 147 and 599 mg/kg/day in males and 0, 36.8, 185 and 758 mg/kg/day in females, at 0, 200, 1000 and 4000 ppm, respectively. Mortality and clinical signs were checked daily. Additionally, detailed physical examinations including palpation for masses were performed weekly throughout treatment. Body weight and food consumption were measured weekly for the first 13 weeks of the study, then monthly thereafter. Haematology determinations were performed at approximately 6, 12 and 18 months from designated animals. Where possible, blood smears were prepared from moribund animals just before sacrifice. All animals were subjected to necropsy, with selected organs weighed at scheduled interim and final sacrifice. Designated tissues were fixed and examined microscopically. In addition, urinary bladder stones sampled from five males at 4000 ppm were analyzed for the presence of test substance.

At 4000 ppm:

The mortality incidence over 18 months was higher (44%) in males than in the corresponding control group (22%), the effect being statistically significant. The higher mortality rate was largely due to the presence of stones within the urinary tract. Treatment-related clinical signs consisted of an increased incidence of generalized soiled fur in both sexes and an increased incidence of skin lesions, principally in the anogenital region, abnormal penis and wasted appearance in males. Abnormal penis was not associated with any intrinsic histopathological findings, but was associated with chronic ulcerative dermitis and/or an abscess in the preputial gland in the majority of cases at the microscopic examination. Mean body weight was reduced by 3% on Study Day 176 in males; this slight reduction in mean body weight continued thereafter resulting in a 5% lower body weight at the end of the study on Study Day 540, when compared with the controls. Mean cumulative body weight gain in males was statistically significantly reduced throughout most of the study, resulting in an overall mean cumulative body weight gain reduction of 15% by Study Day 540, when compared with the controls.

Mean body weight and body weight gain were unaffected by treatment in females. Mean food consumption was comparable to the controls in both sexes. Haematology parameters were unaffected by treatment.

At the 28-week interim sacrifice there was no treatment-related organ weight, macroscopic or microscopic findings.

There were 22/50 male and 9/50 female unscheduled deaths for animals allocated to the carcinogenicity phase of the study. Treatment-related macroscopic findings at unscheduled sacrifice consisted of stones found in the urinary bladder of 19/22 males and 3/9 females. Thickening of the mucosa in the urinary bladder was observed in 8/22 males and 2/9 females. This finding was often correlated with urothelial hyperplasia and/or interstitial oedema at the microscopic examination. Pelvic dilatation of the kidney was observed in 4/22 males, and was correlated with treatment-related pelvic dilatation at the microscopic examination. Lesions in the anogenital region were observed in 8/22 males. This finding was often correlated with chronic ulcerative dermatitis microscopically.

At the 18-month terminal sacrifice of the carcinogenicity phase of the study, terminal body weight was 4% lower in males than in the controls. There was no effect on organ weights. Treatment-related macroscopic findings consisted of stones found in the urinary bladder of 20/28 males and 14/41 females. Thickening of the mucosa in the urinary bladder was noted in 10/28 males and 7/41 females. At the microscopic examination, treatment-related effects were found in the urinary bladder, kidney, prostatic urethra, ureter, skin and bone marrow. In the urinary bladder, histological confirmation of the stones observed macroscopically was made with the males being more affected than females. The presence of the following various stone-induced findings, secondary to chronic irritation, was observed in both sexes:

- hyperplastic changes (simple and/or nodular/glandular urothelial hyperplasia),
- inflammatory changes (interstitial oedema, suburothelial and/or serosal mixed cell infiltrate, intramuscular inflammatory cell infiltrate and induced arteritis),
- focal/multifocal adenomyosis in a few treated males.

In the kidney, a higher incidence and severity of unilateral and/or bilateral pelvic dilatation in both sexes was observed. This finding was considered to be secondary to the stone-induced urinary obstruction. In the prostatic urethra, minimal to moderate urothelial hyperplasia was observed in males. In the ureter, simple urothelial hyperplasia was observed in 2/9 males. In the skin, a higher incidence of chronic ulcerative dermatitis was observed in males. Chronic ulcerative dermatitis is a spontaneous disease commonly observed in the C57BL/6J mouse. As this finding was located in the anogenital region or surrounding area, it was considered to be most likely related to a stone-induced dysuria and thus indirectly treatment-related. In the bone marrow, a higher incidence in both sexes and severity in males only of myeloid hyperplasia was observed. This finding was considered to be secondary to the chronic inflammation of the urinary bladder and chronic ulcerative dermatitis.

Neoplastic changes comprised of a low number of tumours of the transitional cell epithelium (papilloma and/or carcinoma) which were observed in the urinary bladder of 1/50 males and 3/49 females and in the prostatic urethra of 1/50 males. These finding were considered to be secondary to the chronic hyperplastic changes due to the presence of stones in the urinary bladder.

Analysis of urinary bladder stones taken from five high dose males at terminal sacrifice revealed that the composition of the stones consisted of approximately 70 to 75% of test material.

Given the findings at the high dose of 4000 ppm of increased mortality and decreased body weight, the maximum tolerated dose (MTD) was therefore considered to have been exceeded for both sexes at this dose level.

At 1000 and 200 ppm:

No treatment-related changes were observed in either sex.

In conclusion, dietary administration of BYH 18636 over an 18-month period to the C57BL/6J mouse, at dose levels up to 4000 ppm (equivalent to 599 mg/kg/day in males and 758 mg/kg/day in females) produced transitional cell epithelium tumours in the urinary bladder in one male and three females and

in the prostatic urethra in one male. The incidence of these tumours was very low and was considered to be secondary to the chronic hyperplastic changes resulting from chronic irritation due to the presence of stones in the urinary bladder. Thus, BYH 18636 was considered not to be directly carcinogenic to the mouse. In addition, this dose level was considered to have exceeded the maximum tolerated dose (MTD).
The No Observed Effect Level (NOEL) was 1000 ppm for both sexes (equivalent to 147 mg/kg/day in males and 185 mg/kg/day in females).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636
Description: White powder
Lot/Batch: Mix-batch 702-73-06-0001
Purity: 96.4% (analyzed in December, 2003); 96.2% (analyzed in May, 2004); 96.4% (analyzed in June, 2005)
CAS: 317815-83-1
Stability of test compound: Stable in rodent diet at 50 and 15 000 ppm over a 102-day period at ambient temperature or over a 92-day freezing period followed by 10 days at ambient temperature

2. Vehicle and /or positive control: None

3. Test animals:
Species: Mouse
Strain: C57BL/6 J
Age: 6 weeks approximately
Weight at dosing: 20.1-20.2g for male mean group weight
16.0-16.2g for female mean group weight
Source: Charles River Laboratories, St Germain sur L'Arbresle, France
Acclimation period: 15 days
Diet: Certified rodent powdered and irradiated diet A04C-10 P1 from S.A.F.E. (Scientific Animal Food and Engineering, Augy, France), *ad libitum* except during designated time period
Water: Tap water, *ad libitum* except during designated time period
Housing: Animals were caged individually in suspended stainless steel wire mesh cages. During the first week of acclimatization, the animals were housed by sex in groups of 3.
Environmental conditions –
Temperature: 22 ± 2°C
Humidity: 55 ± 15%
Air changes: Approximately 10 to 15 changes per hour
Photoperiod: Alternating 12-hour light and dark cycles (7 am- 7 pm)

B. STUDY DESIGN:

1. In life dates: March 31, 2004 to October 26, 2005 at Bayer CropScience, Sophia Antipolis, France

2. Animal assignment and treatment

On the day of randomization, animals were allocated to dose groups using a computerized randomization procedure that ensured a similar body weight distribution for each sex. The acceptable body weight range for each sex was ± 20% of the mean body weight on the day of

randomization. Any animal deemed unsuitable for selection based on weight, clinical findings or health status was not used for the study.
Animals were assigned to the test groups noted in the following table. Control animals received untreated diet. All other groups received the appropriate dietary concentrations at a constant (ppm) level.

Table IIA 5.5.3-1 Study design

Test group	Concentration in diet (ppm)	Dose per animal (week period 1 to 80)		Animals assigned/sex	
		Male (mg/kg bw/day)	Female (mg/kg bw/day)	Main study 78 weeks	Interim sacrifice 28 weeks
1	0	0	0	50	10
2	200	29.2	36.8	50	10
3	1000	147	185	50	10
4	4000	599	758	50	10

On Day 2 of the acclimatization phase, each animal was identified by a micro identification implant from Biomedic Data Systems (P.O. Box 217, 6660 AE Elst, Pascalweg 24, 6662 MX Elst, The Netherlands).

Dose selection: the dose levels were selected based on the results from a previous 90-day dietary study in the mouse (SA 03086), where dietary administration of up to 4000 ppm resulted in a urinary bladder calculus observed in one male, which was associated with a marked submucosal inflammatory cell infiltration, minimal diffuse urothelial inflammation and moderate diffuse urothelial hyperplasia of the urinary bladder.

3. Diet preparation and analysis

BYH 18636 was incorporated into the diet by dry mixing to provide the required concentrations. The test substance formulations were prepared to cover the dietary requirements over 8 weekly periods. When not in use, the diet formulations were stored at ambient temperature. The stability of the test substance in the diet had been demonstrated in the rat 90-day study, where BYH 18636 was found to be stable in the diet at 50 and 15000 ppm over a 102-day period at ambient temperature.
The homogeneity of the test substance in the diet was verified at each concentration on the first formulation (F1) and on formulation F6 at 200 and 4000 ppm, to demonstrate adequate formulation procedures. The results obtained from the homogeneity checks were taken as measured concentration. In addition, the concentration of test substance in the preparations at all dose levels was verified prior to administration to the animals for the following formulations: F3, and F9 and at the intermediate dose level of 1000 ppm on formulation F6. Homogeneity checks were between 93 to 99% of nominal concentration and concentration checks were between 91 to 97% of nominal concentration. Results were within the in-house target range of 85 to 115% of nominal concentration and were therefore considered to be acceptable.

Table IIA 5.5.3-2 Homogeneity and concentration of test material

	Nominal concentrations (ppm)	Analysed concentrations (ppm)	% of nominal concentration
Homogeneity (first preparation)	200	H1 193	97
		H2 189	95
		H3 193	97
		M1 193	97
		M2 190	95
		M3 189	95
		B1 185	93
		B2 192	96
		B3 194	97
	1 000	H1 972	97
		H2 959	96
		H3 955	96
		M1 955	96
		M2 958	96
		M3 965	97
		B1 967	97
		B2 969	97
		B3 961	96
	4 000	H1 3795	95
		H2 3860	97
		H3 3821	96
		M1 3846	96
		M2 3847	96
		M3 3862	97
		B1 3829	96
		B2 3732	93
		B3 3812	95
Homogeneity (sixth preparation)	200	H1 197	99
		H2 194	97
		H3 193	97
		M1 192	96
		M2 193	97
		M3 191	96
		B1 194	97
		B2 188	94
		B3 190	95
	4 000	H1 3816	95
		H2 3787	95
		H3 3866	97
		M1 3824	96
		M2 3813	95
		M3 3868	97
		B1 3838	96
		B2 3856	96
		B3 3694	92

H = surface; M = middle; B = bottom of the preparation; three samples were taken at each level (H, M, B) of the preparation.

	Nominal concentrations (ppm)	Analysed concentrations (ppm)	% of nominal concentration
Concentration (first formulation)	200 1000 4000	191 962 3823	95 96 96
Concentration (third formulation)	200 1000 4000	189 960 3668	95 96 92
Concentration (sixth formulation)	200 1000 4000	192 951 3818	96 95 95

4. Statistics

Means and standard deviations were calculated for each group and per time period. The Bartlett test was performed to compare the homogeneity of group variances.

- If the Bartlett test was not significant (p>0.05), means were compared using the analysis of variance (ANOVA), which was followed by Dunnett test (2-sided) if ANOVA indicated significance.

- If the Bartlett test was significant (p≤ 0.05) (for body weight change parameters, terminal body weight, absolute and relative organ weight parameters, haemoglobin concentration, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, %neutrophils, %lymphocytes), group means were compared using the non-parametric Kruskal-Wallis test, which was followed by the Dunn test (2-sided) if the Kruskal-Wallis test indicated significance.

- If the Bartlett test was significant (p≤ 0.05) (for body weight and average food consumption/day parameters), data were transformed using the log transformation. If the Bartlett test on log transformed data was not significant (p>0.05), means were compared using the ANOVA on log transformed data, which was followed by the Dunnett test (2-sided) on log transformed data if ANOVA indicated significance. If the Bartlett test was significant (p≤ 0.05) even after log transformation, group means were compared using the non-parametric Kruskal-Wallis test, which was followed by the Dunn test (2-sided), if the Kruskal-Wallis test indicated significance.

- If the Bartlett test was significant (p≤ 0.05) (for haematology parameters such as red blood cell count, platelet count, white blood cell count, neutrophil count, lymphocyte count), data were transformed using the square root transformation. If the Bartlett test on square root transformed data was not significant (p>0.05), means were compared using the ANOVA on square root transformed data, which was followed by the Dunnett test (2-sided) on square root transformed data, if ANOVA indicated significance.
- If the Bartlett test was significant (p≤ 0.05) even after square root transformation, group means were compared using the non-parametric Kruskal-Wallis which was followed by the the Dunn test (2-sided), if Kruskal-Wallis test indicated significance.

If one or more group variance(s) equaled 0, means were compared using non-parametric procedures. Group means were compared at the 5% and 1% levels of significance. Statistical analyses were carried out using Path/Tox System V4.2.2. (Module Enhanced Statistics).

For survival analysis:
Adjusted mortality rates were estimated using Kaplan-Meier estimation procedures. Kaplan-Meier estimates were calculated separately for each sex and treatment group. Mortalities which were the result of animals dying following accidents (accidental trauma) or at scheduled

sacrifice were considered to be censored observations.
Statistical significance of differences in survival rates between treated and control groups and dose-related trend in survival were assessed using Cox's and Tarone's tests on life table data. Probability values presented were two-sided for pairwise comparisons and trend test. Group mortality rates were compared at the 5% and 1% levels of significance. Survival analyses were performed on the carcinogenicity phase.

For neoplastic and non-neoplastic findings:
When the incidences of the 200 and 1000 ppm treated groups were equal to 0, only the 4000 ppm treated group was compared to the control group and no trend test was performed.
Not adjusted analyses:
Selected lesions were analyzed by Cochran-Armitage method for trend (1-sided) and the Fisher's exact test (1–sided) for control versus treatment comparisons. Cochran-Armitage trend significance was reported when there was existence of any monotone response in the incidence data (that is there was a relationship between the response and the dose represented by a regression line that was continually increasing (or decreasing), but perhaps not in a straight line).
Survival adjusted analyses:
Further survival adjusted analyses, considering any possible intercurrent mortality differences due to the competing toxicity among the treated groups, were performed on lesions. For non-palpable tumours, each tumour was categorized as fatal if the tumour was a factor contributing towards the death of the animal, incidental otherwise.
Incidental tumours and non-neoplastic lesions data were analyzed by logistic regression of tumour prevalence. Logistic regression analysis was based on the assumption that the diagnosed lesions were not directly responsible for the animal's death. Treated and control group lesion rates and dose-related trends were compared using the corrected score test. Fatal tumours were analyzed by the life-table test. The life-table test was based on the assumption that all lesions were fatal. Statistical significance of differences in incidences between treated and control groups and dose-related trends were investigated using Cox's and Tarone's tests.
Trend tests were conducted firstly including all groups. When both the trend test including all the dose levels and only the high dose group were significant, a second trend test excluding the high dose group was also done.

Group incidences were compared at the 5% and 1% levels of significance. All finding analyses were performed on the carcinogenicity phase.

C. METHODS:

1. Clinical signs and mortality

Animals were checked for mortality twice daily (once daily on weekends or public holidays). Observed clinical signs were recorded at least once daily for all animals. Detailed physical examinations including palpation for masses were performed weekly throughout the study.

2. Body weight

Each animal was weighed at least weekly during the acclimatization period then weekly for the first 13 weeks of study, approximately every 4 weeks thereafter and prior to necropsy. Body weight recorded prior to necropsy are referred to as terminal body weight.

3. Food consumption and compound intake

Food consumption was recorded weekly during the first 13 weeks of treatment, and once approximately every 4 weeks thereafter. The weekly mean achieved dosage intake in mg/kg body weight/day for Weeks 1 to 13, then 1 week per month thereafter was calculated as follows:

$$\text{Achieved dosage intake (mg/kg body weight/day)} = \frac{\text{Dose level (ppm) x Group mean food consumption (g/day) per week}}{\text{Group mean body weight (g) at end of week}}$$

The monthly and overall mean achieved dosage intake for the 18 months of treatment were derived from the weekly data.

4. Ophthalmological examination

Not evaluated in the present study. Not required for carcinogenicity studies based on Guideline 870.4200 & OECD 451.

5. Haematology and clinical chemistry

Blood was sampled from Isoflurane anaesthetized animals by puncture of the retro-orbital venous plexus after overnight fasting. Blood was collected in tubes containing EDTA (0.5 ml).
At terminal sacrifice, blood smears were prepared for all animals not sampled for haematology. When possible, a blood smear was prepared for the moribund animals, just before sacrifice.

a. Haematology

Haematology was performed on all the surviving animals of the interim sacrifice groups and on the first ten surviving animals of the terminal sacrifice groups on Weeks 29 or 30.
Haematology was performed on the first twenty surviving suitable mice of the terminal sacrifice groups on Weeks 53 or 54 and prior to necropsy on Week 79. The following parameters were measured: haematocrit, haemoglobin, leukocyte count, erythrocyte count, platelet count, leukocyte differential count, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular volume.

A blood smear was prepared and stained with Wright stain**.** It was examined only when the results of Advia 120 determinations were abnormal.
For moribund and terminal sacrificed animals, the blood smears were stained with Wright stain for possible differential white blood cell determination.

b. Clinical Chemistry

Not evaluated in the present study.
Not required for carcinogenicity studies based on Guideline 870.4200 & OECD 451.

c. Urinalysis

Not evaluated in the present study.
Not required for carcinogenicity studies based on Guideline 870.4200 & OECD 451.

6. Sacrifice and pathology

On study Days 202 to 204 (Weeks 29 to 30) for the 28-week interim kill, and on study Days 547 to 560 for the carcinogenicity phase, all surviving animals dedicated to the interim sacrifice group and carcinogenicity phase group, respectively, were sacrificed by exsanguination under deep anaesthesia (Isoflurane). Animals were fasted overnight prior to sacrifice.
All animals, including animals at scheduled sacrifice, found dead, or sacrificed during the course of the study, were necropsied. The necropsy included the examination of all major organs, tissues and body cavities. All significant macroscopic findings were recorded.

Adrenal gland, brain, epididymides, heart, kidney, liver, lung, ovary, spleen, testis, and uterus (including cervix) were weighed fresh at scheduled sacrifice only. Paired organs were weighed together.
The following organs or tissues were sampled: adrenal gland, aorta, articular surface (femoro-tibial), bone (sternum), bone marrow (sternum), brain, epididymis, oesophagus, exorbital (lachrymal) gland, eye and optic nerve, gall bladder, harderian gland, heart, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidney, larynx/pharynx, liver, lung, lymph nodes (submaxillary, mesenteric), mammary gland, nasal cavities, ovary, pancreas, pituitary gland, prostate gland, sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, submaxillary (salivary) gland, testis, thymus, thyroid gland (with parathyroid), tongue, trachea, urinary bladder, uterus (with cervix), vagina. Duplicate femoral bone marrow smear were prepared from sacrificed animals (except on weekends and public holidays), one of which was stained with May-Grünwald Giemsa, but not examined as no relevant changes were observed in haematology or bone marrow histology. The second smear was stored unstained for possible examination. Tissues samples were fixed by immersion in neutral buffered 10% formalin with the exception of the eye, optic nerve, Harderian gland, epididymis and testis that were fixed in Davidson's fixative.
In addition, urinary bladder stones were retained from five males at 4000 ppm at terminal necropsy and were analyzed for the presence of test material.
Histopathological examinations were performed on liver, kidney, urinary bladder and significant macroscopic findings from all the animals at the interim sacrifice. At the final sacrifice, histopathological examinations were performed on all organs and tissues embedded, including gross abnormalities, from all animals in all groups including decedents.

II. RESULTS AND DISCUSSION

A. Observations:

1. Mortality

There was no treatment-related effect on mortality rate in animals allocated to the 28-week interim sacrifice group. One male at 200 ppm was found dead on Study Day 61 and was noted to have a small thymus and a small spleen. One male at 1000 ppm was humanely killed on Study Day 167 with a wound on the back, and was noted to have an enlarged spleen and prominent mammary gland.

In animals allocated to the carcinogenicity phase of the study, the mortality incidence over 18 months was statistically significantly higher ($p \leq 0.05$) in males at 4000 ppm than in the corresponding control group. The increase was due to the number of males at 4000 ppm that were killed for humane reasons due to their poor condition, the majority of which (10/17) had skin lesions around the anogenital region on the day of sacrifice.

Table IIA 5.5.3-3: Mortality incidecence of animals of the carcinogenicity phase (unscheduled deaths)

Sex	Males				Females			
Dose level of BYH 18636 (ppm)	0	200	1000	4000	0	200	1000	4000
Number of animals	50	50	50	50	50	50	50	50
Killed for humane reasons	5 (10%)	7 (14%)	5 (10%)	17 (34%)	6 (12%)	11 (22%)	3 (6%)	9 (18%)
Found dead	6 (12%)	9 (18%)	6 (12%)	5 (10%)	0 (0%)	3 (6%)	1 (2%)	0 (0%)
Total number of unscheduled deaths	11 (22%)	16 (32%)	11 (22%)	22* (44%)	6 (12%)	14 (28%)	4 (8%)	9 (18%)

*: p≤ 0.05 Percentage mortality in parentheses.

2. Clinical signs of toxicity and neurotoxicity assessment

At 4000 ppm, treatment-related signs consisted of an increased incidence of generalized soiled fur in both sexes and an increased incidence of skin lesions, principally in the anogenital region, abnormal penis and wasted appearance in males only. Abnormal penis was not associated with any intrinsic histopathological findings, but was associated with chronic ulcerative dermatitis and/or an abscess in the preputial gland in the majority of cases at the microscopic examination.

The incidence of abnormal penis was marginally higher in males at 1000 and 200 ppm, however, in the absence of any other treatment-related clinical signs or corresponding histopathology findings, this finding was considered to be incidental at these two dose levels.

All other clinical observations were considered to be incidental.

Table IIA 5.5.3-4: Incidence of treatment-related clinical signs (first day of appearance)

Sex	Male				Females			
Dose level of BYH 18636 (ppm)	0	200	1000	4000	0	200	1000	4000
Group size	60	60	60	60	60	60	60	60
Skin lesion	5 (341)	5 (117)	6 (55)	13 (145)	2 (334)	5 (160)	2 (357)	4 (335)
Skin lesion – anogenital region	*2 (341)*	*3 (117)*	*3 (229)*	*11 (153)*	*0*	*0*	*0*	*0*
Soiled fur, generalized	2 (363)	1 (440)	0	16 (243)	0	0	1 (425)	7 (412)
Wasted appearance	0	1 (453)	2 (55)	7 (243)	3 (182)	3 (76)	1 (425)	1 (412)
Penis abnormal	12 (285)	17 (103)	20 (166)	23 (117)	-	-	-	-

B. BODY WEIGHT AND BODY WEIGHT GAIN:

At 4000 ppm in males there was a slight reduction of 3% in mean body weight on Study Day 176 which was statistically significant (p≤ 0.01). This trend continued for the remainder of the study, the effect being statistically significant (p≤ 0.05 or p≤ 0.01) at most time points resulting in a reduction of 5% in comparison with the controls at the end of the study on Study Day 540. Mean cumulative body weight gain was statistically significantly reduced (p≤ 0.05 or p≤ 0.01) throughout most of the study, resulting in an overall mean cumulative body weight gain reduction of 15% by Study Day 540, when compared with the controls.
Mean body weight and mean cumulative body weight gain were comparable to the controls at 4000 ppm in females and in both sexes at 1000 and 200 ppm.

Table IIA 5.5.3-5: Mean body weights (BW) and cumulative body weight gains (BWG) (g)

Dose level of BYH 18636 (ppm)	0	200	1000	4000
Males				
Initial BW (Day 1) (%C)	**20.1**	**20.2 (100)**	**20.2 (100)**	**20.2 (100)**
BW Week 1 (Day 8) (%C)	21.3	21.1 (99)	21.4 (100)	21.2 (100)
BW Week 13 (Day 92) (%C)	26.7	27.2** (102)	26.5 (99)	26.4 (99)
BW Week 25 (Day 176) (%C)	28.7	28.7 (100)	28.1* (98)	27.9** (97)

BW Week 53 (Day 372) (%C)	30.1	30.3 (101)	30.1 (100)	28.8** (96)
Final BW Week 77 (Day 540) (%C)	**31.7**	**32.9* (104)**	**31.9 (101)**	**30.2** (95)**
BWG Weeks 1-3 (Days 1 to 22) (%C)	**2.8**	**2.8 (100)**	**2.6 (93)**	**2.7 (96)**
BWG Weeks 4-13 (Days 22 to 92) (%C)	3.8	4.2** (111)	3.7 (97)	3.5* (92)
BWG Weeks 14-25 (Days 92 to 176) (%C)	2.1	1.5** (71)	1.6** (76)	1.5** (71)
BWG Weeks 26-53 (Days 176 to 372) (%C)	1.4	1.6 (114)	1.9 (136)	0.9 (64)
BWG Weeks 54-77 (Days 372 to 540) (%C)	1.4	2.5** (179)	1.8 (129)	1.4 (100)
Overall BWG (Days 1 to 540) (%C)	**11.7**	**12.6* (108)**	**11.7 (100)**	**10.0** (85)**
Females				
Initial BW (Day 1) (%C)	**16.2**	**16.1 (99)**	**16.0 (99)**	**16.1 (99)**
BW Week 1 (Day 8) (%C)	17.2	17.3 (101)	17.3 (101)	17.1 (99)
BW Week 13 (Day 92) (%C)	21.9	22.1 (101)	22.1 (101)	21.9 (100)
BW Week 25 (Day 176) (%C)	23.0	23.3 (101)	23.4 (102)	23.1 (100)
BW Week 53 (Day 372) (%C)	25.5	25.9 (102)	26.2 (103)	25.2 (99)
Final BW Week 77 (Day 540) (%C)	**26.5**	**26.7 (101)**	**26.3 (99)**	**26.0 (98)**
BWG Weeks 1-3 (Days 1 to 22) (%C)	**3.0**	**2.9 (97)**	**2.9 (97)**	**2.7 (90)**
BWG Weeks 4-13 (Days 22 to 92) (%C)	2.8	3.0 (107)	3.2** (114)	3.2** (114)
BWG Weeks 14-25 (Days 92 to 176) (%C)	1.1	1.2 (109)	1.3 (118)	1.2 (109)
BWG Weeks 26-53 (Days 176 to 372) (%C)	2.5	2.5 (100)	2.8 (112)	2.0 (80)
BWG Weeks 54-77 (Days 372 to 540) (%C)	1.1	0.5 (45)	0.3 (27)	1.0 (91)
Overall BWG (Days 1 to 540) (%C)	**10.4**	**10.3 (99)**	**10.3 (99)**	**10.0 (96)**

%C: % *vs* control.
* Statistically different ($p \leq 0.05$) from the control. ** Statistically different ($p \leq 0.01$) from the control.

D. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. Food consumption

Food consumption was not affected by treatment at any dose level in either sex, throughout the course of the study.

2. Achieved dosage

Table IIA 5.5.3-6: Mean achieved dietary intake of BYH 18636 (mg/kg/day)

Sex	Males			Females		
Dose level of BYH 18636 (ppm)	200	1000	4000	200	1000	4000
Weeks 1-13	34.8	177	704	44.1	219	891
Weeks 1-52	31.3	158	638	39.9	199	816
Weeks 1-80	29.2	147	599	36.8	185	758

3. Food efficiency

Not evaluated in this study.

E. OPHTHALMOSCOPIC EXAMINATION:

Not evaluated in this study.

F. BLOOD ANALYSES:

1. Haematological findings

After BYH 18636 administration up to 4000 ppm, no relevant treatment-related change was noted.

In view of their incidental occurrence and/or their low amplitude, the few statistically significant differences observed throughout the study were considered to be incidental and not treatment-related.

2. Clinical chemistry findings

Not evaluated in this study.

G. URINALYSIS:

Not evaluated in this study.

H. SACRIFICE AND PATHOLOGY:

1. Organ weight – 28-week interim sacrifice

There was no relevant change in terminal body weight of treated animals when compared to control animals. There were no treatment-related changes in organ weights.

2. Organ weight – 18-month carcinogenicity phase

At 4000 ppm in males, mean terminal body weight was lower and statistically different (-4%, p≤ 0.01) when compared to controls.

Table IIA 5.5.2-7: Mean terminal body weight changes at final sacrifice (% change when compared to controls)

Sex	Males				Females			
Dose level of BYH 18636 (ppm)	0	200	1000	4000	0	200	1000	4000
Mean terminal body weight (g)	27.5	27.7 NC	27.5 NC	26.3** (- 4 %)	23.0	23.6 NC	23.1 NC	22.9 NC

NC: no relevant change, *: p≤ 0.05; **: p≤ 0.01

There were no treatment-related changes in organ weights at any dose level.

3. Gross pathology – 28-week interim sacrifice

a. Unscheduled deaths:

Two animals died prematurely prior to the scheduled 28-week interim sacrifice.
One male at 200 ppm was found dead on Study Day 61, with a small thymus and a small spleen. Another male at 1000 ppm was killed for humane reasons on Study Day 167, with a wound on the back, an enlarged spleen and prominent mammary gland.

b. Terminal sacrifice:
All gross pathology changes were considered as incidental and not treatment-related.

4. Gross pathology – 18-month carcinogenicity phase

a. Unscheduled deaths:
Ninety-three animals died or were killed prematurely before the end of the study. A statistically significantly higher incidence of unscheduled deaths was observed in males at 4000 ppm. This increase was attributable to the high number of males at 4000 ppm (17/22) which were killed for humane reasons, the majority of these animals had skin lesions in the anogenital region on the day of sacrifice.

Stones were found in the urinary bladder of 19/22 males and 3/9 females at 4000 ppm. The stones were often multiple (0.1 to 0.6 cm in diameter), yellow (mainly), greenish or white and firm. Thickened mucosa was observed in 8/22 males and 2/9 females at 4000 ppm. This finding was often correlated with urothelial hyperplasia and/or interstitial oedema at histopathology and was considered to be treatment-related.

Table IIA 5.5.3-8: Incidence of macroscopic changes in the urinary bladder, unscheduled sacrifice of the carcinogenicity phase

Sex	Males				Females			
Dose level of BYH 18636 (ppm)	0	200	1000	4000	0	200	1000	4000
Stones	1/11	0/16	0/11	19/22	0/6	0/14	0/4	3/9
Thickened mucosa	0/11	0/16	0/11	8/22	0/6	0/14	0/4	2/9

Pelvic dilatation of the kidney was observed in 4/22 males at 4000 ppm. This finding was correlated with treatment-related pelvic dilatation at histopathology.

Table IIA 5.5.3-9: Incidence of macroscopic changes in the kidney, unscheduled sacrifice of the carcinogenicity phase

Sex	Males				Females			
Dose level of BYH 18636 (ppm)	0	200	1000	4000	0	200	1000	4000
Pelvic dilatation	0/11	0/16	0/11	4/22	0/6	1/14	0/4	0/9

At 4000 ppm, lesions were observed in the skin of 8/22 males compared with no cases in the controls. The localization was always in the anogenital region. This finding was often correlated with chronic ulcerative dermatitis at histopathology.

At 4000 ppm, higher incidences of dark liver (10/22 males), pale kidney (6/22 males) and dilatation of ureter(s) (5/22 males) were observed at necropsy. As these gross observations

were not correlated with relevant histopathological findings, they were considered to be incidental and not treatment-related.

b. Terminal sacrifice:

Stones were found in the urinary bladder of 20/28 males and 14/41 females at 4000 ppm. The stones were single (0.3 to 1 cm in diameter) or multiple (0.2 to 0.8 cm in diameter), greenish (mainly), yellow or white and firm. Thickened mucosa was observed in 7/41 females and 10/28 males.

Table IIA 5.5.3-10: Incidence of macroscopic changes in the urinary bladder, scheduled sacrifice of the carcinogenicity phase

Sex	Males				Females			
Dose level of BYH 18636 (ppm)	0	200	1000	4000	0	200	1000	4000
Stones	0/39	0/34	0/39	20/28	0/44	0/36	0/46	14/41
Thickened mucosa	0/39	0/34	0/39	10/28	0/44	0/36	0/46	7/41

All other macroscopic changes were considered to be incidental and not treatment-related.

5. Microscopic pathology – 28-week interim sacrifice

a. Unscheduled deaths:

All the microscopic findings observed were considered as incidental and the unscheduled deaths were considered not to be treatment-related.

b. Terminal sacrifice:

All the microscopic findings observed were considered as incidental and not to be treatment-related.

6. Microscopic pathology – 18-month carcinogenicity phase

a. Unscheduled deaths:

The presence of urinary stone(s) was the cause of death for 3/22 males and 2/9 females at 4000 ppm and was considered to be treatment-related.

A higher incidence of males killed for humane reasons due to chronic ulcerative dermatitis in the anogenital region was observed at 4000 ppm, this condition was considered to be treatment-related.

Table IIA 5.5.3-11: Incidence of factor(s) related to death, unscheduled sacrifice of the carcinogenicity phase

Sex	Males				Females			
Dose level of BYH 18636 (ppm)	0	200	1000	4000	0	200	1000	4000
Urinary stone(s)	0/11	0/16	0/11	3/22	0/6	0/14	0/4	2/9

Chronic ulcerative dermatitis	1/11	5/16	2/11	9/22	1/6	3/14	0/4	0/9

b. Terminal sacrifice:

Treatment-related effects of BYH 18636 were found in the urinary tract (urinary bladder, prostatic urethra and ureter), kidney, skin and bone marrow.

Non-neoplastic findings (all animals)

In the urinary bladder, histological confirmation of the macroscopic stones was made in both sexes at 4000 ppm. The males were more affected than females. The stones were large, round to oval-shaped structures made of concentric eosinophilic lamellae, often embedded in the neck of the urinary bladder.
The presence of various stone-induced findings, secondary to chronic irritation, was observed in both sexes at 4000 ppm:

- hyperplastic changes (simple and/or nodular/glandular urothelial hyperplasia),
- inflammatory changes (interstitial oedema, suburothelial and/or serosal mixed cell infiltrate, intramuscular inflammatory cell infiltrate and induced arteritis),
- focal/multifocal adenomyosis in a few treated males.

Table IIA 5.5.3-12: Incidence and severity of microscopic changes in the urinary bladder, all animals, carcinogenicity phase

Sex	Males				Females			
Dose level of BYH 18636 (ppm)	0	200	1000	4000	0	200	1000	4000
Number of animals	49	49	50	50	48	49	47	49
Stone(s): intraluminal								
Minimal	0	0	0	6	0	0	0	2
Slight	0	0	0	1	0	0	0	3
Moderate	0	0	0	13	0	0	0	2
Marked	0	0	0	11	0	0	0	5
Severe	0	0	0	1	0	0	0	3
Total	**0**	**0**	**0**	**32****	**0**	**0**	**0**	**15****
Stone(s): only noted at necropsy	**1**	**0**	**0**	**9***	**0**	**0**	**0**	**5***
Total incidence of animals with stones	**1**	**0**	**0**	**41****	**0**	**0**	**0**	**20****
Urothelial hyperplasia: simple: multifocal/diffuse								
Minimal	0	0	1	14	0	0	0	10
Slight	0	0	0	22	0	0	0	10
Moderate	0	0	0	2	0	0	0	0
Total	**0**	**0**	**1**	**38****	**0**	**0**	**0**	**20****
Urothelial hyperplasia: nodular/glandular: multifocal/diffuse								
Minimal	0	0	0	12	0	0	0	2
Slight	0	0	0	9	0	0	0	4
Moderate	0	0	0	2	0	0	0	6
Marked	0	0	0	0	0	0	0	1
Total	**0**	**0**	**0**	**23****	**0**	**0**	**0**	**13****
Interstitial oedema: diffuse								
Minimal	0	0	0	16	0	0	0	8
Slight	0	0	0	12	0	0	0	5
Moderate	0	0	0	6	0	0	0	0
Total	**0**	**0**	**0**	**34****	**0**	**0**	**0**	**13****
Suburothelial mixed cell infiltrate: focal/multifocal								
Minimal	1	0	0	21	1	0	0	7
Slight	0	0	0	18	0	0	0	9
Moderate	0	0	0	2	0	0	0	3
Total	**1**	**0**	**0**	**41****	**1**	**0**	**0**	**19****
Intramuscular inflammatory cell infiltrate: focal/multifocal								
Minimal	0	0	0	24	0	0	0	12
Slight	0	0	0	10	0	0	0	7
Total	**0**	**0**	**0**	**34****	**0**	**0**	**0**	**19****
Serosal mixed cell infiltrate: focal/multifocal								
Minimal	0	0	0	3	0	0	0	5
Slight	0	0	0	3	0	0	0	1
Total	**0**	**0**	**0**	**6***	**0**	**0**	**0**	**6***
Induced arteritis								
Minimal	0	0	0	4	0	0	0	3
Total	**0**	**0**	**0**	**4**	**0**	**0**	**0**	**3**
Adenomyosis: focal/multifocal								
Minimal	0	0	0	1	0	0	0	0
Slight	0	0	0	1	0	0	0	0
Total	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**

*: $p \leq 0.05$; **: $p \leq 0.01$

In the kidney, a higher incidence and severity of unilateral and/or bilateral pelvic

dilatation in both sexes was observed at 4000 ppm. This finding was considered to be secondary to the stone-induced urinary obstruction.

Table IIA 5.5.3-13: Incidence and severity of microscopic changes in the kidney, all animals, carcinogenicity phase

Sex	Males				Females			
Dose level of BYH 18636 (ppm)	0	200	1000	4000	0	200	1000	4000
Number of animals	50	50	50	50	50	50	49	50
Pelvic dilatation: unilateral								
Minimal	0	0	0	5	0	0	1	3
Slight	1	0	0	3	1	1	0	1
Moderate	0	0	0	0	0	0	0	1
Marked	0	0	0	0	0	0	1	0
Severe	0	0	1	0	0	0	0	1
Total	**1**	**0**	**1**	**8***	**1**	**1**	**2**	**6**
Pelvic dilatation: bilateral								
Minimal	0	1	0	3	0	0	0	2
Slight	0	0	0	4	0	0	0	1
Total	**0**	**1**	**0**	**7***	**0**	**0**	**0**	**3**
Pelvic dilatation: unilateral/bilateral								
Minimal	0	1	0	8	0	0	1	5
Slight	1	0	0	7	1	1	0	2
Moderate	0	0	0	0	0	0	0	1
Marked	0	0	0	0	0	0	1	0
Severe	0	0	1	0	0	0	0	1
Total	**1**	**1**	**1**	**15****	**1**	**1**	**2**	**9****

*: $p \leq 0.05$; **: $p \leq 0.01$

In the prostatic urethra, minimal to moderate urothelial hyperplasia was observed at 4000 ppm in males and was considered to be treatment-related.

Table IIA 5.5.3-14: Incidence and severity of microscopic changes in the urethra (prostate), all males, carcinogenicity phase

Sex	Males			
Dose level of BYH 18636 (ppm)	0	200	1000	4000
Number of animals	49	50	48	50
Urothelial hyperplasia: urethra				
Minimal	0	0	0	3
Slight	0	0	0	1
Moderate	0	0	0	1
Total	**0**	**0**	**0**	**5***

*: $p \leq 0.05$

In the ureter (preserved when macroscopic findings were present at necropsy), simple urothelial hyperplasia was observed in 2/9 males at 4000 ppm and was considered to be treatment-related.

In the skin, a statistically significantly higher incidence of chronic ulcerative dermatitis was observed in males at 4000 ppm. Chronic ulcerative dermatitis is a spontaneous disease commonly observed in the C57BL/6J mouse. As this finding was located in the anogenital region or surrounding area, it was considered to be probably related to a stone-induced dysuria and thus indirectly treatment-related.

Table IIA 5.5.3-15: Incidence and severity of microscopic changes in the skin, all animals, carcinogenicity phase

Sex	Males				Females			
Dose level of BYH 18636 (ppm)	0	200	1000	4000	0	200	1000	4000
Number of animals	49	48	49	48	48	50	48	47
Chronic ulcerative dermatitis								
Slight	0	0	1	0	1	1	0	0
Moderate	0	2	1	7	0	2	0	0
Marked	1	3	1	3	0	0	0	0
Total	**1**	**5**	**3**	**10***	**1**	**3**	**0**	**0**

*: $p \leq 0.05$

In the bone marrow, a higher incidence and severity (males only) of myeloid hyperplasia was observed in both sexes at 4000 ppm. This finding was considered to be secondary to the chronic inflammation of the urinary bladder and chronic ulcerative dermatitis.

Table IIA 5.5.3-16: Incidence and severity of microscopic changes in the bone marrow, sternum, all animals, carcinogenicity phase

Sex	Males				Females			
Dose level of BYH 18636 (ppm)	0	200	1000	4000	0	200	1000	4000
Number of animals	50	50	50	50	50	50	49	50
Myeloid hyperplasia: diffuse								
Minimal	2	1	1	9	3	3	4	7
Slight	1	2	0	2	2	0	0	2
Moderate	1	2	0	6	0	0	0	0
Marked	0	1	0	0	0	0	0	0
Total	**4**	**6**	**1**	**17***	**5**	**3**	**4**	**9**

*: $p \leq 0.05$

All other non neoplastic findings were those commonly observed in this strain and age of mouse kept under monitored environmental conditions and were considered to be incidental in origin.

Neoplastic findings (all animals)

At 4000 ppm, tumours of the transitional cell epithelium (papilloma and/or carcinoma) were observed in the urinary bladder of both sexes and the prostatic urethra in males, and were considered to be treatment-related. The incidence of these tumours was very low and was considered to be secondary to the chronic hyperplastic changes.

Table IIA 5.5.3-17: Incidence of neoplastic microscopic changes in the urinary bladder, all animals, carcinogenicity phase

Sex	Males				Females			
Dose level of BYH 18636 (ppm)	0	200	1000	4000	0	200	1000	4000
Number of animals	49	49	50	50	48	49	47	49
M-Transitional cell carcinoma	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
B-Transitional cell papilloma	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**2**
Total number of Transitional cell tumours	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**3**

Table IIA 5.5.3-18: Incidence of neoplastic microscopic changes in the urethra (prostate), all animals, carcinogenicity phase

INCIDENCE OF NEOPLASTIC MICROSCOPIC CHANGES IN THE URETHRA (PROSTATE), ALL ANIMALS, CARCINOGENICITY PHASE				
Sex	Males			
Dose level of BYH 18636 (ppm)	0	200	1000	4000
Number of animals	49	50	48	50
M-Urethral transitional cell carcinoma	**0**	**0**	**0**	**1**

The other tumours in treated animals were those commonly observed in this strain and age of mouse kept under monitored environmental conditions and were considered to be incidental in origin.

III. CONCLUSION

In conclusion, dietary administration of BYH 18636 over an 18-month period to the C57BL/6J mouse, at dose levels up to 4000 ppm (equivalent to 599 mg/kg/day in males and 758 mg/kg/day in females) produced transitional cell epithelium tumours in the urinary bladder in one male and three females and in the prostatic urethra in one male. The incidence of these tumours was very low and was considered to be secondary to the chronic hyperplastic changes resulting from chronic irritation due to the presence of stones in the urinary bladder. Thus, BYH 18636 was considered not to be directly carcinogenic to the mouse. In addition, this dose level was considered to have exceeded the maximum tolerated dose (MTD).

The No Observed Effect Level (NOEL) was 1000 ppm for both sexes (equating to 147 mg/kg/day in males and 185 mg/kg/day in females).

(Wason S., 2006)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable The study (SA04062) is fully compliant with OECD 451 Mortality of top dose males was slightly higher than the other groups as a consequence of treatment. However the survival of groups of animals in this study exceeded 50% and is therefore considered to be adequate for the assessment of carcinogenicity. Treatment-related findings were confined to the top dose level in both sexes. The urinary bladder and kidney were identified as the target organs of toxicity, with secondary findings in the skin. Incidences of these findings at the next lowest dose level of 1000 ppm are either within the concurrent control range or are only marginally increased. The tumorigenesis in the transitional epithelium is considered to be treatment-related and clearly secondary to the hyperplasia of this tissue associated with the presence of urinary stones. The tumours occur with low incidence but are not seen in any other groups, are not reported in the background range and are therefore considered to be related to treatment.
Conclusions	Evidence of carcinogenicity was seen in both sexes at the high dose level of 4000 ppm, equivalent to a mean intake of 599 and 758 mg/kg bw/d in males and females respectively. A NOAEL of 1000 ppm (equivalent to mean intakes of 147 and 185 mg/kg bw/d in males and females respectively) can be determined for this study based on the findings at the top dose level. A NOAEL for carcinogenicity of 1000 ppm can also be determined, based on the incidences of benign and malignant transitional cell tumours seen in both sexes at the top dose level. The NOAELs for this study are therefore the same as proposed by the Notifier.

Mechanism of action and supporting data (IIA 5.5.4)

(This text has been copied from the Applicant's dossier)

All the effects seen in the urinary tract (combination of hyperplastic and inflammatory changes) following BYH 18636 administration to rodents and dogs were considered to be due to a single and chronic irritative mechanism, resulting from the formation of stones (which were observed on a number of animals). These hyperplastic changes lead to transitional cell epithelium tumours in the urinary bladder in one male and three females and in the prostatic urethra in one male in the mouse oncogenicity study. These tumours were only observed at the highest dose and at very low incidence.

It is well established by a number of rodent bioassays that a major consequence of a foreign body in the bladder lumen is abrasion of the mucosa surface resulting in erosion and ulceration. This frequently is accompanied by an acute inflammatory reaction, but is always accompanied by marked regenerative hyperplasia *(DeSesso, I.M., 1989,-Confounding factors in direct bladder exposure studies-, Comments Toxicol., 3, 317-334*). Interestingly, the formation of proliferative lesions produced by these foreign materials are reversible until the actual appearance of carcinomas. This was demonstrated following the administration of uracil in rodents (*Shirai T., Fukushima S., Tagawa Y., Okumura M. & Ito N., 1989, -Cell proliferation induced by Uracil-calculi and subsequent development of reversible papillomatosis in the rat urinary bladder-, Cancer Res., 49, 378-383*). The major factor, related to calculus formation is the dose of administration of the chemical (*Cohen S.M. & Ellwein L.B., 1992, -Risk assessment based on high-dose animal exposure experiments-, Chem. Res.*

Toxicol., 5, 742-748). An adequate amount of the chemical needs to be administered to generate a sufficently high concentration in the urine to lead to precipitation and calculus formation. The dose effect is the greatest fundamental factor in interpreting the results of these rodent bioassays for extrapolation to potential carcinogenic hazard in humans. Calculi in humans are also associated with slightly increased risk of bladder tumour development, although humans appear to be considerably less sensitive than rodents. This is probably related to the upright stature of humans. Formation of a calculus may lead to obstruction of the urinary tract. This is associated with either eventual passage of the calculus or removal by a urologist. Thus, exposure is relatively brief (*Burin G.J., Gibb H.J. & Hill R.N.,1995, -Human Bladder Cancer: Evidence for a potential irritation-induced mechanism-, Fd Chem. Toxic., 33, 785-795 ; Cohen.S.M. & Lawson T.A., 1995, Rodent bladder tumors do not always predict for humans-, Cancer Letters, 93, 9-16*).

When considering the potential carcinogenic hazard to humans, calculus formation is the only potentially carcinogenic mechanism involved with BYH 18636, as the battery of genotoxicity testing has clearly demonstrated that BYH 18636 is not a genotoxic compound. Calculus formation is clearly a threshold phenomenon, with a certain minimun concentration of chemical required for the formation of the calculus. If this concentration is not attained, calculi do not form and no consequent regenerative or tumourigenic potential can be realized. A very low incidence of tumours has been observed in mice only at high doses. Humans will be never exposed at such high doses. When considering the highest TMDI of 0.005358 mg/kg/day found in infants, there is a margin of safety of 27 435 fold over the non-tumourigenic dose level of 147 mg/kg/day obtained in the mouse oncogenicity study. Therefore, it can be considered that BYH 18636 presents no oncogenic hazard to humans.

B.6.6 Reproductive and developmental toxicity studies (IIA 5.6)

B.6.6.1 Multi-generation study in the rat (IIA 5.6.1)

Two-generation reproductive toxicity in the rat (IIA 5.6.1)

Report:	**KIIA 5.6.1/01, Eiben R., 2006 (MRID # 47070132)**
Title:	BYH 18636 – Two-generation Reproduction Study in Wistar Rats by Administration in the Diet
Report No & Document No	AT 03180 M-275585-01-2
Guidelines:	OECD 416 (2001); EPA Health Effects Test Guideline (OPPTS 870.3800; 1998); M.A.F.F. in Japan notification 12 Nousan N°8147 (2000) guidelines.
GLP	Yes (certified laboratory)

Executive Summary:

BYH 18636 (Batch 702-73-06-0001, 96.4% of purity) was administered via the diet to Wistar rats over two generations to evaluate its potential effects on the entire reproductive process. There were 25 male and 25 female parental rats per group per generation. The dietary concentrations of BYH 18636 were: 0 (control), 500, 2500, or 10000 ppm. The achieved doses in mg/kg/day, as calculated during the pre-mating phase, were:

F0: 46.0, 245.0, 945.8 mg/kg in males and 55.6, 263.7 or 968.4 mg/kg in females.

F1: 50.2, 260.5 or 992.1 mg/kg in males and 68.0, 353.1 or 1284.0 mg/kg in females.

Test diets were fed continuously to male and female rats in the F0 parental generation throughout premating, mating, gestation, and lactation periods. After weaning of the F1 generation at 4 weeks of age, selected weanlings were maintained in their same dietary groups through maturation, mating, gestation, and lactation. The final necropsy took place when the F2 offspring were weaned at 4 weeks of age.

Body weights, food consumption and clinical signs were monitored regularly. Reproductive parameters such as mating performance, fertility, duration of pregnancy, oestrus cycling and sperm analyses were examined in F0 and F1 rats. Litter parameters such as litter size, gender ratio, pup weight, pup viability, body weight gain and clinical signs were studied in F1 and F2 offspring.

Developmental milestones were evaluated in F1 weanlings. At necropsy, implantation sites in F0 and F1 females were recorded. Selected organs were weighed in adult rats and weanlings and histopathology including ovarian follicle staging (only F1) was performed in a number of organs of F0 and F1 parental rats.

At 10000 ppm:
Treatment-related clinical findings such as tilted head, sunken flanks, emaciation and/or increased urination were noted in a few animals. One high dose F1 male died as a result of treatment. The body weights of F1 females were depressed towards the end of lactation. No treatment effect on the food intake of the parental rats was seen. At necropsy the occurrence of stone/s, pelvic dilation/s and/or thickening/s was observed in the kidneys, ureters (females only) and urinary bladder. These findings were histopathologically correlated with several degenerative and reactive lesions in these organs and the urethra mainly as a consequence of urolithiasis. Higher absolute and relative weights of the kidneys were seen in F0 and F1 females than at 0 ppm. In F0 males the periportal fat accumulation in the liver was slightly decreased. Furthermore, F1 males exhibited reduced absolute and relative liver weights without histopathology correlates.

Examinations on sperm and oestrus cycling as well as histopathology on ovarian follicles, corpora lutea and other reproductive organs revealed no substance-related effects. The parameters of reproductive performance (insemination, fertility, gestation and rearing indices as well as gestation length) were not influenced by BYH 18636.
The litter data such as mean number of implantations and prenatal loss, live birth, viability and lactation indices, sex distribution, number of pups born, and litter size were not affected by the test substance. No treatment-related clinical findings were seen in F1 and F2 pups. There were no effects on pup body weight or developmental milestones. At pup or weanling necropsies slightly more F2 pups without a milk spot were seen as well as more F1 and F2 weanlings exhibited dilated and/or enlarged kidneys. There were stones in the kidneys and urinary bladder of few F2 weanlings.

At 2500 ppm:
Treatment-related findings were limited to dilated and/or stones in the ureters of one female from the F0 generation.

At 500 ppm:
No treatment-related findings were observed.

Thus, under the conditions described the NOAEL for parental reproductive parameters was 10000 ppm.
The parental systemic LOAEL in rats was 2500 ppm (corresponding to 245 mg/kg bw/day in males and 264 mg/kg bw/day in females), and the parental systemic NOAEL in rats was 500 ppm (46 mg/kg bw/day in males, 56 mg/kg bw/day in females), based on occurrence of dilation/s and stone/s in the ureter of an F0 female.
The offspring NOAEL (=NOEL) was at 2500 ppm (270 mg/kg bw/day), based on clinical signs and macroscopic findings in the kidney and the urinary bladder of F1 and /or F2 pups.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636

Description:	white powder
Lot/Batch:	702-73-06-0001
Purity:	96.4 %
CAS:	317815-83-1
Stability of test compound:	Stable in dietary formulations at concentrations of 10 and 7000 ppm for a period of 14/15 days at animal room temperature or after freezer storage.

2. Vehicle and /or positive control: None

3. Test animals:

Species:	Rat
Strain:	Wistar Crl: (WI) WU BR
Age:	F0: 5 to 6 weeks; F1: 4 weeks
Weight at dosing:	F0 Males: 99-129 g ; F0 Females: 105- 136 g F1 Males: 71-119 g ; F1 Females: 57-109 g
Source:	Charles River GmbH, 97633 Sulzfeld, Germany
Acclimation period:	About one week.
Diet:	Kliba 3883.9.25 *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Singly except when co-housed for matings or with litters in Makrolon cages Type IIIh on low-dust soft-wood shavings or nesting material (day 20 p.c. to 14 p.p.).
Environmental conditions –	
Temperature:	23 ± 2°C
Humidity:	55 ± 5%
Air changes:	At least 10 changes per hour
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN:

1. In life dates: July 5, 2004 to April 18, 2005 at Bayer Healthcare AG.

2. Animal assignment and treatment

The F0 animals (25 males and 25 females per group) were randomly allocated to the individual study groups, then were pre-treated with the compound for about 10 weeks before they were mated. Within the weeks 8 – 10 of the pre-mating period examinations on œstrus cycle were performed. Then the mating period of 3 weeks followed.

Mating procedure: the first F0 or F1 male was caged overnight with the first F0 or F1 female from the same test group and so on or a maximum of 12 times during the three-week mating period until sperm were observed in vaginal smears taken the next morning. Inseminated females were not further co-housed. F0 and/or F1 females found sperm-positive after the first mating day but not shown to be pregnant (lack of weight gain within 14 days following insemination) were co-housed again over one week with the same male without checking insemination or measuring body weight and food intake during possible further pregnancy.

After a gestation period of about 22 days litters were born and four days after birth the F1 litters were reduced to eight pups (of equal gender ratio where possible). Pups found in a moribund state on day 4 were excluded from lactation. This was done to investigate possible malformations and to prevent cannibalism during the further rearing period. The selected F1 pups were reared to an age of four weeks. At weaning, one male and one female per litter were chosen for further treatment and to produce the F2 generation. Weanlings not chosen, as well as F0 females were necropsied. F0 males were necropsied after the mating period partly in the course of spermatological investigations.

The weaned F1 offspring were treated further with the compound for at least 10 weeks (including a three-week period for oestrus cycle determination) and then co-housed for mating. The procedures during the mating, pregnancy and lactation period of F1 rats were the same as described for F0 rats. The F1 parental animals were killed as scheduled after their F2 litters had been weaned at about day 28 post partum (p.p.) as described for F0 rats.

3. Test substance dosage formulations and analysis

Dose selection was based on results of a one-generation pilot study where rats received 0, 1000, 5000 and 10000 ppm in the diet.
At 5000 and 10000 ppm F0 female body weight gain was decreased during gestation and lactation. 10000 ppm females consumed more food and water during lactation than controls. At 10000 ppm the number of implantation sites and the total number of pups born were reduced and pup weights were slightly reduced from day 14 p.p. onwards.
Thus, in that study the NOEL for parental animals was 10000 ppm in males and 1000 ppm in females. The NOAEL for reproductive parameters was 5000 ppm.
Therefore, for the full 2-generation reproduction study, doses were 0, 500, 2500 and 10000 ppm.

Table 5.6.1-1 Study design and animal assignement

Test group	Dose in diet [a] (ppm)	Animals/group			
		F0 Males	F0 Females	F1 Males	F1 Females
Control	0	25	25	25	25
Low (LDT)	500	25	25	25	25
Mid (MDT)	2500	25	25	25	25
High (HDT)	10000	25	25	25	25

a Diets were administered from beginning of the study until sacrifice

Dietary formulations were prepared at least weekly by mixing appropriate amounts of test substance with diet using a pre-mixture. A mixing granulator was used. The formulations were used at room temperature. Prior to the start of the study, stability and homogeneity of the test substance in the same diet as used in the study were evaluated. Dietary levels ranged between 10 and 10,000 ppm and the content as a % of nominal values ranged from 90 to 114% for homogeneity as well as stability over 14/15 days at room temperature or in the freezer. The test substance concentration in 10 ppm mixtures was within the range of ± 20% of their initially measured concentration after storage at animal room temperature for up to 15 days. Values were 105 - 109 % of nominal concentration. The test substance concentration in 10000 ppm mixtures was within the range of ± 20% of the initially measured concentration after storage at animal room temperature for up to 14 days and after 14 days of freezer storage. Values were 107% and 104 % of nominal concentration.

Checks on homogeneity (low and high concentration only) and concentrations were done at several times during the study. The test substance concentrations measured at six time points were in the range of ± 20% of the nominal concentrations (104 – 120%) except that one of the 2500 ppm mixtures prepared on September 23, 2005 was at 134% of nominal concentration and this was considered not to influence the study outcome. The low and high concentrations were proven to be homogenous in these formulations.

Table 5.6.1-2: Analytical results

	Nominal value in ppm	Content in ppm	% of nominal value	% RSD
Homogeneity, before start of the study	10 ppm, mixture A	9.47	95	4.84
	10 ppm, mixture B	9.46	95	1.59
	10 ppm, mixture C	9.86	99	2.57
	7000 ppm, mixture A	7261	104	2.88
	7000 ppm, mixture B	7169	102	4.39
	7000 ppm, mixture C	7384	105	1.71
Stability, before the start of the study 15 days at room	10 ppm, mixture A	10.93	109	-
	10 ppm, mixture B	10.46	105	-
	10 ppm, mixture C	10.63	106	-
	7000 ppm, mixture A	6065	87	-

temperature	7000 ppm, mixture B	5943	85	-
	7000 ppm, mixture C	5757	82	-
Stability, before the start of the study 15 days at a deep freezer	10 ppm, mixture A	11.22	112	-
	10 ppm, mixture B	10.92	109	-
	10 ppm, mixture C	11.54	115	-
	7000 ppm, mixture A	6584	94	-
	7000 ppm, mixture B	6667	95	-
	7000 ppm, mixture C	7131	102	-
Formulations prepared on July 01, 2004	500	518	104	1.40
	2500	2596	104	Content only
	10000	10648	106	0.5
Formulations prepared on Sep. 23, 2004	500	540	108	1.76
	2500	3353	134*	Content only
	10000	11450	115	3.62
Formulations prepared on Sep. 30, 2004	2500	2633	105	Content only
Formulations prepared on Dec. 02, 2004	500	528	106	4.81
	2500	2697	108	Content only
	10000	10718	107	3.50
Formulations prepared on Feb. 17, 2005	500	534	107	2.60
	2500	2760	110	Content only
	10000	11059	111	4.21
Formulations prepared on Apr. 07, 2005	500	601	120	4.94
	2500	2958	118	Content only
	10000	11493	115	4.41

* Value outside of the defined limits (± 20%).

4. Statistics

a) Analysis of Variance (ANOVA) and in case of significant results Dunnett's test as post hoc test were performed for:

- Body weights and body weight gains
- Food consumption
- Number of implantation sites per female
- Number of viable pups per female
- Organ weights at necropsy
- Time to insemination
- Life birth, viability and lactation index

b) 2 by N CHI^2 test; in case of significant differences Fisher's exact test with Bonferroni correction for:

- Number of viable pups per group based on the number of implantations
- Insemination, fertility, gestation and rearing indices

c) Kruskall-Wallis test and in case of significant differences Dunnett's test for:

- Number of prenatal losses per litter

The sperm and spermatid count data were not evaluated statistically because there were no meaningful differences between the high dose and control groups.

Generally, differences between the control group and groups treated with the test substance were considered as statistically significant when $p \leq 0.05$.

Follicle counts were evaluated statistically using the Wilcoxon Mann-Whitney U test.

Females without implantation sites and females and males, which died or which had to be sacrificed in moribund condition, were excluded from statistical evaluation of post-mating parameters. Animals which spilled their food, if any, were excluded from calculation of the means for food intake at the respective days.

The mean pup weight of each individual litter was used as the basis for calculating the mean pup weight of the dose groups. The mean litter size calculation was based on the number of female animals with living pups. Body weight data of F0 rats at study start are given under week 1.

C. METHODS:

1. F0/F1 parental animals

a. Mortality and clinical signs

Mortality and morbidity of the animals were checked twice daily (once daily on weekends and public holidays). Clinical findings were recorded individually daily. A detailed clinical examination was done prior to the first administration in the diet and then weekly and during the pregnancy and lactation periods as follows:

Pregnancy on day 0 (=day of a sperm positive smear or vaginal plug) 7, 14 and 20. Lactation on day 0 (day of birth), 4, 7, 14, 21 and 28.

b. Body weight

Body weights were recorded directly prior to the first administration and weekly thereafter up to necropsy (for males and not pregnant females) and during the pregnancy and lactation periods as follows:

During pregnancy on day p.c. 0, 7, 14 and 20. (p.c.: post coitum)
During lactation on day p.p. 0, 4, 7, 14, 21 and 28. (p.p.: post partum)
On the day of scheduled necropsy.

For females found to be not pregnant, only individual data are reported from the date of sperm positive findings up to necropsy. Data of remated females are not reported at all.

c. Food consumption

The individual food consumption was measured as follows:
- Males: Weekly from week 1 up to necropsy (except during mating period).
- Females: Weekly from week 1 up to mating.
  During pregnancy day p.c. 0-7; 7-14; 14-20.
  During lactation day p.p. 0-4; 4-7.

From these food intake data the intake of the test compound was calculated per animal and/or per kg body weight per day, week or for a given period.

d. Determination of insemination

During the mating periods vaginal smears were taken in the morning after rats had been co-housed overnight to determine time of insemination and gestation length. The date when sperm or a vaginal plug was found was taken as gestation day 0 for calculating the gestation length. Females which exhibited marked weight gains although insemination had not been established were not further co-housed. No duration of pregnancy could be determined for these animals.

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

$$\text{Insemination index (\%)} = \frac{\text{No. of sperm positive females*}}{} \times 100$$

No. of females co-housed with a male

$$\text{Fertility index (\%)} = \frac{\text{No. of pregnant females}}{\text{No. of sperm positive females*}} \times 100$$

$$\text{Gestation index (\%)} = \frac{\text{No. of females completing delivery}}{\text{No. of pregnant females}} \times 100$$

$$\text{Rearing index (\%)} = \frac{\text{No. of females rearing a litter up to day 21 p.p.}}{\text{No. of females that delivered a litter}} \times 100$$

* including pregnant females (with implantations), which were not sperm positive.

e. Oestrus cycle evaluation

Oestrus cycle length determination was done by evaluation of vaginal smears received daily over 19 consecutive days directly prior to the mating period. The smears were examined microscopically for large serrated cells indicating that œstrus had occurred. This data was used to determine the œstrus cycle length and whether females were cycling properly.

f. Sperm analysis

Spermatological investigations were performed in all surviving F0 and F1 males of the 0 and 10000 ppm groups on the day of necropsy. Sperm motility was also studied in 500 and 2500 ppm F0 rats.

- Determination of spermatozoa motility and viability

Sperm samples were collected from the right cauda epididymis and suspended in HAM`s F10 tissue culture medium (37°C). Sperm motility was recorded on 100 spermatozoa during minute 1 and minute 5 after preparation of the sample using semi-dark field microscopy. Furthermore, the difference between motility recorded during the first minute and that measured during the fifth minute was calculated.

- Determination of spermatozoa morphology

Spermatozoa morphology was evaluated in a formalin citrate fixed and Eosin G stained sperm sample collected from the right cauda epididymis. Morphological changes of the head, upper and middle tail were evaluated on 200 spermatozoa using the microscope as mentioned above.

- Determination of spermatozoa in epididymis

Determination of spermatozoa density was performed in a suspension (0.9% NaCl) of minced cauda epididymis tissue by counting of spermatozoa in a haemocytometer and calculation of spermatozoa density per mg epididymis. Each sample was counted in 5 tertiary squares of two secondary squares. All 10 values were averaged and taken for calculating spermatozoa density per mg epididymis as follows:

$$\text{No. of sperms per mg epididymis} = \frac{\text{10,000* x mean sperm count}}{\text{0.004** x weight of sample}}$$

* 10,000 mm^3 = 10 ml dilution medium;
** 0.004 mm^3 volume of one tertiary square

- Determination of homogenization resistant spermatid heads in the testis

Determination of spermatid head density was performed in a suspension of homogenized testis tissue by counting of spermatid heads in a haemocytometer. Each sample was counted in 5 tertiary squares of two secondary squares. All 10 values were averaged and taken for calculating spermatid head density per mg testis as follows:

$$\text{No. of spermatid heads per mg testis} = \frac{20{,}000^{*} \times \text{mean spermatid heads}}{0.004^{**} \times \text{weight of sample}}$$

* 20,000 mm^3 = 20 ml dilution medium;
** 0.004 mm^3 volume of one tertiary square

2. Litter observations

The following litter observations (X) were made:

Table 5.6.1-3: F1 and F2 Litter observations

Observation	Time of observation (lactation day)							
	Day 0	Day 4 a)	Day 4 b)	Day 7	Day 14	Day 21	Day 28	F1 Post weanlings
Number of live pups (1)	X	X	Xy	X	X	X	X	
Pup weight (2)	X	X	Xy	X	X	X	X	
External alterations (=clinical and necropsy observation) (3)	X	X	Xy	X	X	X	X	
Number of dead pups (4)	X	X	Xy	X	X	X	X	
Ano-genital measurements (only F2) (5)								
Sex of each pup (M/F) (6)	X	X	Xy					
Developmental milestones								X

a) Before culling
b) After culling
Xy Same data as recorded before culling.

a. Culling

Litter size was reduced by random selection to 8 pups (equal sex distribution) on day 4 p.p. Eliminated pups (dead/alive) were examined for external defects.

b. Weaning of pups

All pups were weaned on day 28 p.p. This could have been postponed up to two days later in case that the day 28 was a Saturday, Sunday or holiday.

c. Selection of weaned F1 pups for further treatment

One F1 male and one F1 female per litter were randomly selected for further treatment at the same dose level (using a computer generated random number table). In cases where this procedure was not possible (severe clinical findings, missing pups or if pup weight differed extremely from the group mean) another pup or pups of other litters were chosen.

d. Developmental milestones in F1 weanlings

In all F1 weanlings selected for further treatment the age and body weight when balano-preputial separation or vaginal opening occurred was recorded. This was done by investigating daily the preputium (from day 38 p.p. onwards) and vagina (from day 28 p.p. onwards) up to the time point when the balano-preputial separation or vaginal opening occurred. Time of vaginal opening and balano-preputial separation of F1 weanlings was recorded together with actual body weight.

e. Selection procedure for F1 co-housings

As far as possible the first F1 male was co-housed with a female taken from a litter with a different number. Using this procedure sibling matings among the F1 generation were excluded as far as possible.

f. Offspring viability indices

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

$$\text{Live birth index (\%)}^{\S} = \frac{\text{No. of live pups at birth}}{\text{total No. of pups born}} \times 100$$

$$\text{Viability index (\%)}^{\S} = \frac{\text{No. of live pups on day 4 pre-culling}}{\text{No. of live pups born}} \times 100$$

$$\text{Lactation index (\%)}^{\S} = \frac{\text{No. of live pups after three weeks}}{\text{No. of live pups after four days (after culling)}^{**}} \times 100$$

** moribund pups that died during the course of culling were not included.
§ index calculation from litter means.

3. Postmortem observations

a. F0 and F1 parental animals

- Macroscopic post-mortem examination

All parental rats were killed by exsanguination under CO_2 narcosis.

Males and non pregnant females: When no longer needed for matings.
Females with pups: At weaning of 28 days old pups or up to 2 days later.

Macroscopic investigations were done on all rats. Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera.
Implantation sites were counted after staining with 10% aqueous ammonium sulfide. In cases where implausible discrepancies existed between the number of observed implantation sites and the number of pups delivered (for example more pups than implantation sites) the number of pups was taken as the number of implantation sites. Rats found dead or which had to be killed in moribund condition were necropsied as early as possible in the same way.

- Organ weights and tissue collection

The following tissues of adult F0 and F1 rats were fixed in 10% formalin with the exception of kidneys and testes, which were fixed in Davidson's solution. The organs marked with Y in the table below were weighed at necropsy.

Table 5.6.1-4: List of tissues collected

X		Abnormalities	X	Y	Seminal vesicles and coagulating glands
X	Y	Adrenals	X		Skin in mammary region
X	Y	Brain	X	Y	Spleen
X	Y[4)]	Epididymides [3)]	X	Y	Testes [3)]
X		Oesophagus	X	Y	Thyroid/Parathyroids [2)]
X	Y	Kidneys	X		Trachea
X		Larynx	X		Ureters
X	Y	Liver	X		Urinary bladder (instillation fixed)
X	Y	Ovaries and oviducts	X	Y	Uterus (incl. cervix)
X	Y	Pituitary gland [1)]	X		Vagina
X	Y	Prostate	X		Head with scull cap
X		Physical identifier			

1) fixation *in situ* and then isolated
2) fixed together with trachea, oesophagus and larynx. One of the paired organs was then isolated for weighing
3) in case of spermatological investigations left organ only
4) left organ only
X organ evaluated histopathologically at least in control and high dose
Y organ was weighed

- **Histopathological evaluations**

The organs marked with X in the table above were microscopically examined at least in the control and high dose group. This included also all F0/F1 rats which died intercurrently (as far as possible) and those which were killed moribund. Organs altered possibly by the treatment and any gross and/or microscopically changed lesions were examined in all rats. Staging of ovarian follicles was done in F1 females (control and high concentration) only. All reproductive organs of mating pairs suspected of reduced fertility were investigated.

b. Offspring

- **Macroscopic post-mortem examination**

In case of sacrifices prior to weaning, pups were killed under CO_2 narcosis. Weanlings on day 28-30 p.p. were killed by cervical dislocation under CO_2 narcosis.

- All pups culled for standardization of litter size, found dead or killed moribund during lactation were necropsied and investigated macroscopically with particular attention on the organs of reproduction except for cases of autolysis or cannibalism. This included also visible skeletal abnormalities as far as possible.
- All F1 weanlings not selected for further treatment were killed when they were at least 4 weeks old and examined macroscopically.
- All F2 weanlings were killed after a 4 week lactation period and examined macroscopically.

- **Preservation of tissues**

Grossly abnormal tissues if any were fixed in all pups/weanlings. In F1 and F2 weanlings brain, spleen, thymus, uterus, urinary bladder (instillation fixed), ureters, urethra and kidneys of one male and one female out of the firstly necropsied 5 litters per group were fixed in 10% formalin.

- **Organ weights**

The brain, spleen, thymus and uterus of one male and one female per F1/F2 litter were trimmed and weighed as soon as possible after dissection. The ratio of organ weights to body

weights was calculated. Therefore, all these weanlings were weighed at day of necropsy.

II. RESULTS AND DISCUSSION

A. PARENTAL ANIMALS:

1. Mortality and clinical signs

One 0 ppm F0 female was killed in moribund condition. This rat had a poor general condition caused by a prolonged parturition, which was due to endometritis and bacterial placentritis as well as inflammatory oedema of the cervix and vagina.
One 500 ppm F0 female showing signs of poor general condition and a mass on the neck (malignant basal cell carcinoma in the subcutis) had to be killed in moribund condition at the end of the pre-mating period. This death was considered not to be caused by the treatment.

One 10000 ppm F1 male, which had shown clinically emaciation and reduced water intake, was found dead at the end of the pre-mating period. As this animal exhibited morphological degenerative findings in the kidneys (papillary necroses, transitional cell hyperplasia and tubulus dilations), ureters (hypertrophy) and urinary bladder (transitional cell hyperplasia), which had been identified as treatment-related, the death of this animal is considered to be due to the test substance.

Up to 10000 ppm (F0) or 2500 ppm (F1) no clinical findings attributable to the treatment were evident. 10000 ppm F1 males showed titled head, sunken flanks and/or emaciation with higher incidences than in the other groups. In two 10000 ppm F1 females increased urination was noted during the lactation.

Table 5.6.1-5: Mortality and incidence of treatment-related clinical signs

Observations[#)]	Dietary concentration (ppm)							
	0	500	2500	10000	0	500	2500	10000
	F0 Males				F1 Males			
Mortality								
No. of unscheduled deaths	0	0	0	0	0	0	0	1
Clinical findings (premating)								
Tilted head	0	0	0	0	0	0	0	2
Sunken flanks	0	0	0	0	0	0	0	1
Emaciation	0	0	0	0	0	0	0	3
	F0 Females				F1 Females			
Mortality								
No. of unscheduled deaths	1	1	0	0	0	0	0	0
Clinical findings (lactation)								
Increased urination	0	0	0	0	0	0	0	2

[#)] Numbers of animals affected is given, if not mentioned otherwise./ Detected at regular examinations.

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2. Body weight and food consumption

At the end of the pre-mating periods no statistically significant changes in body weights or body weight gain were noted up to 10000 ppm in F0 and F1 rats. However, there was about 7% lower body weight at necropsy in 10000 ppm F1 females indicating a slight effect on this parameter at the high dose.

The food intake during the pre-mating period was not influenced by the treatment up to 10000

ppm in F0 and up to 2500 ppm in F1 rats. At 10000 ppm F1 males and females ingested about 8% less diet than controls.

Table 5.6.1-6:Mean (±SD) body weight and food consumption – pre-mating

Observations (study week)	Dietary concentration (ppm)			
	0	500	2500	10000
F0 Males (during pre-mating)				
Mean body weight (g) week 11 (about end of premating)	**377.8** ±29.66	**375.4** ±14.67	**357.2*** ±30.50	**371.3** ±33.36
Mean weight gain (g) weeks (1-11)	**264.6** ±27.20	**260.9** ±14.32	**245.8** ±25.33	**257.5** ±30.42
Mean food consumption (g/kg body weight/day) weeks (1-9)	**97.8** ±37.14	**91.9** ±31.71	**98.0** ±35.55	**94.6** ±35.31
F0 Females (during pre-mating)				
Mean body weight (g) week 10 (about end of premating)	**221.1** ±16.59	**225.8** ±18.54	**219.8** ±16.04	**220.3** ±18.02
Mean weight gain (g) weeks (1-10)	**102.0** ±13.32	**106.4** ±18.45	**101.1** ±13.25	**99.8** ±14.96
Mean food consumption (g/kg body weight/day) weeks (1-9)	**97.3** ±25.12	**111.1** ±19.97	**105.5** ±21.96	**96.8** ±20.47
F1 Males (during pre-mating)				
Mean body weight (g) week of age 15 (end of premating)	**386.1** ±38.93	**380.2** ±28.44	**379.2** ±25.40	**375.8** ±31.14
Mean weight gain (g) weeks of age 5-15	**285.3** ±32.45	**281.4** ±23.28	**280.2** ±23.45	**276.4** ±22.37
Mean food consumption (g/kg body weight/day) week of age 5-15	**107.2** ±52.64	**100.4** ±46.71	**104.2** ±54.05	**99.2** ±49.98
F1 Females (during pre-mating)				
Mean body weight (g) week 14 of age (end of premating)	**212.9** ±14.76	**209.0** ±22.12	**210.9** ±13.19	**205.2** ±16.78
Mean weight gain (g) weeks of age 5-14	**125.4** ±14.13	**120.8** ±19.68	**125.4** ±12.63	**118.0** ±11.90
Mean food consumption (g/kg body weight/day) week of age 5-14	**140.0** ±53.73	**135.9** ±45.19	**141.2** ±56.58	**128.4** ±42.22

* Statistically different from control, $p \leq 0.05$.
** Statistically different from control, $p \leq 0.01$.

No statistically significant body weight decrease was noted in pregnant or lactating F0 females up to 10000 ppm and F1 females up to 2500 ppm. During the gestation period (day 14 to 20 p.c.) F1 dams receiving 10000 ppm gained less ($p \leq 0.05$) weight compared to controls.
The relatively low body weight gain of 2500 ppm F1 females during lactation (day 0-4 p.p.) is considered as incidental due to the lack of dose dependency.

The mean food intake was not changed statistically significantly up to 10000 ppm.

Table 5.6.1-7:Mean (±SD) body weight and food consumption – gestation or lactation

Observations (study week)	Dietary concentration (ppm)			
	0	500	2500	10000
F0 Females (during gestation or lactation)				
Mean body weight on day 20 p.c. (g)	**309.6** ±20.43	**324.4** ±21.66	**317.3** ±20.15	**311.8** ±26.49
Mean weight gain day 14 to 20 p.c. (g)	**51.5** ±8.45	**53.7** ±7.71	**54.2** ±8.61	**51.8** ±10.91
Mean food consumption day 14 to 20 p.c. (g/kg body weight/day)	**68.6** ±11.04	**72.2** ±14.55	**72.7** ±9.64	**69.7** ±8.10
Mean body weight day 4 p.p. (g)	**251.2** ±21.54	**258.9** ±21.34	**254.7** ±17.04	**250.6** ±20.49
Mean weight gain day 0 to 4 p.p. (g)	**9.7** ±9.90	**12.1** ±13.02	**9.6** ±12.81	**13.4** ±12.49
Mean food consumption day 0 to 4 p.p. (g/kg body weight/day)	**110.7** ±30.47	**115.6** ±38.69	**108.2** ±29.13	**117.6** ±30.42
F1 Females (during gestation or lactation)				
Mean body weight on day 20 p.c. (g)	**317.4** ±23.17	**313.2** ±25.17	**312.6** ±19.43	**304.5** ±25.18
Mean weight gain day 14 to 20 p.c. (g)	**53.9** ±7.21	**52.0** ±5.74	**54.8** ±6.72	**48.9*** ±7.54
Mean food consumption day 14 to 20 p.c. (g/kg body weight/day)	**78.3** ±19.96	**80.9** ±13.85	**90.6** ±30.38	**74.2** ±9.04
Mean body weight day 4 p.p. (g)	**250.3** ±16.68	**251.3** ±24.07	**248.2** ±18.75	**239.9** ±20.41
Mean weight gain day 0 to 4 p.p. (g)	**8.2** ±14.25	**8.0** ±12.24	**2.8** ±11.02	**10.3** ±11.01
Mean food consumption day 0 to 4 p.p. (g/kg body weight/day)	**184.5** ±58.50	**171.8** ±61.82	**184.1** ±64.17	**183.3** ±59.55

* Statistically different from control, $p \leq 0.05$.
** Statistically different from control, $p \leq 0.01$.

3. Test substance intake

Based on food consumption and body weight data, the doses expressed as mean daily mg test substance/kg body weight during the pre-mating period are presented in table 5.6.1-8. The values for the F0 and F1 generation are considered to be representative of the test substance intake for the entire study. For completeness the values for test consumption during part of gestation and part of lactation are presented in Table 5.6.1-9.

Table 5.6.1-8: Mean (±SD) test substance intake during pre-mating (mg/kg body weight/day)

Observation period	Male			Female		
	Dietary concentration (ppm)					
	500	2500	10000	500	2500	10000
F0 (study week 1-9)	**46.0** ±15.86	**245.0** ±88.88	**945.8** ±353.08	**55.6** ±9.99	**263.7** ±54.91	**968.4** ±204.75
F1 (week of age 5-15 (M), 5-14 (F))	**50.2** ±23.35	**260.5** ±135.13	**992.1** ±499.84	**68.0** ±22.59	**353.1** ±141.44	**1284.0** ±422.16

Table 5.6.1-9: Mean (±SD) test substance consumption – gestation and lactation

Observation in study days	Dietary concentration (ppm)		
	500	2500	10000
F0 Females			
Mean test substance consumption day 14 to 20 p.c. (mg/kg body weight/day)	**36.1** ±7.28	**181.6** ±24.10	**696.8** ±81.04
Mean test substance consumption day 0 to 4 p.p. (mg/kg body weight/day)	**57.8** ±19.34	**270.4** ±72.84	**1175.7** ±304.20
F1 Females			
Mean test substance consumption day 14 to 20 p.c. (mg/kg body weight/day)	**40.4** ±6.92	**226.6** ±75.95	**742.2** ±90.45
Mean test substance consumption day 0 to 4 p.p. (mg/kg body weight/day)	**85.9** ±30.91	**460.3** ±160.42	**1832.8** ±595.46

4. Reproductive function

a. Oestrus cycle length and periodicity

Results from the evaluation of vaginal smears performed at the end of pre-mating phases indicated that there were no biologically relevant effects on the œstrous cycle up to 10000 ppm in F0 or F1 rats

b. Sperm measurements

There were no biologically relevant effects on sperm parameters (epididymal sperm count, sperm motility and morphology and testicular spermatids counts) in F0 nor in F1 rats at 10000 ppm.

Because there were three spermless 10000 ppm F0 males, sperm motility was also investigated in the low and mid dose. As these analyses did not reveal dose dependence in the number of spermless males and 10000 ppm F1 males were similar to controls in sperm parameters, the occurrence of five or three spermless F0 males at 2500 or 10000 ppm is considered as incidental.

c. Reproductive performance

The insemination, fertility, gestation and rearing indices as well as gestation length and number of litters born were not changed by the treatment up to 10000 ppm in both

generations.

Table 5.6.1-10: Reproductive Performance (means and ±SD)

	F0 Generation a)				F1 Generation b)			
	Dietary concentration (ppm)							
Observation	**0**	**500**	**2500**	**10000**	**0**	**500**	**2500**	**10000**
Insemination index	**96.0**	**100.0**	**92.0**	**80.0**	**92.0**	**100.0**	**100.0**	**100.0**
Fertility index	**100.0**	**100.0**	**87.0**	**100.0**	**95.7**	**96.0**	**92.0**	**88.0**
Gestation index	**95.8**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Gestation length (days)	**22.11** ±0.567	**21.89** ±0.459	**21.67** ±0.900	**21.89** ±0.583	**21.86** ±0.478	**21.67** ±0.482	**21.52** ±0.750	**22.09** ±0.526
Co-housed females	**25**	**24**	**25**	**25**	**25**	**25**	**25**	**25**
Matings until day 0 p.c.	**3.8** ±4.17	**3.8** ±4.05	**4.9** ±4.19	**2.4** ±2.12	**4.2** ±3.49	**4.3** ±4.08	**4.0** ±2.30	**3.7** ±2.62
	F0 Males				**F1 Males**			
Number co-housed	**25**	**24**	**25**	**25**	**25**	**25**	**25**	**24**
Number fertile	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Intercurrent deaths	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
	F0 Females				**F1 Females**			
Number co-housed	**25**	**24**	**25**	**25**	**25**	**25**	**25**	**25**
Number fertile	**24**	**24**	**20**	**20**	**22**	**24**	**23**	**22**
Intercurrent deaths	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
Number of litters 1)	**23**	**24**	**20**	**20**	**22**	**24**	**23**	**22**
Rearing index	**100.0**	**100.0**	**95.0**	**100.0**	**95.5**	**87.5**	**100.0**	**100.0**

- Not to be determined
1) With live born pups
* Statistically different from control, p≤ 0.05.
** Statistically different from control, p≤ 0.01.

5. Parental post-mortem results

a. Organ weights

F0 rats: Organ measurements revealed a statistically significant increase in absolute and relative weights of kidneys (about 15 %) of F0 females receiving 10000 ppm, a possible treatment-related effect.

There were also slightly higher (p≤ 0.01) relative liver weights in 10000 ppm F0 females than in controls. As the deviation to the control mean was less than 10 % this finding was considered not to reflect a treatment-related effect.

In F0 females absolute (500 ppm onwards) and relative (10000 ppm) weights of the thyroids were statistically higher than at 0 ppm. This finding was considered not to be treatment-related because 1) individual values of this parameter vary widely, 2) the absolute control mean was very low compared with historical control means and control means of F1 rat in this study, 3) the deviations between means of the treatment groups and the control mean were very small, 4) the means in the treatment groups were comparable with that of historical controls and 5) thyroid weights of F1 rats were clearly not effected.

The remaining organ weights of F0 rats were not significantly changed up to 10000 ppm.

Table 5.6.1-11: Significant changes in mean absolute organ weights of parental F0 animals (±SD)

Observation	Absolute Weights (mg)							
	Dietary concentration (ppm)							
	0		500		2500		10000	
	F0 Males							
Body weight (g)	**425.0**	±29.40	**421.6**	±17.38	**403.2**	±29.99	**417.5**	±38.76
Kidneys	**2965**	±230.6	**2796**	±193.6	**2667***	±280.5	**3012**	±673.4
Liver	**15666**	±1505.1	**14875**	±915.1	**14411***	±1472.3	**15429**	±2208.4
Thyroid a)	**13**	±3.5	**14**	±2.3	**15**	±3.9	**13**	±4.7
	F0 Females							
Body weight (g)	**250.4**	±16.56	**262.3***	±18.66	**256.9**	±14.68	**247.3**	±17.08
Kidneys	**2058**	±144.5	**2212**	±185.7	**2140**	±182.0	**2357****	±480.0
Liver	**11937**	±1609.9	**12870**	±1267.9	**12536**	±1523.4	**12920**	±1756.0
Thyroid a)	**9**	±3.8	**12***	±3.7	**12***	±2.9	**13****	±3.7

a) One thyroid with parathyroid weighed after fixation.
* Statistically different from control, p≤ 0.05.
** Statistically different from control, p≤ 0.01.

Table 5.6.1-12: Significant changes in mean relative organ weights of parental F0 animals (±SD)

Observation	Relative organ weights in mg/100 g body weight							
	Dietary concentration (ppm)							
	0		500		2500		1000	
	F0 Males							
Body weight (g)	**425.0**	±29.40	**421.6**	±17.38	**403.2**	±29.99	**417.5**	±38.76
Kidneys	**698.5**	±44.57	**663.6**	±43.13	**661.0**	±43.67	**723.0**	±153.86
Liver	**3685.5**	±240.61	**3528.6**	±164.36	**3570.5**	±200.15	**3686.4**	±315.95
Thyroid a)	**3.1**	±0.81	**3.3**	±0.56	**3.7**	±0.91	**3.1**	±1.23
	F0 Females							
Body weight (g)	**250.4**	±16.56	**262.3***	±18.66	**256.9**	±14.68	**247.3**	±17.08
Kidneys	**823.5**	±55.87	**846.9**	±62.33	**833.7**	±58.33	**956.4****	±200.82
Liver	**4762.5**	±523.69	**4905.7**	±358.67	**4887.2**	±575.94	**5221.6***	±608.46
Thyroid a)	**3.7**	±1.41	**4.6**	±1.46	**4.7**	±1.19	**5.3****	±1.46

a) One thyroid with parathyroid weighed after fixation.
* Statistically different from control, p≤ 0.05.
** Statistically different from control, p≤ 0.01.

F1 rats: In males statistically significantly lower weights of the kidneys were found at 2500 ppm (absolute) or from 500 ppm onwards (relative). As a dose dependence is missing and the deviations were relatively small, this parameter was considered not to be affected by the treatment.

In 10000 ppm F1 females, statistically significantly increased absolute (8 %) and relative (17 %) weights of the kidneys were observed compared to those at 0 ppm.

At 10000 ppm reduced absolute (14 %) and relative (13 %) liver weights occurred in 10000 ppm F1 males.
The remaining organ weights of F1 rats were not significantly changed up to 10000 ppm.

Table 5.6.1-13: Significant changes in mean absolute organ weights of parental F1 animals (±SD)

Observation	Absolute Weights (mg)							
	Dietary concentration (ppm)							
	0		500		2500		10000	
	F1 Males							
Body weight (g)	**444.0**	±43.70	**446.5**	±29.95	**443.2**	±36.28	**441.6**	±32.77
Kidneys	**3138**	±308.2	**2973**	±256.5	**2867****	±319.5	**2951**	±249.1
Liver	**18813**	±3627.0	**17996**	±1974.0	**17446**	±1569.4	**16129****	±1419.7
Thyroid a)	**14**	±3.6	**14**	±4.0	**15**	±3.5	**14**	±3.7
	F1 Females							
Body weight (g)	**259.4**	±16.35	**258.4**	±20.65	**254.5**	±17.20	**241.0****	±19.28
Kidneys	**2121**	±148.9	**2165**	±191.1	**2034**	±172.0	**2291***	±323.4
Liver	**14154**	±1909.2	**14410**	±2278.7	**13840**	±2102.6	**12876**	±1556.5
Thyroid a)	**11**	±3.6	**12**	±3.0	**10**	±2.8	**12**	±4.1

a) One thyroid with parathyroid weighed after fixation
* Statistically different from control, p≤ 0.05.
** Statistically different from control, p≤ 0.01

Table 5.6.1-14: Significant changes in mean relative organ weights of parental F1 animals (±SD)

	Relative organ weights in mg/100 g body weight							
	Dietary concentration (ppm)							
Observation	0		500		2500		10000	
	F1 Males a)							
Body weight (g)	**444.0**	±43.70	**446.5**	±29.95	**443.2**	±36.28	**441.6**	±32.77
Kidneys	**710.3**	±67.63	**666.6***	±50.76	**647.8****	±57.89	**669.5***	±47.44
Liver	**4203.3**	±598.03	**4032.2**	±3727.6	**3948.2**	±3488.1	**3655.1****	±218.93
Thyroid a)	**3.1**	±0.77	**3.3**	±0.93	**3.5**	±0.95	**3.1**	±0.95
	F1 Females a)							
Body weight (g)	**259.4**	±16.35	**258.4**	±20.65	**254.5**	±17.20	**241.0****	±19.28
Kidneys	**817.8**	±25.90	**838.6**	±52.47	**800.7**	±63.54	**958.1****	±175.89
Liver	**5461.7**	±695.30	**5563.2**	±665.19	**5427.2**	±673.52	**5350.0**	±571.69
Thyroid a)	**4.1**	±1.39	**4.5**	±1.25	**4.0**	±1.18	**5.1**	±1.59

a One thyroid with parathyroid weighed after fixation
* Statistically different from control, p≤ 0.05.
** Statistically different from control, p≤ 0.01.

b. Pathology

- **Macroscopic examination**

At necropsy no macroscopic findings due to the treatment were observed at 500 ppm (F0) or up to 2500 ppm (F1). At 2500 and/or 10000 ppm toxicologically relevantly increased incidences in macroscopic changes of the kidneys, ureters and urinary bladder were found as follows:
In the kidneys of 10000 ppm F0 and F1 females there were pelvic dilations and/or stone/s. Pelvic dilation was also observed at 500 and 2500 ppm but at a lower incidence and was therefore considered not to be treatment-related at 500 and 2500 ppm.
In the ureters dilations and/or stones were seen in F0 (2500 and 10000 ppm) and F1 females (10000 ppm).
In the urinary bladder stones and/or thickenings were detected in 10000 ppm F1 males and females.

Table 5.6.1-15: Significant necropsy findings of parental F0 and F1 rats

	No of Animals Affected (No of rats investigated each =25)							
	Dietary concentration (ppm)							
Observation	0	500	2500	10000	0	500	2500	10000
	F0 Males				F1 Males			
Urinary bladder								
- Stone/s	-	-	-	-	-	-	-	1
- Thickened	-	-	-	-	-	1	-	2
	F0 Females				F1 Females			
Kidneys								
- Pelvic dilation/s	-	3	3	6	-	-	1	9
- Stones	-	-	-	-	-	-	-	3
Ureters								
- Dilation/s	-	-	1	6	-	-	-	11
- Stones	-	-	1	2	-	-	-	2
Urinary bladder								
- Stone/s	-	-	-	-	-	-	-	3
- Thickened	-	-	-	-	-	1	-	7

- **Microscopic examination**

Microscopically, administration of BYH 18636 led to lesions which were predominantly restricted to the urinary system. The changes affected high dose animals of both generations. However, in general the females and the F1-generation were more severely affected. The alterations were characterized by degenerative and reactive changes, which were caused by a urolithiasis. This urolithiasis could be noticed macro- and/or microscopically. However, there were also animals with pronounced changes without the obvious presence of stones. Some of the changes were also observed in control rats and/or animals of the low and mid dose group. However, the incidence and/or severity of the findings increased in rats of the high dose group of both generations.
Degenerative changes were observed in the kidneys (pyelitis and/or pelvic degeneration, pyelonephritis, dilation of cortical and/or medullary tubules, pelvic dilation, dilation of papillary tubules, inflammation, basophilic tubules and interstitial fibrosis), the ureters (oedema, inflammatory cell infiltration) and the urinary bladder (submucosal oedema and infiltration).
Reactive changes occurred predominantly as transitional cell hyperplasia in the kidneys, ureters, urinary bladder and urethra (one high dose male of each generation) as well as dilation and/or hypertrophy of the ureters.

In the F0-generation the periportal fat accumulation in the liver of high dose males was slightly decreased. However, the meaning of this finding is unclear.

With respect to pathology, the test compound had no toxicological effect on the

reproductive organs.

Table 5.6.1-16: Remarkable histopathological findings of parental F0 and F1 rats

	No. of Animals Affected (No. of rats investigated each =25)							
	Dietary concentration (ppm)							
Observation	0	500	2500	10000	0	500	2500	10000
	F0 Males				F1 Males			
Kidneys								
- Tubulus dilation /papilla	-	-	-	3	-	-	-	4
- Pyelitis/pelvic degeneration	1	-	-	4	1	-	-	1
- Pyelonephritis	-	-	-	1	-	-	-	-
- Urolithiasis	-	-	-	4	-	-	-	3
Ureters								
- Dilation	3	1	4	8	3	5	7	2
- Hypertrophy	-	-	-	3	-	1	-	3
Urinary bladder								
- Transitional cell hyperplasia	3	2	2	5	2	4	6	6
- Submucosal infiltration	2	2	2	4	2	5	5	13
- Submucosal oedema	-	-	-	4	-	-	-	2
- Urolithiasis	-	-	-	2	-	-	-	2
Urethra								
- Transitional cell hyperplasia	-	-	-	1	-	-	-	1
Liver								
- Fat accumulation periportal	17	18	12	9	15	not done	not done	15
	F0 Females				F1 Females			
Kidneys								
- Basophilic Tubules	3	2	6	16	3	2	6	13
- Inflammation	2	-	-	8	-	-	-	-
- Tubulus dilation / cort./med.	1	-	1	9	1	1	1	11
- Pyelitis/pelvic degeneration	2	-	1	2	-	1	2	9
- pyelonephritis	-	-	-	-	-	-	-	4
- Tubulus dilation / papilla	-	-	-	-	-	-	-	9
- Transitional cell hyperplasia	2	2	5	10	1	5	1	13
- Pelvic dilation	1	3	3	9	1	3	2	12
- Intersticial fibrosis	-	-	-	-	1	1	1	7
- Urolithiasis	-	-	-	6	-	-	-	9
Ureters								
- Dilation	1	3	4	7	2	3	3	9
- Hypertrophy	-	1	1	6	-	1	-	12
- Transitional cell hyperplasia	-	-	-	6	-	-	-	8
- Oedema	-	-	-	3	-	-	-	1
- Inflammat. cell infiltration	-	-	-	5	-	-	1	5
- Submucosal infiltration	-	-	-	-	-	-	-	2
- Urolithiasis	-	-	-	1	-	-	-	-
Urinary bladder								
- Transitional cell hyperplasia	-	1	-	7	-	1	-	11
- Submucosal infiltration	-	1	-	2	-	1	-	12
- Submucosal Edema	-	-	-	1	-	1	-	4

\- No finding

B. OFFSPRING:

1. Viability, clinical signs and ano-genital distance

The number of implantation sites, the live birth, viability and lactation indices, the number pups

born, the sex distribution, the litter sizes during lactation as well as prenatal loss were not altered by the treatment up to 10000 ppm.
The lower number of F1 pups born at 2500 and 10000 ppm compared with those born at 0 and 500 ppm is based on the fact that in these groups more spermless males were found than in the other groups, which was as discussed above an incidental finding.
As there were no effects on the pup numbers in the F2 generation up to 10000 ppm, the changes observed in the F1 genenration was considered not to be treatment-related up to 10000 ppm.

Table 5.6.1-17: Significant changes in litter parameters of F0 and F1 generations (means and partly ±SD)

Observation	Dietary concentration (ppm)			
	0	500	2500	10000
	F0→F1 Pups			
Mean implantations [a)]	12.00 ±1.907	12.54 ±1.503	11.90 ±2.808	12.00 ±1.686
Mean prenatal loss [a)]	1.17 ±0.834	0.79 ±1.062	0.90 ±0.968	1.15 ±1.137
Number born	249	282	220	217
Number born dead	2	1	4	1
Live birth index	99.24	99.48	98.13	99.62
	F1→F2 Pups			
Mean implantations [a)]	12.41 ±1.221	11.88 ±1.454	11.91 ±1.379	11.82 ±1.006
Mean prenatal loss [a)]	1.09 ±1.065	0.75 ±0.944	0.87 ±1.014	0.86 ±0.990
Number born	249	267	254	241
Number born dead	10	0**	1*	0**
Live birth index	95.52	100.00	99.64	98.64

a) Per litter.
* Statistically different from control, $p \leq 0.05$.
** Statistically different from control, $p \leq 0.01$.

Clinical observations on pups revealed no remarkable increase of clinical symptoms up to 10000 ppm in F1 and F2 pups.

2. Body weight

There was no treatment-related change in offspring body weights and litter weights up to 10000 ppm in any generation.

3. Sexual maturation (F1) and measurements of ano-genital distance in F2 pups

The balano-preputial separation and vaginal opening in F1 post weanlings were unaffected up to 10000 ppm.

4. Offspring post-mortem results

a. Organ weights

There were no toxicologically relevant changes in absolute or relative organ weights observed in male and female F1 or F2 pups up to 10000 ppm.
Reduced absolute spleen weights of male and female 10000 ppm F2 weanlings are based on body weight difference as corresponding relative weights were uneffected up to 10000 ppm. As the deviations to control values were small, an effect of the test substance was not assumed.

b. Macroscopic examinations

Up to 2500 ppm no remarkable incidences of macroscopic findings were found at pup or weanling necropsies. At 10000 ppm slightly more F2 pups without a milk spot were noted. More 10000 ppm F1 and F2 weanlings exhibited dilated and/or enlarged kidneys than in the other groups. In addition, there were stones in the kidney and urinary bladder of a few F2 weanlings at 10000 ppm.

Table 5.6.1-18: Significant macroscopic findings in F1 and F2 weanlings

Observation males and females pooled	**Dietary concentration (ppm)**							
	0	**500**	**2500**	**10000**	**0**	**500**	**2500**	**10000**
	F1 Pups/Weanlings				**F2 Pups/Weanlings**			
Stomach								
no milk in stomach								
affected pups	**0**	**0**	**0**	**0**	**3**	**4**	**3**	**7**
affected pups per litter (%)	**0.00**	**0.00**	**0.00**	**0.00**	**2.12**	**2.03**	**1.40**	**3.44**
Kidney								
stone/s								
affected pups	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
affected pups per litter (%)	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.41**
dilated and/or enlarged								
affected pups	**1**	**0**	**1**	**2**	**0**	**0**	**0**	**2**
affected pups per litter (%)	**0.48**	**0.00**	**0.42**	**0.90**	**0.00**	**0.00**	**0.00**	**0.83**
Urinary bladder								
stone/s								
affected pups	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**
affected pups per litter (%)	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.83**

III. CONCLUSION

Under the conditions described the NOAEL for parental reproductive parameters was 10000 ppm.
The parental systemic LOAEL in rats was 2500 ppm (corresponding to 245 mg/kg bw/day in males and 264 mg/kg bw/day in females). The parental systemic NOAEL in rats was 500 ppm (46 mg/kg bw/day in males, 56 mg/kg bw/day in females), based on occurrence of dilation/s and stone/s in the ureter of a F0 female.
The offspring NOAEL (=NOEL) was at 2500 ppm (270 mg/kg bw/day), based on clinical signs and macroscopic findings in the kidney and the urinary bladder of F1 and/or F2 pups.

Eiben R., 2006

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable The study is fully compliant with OECD 416 (2001) Occasional deviations from acceptable limits in the homogeneity of the test material in the diet are not considered to affect the integrity of the study. There is no evidence for reproductive toxicity in this study at up to and including the top dose level of 10000 ppm. It is noted that the results of the one-generation pilot reproductive toxicity study (in 10 animals/sex/group treated for 4 weeks prior to mating) indicate a reduction in the number of implantation sites, the total number of pups born and pup weight gain at the highest dose level of 10000 ppm. However these parameters were not affected by treatment with 10000 ppm in the main study, using 25 rats/sex./group. The reduced insemination index (Table 5.6.1-10) in 10000 ppm F_0 males of 80% does not lie within the laboratory's historical control range (88.9-100%; mean 97.7%) reported for 16 studies. Of the five males not mating in this group, three are those with observations of 'no sperm'. This finding is not considered to be related to treatment in the absence of similar findings in the F_1 generation. The lower total number of F_1 pups seen at 2500 and 10000 ppm is considered to be secondary to the number of F_0 males with 'no sperm' seen in these dose groups; litter size was unaffected by treatment. However the incidence of 'no sperm' (1, 0, 5, 3) is not considered to be related to treatment in the absence of a dose-response relationship or similar findings in F_1 males. The treatment-related findings in this study are comparable to those seen in other studies in the rat and are consistent with the mode of action of thiencarbazone-methyl. Effects at the high dose level are associated with urolithiasis: mortality in one male at this dose level was secondary to renal necrosis. Findings in other parental animals in this dose group were less severe but were largely limited to characteristic histopathological changes in the urinary system. Slight reductions in weight gain and food consumption and increased kidney weight (in females) may also be related to the mode of action. Similar macroscopic and histopathological findings seen in 500 ppm females are not considered to be clearly related to treatment due to their low incidence and/or a lack of a dose-response relationship. The statistically significantly lower absolute spleen weights in F2 pups at 10000 ppm is not considered to be of toxicological significance in the absence of similar effects on relative weights and in the absence of pathological correlates seen in this study or in other studies.
Conclusions	No evidence of reproductive toxicity was seen under the conditions of this study at the highest dose level of 10000 ppm. A reproductive NOAEL of 10000 ppm (equivalent to mean achieved pre-mating dietary intakes of 946 and 968 mg/kg bw/d in males and females respectively) can therefore be determined for this study. A parental NOAEL of 2500 ppm for males (equivalent to 245 mg/kg bw/d) can be determined for this study based on the mortality, signs of toxicity and histopathological findings in the kidney, ureter and bladder seen at the top dose level of 10000 ppm. A parental NOAEL for females of 500 ppm(equivalent to 56 mg/kg bw/d) can be determined, based on the histopathological findings in the kidney and ureter seen at 2500 ppm.

	A NOAEL for offspring of 2500 ppm (equivalent to 245 and 264 mg/kg bw/d in males and females respectively) can be determined, based on the macroscopic findings in the kidney and bladder at the top dose level. The Notifier proposes a NOAEL for offspring of 2500 ppm, but states that this dose level is equivalent to an intake of 270 mg/kg bw/d (the intake of maternal animals during the lactation period) rather than the RMS' figure which refers to intake during the pre-mating period.

Separate male and female studies (IIA 5.6.2)

Results of the two-generation study did not show any sex-related findings. Therefore, specific studies on males and females were not triggered.

Three segment designs (IIA 5.6.3)

In the two-generation study, no effects on reproductive parameters occurred. Therefore, this type of study is not required.

Dominant lethal assay for the male fertility (IIA 5.6.4)

In the two-generation study, no effects on reproductive parameters occurred. Therefore, this type of study is not required.

Cross-matings of treated males with untreated females and *vice versa* (IIA 5.6.5)

In the two-generation study, no effects on reproductive parameters occurred. Therefore, this type of study is not required.

Effects on spermatogenesis (IIA 5.6.6)

In the two-generation study, no effects on reproductive parameters occurred. Therefore, this type of study is not required.

Effects on oogenesis (IIA 5.6.7)

In the two-generation study, no effects on reproductive parameters occurred. Therefore, this type of study is not required.

Sperm motility, mobility and morphology (IIA 5.6.8)

In the two-generation study, sperm motility, mobility and morphology were assessed. Since there were no effects on sperm parameters, further studies were not required.

Investigation of hormonal activity (IIA 5.6.9)

As no effects on the reproductive parameters were seen in the two-generation study, no investigations on the hormonal activity were required.

B.6.6.2 Developmental toxicity in the rat (IIA 5.6.1)

Teratogenicity test by the oral route in the rat (IIA 5.6.10)

Report:	**KIIA 5.6.10/01, Langewische F., 2005 (MRID # 47070130)**
Title:	BYH 18636, Developmental toxicity study in rats after oral administration
Report No &	AT02339
Document No	M-257678-01-2
Guidelines:	OECD 414 (2001); EEC Directive 88/302; EPA Health Effects Test Guideline (OPPTS 870.3700; 1998); MAFF in Japan 12-Nousan N° 8147 (2001)
GLP	Yes (certified laboratory)

Executive summary:
In a developmental toxicity study, BYH 18636 (Mix-batch 702-73-06-0001, 96.2% of purity) was administered daily by gavage from gestation day (GD) 6 to 19 at the following doses: 0, 50, 200 and 1000 mg/kg/day in 0.5% aqueous carboxymethylcellulose. All animals were examined twice daily except once daily on weekends, on public holidays and on gestation day 20. Body weight was determined on day 0 and daily from GD 6 to 20. Food consumption was determined during the intervals GD 0-3, 3-6, 6-9, 9-12, 12-15, 15-18 and 18-20. The foetuses were delivered by Caesarean section on GD 20. The females were subjected to gross pathological examination. The gravid uterine weight was recorded. The liver and the urinary bladder were fixed in 10% neutral buffered formalin solution and the kidneys were fixed in Davidson's solution. The dams were evaluated for the number of corpora lutea, number and status of implantations (resorption, dead and live fœtuses). Live fœtuses were removed from the uteri, counted, weighed, sexed and examined externally. Half of the fœtuses were processed by scapel and /or razor blade sectioning according to the modified Wilson technique for visceral malformations and other findings deviating from normal. The other half of the fœtuses was processed by the modified Dawson technique with the addition of cartilage staining for findings in abdominal, pelvic and thoracic organs as well as skeletal and cartilage findings.

Appearance, behaviour and mortality were unaffected at dose levels up to and including 1000 mg/kg/day. Treatment-related effects were limited at 1000 mg/kg/day. Decreased food intake of 14% to 23% compared to the control group occurred between GD 6-20 and there was an increased incidence of light coloured faeces, most likely caused by the faecal excretion of the test substance, which was white coloured. Body weight loss occurred between GD 6-7 and overall absolute and corrected body weight gains were also decreased at this dose level (decrease of 26% and 51% respectively compared to the control group). Increased incidences of urinary bladders, urethras and dilated ureters, each filled with yelowish sediment, in one case additionally with a pale kidney or a caudal end of one kidney swollen and light brown discoloured occurred at necropsy.

The gestation rate, appearance of placentas, post-implantation loss and correspondingly the number of fœtuses and fœtal sex distribution were unaffected by treatment with BYH 18636 up to and including 1000 mg/kg/day. Placental weight was marginally decreased and fœtal weight was decreased at 1000 mg/kg/day. The incidences and types of fœtal malformations and external and visceral deviations were unaffected by treatment up to 1000 mg/kg/day. Evaluation of skeletal deviations resulted in retarded ossification of several localization (distal phalanges of digits and toes, metacarpals, sternebrae, sacral and caudal vertebrae) at 1000 mg/kg/day, correlating to decreased foetal weights. A treatment-related effect for an increased incidence of wavy ribs cannot be excluded at 1000 mg/kg/day due to maternal toxicity at this dose level.

The NOAEL for maternal toxicity was 200 mg/kg/day, based on body weight loss, decreased placenta weight and yellowish sediment in the urinary bladder, urethras and dilated ureters. The NOAEL for developmental toxicity was also 200 mg/kg/day, based on retarded ossification of several localizations, correlating to decreased fœtal weights.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636
Description: White powder
Lot/Batch: Mix-batch 702-73-06-0001
Purity: 96.2%
CAS: 317815-83-1
Stability of test compound: Stable after 8 days at room temperature. Formulations prepared for three to seven days of use.

2. Control materials:
Negative: None
Solvent: 0.5% aqueous carboxymethylcellulose
Positive: None

3. Test animals:
Species: Rat
Strain: Hsd Cpb: WU
Age: 14 to 16 weeks approximately
Weight at dosing: 469 to 571 g for the males (only for mating)
201 to 250 g for the females
Source: Harlan/Winkelmann GmbH, 33178 Borchen, Germany
Number of animals per dose: 25 females
Acclimation period: 7 days before mating
Diet: Standard rat diet (Provimi Kliba Maus/Ratte-Haltung-GLP, Art.-N°: 3883.0.15, supplied by Promimi Kliba SA, 4303 Kaiseraugst, Switzerland), *ad libitum*
Water: Tap water, *ad libitum*
Housing: The females were caged individually from GD 0 in type III Makrolon cages on low-dust wood shavings. The males were kept in groups of 2-3 males in type IV Makrolon cages.
Environmental conditions –
Temperature: 22 ± 2°C
Humidity: 50 ± 20%
Air changes: At least 10 changes per hour
Photoperiod: Alternating 12-hour light and dark cycles

4. Test compound concentrations: 0, 50, 200 and 1000 mg/kg/day based on a previous range-finding study

B. TEST PERFORMANCE

Bayer Health Care AG conducted the experimental phase of the study during the period of April 14 to September 28, 2004.

1. Animal assigment and treatment
The males were used for mating only and were not treated. The animals were mated by placing two females overnight into a type III cage together with one male. After insemination was ascertained, 25 females each were allocated to four experimental groups according to a randomization plan generated on a personal computer.

The females were treated by gavage once daily between 06:00 and 12:30 from GD 6 to 19. The females of all experimental groups received a uniform administration volume of 10 ml/kg body weight/day. The females of the control group received vehicle (0.5% aqueous

carboxymethylcellulose), only. The following doses referring to BYH 18636 were administered once daily corresponding to the current body weight: 0, 50, 200 and 1000 mg/kg/day.

2. Test substance formulations and analysis

Administration formulations were prepared using 0.5% aqueous carboxymethylcellulose as the vehicle. Fresh formulations for each concentration were prepared after three to at a maximum seven days of use. The administration formulations were stored at room temperature for the duration of use.

Homogeneity and stability after 8 days at room tenperature were checked before the start of the study at 3 and 100 mg/ml, covering the concentration range used. The content varied from 90 to 105% of the nominal concentration. Homogeneity and content checks were carried out during the study at 5, 20 (content checks only) and 100 mg/ml. The percentage of the nominal concentration varied from 90 to 110%. These results demonstrated no meaningful deviation of the test substance content from the nominal value and homogeneous distribution was confirmed.

7. Statistics

Except for the fœtal visceral and skeletal observations, all data were recorded online. The fœtal visceral and skeletal observations of the females were recorded on paper sheets, serving as raw data.

Females without implantation sites were excluded from any statistical evaluation; females with total resorptions were excluded from statistical evaluation of maternal food intake, body weight data and uterine weight.

Differences between the control and BYH 18636 groups were considered to be significant when $p < 0.05$. The following methods were applied:

- analysis of variance (ANOVA), and in case of significant results Dunnett's test as posthoc test for: food consumption, body weights, body weight gains and corrected body weight gains, uterine weights, number of corpora lutea per female, number of implantations per female, number of live foetuses per female and as percentage of implantations per female, placental weights per female, fœtal weights per female.

- 2 by N CHI^2 test; in case of significant differences Fisher's exact test with Bonferroni correction for: fertility and gestation rate, number of implantations per group, number of pre-implantation losses per group, number of post-implantation losses, early resorptions, late resorptions or dead foetuses per group, number of live foetuses per group in percent of implantations, number of male or female foetuses or foetuses with undeterminable sex per group, number of placentas with findings or litters with placental findings per group, number of foetuses or litters with external, visceral and skeletal findings, number of foetuses or litters with malformations. Skeletal localizations with mechanical damage in single foetuses were excluded from the calculation of percentages of affected localizations.

- Kruskal-Wallis test and in case of significant differences Dunn's test for: number of pre-implantation losses per female, number of post-implantation losses, early resorptions, late resorptions or dead foetuses per female, number of male or female foetuses or foetuses with undeterminable sex per female, proportion of placental, fœtal external and fœtal visceral findings per female.

C. METHODS

1. Clinical examination of females

From GD 0 to 20 all animals were inspected twice daily (only once daily on weekends, on public holidays and on GD 20).

2. Body weight
The body weights of the animals were determined on GD 0 and daily from GD 6 to 20. Corrected body weight gain was calculated by substracting the weight of the uterus on GD 20 from the body weight gain over the period from GD 0 to 20.

3. Food intake
The food consumption was determined for the following intervals: GD 0-3, 3-6, 6-9, 9-12, 12-15, 15-18 and 18-20.

4. Necropsy
The females were subjected to gross pathological examination at the time of Caesarian section on GD 20 without knowledge of treatment groups. The livers and urinary bladders of the females were fixed in 10% neutral buffered formalin solution after necropsy and the kidneys were fixed in Davidson's solution.

5. Investigation at cesarian section
Caesarian sections were performed on GD 20 without knowledge of treatment groups. The females were sacrificed by cardiotomy under deep carbon dioxide anesthesia. The following parameters were determined and assessed at Caesarian section without knowledge of treatment groups: number of corpora lutea, number of implantations (in females without visible implantation sites after staining of the uterus with a solution of 10% ammonium sulfide), uterine weights, individual weight and appearance of placentas, number of early resorptions (only implantation site visible), late resorptions (fœtal or placental remnant visible), and dead fœtuses (fœtuses without signs of life, but without maceration), number of live fœtuses, sex of live fœtuses, individual weight of live fœtuses, external malformations and other findings deviating from normal, visceral malformations and other findings deviating from normal (evaluation of about half of the fœtuses by scalpel and/or razor blade sectioning according to the modified Wilson technique), findings in abdominal, pelvic and thoracic organs as well as skeletal and cartilage findings by the modified Dawson technique with the addition of cartilage staining (cartilage staining with alcian blue GX and skeletal staining with alizarin red S). Every other fœtus within a litter was prepared for either skeletal or visceral evaluation.

Fœtuses were sacrificed by carbon dioxide asphyxia.

Table IIA 5.6.10-1: Number of fœtuses examined by standardized methods

Doses (mg/kg/day)	Total number of fœtuses	Fœtuses investigated according to modified Wilson method	Fœtuses investigated according to modified Dawson/Inouye method
0	219	107	112
50	284	138	146
200	276	130	146
1000	225	107	118

II. RESULTS AND DISCUSSION

A. OBSERVATIONS AND MORTALITY

No mortality and no treatment-related findings up to 1000 mg/kg/day were observed during the study.

B. BODY WEIGHT

Marginal body weight loss occurred at 1000 mg/kg/day between GD 6 and 7, related to the decrease in food consumption. Body weight gain during the treatment period GD 6-19 and during gestation GD0-20 was statistically significantly decreased at 1000 mg/kg/day (decrease of 33% and 26% respectively compared to the control group). Mean corrected body weight gain was also statistically significantly decreased at 1000 mg/kg/day (decrease of 51%) in comparison to the current control. The marginally decreased mean corrected body weight gain in the 200 mg/kg/day group was considered not to be a treatment-related effect, because this value layed within the normal range of scattering for the rat strain used (within historical control data).

Table IIA 5.6.10-2: Mean body weight gain

Intervals	Doses (mg/kg/day)			
	0	50	200	1000
Mean body weight gain				
GD 6 to 19	75.8	84.4	74.0	50.8 **
GD 0 to 20	110.1	119.1	106.0	81.8 **
Corrected body weight gain				
GD 0 to 20	47.4	44.9	40.8	23.0 **

Statistically significant difference to control ** = p< 0.01

C. FOOD INTAKE

Statistically significantly decreased food intake (decrease of 14% to 23% compared to the control group) was observed at 1000 mg/kg/day from GD 6 to 20. An increased incidence of light coloured faeces occurred in this dose group, most likely caused by faecal excretion of the test substance, which was white coloured.

No effect was reported up to 200 mg/kg/day.

Table IIA 5.6.10-3: mean food intake

Intervals	Doses (mg/kg/day)			
	0	50	200	1000
GD 0-3	20.7	20.4	20.3	20.7
GD 3-6	21.6	21.2	20.8	21.7
GD 6-9	21.4	21.2	20.5	18.3 **
GD 9-12	22.3	22.2	20.8	17.8 **
GD 12-15	22.2	22.3	20.8	18.0 **
GD 15-18	25.1	25.1	23.7	20.8 **
Gd 18-20	25.5	25.2	23.5	19.6 **

Statistically significant difference to control ** = p< 0.01

D. NECROPSY

Yellowish sediments were observed in the urinary bladder of twelve females treated at 1000 mg/kg/day. Two of these females had also urethra filled with yellowish sediment and one had a pale kidney unilaterally. Two further females from this group had unilateral dilated ureters with an inlet of yellowish sediment, in one case together with the caudal end of one kidney swollen and light brown discoloured. No other treatment-related gross pathological findings occurred at 1000 mg/kg/day, 200 or 50 mg/kg/day.

E. GENERAL REPRODUCTION DATA

As shown in the following table, the fertility rate, mean numbers of corpora lutea, pre-implantation losses and implantation sites in the different groups did not differ significantly.

Table IIA 5.6.10-4: general reproduction data

Dose (mg/kg/day)	0	50	200	1000
Inseminated females	25	25	25	25
Inseminated females evaluated	25	25	25	25
Females with implantations	21	22	24	21
In % of those inseminated	84	88	96	84
Mean values per female with implantation sites				
Corpora lutea	14.0	15.0	14.4	13.4
Pre-implantation loss	2.6	1.6	2.0	2.0
Implantations	11.5	13.3	12.4	11.5

F. EFFECTS ON INTRAUTERINE DEVELOPMENT

1. Gestation rate

The gestation rate (number of females with viable foetuses as a percentage of the number of females with implantations) was unaffected up to and including 1000 mg/kg/day.

Table IIA 5.6.10-5: Gestation rate

Dose (mg/kg/day)	Females with viable fœtuses		Females with total resorption
	N	In % of females with implantations	
0	**20**	**95.2**	**1**
50	**22**	**100**	**0**
200	**24**	**100**	**0**
1000	**20**	**95.2**	**1**

2. Weight and appearence of placentas

The weight of placentas was marginally decreased at 1000 mg/kg/day (see table IIA 5.6.10-5). No other treatment-related effect was observed in any group.

3. Post-implantation loss, number and sex of foetuses

No treatment-related effect was observed up to and including 1000 mg/kg/day.

4. Fœtal weight

Fœtal weight was statistically significantly decreased (decrease of 9 % compared to the control group) at 1000 mg/kg/day. No effect was observed at 200 and 50 mg/kg/day.

Table IIA 5.6.10-6: Mean values of the parameters of intrauterine development

Dose (mg/kg/day)	**0**	**50**	**200**	**1000**
Nb of females with implantations (a)	**21**	**22**	**24**	**21**
Nb of females with viable fœtuses (b)	**20**	**22**	**24**	**20**
Mean placental weight per female (g) [b]	**0.59**	**0.59**	**0.64**	**0.55**
Mean number of live fœtuses [b]	**10.9**	**12.9**	**11.5**	**11.3**
Mean post-implantation loss per female [a]	**1.1**	**0.4**	**0.9**	**0.8**
Mean post-implantation loss per female [b]	**0.6**	**0.4**	**0.9**	**0.3**
Mean fœtal weight per female in (g) [b]	**3.69**	**3.61**	**3.56**	**3.35 ****

Statistically significant difference to control ** = $p<0.01$
a: data referring to the females with implantations
b: data referring to the females with viable fœtuses

5. Fœtal malformation

The total number of foetuses or litters with malformations was unaffected by treatment up to and including 1000 mg/kg/day.

6. Fœtal external and visceral deviations

The incidence of fœtuses, showing slight dilation or dilation of the renal pelvis was statistically singificantly increased at 200 mg/kg/day. A treatment-related effect was not assumed due to a lack of dose dependency and because the value was comparable with previous data of different unaffected study groups. In addition, the incidence was also likely related to the higher litter sizes at the dose groups, resulting in retarded development. Furthermore, slightly increased incidences of slight dilation of ureters occurred at 50, 200 and 1000 mg/kg/day. However, this was considered not to be treatment-related due to a lack of statistical significance, of dose dependency, when calculation was done on a litter basis, and because the values layed within the range of historical control data or the range of previous unaffected study groups.

Therefore, the incidences and types of fœtal external and visceral deviations were unaffected by treatment up to and including 1000 mg/kg/day.

7. Fœtal skeletal and cartilaginous deviations

A few skeletal retardations and variations were statistically significant at 50 mg/kg/day (retarded ossification of distal phalanges of digits or toes compared to controls) and at 200 mg/kg/day (retarded ossification of distal phalanges of digits, metacarpals, 4th caudal vertebral bodies, and nasal bones bilateral together with a decreased incidence of Dumbbell shaped 11th thoracic vertebral bodies compared to controls). A toxicological relevance of these findings was excluded due to the lack of statistical significance on a litter basis, or a lack of dose dependency or because the incidences layed within the range of historical values.

At 1000 mg/kg/day, statistically significant findings on a fœtal basis included retarded ossification of distal phalanges of digits or toes, 5th metacarpals bilateral, 5th and 6th sternebrae, 4th sacral vertebral arches bilateral, 4th and 5th caudal vertebral bodies. These findings were considered treatment-related as several localizations were affected and as the fœtal weight was also decreased at 1000 mg/kg/day. Furthermore, a statistically significantly increased incidence of wavy ribs occurred at 1000 mg/kg/day, for which a treatment-related effect cannot be excluded due to maternal toxicity at this dose level. The statistically significantly decreased incidence of 14th ribs at 1000 mg/kg/day was considered to be a chance finding.

Evaluation of fœtal cartilaginous tissue revealed no treatment-related findings up to and including 1000 mg/kg/day.

III. CONCLUSION

The NOAEL for maternal toxicity was 200 mg/kg/day, based on body weight loss, decreased placenta weight and yellowish sediment in the urinary bladder, urethras and dilated ureters. The NOAEL for developmental toxicity was also 200 mg/kg/day, based on retarded ossification of several localizations, correlating to decreased fœtal weights.

(Langewische F., 2005)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: totally reliable The study (T4062961) is fully compliant with OECD 414 (2001): the numbers of litters in each group meet the minimum guideline requirements. Dosing from Day 6-19 of gestation is acceptable. PSD has concerns regarding the use of the Bonferroni correction: the Notifier has therefore provided a reanalysis of the statistical significance of the relevant parameters without the use of this correction (Lasserre-Bigot, 2007); this data is reflected in the table below. Maternal bodyweight effects were seen following administration of the first dose and should therefore be considered as a basis for derivation of the acute reference dose. Treatment-related foetal effects were mainly limited to the 1000 mg/kg bw/d dose group. Findings are indicative of delayed skeletal ossification secondary to maternal toxicity, and include incomplete or absent ossification of the distal phalanx digits, metacarpals, the 5th and 6th sternebrae and the sacral vertebral arches. Isolated (but statistically significant) increases in the incidence of unossified 5th left distal phalanx digits seen at 50 and 200 mg/kg bw/d are not considered to be clearly related to treatment in the absence of a dose-response relationship in these groups; it is also notable that the foetal and litter incidences of this finding in the concurrent control group is at the high end of the historical control range. A statistically significant increase in the incidence of unossified 5th right metacarpal at 200 mg/kg bw/d is not considered to be treatment-related in the absence of similar treatment-related effects on other bones (incidence shown below), including the 5th left metacarpal. The incidence of this finding only marginally exceeds the historical control range and is associated with a low concurrent control value (additional historical data provided: Lasserre-Bigot, 2007). A significantly increased incidence of wavy ribs was seen at 1000 mg/kg bw. The foetal incidence is clearly within the historical control range, although the litter incidence slightly exceeds the historical control range. The concurrent control values for this finding are noted to be unusually low and lie outside the historical control range. The apparently increased incidences of dilated renal pelvis and ureter in the treated groups compared to controls are not considered to be related to treatment in the absence of statistical significance and/or a clear dose-response relationship.

Rat developmental toxicity study: additional findings

Parameter	Dose level (mg/kg bw/d)				
	0	50	200	1000	Historical
Maternal weight gain Day 6-7	2.5	3.0	1.9	-0.8	-
	Foetal findings: % foetal incidence [% litter incidence]				
Renal pelvis dilated	5.9 [35.0]	7.4 [63.6]	12.7* [79.2*]	8.9 [60.0]	0.8-13.3 [10.0-75.0]
Ureter dilated	3.2 [30.0]	4.9 [45.5]	5.1 [41.7]	6.7 [50.0]	0-5.6 [0-47.4]
Unossified 5th right distal phalanx digits	16.1 [50.0]	26.0 [72.7]	21.9 [66.7]	33.9** [80.0]	0-15.2 [0-50.0]
Unossified 5th left distal phalanx digits	10.7 [45.0]	26.7** [68.2]	25.3** [66.7]	33.1** [80.0]	0-11.6 [0-50.0]
Unossified 5th right metacarpal	5.4 [25.0]	12.3 [45.5]	15.8* [58.3]	28.0** [80.0**]	2.5-15.1 [13.6-61.9]
Unossified 5th left metacarpal	9.8 [40.0]	13.7 [54.5]	19.9 [66.7]	31.4 [80.0]	3.3-18.2 [9.1-61.9]
Wavy ribs	1.8 [10.0]	2.1 [9.1]	3.4 [16.7]	10.2* [50.0*]	2.8-14.6 [10.0-42.9]

**significantly different to controls p≤0.05; **≤0.01*
Historical control range: 15 studies performed 2002-2005

Conclusions

No evidence of teratogenicity was seen in this study.

A NOAEL for maternal toxicity of 200 mg/kg bw/d can be determined for this study, based on the reduced weight gain and food consumption seen at the top dose level of 1000 mg/kg bw/d. A NOAEL for developmental toxicity of 200 mg/kg bw/d can also be determined, based on the slightly (but significantly) reduced mean foetal weight and the increased incidences of a number of skeletal variations seen at 1000 mg/kg bw/d.

The Notifier proposes the same NOAELs for this study.

B.6.6.3 Developmental toxicity in the rabbit (IIA 5.6.1)

Teratogenicity test by the oral route in the rabbit (IIA 5.6.11)

Report:	**KIIA 5.6.11/01, Wason S., 2006 (MRID # 47070131)**
Title:	BYH 18636, Developmental toxicity study in the rabbit by gavage
Report No &	SA 03350
Document No	M-263644-01-2
Guidelines:	OECD 414 (2001); EEC Directive 2004/73/EC; EPA Health Effects Test Guideline (OPPTS 870.3700; 1998); MAFF in Japan 12-Nousan N° 8147 (2000)
GLP	Yes (certified laboratory)

Executive summary:

Time-mated female New Zealand White Rabbits were exposed to BYH 18636 (batch number mix-batch 702-73-06-0001, a white powder, 96.2% to 96.4% w/w purity) by gavage from Gestation Day (GD) 6 to 28. The doses given were 0, 50, 125 and 500 mg/kg/day in suspension in aqueous solution of 0.5% methylcellulose 400 in a volume of 4 ml/kg. The time-mated females were allocated to four groups (25 per group), the day of mating being Day 0 of gestation.

Maternal body weights were recorded for all surviving females on GD 3, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 29. Food consumption was measured for all surviving females during the intervals GD 3-6, 6-8, 8-10, 10-14, 14-18, 18-22, 22-26 and 26-29. Clinical observations were recorded daily. At scheduled sacrifice, on GD 29, the gravid uterine weight and the number of ribs were recorded and

the dams were evaluated for number of corpora lutea, number and status of implantations (resorptions, dead and live fœtuses). The urinary bladder and kidney were retained from all females. Live fœtuses were removed from the uteri, counted, weighed and examined externally. The heads of fœtuses from approximately half of each litter were immersed in Bouin's fluid and the internal structures examined after fixation. The bodies of all fœtuses were dissected for soft tissue anomalies and sexed. Fœtuses were eviscerated, skinned and fixed in absolute ethanol before staining. A modification of the Staples and Schnell technique was applied and a subsequent skeletal examination was performed.

At 500 mg/kg/day
One female was killed for humane reasons on GD 15 following a marked loss in body weight and poor food consumption. Clinical signs comprised of no/few faeces and yellow sediment in the urine. No macroscopic findings were observed at autopsy. At this dosage, treatment-related clinical signs consisted of an increased incidence of dams with few faeces, yellow sediment in the urine for all dams and red traces under the tray of 2/25 females. There was a mean body weight loss of 0.04 kg compared with a loss of 0.01 kg in the control group between GD 6 to 8. Thereafter, mean body weight gain tended to be lower in the high dose group compared with controls, resulting in an overall reduction in body weight gain of 45%. Maternal corrected body weight change was more pronounced at 500 mg/kg/day (-0.29 kg) compared with the controls (-0.17 kg). Mean food consumption was reduced throughout treatment by between 11 and 19%, compared with the controls, the effect being most pronounced between GD 8 and 10. At autopsy, one female had white sediment in the kidney, one female yellow sediment in the kidney and urinary bladder and one female yellow sediment in the bladder.

At Caesarean section, adjusted mean fœtal body weight, taking into account the difference in mean litter sizes across the groups, was statistically significantly reduced for female fœtuses at the high dose level, where female fœtal body weight was reduced by 8% compared with the control group. No other litter parameters were affected.

There were no treatment-related external fœtal findings at this dosage. However, the mean percentage of runt fœtuses was 23% compared with 6.5% in the control. The only visceral fœtal finding considered possibly to be treatment-related was white sediment in the kidney (unilateral) of one fœtus. This finding is classified as an anomaly. At the skeletal examination, there were no treatment-related findings.

At 125 mg/kg/day
Treatment-related clinical signs in the dams consisted of yellow sediment in the urine noted in 4/25 females. No other maternal parameters were affected.

There were no treatment-related effects on litter parameters. There were no treatment-related external, visceral or skeletal fœtal findings at this dosage.

At 50 mg/kg/day
No treatment-related maternal or fœtal findings were noted at 50 mg/kg/day.

In conclusion, a dose level of 500 mg/kg/day BYH 18636 administered to the pregnant female New Zealand White rabbit caused clear maternal toxicity, as evidenced by mortality, weight loss, reduced food consumption and sediment in the kidney and urinary bladder. Fœtal toxicity at this dose level comprised of reduced body weight in females, increased incidence of runt fœtuses and white sediment in the kidney of one fœtus.

The No Observed Adverse Effect Level (NOAEL) in the dam was 125 mg/kg/day, where the only treatment-related finding consisted of yellow sediment in the urine, and a No Observed Effect Level (NOEL) in terms of fœtal development was 125 mg/kg/day, based on increased incidence of runt fœtuses and white sediment in the kidney (unilateral) of one fœtus.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636
Description: White powder
Lot/Batch: Mix-batch 702-73-06-0001
Purity: 96.4% (December 01, 2003), 96.2% (May 25, 2004)
CAS: 317815-83-1
Stability of test compound: Stable for 28 days

2. Control materials:
Negative: None
Solvent: 0.5% aqueous carboxymethylcellulose
Positive: None

3. Test animals:
Species: Rabbit
Strain: New Zealand White Crl:KBL(NZW)
Age: 18 weeks approximately
Weight at dosing: 3.41 to 3.47 kg for the pregnant females
Source: Charles River Laboratories, Châtillon-sur-Chalaronne, France
Number of animals per dose: 25 females
Acclimation period: 4-5 days before dosing
Diet: Laboratory animal pellets 110C-10 from SAFE (Scientific Animal Food Engineering, Route de Saint Bris, Augy, France), *ad libitum*
Water: Water from municipal supply, *ad libitum*
Housing: housed individually in polycarbonate cages on a perforated cage floor

Environmental conditions –
Temperature: 17 to 21 °C
Humidity: 40 to 70 %
Air changes: 10-15 changes per hour
Photoperiod: 16-hour light, 8-hour dark cycles (5 am - 9 pm)

4. Test compound concentrations: 0, 50, 125 and 500 mg/kg/day based on a previous range-finding study

B. STUDY DESIGN

1. In life dates: Bayer CropScience SA, France conducted the experimental phase of the study during the period of April 27 to September 16, 2004.

2. Animal assigment and treatment
Stock males from the same strain were used by the supplier to mate naturally nulliparus females. The day of insemination was designated as gestation day 0 (GD 0). On each day of mating, females were allocated to four experimental groups including one control and three treated groups using a computerized randomization procedure. Body weight means were checked to ensure similar means among all groups.

The doses were administered daily by gavage at a volume of 4 ml/kg to each female from GD 6 to GD 28 inclusive based on the animal's most recent body weight. Control animals received an equivalent volume of vehicle alone (methylcellulose). The suspensions were mixed continuously before and during treatment with an electromagnetic stirrer.

Table 5.6.11-1: Experimental design

Group	Test substance	Dose levels mg/kg/day	Concentration* g/l	Volume ml/kg	Number of animals
1	Vehicle	0	0	4	25
2	BYH 18636	50	12.50	4	25
3		125	31.25	4	25
4		500	125.0	4	25

* In terms of material as supplied.

3. Test substance formulations and analysis

The appropriate amount of test substance was periodically (six formulations at 12.5 and 125 g/l and seven formulations at 31.25 g/l) suspended (w/v) in an aqueous solution of methylcellulose 400 (Fluka, Mulhouse, France) at 0.5% and stored at approximately 5°C (± 3°C). Homogeneity of the suspensions was checked during the first formulation for the lowest and highest concentrations and on the seventh formulation at the middle concentration. All concentrations were checked for all formulations. Stability of BYH 18636 in suspension in the vehicle was determined before the start of the study at 0.1 and 250 g/l in 0.5% aqueous methylcellulose in a previous study, and was found to be stable for 28 days under similar conditions to those of the current study.

4. Statistics

Means and standard deviations for all maternal, litter and foetal parameters were calculated for each group. Statistical analyses were performed on the following variables using TERATEST Version 1.0.

Maternal endpoints: body weight change calculated according to time periods, calculated corrected body weight change, average food consumption calculated according to time periods.

Litter based and fœtal endpoints: number of corpora lutea, number of implantation sites, number of resorption (early and late), pre- and post-implantation loss percentages, fœtal sex, fœtal death status, fœtal body weight.

Homogeneity of variances between control and treated groups was evaluated using the Bartlett test. If not significant, means were compared using the analysis of variance (ANOVA), which was followed by the Dunnett test (2-sided) if ANOVA indicated significance.

If the Bartlett test was significant (for body weight change, corrected body weight change, number of corpora lutea, number of implantation sites or number of resorptions parameters), group means were compared using the non parametric Kruskal-Wallis test, which was followed by the Dunn test (2-sided) if the Kruskal-Wallis test indicated significance.

If the Bartlett test was significant, a log transformation (for food consumption) or an arcsine root transformation (for pre- or post-implantation loss percentages) was performed. If the Bartlett test on transformed data was not significant, means were compared using the analysis of variance (ANOVA), which was followed by the Dunnett test (2-sided) if ANOVA indicated significance. If the Bartlett test on transformed data was significant, group means were compared using the non parametric Kruskal-Wallis test, which was followed by the Dunn test (2-sided) if the Kruskal-Wallis test indicated significance.

If the Bartlett test was significant for fœtal sex (male vs. female fœtuses) and fœtal death status (live vs. dead fœtuses) endpoints, control group and each exposed group were compared using the Chi-square test (for fœtal sex parameter) or the Fisher Exact test (2-sided) (for fœtal death status parameter). Death status was analyzed both using the fœtus as the statistical unit and using the

litter as the statistical unit.

For litter-based fœtal body weight (combined sexes and per sex) endpoint, if the Bartlett test was not significant, means were compared using a mixed linear model where the treatment was incorporated in the model as a fixed effect and the dam was incorporated in the model as a random effect. If the F test of fixed effect was not significant, the group means were considered homogeneous and no further analysis was performed. If the F test of fixed effect was significant, means of the exposed groups were compared to the mean of the control group using the Dunnett test (2-sided). If the Bartlett test was significant, a log transformation of the data was performed. Dependent upon the result of the Bartlett test on transformed data, means were compared using the analysis of variance (ANOVA) or the non parametric Kruskal-Wallis test.

If one or more group variance(s) equaled 0, means were compared using non parametric procedures.

The homogeneity of group variances, results of the ANOVA or the Kruskal-Wallis tests were evaluated at the 5% level of significance. Group means were compared at the 5% and 1% levels of significance.

C. METHODS

1. Clinical examination of females

All rabbits were observed daily for clinical signs and twice daily for mortality (except once daily on weekends and public holidays).

2. Body weight

Body weights were measured on GD: 3, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 29.

3. Food intake

Full feeder weights were measured on GD: 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 28.
Empty feeder weights were measured on GD: 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 29.

4. Investigation at Caesarian section

Caesarean sections

On GD 29, surviving females were killed by intravenous injection of Dolethal® for examination of their uterine content. Each female was first subjected to macroscopic examination of the visceral organs and the number of ribs was recorded. In the case of no visible uterine implants, but with corpora lutea, uterine horn(s) were immersed in a 20% solution of ammonium sulfide. The urinary bladder and kidney were taken from all females and preserved in 10% neutral buffered formalin, for possible future microscopic examination. Tissues and carcasses were then discarded.

The reproductive tract was weighed (gravid uterine weight), dissected out and the following parameters recorded: number of corpora lutea, number of implantation sites.
Number and localisation of resorption sites (classified as early and late), number of live and dead fœtuses, individual weights of live fœtuses.

Dead fœtuses were defined as fœtuses showings distinct digits visible on fore and hind-paws. Runt fœtuses were defined as live fœtuses weighing less than 28 g at cesarean section of the dam. All the live fœtuses were killed by subcutaneous injection of Dolethal® and subjected to an external examination.

Fœtal examination
All data were recorded without knowledge of treatment group. After visceral examination of the fœtuses, the head of fœtuses from approximately half of each litter was immersed in Bouin fluid and the internal structures examined after fixation. The bodies of all fœtuses were dissected for soft tissue anormalies and sexed. Then the fœtuses were fixed in absolute ethanol before staining. A modification of the Staples and Schnell staining technique was used and a subsequent skeletal examination was performed.

II. RESULTS AND DISCUSSION

A. MATERNAL OBSERVATIONS

1. MORTALITY
There was one mortality during the course of the study which occurred at 500 mg/kg/day. One female treated at 500 mg/kg/day was killed for humane reasons on GD 15, following a marked loss in body weight from the commencement of treatment, associated with no food intake from GD 8 onwards. Clinical signs consisted of no/few faeces and yellow sediment in the urine. There were no macroscopic findings at autopsy.

2. CLINICAL SIGNS
At 500 mg/kg/day, treatment-related clinical signs consisted of an increased incidence of dams noted with few faeces: 10/25 females compared with 1/25 females in the control group, yellow sediment in the urine for all dams, and red traces under the tray of two females, on GD 8 and/or 9.

At 125 mg/kg/day, treatment-related signs were confined to 4/25 females noted with yellow sediment in the urine.

No treatment-related clinical signs were observed at 50 mg/kg/day.

3. PREGNANCY RATE
Pregnancy rate was unaffected by the treatment.

B. BODY WEIGHT
At 500 mg/kg/day, there was a mean body weight loss of 0.04 kg compared with a loss of 0.01 kg in the control group, between GD 6 and 8. Thereafter, mean body weight gain tended to be less at the high dose compared with the controls, resulting in an overall reduction in body weight gain of 45% by GD 29, the effect being statistically significant ($p<0.01$).

Mean body weight change at 125 and 50 mg/kg/day was comparable with the controls.

Maternal corrected body weight change (maternal body weight change independent of the uterine weight), was more pronounced at 500 mg/kg/day (-0.29 kg) compared with the controls (-0.17 kg), the effect being statistically significant ($p<0.05$).

Maternal corrected body weight change at 125 mg/kg/day was comparable with the controls. At 50 mg/kg/day, the effect was more pronounced, but as the magnitude of the change did not occur in a dose-related manner and was not statistically significant, this finding was considered to be incidental.

Table 5.6.11-2: Mean maternal body weight or body weight gain of all pregnant females

Maternal body weight (kg)	Dose levels (mg kg.day)				
		0	50	125	500
Gestation day 3	Mean N	3.43 ± 0.247 (23)	3.46 ± 0.231 (24)	3.43 ± 0.206 (25)	3.50 ± 0.209 (25)
Gestation day 6	Mean N	3.43 ± 0.236 (23)	3.42 ± 0.227 (24)	3.41 ± 0.222 (25)	3.47 ± 0.228 (25)
Gestation day 8	Mean N	3.42 ± 0.235 (23)	3.40 ± 0.227 (24)	3.38 ± 0.215 (25)	3.43 ± 0.239 (25)
Gestation day 10	Mean N	3.45 ± 0.241 (23)	3.42 ± 0.226 (24)	3.40 ± 0.222 (25)	3.44 ± 0.255 (25)
Gestation day 12	Mean N	3.47 ± 0.244 (23)	3.43 ± 0.229 (24)	3.43 ± 0.228 (25)	3.45 ± 0.276 (25)
Gestation day 14	Mean N	3.49 ± 0.227 (23)	3.46 ± 0.224 (24)	3.47 ± 0.218 (25)	3.47 ± 0.285 (25)
Gestation day 16	Mean N	3.54 ± 0.242 (23)	3.51 ± 0.221 (24)	3.50 ± 0.234 (25)	3.53 ± 0.270 (24)
Gestation day 18	Mean N	3.56 ± 0.244 (23)	3.52 ± 0.218 (24)	3.52 ± 0.246 (25)	3.56 ± 0.257 (24)
Gestation day 20	Mean N	3.58 ± 0.239 (23)	3.54 ± 0.220 (24)	3.54 ± 0.249 (25)	3.58 ± 0.267 (24)
Gestation day 22	Mean N	3.61 ± 0.236 (23)	3.56 ± 0.227 (24)	3.58 ± 0.254 (25)	3.59 ± 0.282 (24)
Gestation day 24	Mean N	3.64 ± 0.234 (23)	3.59 ± 0.214 (24)	3.62 ± 0.255 (25)	3.61 ± 0.298 (24)
Gestation day 26	Mean N	3.68 ± 0.238 (23)	3.61 ± 0.206 (24)	3.65 ± 0.253 (25)	3.62 ± 0.317 (24)
Gestation day 29	Mean N	3.74 ± 0.259 (23)	3.66 ± 0.212 (24)	3.72 ± 0.267 (25)	3.65 ± 0.352 (24)
Maternal body weight changes : gestation days 6 to 29	Mean N	0.31 ± 0.140 (23)	0.24 ± 0.176 (24)	0.32 ± 0.159 (25)	0.17** ± 0.197 (24)
Maternal corrected body weight changes	Mean N	-0.17 ± 0.167 (23)	-0.25 ± 0.145 (24)	-0.19 ± 0.149 (25)	-0.29* ± 0.157 (24)

*: $\alpha = 0.05$ **: $\alpha = 0.01$

C. FOOD INTAKE

At 500 mg/kg/day, food consumption was reduced by between 11 and 19% compared with the controls. The effect was most pronounced between GD 8 and 10 where food consumption was reduced by 19% and was statistically significant ($p<0.01$).

Food consumption at 125 and 50 mg/kg/day was similar to the controls.

D. NECROPSY

At 500 mg/kg/day, one female had white sediment in the kidney, one female yellow sediment in the kidney and bladder and one female yellow sediment in the bladder. At 125 or 50 mg/kg/day, there were no treatment-related macroscopic findings.

The few other findings noted were considered to be incidental as they occurred in isolation and/or in a non dose-related manner.

E. LITTER DATA

Mean fœtal body weight for the combined sexes and for the individual sexes were lower in all three treatment levels, though not in a dose-related manner. However, the total number of fœtuses per group and mean number of live fœtuses per litter was considerably higher in the treated groups compared with the controls. Once an adjustment was made to take this factor into account, statistically significant effects ($p<0.05$) were confined to female fœtuses at 500 mg/kg/day, where there was an 8% reduction in body weight in comparison with the controls.

No treatment-related effect was observed for the remaining litter parameters including: early and late resorptions, fœtal death status and percentage of male fœtuses.

Table 5.6.11-3: Mean fœtal weights

Parameters		Dose levels (mg/kg/day)			
		0	50	125	500
Nb live fœtuses per litter	Mean ± SD (N)	8.7 ± 2.4 (23)	10.1 ± 2.4 (24)	9.9 ± 2.6 (25)	9.5 ± 3.1 (24)
Nb live fœtuses		201	243	248	228
Fœtal weight (g) [a]	Mean ± SD (Nb fœtuses)	36.7 ± 5.9 (201)	32.5^{++} ± 6.5 (243)	34.7^{++} ± 6.2 (248)	32.2^{++} ± 7.1 (228)
Male f œtal weight (g) [a]	Mean ± SD (Nb males)	36.9 ± 5.7 (96)	32.3 * ± 6.5 (128)	34.9 ± 6.3 (133)	32.5 * ± 7.1 (121)
Female f œtal weight (g) [a]	Mean ± SD (Nb females)	36.4 ± 6.0 (105)	32.8 * ± 6.7 (115)	34.5 ± 6.0 (115)	31.9 * ± 7.1 (107)
Adjusted mean weight estimate (g) [b]		34.99	33.13	35.08	32.68
Adjusted mean male weight estimate (g) [b]		35.23	33.46	35.17	32.96
Adjusted mean female weight estimate (g) [b]		35.01	32.68	35.10	32.27 *

a: Statistical analysis on mean fœtal weights; ++: $\alpha = 0.01$ with Dunn test; *: $\alpha = 0.05$ with Dunnett test
b: Statistical analysis on adjusted mean fœtal weight using the total number of live fœtuses per litter as covariance; *: $\alpha = 0.05$ with Dunnett test

F. FŒTAL NECROPSY FINDINGS

1. External observation

There were no treatment-related malformations, anomalies, or variant findings at any dose levels.

The number of runt fœtuses (BW<28.0g) was increased at 500 mg/kg/day, where the mean percentage of fœtuses classified as runts was 23% and the percentage of litters affected was 62.5%, compared with 6.5% and 34.8%, respectively, in the control group.

2. Visceral observation

There were no treatment-related and dose-dependent malformations findings.

There was white sediment in the kidney, classified as an anomaly, being observed in one fœtus each at 500 and 50 mg/kg/day. At 500 mg/kg/day, this finding could possibly be related to the test compound as this finding was also seen in the dams. At 50 mg/kg/day, this finding was considered to be fortuitous as this fœtal observation occurred in isolation, was not seen in the dams at this dosage, and in addition there were no incidences at the dam or fœtal level at the higher dose level of 125 mg/kg/day.

There were no treatment-related, statistically significant, and dose-dependent variant findings.

3. Skeletal observation

There were no treatment-related, statistically significant, and dose-dependent malformations, anomalies, or variant findings at any dose level.

III. CONCLUSION

The No Observed Adverse Effect Level (NOAEL) in the dam was 125 mg/kg/day, where the only treatment-related finding consisted of yellow sediment in the urine, and a No Observed Effect Level (NOEL) in terms of fœtal development was 125 mg/kg/day, based on increased incidence of runt fœtuses and white sediment in the kidney (unilateral) of one fœtus.

(Wason S., 2006)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: Totally reliable The study (SA03350) is fully compliant with OECD 414 (2001). The dosing period (Days 6-28) is considered to be appropriate and the number of litters evaluated per dose group exceeds the minimum specified in the guideline. Maternal bodyweight and food consumption effects seen during the early dosing period should be considered for use in derivation of the ARfD. The foetal and litter incidences of runts (defined as foetuses of weight <28 g) were clearly increased at the top dose level compared to the concurrent control values and historical control range (foetal incidence 5.2-16.3%; litter incidence 25.0-54.2%). Findings at 50 mg/kg bw/d, although exceeding the historical control range, are not considered to be treatment-related in the absence of similar findings at 125 mg/kg bw/d and in the absence of a dose-response relationship. Mean foetal weights were significantly lower in all treated groups, however findings are considered likely to be influenced by the relatively low mean litter size in the control group. Adjusted foetal weights indicate that a treatment-related effect is limited to the top dose level. The incidence of the malformation ventricular septal defect was higher in foetuses at 125 and 500 mg/kg bw/d. Values are clearly within the laboratory's historical control range for this finding (foetal incidence 0-1.3%; litter incidence 0-12.5%) and are therefore not considered to be clearly related to treatment. The litter incidence of (unilaterally or bilaterally) misshapen thymus was higher at 125 and 500 mg/kg bw/d, however values are clearly within the laboratory's historical control range for this finding (20.8-57.1%), and are associated with a low concurrent control value. The foetal incidences were comparable in all groups. Findings are not considered to be treatment-related. The litter incidence of short innominate artery was slightly higher at 500 mg/kg bw; this value exceeds that laboratory's historical control range for this finding, however it is notable that the concurrent control incidence also exceeds the historical control range. The foetal incidences of this finding are comparable in all groups. The increased incidence seen at 500 mg/kg bw/d is therefore not considered to be treatment-related. Higher incidences of incomplete and absent ossification of the hyoid centrum seen in all treated groups are not considered to be related to treatment as the values are within the laboratory's historical control range (foetal and litter incidences of 11.8-24.3% and 28.6-54.5%; 0.7-4.2% and 4.2-20.8% of litters respectively).

The single incidences of white sediment in the kidney in foetuses at 50 and 500 mg/kg bw/d are not considered to be clearly related to treatment in the absence of a dose-response relationship and findings at the intermediate dose level of 125 mg/kg bw/d. The high dose group foetus exhibiting this finding was not from the one female in this group with a similar finding (yellow sediment in the kidney).

Rabbit developmental toxicity study: additional findings

Finding	Dose level (mg/kg bw/d) 0	50	125	500
Maternal weight gain D6-8	-0.01	-0.01	-0.03	-0.04
D6-10	0.02	0.01	0.00	-0.03**
Food consumption D6-8	139.4	131.5	123.7	114.3
Runts (%)	6.5 [34.8]	18.5 [58.3]	9.5 [36.0]	23.0 [62.5]
Litter weight (g)	319.3	328.3	343.5	305.9
	Foetal findings (% foetal incidence [% litter incidence])			
Ventricular septal defect	-	-	0.4 [4.0]	0.8 [4.2]
Misshapen thymus	4.0 [21.7]	2.3 [16.7]	4.9 [36.0]	5.0 [37.5]
Short innominate artery	1.5 [13.0]	1.0 [12.5]	0.9 [8.0]	2.2 [20.8]
25 pre-sacral, 6 lumbar vertebrae	-	1.9 [8.3]	1.7 [8.0]	1.8 [8.3]
Hyoid incomplete ossification	15.1 [39.1]	18.6 [54.2]	11.4 [36.0]	20.4 [54.2]
Hyoid not ossified	0.9 [4.3]	2.7 [8.3]	2.8 [16.0]	3.6 [12.5]

Conclusions

No evidence of teratogenicity was seen in this study. A NOAEL for maternal toxicity of 125 mg/kg bw/d can be determined for this study, based on the clinical signs and the effects on body weight and food consumption seen at the top dose level of 500 mg/kg bw/d. The presence of yellow sediment in the urine seen in this dose group is treatment-related but is not considered to constitute an adverse effect. A NOAEL for developmental toxicity of 125 mg/kg bw/d can also be determined, based on the lower mean pup weights and the increased incidence of runts seen at the top dose level of 500 mg/kg bw/d.

The Notifier has proposed the same NOAELs for this study.

B.6.7 Neurotoxicity studies (IIA 5.7)

Acute neurotoxicity - rat (IIA 5.7.1)

Report:	**KIIA 5.7.1/01, Gilmore R.G., Hoss H.E., 2006 (MRID # 47070148)**
Title:	An acute Oral Neurotoxicity Screening Study with Technical Grade BYH 18636 in Wistar Rats
Report No &	201512
Document No	M-279833-01-1
Guidelines:	OECD 424 (1997); EPA Health Effects Test Guideline (OPPTS 870.6200; 1998); M.A.F.F. in Japan notification 12 Nousan N°8147 (2000) guidelines.
GLP	Yes (certified laboratory)

Executive summary:

Technical grade BYH 18636 was administered by gavage in a single dose to non-fasted young adult Wistar rats. Four dose groups (12 rats/sex/dose level) were administered the test substance at nominal doses of 0 (vehicle), 125, 500 or 2000 mg/kg for both sexes. All 12 rats/sex/dose level were used for neurobehavioural evaluation, with six/sex/dose level used for micropathology. Body weight was measured weekly as a component of the functional observational battery (FOB). Detailed clinical observations for each animal were performed daily throughout the study. Observations for moribundity and mortality were performed at least once daily. Automated measurements of activity (figure-eight maze) and a functional observational battery (FOB) were conducted during the week prior to treatment and on days 0 (the day of treatment, at the time of peak effect), 7 and 14. All animals placed on study were subjected to a gross necropsy. Selected animals (six/sex/dose level) were perfused, the brain was weighed in order to calculate the brain/body weight ratio and skeletal muscle, peripheral nerves, eyes (with optic nerves) and tissues from the central nervous system were examined microscopically for lesions.
Based on analytical results, the actual doses of BYH 18636 used in the study were 0, 131, 512 and 2180 mg/kg for males and females.

Effects attributed to exposure to BYH 18636 were as follows:

2180 mg/kg. In females, there was a decrease in motor (43%) and locomotor (53%) activity on day 0, which resolved by day 7. Clinical signs associated with treatment in both sexes included urine and red nasal staining on days 1-4. Other signs (white perigenital area, white substance on the bedding and/or urine collection tray and, in females only, red substance in the urine collection pan and milky-white urine on the open field) in both sexes are attributed to the excretion of the parent compound. All signs of exposure reversed by day 5.

512 mg/kg. White perigenital area and white substance on the bedding material were evident in both sexes (excreted parent compound). All signs of exposure reversed by day 1.

131 mg/kg. There were no signs related to treatment in either sex.

Conclusions. Compound-related effects following a single oral dose of the test substance were limited to the high dose (2000 mg/kg) males and females. Effects in males and/or females consisted of decreased motor and locomotor activity on the day of treatment, with recovery by the next test occasion, urine and nasal staining, which resolved within five days after treatment. Other findings in high- and mid-dose males and females were attributed to the excretion of the parent compound, which did not represent systemic toxicity or an adverse effect. Based on these findings, a NOAEL of 512 mg BYH 18636/kg body weight was established in both sexes. There was no evidence of neurotoxicity at any dose level and there were no compound-related gross or microscopic lesions at the high dose of 2180 mg/kg.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636
Description: white powder
Lot/Batch: Mix batch 702-73-06-0001
Purity: 96.1% (January, 2005, certified through July, 2005)
CAS: 317815-83-1
Stability of test compound: Stable in the vehicle at the room temperature for 8 days

2. Vehicle and /or positive control: 0.5% methylcellulose/0.4% Tween 80 in deionized water

3. Test animals:
Species: Rat
Strain: Wistar HAN CRL: WI (HAN)
Age: at least 9 weeks
Weight at dosing: 233.8 to 294.4 g for the males and 165.8 to 208.2 g for the females
Source: Charles River Laboratories, Inc., (Raleigh, NC)
Acclimation period: 8 days
Diet: Purina Mills Rodent Lab Chow 5002 in meal form provided for *ad libitum* consumption during the acclimation period and throughout the study except during neurobehavioural testing
Water: Tap water, *ad libitum* except during neurobehavioural testing
Housing: Animals were caged individually in suspended stainless steel wire-mesh cages.
Environmental conditions –
Temperature: 22 ± 4°C
Humidity: 50 ± 20%
Air changes: At least 10 changes per hour
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN:

1. In life dates: April 26, 2005 to May 26, 2005 at Bayer CropScience LP, Stilwell, KS, United States.

2. Animal assignment and treatment
There were 12 animals of each sex per dose group. Animals were assigned using a randomization by weight. Four dose groups (12 rats/sex/dose level) were administered the test substance at nominal doses of 0 (vehicle), 125, 500 or 2000 mg/kg (actual doses; 131, 512 and 2180 mg/kg) for both sexes. The test substance was administered by gavage as a single dose in 0.5% methylcellulose / 0.4% Tween 80 in deionized water, at a dosing volume of 10 ml/kg. The table below summarizes the study design.

Table 5.7.1.-1: Study design and animal assigment

	Dose Group (mg/kg bwt)			
Experimental Parameter	**Control**	**125**	**500**	**2000**
Total number of animals/sex/group	12	12	12	12
Behavioural Testing (FOB, Motor Activity) [a]	12/sex	12/sex	12/sex	12/sex
Neuropathology [b]	6/sex	0/sex	0/sex	6/sex

[a] *FOB and motor activity were assessed prior to dosing and again during days 0, 7 and 14.*
[b] *Tissues from the mid- and low-dose groups were not examined, as no treatment-related neuropathology was noted at the highest dose level.*

The rationale for dose selection was based on the results of an acute oral toxicity study in young adult female Wistar rats. In that study, six fasted female Wistar rats were administered an acute oral (gavage) dose of 2000 mg/kg as an aqueous suspension in 2% cremophor EL in demineralized water, at a dosing volume of 10 ml/kg. Animals were observed for mortality and clinical signs for at least 14 days after treatment. The test substance produced no clinical signs or mortality at the limit dose. These results supported the use of a limit dose (2000 mg/kg) in the neurotoxicity study but provided no information to establish the time of peak effect.

Thus, the results from a study using radio-labeled BYH 18636 were examined to estimate the time of peak effect. In that study, adult male and female Wistar rats (4/sex/dose) received a single oral (gavage) dose of 2 mg/kg and another group of four males received a dose of 100 mg/kg BYH 18636 both in 0.5% aqueous tragacanth, at a dosing volume of 10 ml/kg. In that study, the tmax in plasma occurred 1 hour after treatment in both sexes following administration of 2 or 100 (males only) mg/kg.
Based on these collective results, the doses selected for this study were 0, 125, 500 and 2000 mg/kg for both sexes Based on the estimated time of peak blood concentrations at this dose range, the FOB began approximately one hour (minimum) following dose administration, with the automated test of activity concluding at about three hours after treatment.

3. Diet preparation and analysis
Doses were prepared by suspending the test substance in 0.5% methylcellulose/0.4% Tween 80 in deionized water. The concentration of BYH 18636 in the vehicle was measured using liquid chromatographic (LC) analysis. The homogeneity and stability of BYH 18636 in the vehicle were established using samples at nominal concentrations (10 and 220 mg/ml) that bracketed the range of concentrations used in the present study (nominal 12.5 and 200 mg/ml). Homogeneity was accepted if the percent relative standard deviation (%RSD) was <6%. Each dosing suspension was also analyzed to measure the concentration of BYH 18636.

Homogeneity Analysis: Homogeneity of the test substance in the vehicle was acceptable for the range of concentrations used here, as the 10 mg/ml and 220 mg/ml concentrations had percent relative standard deviations (%RSD) of 0.78% and 0.62%, respectively.

Stability Analysis: The stability (at room temperature conditions) of BYH 18636 in the vehicle was established, with no appreciable decrease in concentration with eight days at room temperature storage for nominal concentrations of 10 mg/ml or 220 mg/ml (equivalent to doses of 100 or 2200 mg/kg, respectively).

Concentration Analysis: Doses of 0, 125, 500 and 2000 mg/kg for males and females ranged from 102% to 109% of the nominal concentrations. Based on these results, the analytically confirmed doses for males and females were 0, 131, 512 and 2180 mg/kg.

4. Statistics:
Statistical evaluations were generally performed using software from either INSTEM Computer Systems or SAS. The level of significance was set at $p \leq 0.05$, with the exception of Bartlett's

test, which was tested at $p \leq 0.001$. In some cases, data were visually screened for potential effects prior to being subjected to statistical analysis. In general, continuous data were initially assessed for equality of variance using Bartlett's test. Group means with equal variances were analyzed further using an Analysis of Variance (ANOVA), followed by a Dunnett's test if a significant F-value was determined in the ANOVA. In the event of unequal variances, these data were analyzed using nonparametric statistical procedures (Kruskal-Wallis ANOVA followed by the Mann-Whitney U test for between-group comparisons). For the FOB, continuous data were first analyzed using a Repeated-Measures ANOVA, followed by a one-way ANOVA if there was a significant interaction between dose group and test day. For days on which there was a significant treatment effect, Dunnett's test was applied to determine which groups, if any, were significantly different from the control group. Categorical data collected in the FOB was analyzed in a similar manner, using General Linear Modeling and Categorical Modeling (CATMOD) Procedures, with post-hoc comparisons using Dunnett's test and an Analysis of Contrasts, respectively.

Motor and locomotor activity (total session activity and activity for each 10-minute interval) was analyzed using ANOVA procedures. Session activity data were analyzed using a Repeated-Measures ANOVA, followed by a one-way ANOVA if there was a significant interaction with test occasion. For days on which there is a significant treatment effect, Dunnett's test was used to determine which, if any, groups were significantly different from the control group. Interval data were subjected to a two-way Repeated-Measures ANOVA, using both test interval and test occasion as repeated measures, followed by a Repeated Measures ANOVA to determine on which days there was a significant treatment by interval interaction. For those days, the data for each interval were subjected to analysis using a one-way ANOVA to determine at which intervals there was a significant treatment effect. For those intervals, Dunnett's test was used to determine which groups, if any, were significantly different from the control group.

Continuous pathology data (e.g., brain weight) was initially evaluated using Bartlett's test to analyze for homogeneity of variance among groups. Groups with homogeneous variances were analyzed further using an ANOVA followed by Dunnett's test for pair-wise comparisons. In the event of non-homogeneous variances, continuous data were analyzed using the nonparametric Kruskal-Wallis test followed by a Mann-Whitney U test for pair-wise comparisons. Micropathology frequency data were screened for potential effects and then evaluated using a Chi-Squared procedure, followed by a one-tailed Fischer's Exact Test in cases of significant variation by the Chi-Square analysis. Micropathology severity grades were not routinely subjected to statistical analysis. Additional statistical tests to assess continuous and frequency data were used when deemed appropriate.

B. METHODS:

1. Mortality and Clinical Observations

Cage-side observations were performed at least once daily (once daily on holidays and weekends) for mortality or clinical signs of moribundity. Detailed physical examinations for clinical signs of toxicity were carried out and recorded daily.

2. Body weight:

Animals were weighed weekly as a component of the FOB. Additionally, all animals were weighed on the day of sacrifice for terminal body weight measurement.

3. Food Consumption:

Food consumption was not measured in this study.

4. Neurobehavioral Assessment:

All animals that were assigned to the study were tested using the FOB and motor activity on four occasions - one week prior to treatment, approximately 1 hour after administration of the dose, and again 7 and 14 days following treatment. The order of testing and assignment of animals to mazes were done in a semi-random manner, such that groups were balanced across test times and test devices, and no animal would be tested more than once in the same maze. On the day of FOB and motor activity testing, the appropriate animals were placed in the sequence that was established for testing on that day. The dose group identification was concealed at that time to ensure that testing would be performed without knowledge of the group assignment. Sets of eight animals (maximum) were evaluated individually using the FOB and then, approximately 30 minutes after the last animal in the set had finished being tested in the FOB, all eight rats were placed individually into the mazes to measure activity.

a. Functional Observational Battery (FOB):

The FOB closely follows the battery of tests described by Moser, with each animal tested individually. The technicians who performed the FOB were "blind" with respect to the animal's group assignment. Observations and measurements (e.g., grip strength and foot splay) for all animals were performed by the same person throughout the study. Inter-observer reliability has been established in order to allow a second person to perform either the observations or measurements, ensuring the consistency of the results of each technician. Studies have been conducted with acrylamide, carbaryl and untreated rats to establish the sensitivity, reliability, and validity of these test procedures, the adequacy of training of technical personnel and to serve as a historical control.

When applicable, observations were scored on intensity as follows: 1) slight (barely perceptible or infrequent) or 2) moderate to severe. Data were collected while the rats were in their home cage, during handling, and in an open field for 2 minutes (in the centre of a flat surface with a perimeter barrier, such as a cart). In addition, reflex and physiologic observations and measurements were made while the animals were sitting on the cart surface following open field observations.

Home cage observations included: posture, piloerection, involuntary motor movements (such as repetitive "chewing" movements of mouth and jaw, tremors, and convulsions), gait abnormalities, vocalizations, decreased activity, repetitive head bobbing, and increased reactivity.

Observations during handling included: ease of removal from cage, reaction to being handled, muscle tone, palpebral closure, lacrimation, salivation, nasal discharge, stains (lacrimal, nasal, perianal, urine, oral), alopecia, emaciation, bite marks, exophthalmia, broken teeth/malocclusion, missing toe nail(s), dehydration, and temperature upon touching (cool-to-touch).

Open field (2 min.) observations included: number of rears, piloerection, respiratory abnormalities, posture, involuntary motor movements, stereotypy (excessive or repetitive behavior), bizarre behaviour, gait abnormalities, vocalizations, arousal level, and amount of excretion.

Reflex and physiologic observations/measurements included: approach response, touch response, auditory response, tail pinch, pupil size at normal lighting, pupil response, righting reflex, grip strength body weight, body temperature, and landing foot splay.

b. Motor and Locomotor Activity:

Motor and locomotor activities were measured approximately 30 minutes after the last animal in the set (8 rats maximum) had finished the FOB. All rats in each set were placed individually into figure-eight mazes and activity was measured for a total of 60 minutes. Eight infrared emitter / detector pairs (three in each of the figure-eight alleys and one in each of the blind alleys) measured activity; each time a beam was interrupted, an activity count was registered. Broad-spectrum background noise (approximately 74 dB(A)) was provided throughout the test to minimize acoustical variations during testing.

Motor and locomotor activities were examined during each of the six, ten-minute intervals. Motor activity was measured as the number of beam interruptions that occurred during the test session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Thus, for locomotor activity, only one interruption of a given beam was counted until the rat relocated in the maze and interrupted one of the other beams. Habituation was evaluated as a decrement in activity during the test session.

5. Sacrifice and Pathology:

All animals placed on study were subjected to a complete gross necropsy. The necropsy involved an examination of all organs, body cavities, cut surfaces, external orifices and surfaces. On day 14, a minimum of six males and six females at each dose level were selected for perfusion and collection of tissues, with replacement, as necessary, if the perfusion was considered inadequate. These animals were deeply anaesthetized using an intraperitoneal dose (50 mg/kg) of pentobarbital and then perfused via the left ventricle with a sodium nitrite (in phosphate buffer) flush followed by Universal fixative (1% (w/v) glutaraldehyde and 4% (w/v) EM-grade formaldehyde) in phosphate buffer. The entire brain and spinal cord, both eyes (with optic nerves) and selected (bilateral) peripheral nerves (sciatic, tibial and sural), the gasserian ganglion, gastrocnemius muscle, both forelimbs, gross lesions in neural tissues or skeletal muscle and physical identifier were dissected from each animal and post-fixed in 10% buffered formalin. The brain was weighed upon removal from the skull, prior to placement into formalin, and the brain/body weight ratio was calculated. Other animals that survived to term were sacrificed by CO_2 asphyxiation without perfusion.

Micropathology examinations were performed on a comprehensive battery of neural tissues from perfusion-fixed control and high-dose rats of both sexes, as follows. Eight coronal sections of the brain and sections from three levels of the spinal cord (cervical, thoracic, lumbar) and the cauda equina were embedded in paraffin and examined utilizing haematoxylin and eosin (H&E). Dorsal root ganglia (including dorsal and ventral root fibres) from the cervical and lumbar swellings and gasserian ganglion were embedded in glycol methacrylate (GMA). Eyes, optic nerves and gastrocnemius muscle were embedded in paraffin and stained using H&E. Peripheral nerve tissues (sciatic, tibial and sural nerves) were embedded in GMA and cut in cross/transverse-section as well as longitudinal section. GMA-embedded tissues were sectioned at 2-3 µm and stained using a modified Lee's stain. The sciatic nerve was also cross-sectioned at approximately 2-3 µm and stained with a modified Lee's stain. In addition, histopathology was performed on any gross lesions collected at necropsy. Tissues from perfusion-fixed animals at the low- and mid-dose levels were not subjected to micropathology unless compound-related lesions were present in the high-dose group.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Mortality

There were no compound-related deaths at any dose level in either sex. No animals were found dead or sacrificed *in extremis* during the course of the study.

2. Clinical signs

Compound-related effects at the nominal 2000 mg/kg dose consisted of red nasal staining in two males and one female on day 1 only and urine staining in one male and two females on days 1-4. Urine staining persisted in high-dose males and females, which compares with urine staining in two control males on day 0 only.

Other clinical signs that were associated with treatment occurred in males and females at the mid- and high-dose levels. In males, this consisted of a white perigenital area (6 mid- and 10 high- dose), white substance on the bedding (8 mid- and 7 high-dose) and white substance in the urine collection tray (five high-dose). In females, this consisted of a white perigenital area (2 mid- and 5 high-dose), a red or white substance in the urine collection pan (one or three high dose animals, respectively), and a white substance on the bedding (3 high-dose females). All of these findings were present in males and females on day 0 only. Samples of the white substance were collected from the perigenital area and the bedding or urine collection trays and analyzed and determined to be the parent compound. The urine samples and the sediment sample from one high-dose male and the urine sample from one high-dose female were analyzed for the presence of the active ingredient or metabolites. Positive and negative ion electrospray (ESI) spectra confirmed the presence of the compound in the samples. Therefore, these findings in mid- and high-dose males and females do not represent a systemic or adverse effect.

Table 5.7.1.-2: Treatment-related clinical Observations

Observation	Dose Level (mg/kg bw)			
	Control	125	500	2000
Males				
Urine Staining	2/12	0/12	0/12	1/12
Red Nasal Staining	0/12	0/12	0/12	2/12
White Substance on Bedding	0/12	0/12	8/12	7/12
White Substance in Urine Collection Tray	0/12	0/12	0/12	5/12
White Perigenital Area	0/12	0/12	6/12	10/12
Females				
Urine Staining	0/12	0/12	0/12	2/12
Red Nasal Staining	0/12	0/12	0/12	1/12
White Substance on Bedding	0/12	0/12	1/12	0/12
White Substance in Urine Collection Tray	0/12	0/12	0/12	3/12
Red Substance in Urine Collection Tray	0/12	0/12	0/12	1/12
White Perigenital Area	0/12	0/12	2/12	5/12

Numbers represent the total number of animals exhibiting the sign at least once/number of animals in each group

B. BODY WEIGHT

Body weight was not affected by treatment in either sex at any dose level.

C. NEUROBEHAVIORAL RESULTS

1. FOB findings

Treatment-related findings were evident in females at the mid- and high-dose levels but not at the lowest dose or in males at any dose level. For females, treatment-related findings were limited to white perigenital area in one mid- and five high-dose animals. This finding was statistically significant in high-dose females. Lastly, milky-white urine was found on the open field in two high-dose females. Both of these findings were present on day 0 and resolved by the next test occasion, seven days after treatment.

Table 5.7.1.-3 : Functional Observational Battery Results

Females	Observation	Dose Level (mg/kg bw)			
		Control	125	500	2000
Day 0	Handling - Stains:				*
	Not Observed	12(100)	12(100)	11(92)	7(58)
	White Perigenital Area, Slight	0(0)	0(0)	1(8)	1(8)
	White Perigenital Area, Moderate/Severe	0(0)	0(0)	0(0)	4(33)
	Open Field Observations:				
	Not Observed	12(100)	12(100)	12(100)	10(83)
	Milky-White Urine on Table, Present	0(0)	0(0)	0(0)	2(17)

Values represent the number of animals and % incidence (in parentheses) with observation.

*n=12 animals/dose level *=p≤05,** p≤01 compared with controls*

2. Motor activity

An examination of inherent variability, using the average pre-treatment values among the four groups of males and females, provides a measure of the magnitude of the difference that should be considered biologically significant. For motor activity, the pre-treatment values for groups that later received the test substance averaged from 12% to 28% higher than controls for males and from 2% to 13% higher than controls for females. For locomotor activity, the pre-treatment values for groups that later received the test substance averaged from 10% to 29% higher than controls for males and from 0.3% to 17% higher than controls for females. As a general guide, these results confirm that differences of + 20% are within the range of normal variability in this laboratory for groups of 10-12 rats/sex/dose level and, therefore, are not biologically significant.

For the overall 60-minute test session, there was a compound-related decrease in session motor (43%) and locomotor (53%) activity in high-dose females on the day of treatment, with complete recovery by the next test occasion (day 7). There was no compound-related effect on session motor or locomotor activity in females at lower doses or in males at any dose.

Table 5.7.1.-4: Summary Session Motor Activity Results (Percent Difference from Control)

MALES				
Nominal Dose	**Pre-treatment**	**Day 0**	**Day 7**	**Day 14**
125 mg/kg	**+ 12**	**+ 8**	**+ 9**	**+ 22**
500 mg/kg	**+ 28**	**+ 23**	**+ 23**	**+ 17**
2 000 mg/kg	**+ 21**	**- 1**	**+ 24**	**+ 24**
FEMALES				
Nominal Dose	**Pre-treatment**	**Day 0**	**Day 7**	**Day 14**
125 mg/kg	**+ 5**	**- 14**	**+ 4**	**- 9**
500 mg/kg	**+ 13**	**+ 6**	**+ 10**	**+ 5**
2 000 mg/kg	**+ 2**	**- 43***	**- 3**	**- 16**

[a] *Percent greater (+) or less (-) than concurrent control for N=12.*

Summary session motor activity was statistically different from control (= p≤0.05; ANOVA).*

Table 5.7.1.-5 Summary Session Locomotor Activity Results (Percent Difference from Control)

MALES

Nominal Dose	Pre-treatment	Day 0	Day 7	Day 14
125 mg/kg	+ 10	+ 9	+ 7	+ 26
500 mg/kg	+ 29	+ 20	+ 16	+ 13
2 000 mg/kg	+ 22	- 9	+ 18	+ 25
FEMALES				
Nominal Dose	Pre-treatment	Day 0	Day 7	Day 14
125 mg/kg	+ 6	- 20	- 3	- 12
500 mg/kg	+ 17	+ 0.3	+ 6	+ 4
2 000 mg/kg	+ 0.3	- 53*	- 11	- 24

[a] *Percent greater (+) or less (-) than concurrent control for N=12 (unless otherwise noted).*
Summary session locomotor activity was statistically different from control (= p≤0.05; ANOVA) .*

Motor and locomotor activity data were subjected to further analysis at each interval on each test day. The only compound-related effect consisted of a slight (non-statistical) decrease in measures of motor and locomotor activity for high-dose females on day 0, at all intervals. These slight differences from control were considered to be due to treatment, since they occurred at the high dose, on the day of treatment, and because cumulative activity (for the entire test session) was statistically different from control (see above). There were no compound-related effects in females at lower dose levels and the lower level of activity in high dose females resolved by the next test occasion. In males, there were no treatment-related effects at any dose level on any test occasion.
Habituation was not affected by treatment with BYH 18636 at any dose level.

Table 5.7.1.-6 Motor activity (total activity counts for session)

Test Day	Dose Level (mg/kg bw)			
	Control	125	500	2000
Males				
Pre-test	452±142	506±182	580±141	547±190
Day 0	459±131	495±179	563±148	455±151
Day 7	486±94	531±101	596±116	605±100
Day 14	476±131	583±153	558±74	591±102
Females				
Pre-test	590±280	621±147	666±165	604±144
Day 0	611±166	525±204	649±169	**347*±211**
Day 7	544±143	565±84	599±173	530±234
Day 14	621±177	567±158	654±193	519±138

Values represent mean ± s.d. for 1 hr Test Session (hh mm ss)
*n=12 *=p≤05, ** p≤.01 compared with controls*

Table 5.7.1.-7 Locomotor activity (total activity counts for session)

	Dose Level (mg/kg bw)

Test Day	Control	125	500	2000
Males				
Pre-test	259±85	285±101	333±83	315±120
Day 0	254±72	278±108	304±77	231±90
Day 7	255±38	273±64	295±62	302±73
Day 14	239±69	301±100	271±63	298±36
Females				
Pre-test	286±122	304±89	334±88	287±106
Day 0	338±103	272±102	339±103	**160***±119
Day 7	281±61	273±45	298±80	249±126
Day 14	316±78	279±81	328±98	239±67

Values represent mean ± s.d. for 1 hr Test Session (hh mm ss)
*n=12 *=p≤.05, ** p≤.01 compared with controls*

D. SACRIFICE AND PATHOLOGY

1. Gross pathology

There were no compound-related gross lesions evident at terminal sacrifice in males or females at any dose level.

2. Terminal body and brain weights

There was no compound-related effect on terminal body weight or brain weight in perfused males or females at any level of exposure.

Table 5.7.1.-8: Absolute and Relative Brain Weights

Weights (g)	Dose Level (mg/kg bw)			
	Control	125	500	2000
Males				
Body wt [a]	308.7±25.2	314.2±24.1	310.4±19.4	308.4±13.8
Brain wt [b]	1.840±0.037	1.842±0.136	1.833±0.096	1.797±0.135
Brain/body wt [b]	0.584±0.040	0.590±0.051	0.597±0.013	0.593±0.033
Female				
Body wt [a]	202.8±10.9	200.8±9.0	205.4±10.8	200.1±13.3
Brain wt [b]	1.708±0.079	1.755±0.087	1.716±0.031	1.718±0.065
Brain/body wt [b]	0.850±0.059	0.859±0.037	0.844±0.019	0.832±0.036

[a] *N=12* * *Statistically different (p ≤ 0.05) from the control.*
[b] *N=6*

3. Neuropathology

There were no compound-related microscopic lesions in the high-dose males or females. Therefore, tissues from animals that received a lower dose of BYH 18636 were not examined.

III. CONCLUSION

In conclusion, compound-related effects following a single oral dose of the test substance were limited to the high dose (2000 mg/kg, nominal concentration) males and females. Effects in males and/or

females consisted of decreased motor and locomotor activity on the day of treatment, with recovery by the next test occasion, urine and nasal stains, which resolved within five days after treatment. Other findings in high- and mid-dose males and females were attributed to the excretion of the parent compound, which did not represent a systemic or adverse effect. There was no evidence of neurotoxicity at any dose level and no compound-related gross or microscopic lesions at the high dose of 2000 mg/kg. These results establish a NOAEL of 512 mg BYH 18636/kg body weight.

(Gilmore R.G., 2006)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: Reliable with restrictions The study is compliant with OECD 424 (1997) with the exception of the following deviations: The OECD guideline states that 'dosing should normally begin as soon as possible after weaning, preferably not later than when the animals are six weeks, and, in any case, before the animals are nine weeks of age'. The study report states that the animals in this study were 'at least nine weeks' old at the time of dosing. This deviation is not considered to affect the integrity of the study in this case. It is noted that there was no indication of neurotoxicity in any of the standard toxicity studies (i.e. those studies required under Directive 91/414/EEC), including in the neurotoxicity assessment performed as part of the 90-day toxicity study (McElligott, 2003). A study of neurotoxicity is therefore not a specific data requirement for this compound according to EU legislation, based on the absence of findings in the other studies and the mode of action of the compound. Food consumption was not measured in this study, whereas the guideline specifies that food consumption should be measured at least weekly. This deviation is not considered to be critical and does not affect the integrity of the study. Food consumption was not affected in most of the other studies in the rat (or other species) and is therefore unlikely to have been a critical parameter in this study. Findings in females at the top dose level and at the time of peak effect (reduced motor and locomotor activity) are considered to be secondary to general toxicity and do not represent specific neurotoxicity. The clinical signs consistent with urinary excretion of the test material (white urine, white staining) seen at 500 and 2000 mg/kg bw are not considered to be of toxicological significance in isolation, however these signs are notable in that they indicate that precipitation/crystallisation of the test material in the urine may occur following the administration of a single dose and that an acute reference dose should be considered for this compound. It is notable that similar findings were not reported in either of the acute oral toxicity studies with thiencarbazone-methyl (Schüngel, 2004; 2006).
Conclusions	No evidence of specific neurotoxicity or neuropathology was seen following the administration of a single oral limit dose of 2000 mg/kg bw under the conditions of this study. A NOAEL of 500 mg/kg bw (actual achieved dose 512 mg/kg bw) can be determined, based on the reduced activity in females and the signs of toxicity (white urine and red nasal staining) in both sexes at the top dose level of 2000 mg/kg bw (achieved dose 2180 mg/kg bw). The Notifier has proposed the same NOAELs.

Delayed neurotoxicity following acute exposure (IIA 5.7.2)

BYH 18636 is not an organophosporous insecticide and there was no indication of neurotoxicity, therefore this type of study is not triggered.

28-day delayed neurotoxicity (IIA 5.7.3)

BYH 18636 is not an organophosporous insecticide and there was no indication of neurotoxicity, therefore this type of study is not triggered.

Subchronic neurotoxicity – rat – 90-day (IIA 5.7.4)

Report:	**KIIA 5.7.4/01, Gilmore R.G., 2006 (MRID # 47070149)**
Title:	A Subchronic Neurotoxicity Screening Study with Technical Grade BYH 18636 in Wistar Rats
Report No &	201518
Document No	M-281238-01-1
Guidelines:	**OECD 424 (1997); EPA Health Effects Test Guideline (OPPTS 870.6200; 1998); M.A.F.F. in Japan notification 12 Nousan N°8147 (2000) guidelines.**
GLP	**Yes (certified laboratory)**

Executive summary:

Technical grade BYH 18636 (Mix batch 702-73-06-0001, 95.7 % - 96.4% of purity) was administered in the diet for 13 weeks to young adult Wistar rats (12/sex/dietary level), using nominal concentrations of 0, 500, 2000 and 6000 ppm for males and females. The top dose level for this study was chosen based on mortality observed in a 90-day toxicity study at a higher dietary level (i.e., 7000 ppm). All 12 rats/sex/dietary level were used for neurobehavioural evaluation, with micropathology performed on selected tissues from 6 rats/sex from control and high-dose groups. Body weight and food consumption determinations, as well as a detailed clinical observation for each animal, were conducted weekly throughout the study. Observations for moribundity and mortality were performed at least once daily. Automated measurements of activity (figure-eight maze) and a functional observational battery (FOB) were conducted the week prior to treatment and during weeks 2, 4, 8 and 13. Ophthalmologic examinations were conducted on all animals prior to shipment release and then again on all study animals during week 12. All animals placed on study were subjected to a gross necropsy. For selected animals, the brain was weighed in order to calculate the brain/body weight ratio and skeletal muscle, peripheral nerves, eyes (with optic nerves) and tissues from the central nervous system were also examined microscopically. Additionally, the urinary bladder and kidneys were collected, weighed (kidneys only) and preserved for histopathologic evaluation.

The mean daily intake of the test substance (mg BYH 18636/kg body wt/day) over approximately 13 weeks at nominal dietary concentrations of 500, 2000 or 6000 ppm, respectively, was 33.1, 137 and 411 mg/kg/day for males and 42.4, 171 and 527 mg/kg/day for females. There were no treatment-related effects attributed to exposure to BYH 18636 at any dietary level in either sex.

Conclusions. Through approximately 13 weeks of continuous dietary exposure to the test substance, there were no treatment-related findings apparent at any dietary level in either sex. Based on these findings, a NOAEL of 6000 ppm was established for the rat (specifically, 411 and 527 mg BYH 18636/kg body wt/day for males and females, respectively).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	BYH 18636
Description:	white powder
Lot/Batch:	Mix batch 702-73-06-0001
Purity:	96.4% (June 2005); 95.7% (November 2005; Expiration date: November 2006)
CAS:	317815-83-1
Stability of test compound:	Stable in the vehicle at 10 and 12000 ppm at the room temperature for 7 days

2. Vehicle and /or positive control: Acetone served as a solvent in the diet preparation process and was allowed to evaporate prior to administration.

3. Test animals:

Species: Rat
Strain: Wistar HAN CRL: WI (HAN)
Age: at least 9 weeks
Weight at dosing: 228.8 – 294.6 g for the males – 135.7 – 172.4 g for the females
Source: Charles River Laboratories, Inc., (Raleigh, NC)
Acclimation period: 9 days
Diet: Purina Mills Rodent Lab Chow 5002 in meal form provided for *ad libitum* consumption during the acclimation period and throughout the study except during neurobiavioural testing
Water: Tap water, *ad libitum* except during neurobiavioural testing
Housing: Animals were caged individually in suspended stainless steel wire-mesh cages.
Environmental conditions –
Temperature: 22 ± 4°C
Humidity: 50 ± 20%
Air changes: At least 10 changes per hour
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN:

1. In life dates: September 07, 2005 to December 21, 2005 at Bayer CropScience LP, Stilwell, KS, United States.

2. Animal assignment and treatment

There were 12 animals of each sex per dose group. Animals were assigned using a randomization by weight. Four dose groups (12 rats/sex/dose level) were administered the test substance at nominal doses of 0 (vehicle), 500, 2000 and 6000 ppm for both sexes (33.1, 137 and 411 mg/kg/day for males and 42.4, 171 and 527 mg/kg/day for females). The table below summarizes the study design.

Table 5.7.4.-1: Study design and animal assigment

Experimental Parameter	Dose Group ppm (mg/kg bw/day)			
	Control	**500 (♂ 33.1) (♀ 42.4)**	**2000 (♂ 137) (♀ 171)**	**6000 (♂ 411) (♀ 527)**
Total number of animals/sex/group	12	12	12	12
Behavioural Testing (FOB, Motor Activity) [a]	12/sex	12/sex	12/sex	12/sex
Neuropathology [b]	6/sex	0/sex	0/sex	6/sex
Ophthalmic Examination [c]	12/sex	12/sex	12/sex	12/sex

[a] *FOB and motor activity was assessed prior to dosing and again during weeks 2, 4, 8 and 13.*
[b] *Tissues from the mid- and low-dose groups were not examined, as no treatment-related neuropathology was noted at the highest dietary level.*
[c] *Ophthalmic examinations were performed prior to dosing and at week 12.*

The rationale for dose selection was based primarily on the results of a 90-day toxicity study, with the test substance administered via the diet at nominal concentrations of 0, 400, 2000, and 7000 ppm to male and female Wistar rats (10 or 20/sex/dietary level). The principal findings from that study were as follows. There were no compound-related findings at the 400 ppm dietary level. There were no adverse treatment-related findings at 2000 ppm. Sulfonamide-like crystals were observed in the urine from 3 and 4 mid-dose males and females, respectively. Findings in animals treated at the 7000 ppm dietary level included one male that was found dead on day 49 and, prior

to death, had a red-soiled anogenital region and red-coloured urine. Macroscopic examination of this animal revealed a urinary tract obstruction. Remaining findings at the highest dietary level were limited to increased alkaline phosphatase activity in males, sulfonamide-like crystals in the urine, a cloudy appearance to the urine and intrapelvic eosinophilic urolithiasis within the kidneys in both sexes. In addition, eosinophilic urolithiasis was observed in the lumen of the urinary bladder and gritty content (stones) was observed macroscopically in males. Lastly, there was a higher incidence of lesions in the urothelial epithelium.

Based on these results, the proposed doses for the present subchronic neurotoxicity study were 0, 500, 2000 and 6000 ppm in both sexes.

3. Diet preparation and analysis

The diet was prepared every other week. Acetone was used to dissolve/suspend the test substance. The test substance/acetone mixture was added to the feed, mixed and the acetone was allowed to evaporate. The control diet was prepared the same way, excluding the test substancey. Feed was available for *ad libitum* consumption for a period of one week prior to changing, at which time any uneaten feed was collected and disposed of by incineration. The concentration of BYH 18636 in the ration was measured by liquid chromatographic analysis. The stability [following both room temperature (~ 22°C), freezer exposure (~ -23°C)] and homogeneity of the test substance in the feed were established by analysis of samples at nominal concentrations of 10 and 12000 ppm. The concentration of the test substance in the ration was measured for the ration that was used during all weeks of the study.

Homogeneity Analysis: Homogeneity of the test substance in the ration was acceptable for concentrations that bracketed those that were used in this study. These concentrations of 10 and 12000 ppm had percent relative standard deviations (%RSD) of 2.80% and 4.11%, respectively.

Stability Analysis: The stability of BYH 18636 in the ration was established at room temperature at dietary concentrations of 10 and 12000 ppm, with no appreciable decrease in concentration with seven days of storage. BYH 18636 was stable at freezer conditions for 28 days, with no appreciable decrease in concentration at 10 and 12000 ppm.

Concentration Analysis: Actual (analytically-determined) concentrations of the active ingredient in the 0, 500, 2000 and 6000 ppm dietary levels used in this study averaged 100% to 101% of the nominal concentration. Based on these results, the mean analytically confirmed dietary levels for this study were 0, 500, 2013 and 6001 ppm for males and females.

4. Statistics:

Statistical evaluations were generally performed using software from either INSTEM Computer Systems or SAS. The level of significance was set at $p \leq 0.05$, with the exception of Bartlett's test, which was tested at $p \leq 0.001$.

In general, continuous data were analyzed using an Analysis of Variance (ANOVA), followed by a Dunnett's test if a significant F-value was determined in the ANOVA. For the FOB, continuous data were first analyzed using a Repeated-Measures ANOVA, followed by a one-way ANOVA if there was a significant interaction between dose group and test week. For weeks in which there was a significant treatment effect, Dunnett's test was applied to determine which groups, if any, were significantly different from the control group. Categorical data collected in the FOB were analyzed in a similar manner, using General Linear Modeling (GLM) and Categorical Modeling (CATMOD) Procedures, with post-hoc comparisons using Dunnett's test and an Analysis of Contrasts, respectively.

Motor and locomotor activity (activity for the entire session and activity for each 10-minute interval) were analyzed using ANOVA procedures. Session activity data were first analyzed using a Repeated-Measures ANOVA, followed by a one-way ANOVA if there was a significant

interaction with test occasion. For weeks in which there was a significant treatment effect, Dunnett's test was used to determine which groups, if any, were significantly different from the control group. Interval data were subjected to a two-way Repeated-Measures ANOVA, using both test interval and test occasion as the repeated measures, followed by a Repeated Measures ANOVA to determine on which weeks there was a significant treatment by interval interaction. For those weeks, the data for each interval were subjected to analysis using a one-way ANOVA to determine at which intervals there was a significant treatment effect. For those intervals, Dunnett's test was used to determine which groups, if any, were significantly different from the control group.

For pathology, continuous data were evaluated initially using Bartlett's Test to analyze for homogeneity of variances among groups. Homogeneous data were further analyzed using an Analysis of Variance (ANOVA) followed by Dunnett's Test for pair-wise comparisons. In the event of non-homogeneous data, statistical analysis was performed using the non-parametric Kruskal-Wallis Test followed by a Mann-Whitney U Test for pair-wise comparisons. Micropathology frequency data were analyzed using a Chi-Square Test followed by a one-tailed Fisher's Exact Test in cases of significant variation by the Chi-Square analysis. A probability value of $p<0.05$ was accepted as significant for all statistical tests with the exception of Bartlett's Test in which a probability value of $p<0.001$ was used.

C. METHODS:

1. Mortality and Clinical Observations

Cage-side observations were performed twice daily (once daily on holidays and weekends) for mortality or clinical signs of moribundity. Detailed physical examinations for clinical signs of toxicity were carried out and recorded daily.

2. Body weight:

Animals were weighed weekly. Additionally, all animals were weighed on the day of sacrifice for terminal body weight measurement.

3. Food Consumption:

Individual food consumption was measured weekly. Daily food consumption was averaged over the duration of the study, based on a per kg body weight. The average daily intake of the active ingredient (a.i..) (mg a.i./kg body weight/day) was calculated using weekly body weight and food consumption data. The general relationship used for this calculation was: [a.i. in feed (ppm)/1,000] x [feed consumed (g/kg body wt/day)] = mg a.i./kg body wt/day. Using this formula, the average consumption of a.i. for males and females that received diets containing analytically-determined concentrations of 0, 500, 2000 and 6000 ppm BYH 18636 was as follows:
0, 33.1, 137 and 411 mg/kg/day, respectively, for males and
0, 42.4, 171 and 527 mg/kg/day, respectively, for females.

4. Neurobehavioral Assessment:

All animals that were assigned to the study were tested using the FOB and motor activity on five occasions - once during the week prior to treatment, and again during weeks 2, 4, 8 and 13. The order of testing and assignment of animals to mazes were done in a semi-random manner, such that groups were balanced across test times and test devices, and no animal would be tested more than once in the same maze. On the day prior to each test day, the appropriate animals were placed in the sequence that was established for testing on that day. The dose group identification was concealed at that time to ensure that testing would be performed without knowledge of the group assignment. Sets of eight animals (maximum)

were evaluated individually using the FOB and then, approximately 30 minutes after the last animal in the set had finished being tested in the FOB, all eight rats were placed individually into the mazes to measure activity.
Each week, testing was staggered over two days for each sex to accommodate the schedule for behavioral testing. Males and females were tested on separate days, with the open field and mazes cleaned during the ensuing interval to reduce the residual scent from the other sex.

a. Functional Observational Battery (FOB):

The FOB closely follows the battery of tests described by Moser, with each animal tested individually. The technicians who performed the FOB were "blind" with respect to the animal's group assignment. Observations and measurements (e.g., grip strength and foot splay) for all animals were performed by the same person throughout the study. Inter-observer reliability has been established in order to allow a second person to perform either the observations or measurements, ensuring the consistency of the results of each technician.
When applicable, observations were scored on intensity as follows: 1) slight (barely perceptible or infrequent) or 2) moderate to severe. Data were collected while the rats were in their home cage, during handling, and in an open field for 2 minutes (in the centre of a flat surface with a perimeter barrier, such as a cart). In addition, reflex and physiologic observations and measurements were made while the animals were sitting on the cart surface following open field observations.

Home cage observations included: posture, piloerection, involuntary motor movements (such as repetitive "chewing" movements of mouth and jaw, tremors, and convulsions), gait abnormalities, vocalizations, decreased activity, repetitive head bobbing, and increased reactivity.

Observations during handling included: ease of removal from cage, reaction to being handled, muscle tone, palpebral closure, lacrimation, salivation, nasal discharge, stains (lacrimal, nasal, perianal, urine, oral), alopecia, emaciation, bite marks, exophthalmia, broken teeth/malocclusion, missing toe nail(s), dehydration, and temperature upon touching (cool-to-touch).

Open field (2 min.) observations included: number of rears, piloerection, respiratory abnormalities, posture, involuntary motor movements, stereotypy (excessive or repetitive behavior), bizarre behaviour, gait abnormalities, vocalizations, arousal level, and amount of excretion.

Reflex and physiologic observations/measurements included: approach response, touch response, auditory response, tail pinch, pupil size at normal lighting, pupil response, righting reflex, grip strength body weight, body temperature, and landing foot splay.

b. Motor and Locomotor Activity:

Motor and locomotor activities were measured approximately 30 minutes after the last animal in the set (8 rats maximum) had finished the FOB. All rats in each set were placed individually into figure-eight mazes and activity was measured for a total of 60 minutes. Eight infrared emitter / detector pairs (three in each of the figure-eight alleys and one in each of the blind alleys) measured activity; each time a beam was interrupted, an activity count was registered. Broad-spectrum background noise (approximately 74 dB(A)) was provided throughout the test to minimize acoustical variations during testing.

Motor and locomotor activities were examined during each of the six, ten-minute intervals. Motor activity was measured as the number of beam interruptions that occurred during the test session. Locomotor activity was measured by eliminating consecutive counts for a

given beam. Thus, for locomotor activity, only one interruption of a given beam was counted until the rat relocated in the maze and interrupted one of the other beams. Habituation was evaluated as a decrement in activity during the test session.

5. Ophthalmology

Pre-exposure and pre-terminal (week 12) ophthalmic exams were conducted on study animals in a semi-darkened room. The pre-exposure examination was used to select animals which did not have ophthalmological defects that could interfere with the interpretation of study results. Animals with such defects were used for vendor surveillance or were sacrificed without necropsy. The pupillary reflex was tested using a penlight or transilluminator with Finnoff, and then a mydriatic agent was applied to each eye to dilate the pupil. After mydriasis, the conjunctiva, cornea and lens were examined with a slit lamp microscope, and the vitreous humor, retina, choroid, and optic disc were examined using an indirect ophthalmoscope HEINE OMEGA

6. Sacrifice and Pathology

All animals placed on study were subjected to a complete gross necropsy. The necropsy involved an examination of all organs, body cavities, cut surfaces, external orifices and surfaces. The first six males and six females at each dietary level were selected for perfusion and collection of tissues, with replacement, as necessary, if the perfusion was considered inadequate. These animals were deeply anaesthetized using an intraperitoneal dose (50 mg/kg) of pentobarbital and then perfused via the left ventricle with a sodium nitrite (in phosphate buffer) flush followed by Universal fixative (1% (w/v) glutaraldehyde and 4% (w/v) EM-grade formaldehyde) in phosphate buffer. The entire brain and spinal cord, both eyes (with optic nerves) and selected (bilateral) peripheral nerves (sciatic, tibial and sural), the gasserian ganglion, gastrocnemius muscle, both forelimbs, gross lesions in neural tissues or skeletal muscle and physical identifier were dissected from each animal and post-fixed in 10% buffered formalin. The brain was weighed upon removal from the skull, prior to placement into formalin, and the brain/body weight ratio was calculated. Other animals that survived to term were sacrificed by CO_2 asphyxiation without perfusion. For all non-perfused animals (six/sex/dietary level) that remained on study until termination, the urinary bladder and kidneys were collected, weighed (kidneys only) and preserved in 10% buffered formalin for histopathologic evaluation. Terminal body weights were performed immediately prior to necropsy to allow for calculation of organ to body weight ratios.

Micropathology examinations were performed on a comprehensive battery of neural tissues from perfusion-fixed control and high-dose rats of both sexes, as follows. Eight coronal sections of the brain and sections from three levels of the spinal cord (cervical, thoracic, lumbar) and the cauda equina were embedded in paraffin and examined utilizing haematoxylin and eosin (H&E). Dorsal root ganglia (including dorsal and ventral root fibres) from the cervical and lumbar swellings and gasserian ganglion were embedded in glycol methacrylate (GMA). Eyes, optic nerves and gastrocnemius muscle were embedded in paraffin and stained using H&E. Peripheral nerve tissues (sciatic, tibial and sural nerves) were embedded in GMA and cut in cross/transverse-section as well as longitudinal section. GMA-embedded tissues were sectioned at 2-3 µm and stained using a modified Lee's stain. The sciatic nerve was also cross-sectioned at approximately 2-3 µm and stained with a modified Lee's stain. In addition, histopathology was performed on any gross lesions collected at necropsy. Tissues from perfusion-fixed animals at the low- and mid-dose levels were not subjected to micropathology unless compound-related lesions were present in the high-dose group.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Mortality

There were no compound-related deaths at any dose level in either sex. One female was found dead during pre-treatment. There were no other animals found dead or sacrificed *in extremis* during the course of the study.

2. Clinical signs

There were no treatment-related findings in either sex at any dietary level.

B. BODY WEIGHT

Body weight and total body weight gain were not affected by treatment in either sex at any dose level.

C. FOOD CONSUMPTION

There were no compound-related effects on food consumption in either sex, at any dietary level.

D. NEUROBEHAVIORAL RESULTS

1. FOB findings

One female was found dead on during the pre-treatment week. This animal had already been tested and could not be replaced.

For the functional observational battery (FOB), compound-related effects were not apparent at any dietary level in either sex. There were a few findings that were statistically different from control but not related to treatment. This included an increased number of urine pools on the open field in low- and high-dose males (1.8 and 1.3, respectively vs. 0.4 for controls) prior to the initiation of treatment. In addition, there was a statistical difference from control during week 4 in mid-dose males and week 13 in low-dose females, which involved differences in posture in the home cage. These differences in posture are not ascribed to treatment, since there was no difference from control at the highest dietary level and all of the observed postures are common and normal in the home cage.

Table 5.7.4.-2: Functional Observation Battery: Statistically Significant Results

	c	Dose Group ppm (mg/kg bw/day)			
MALES	Observations	**Control**	**500 (♂ 33.1) (♀ 42.4)**	**2000 (♂ 137) (♀ 171)**	**6000 (♂ 411) (♀ 527)**
Pre-treatment	Urination: Number of pools (maean ± S.D.)	0.4 ± 0.7	1.8* ± 1.0	0.8 ± 0.08	1.3* ± 1.0
Week 4	Home Cage Posture:			*	
	Standing Normally	3(25)	6(50)	8(67)	7(58)
	Rearing	4(33)	4(33)	4(33)	3(25)
	Sitting or Lying Normally	5(42)	2(17)	0(0)	2(17)
FEMALES					
Week 13	Home Cage Posture:		*		
	Standing Normally	11(92)	5(45)	8(67)	10(83)
	Rearing	1(8)	5(45)	4(33)	1(8)
	Sitting or Lying Normally	0(0)	1(9)	0(0)	1(8)

Values represent the number of animals and % incidence (in parentheses) with observation.

*n=12 animals/dose level, except low- dose females (n=11) *=p ≤ 05 compared with controls*

2. Motor activity

One female was found dead during the pre-treatment week. This animal had already been tested and could not be replaced.

An examination of inherent variability, using the average pre-treatment values among the four groups of males and females, provides a measure of the magnitude of the difference that should be considered biologically significant. For motor activity, the pre-treatment values for groups that later received the test substance averaged from 2% lower to 19% higher than controls for males and from 26% lower to 4% higher than controls for females. For locomotor activity, the pre-treatment values for groups that later received the test substance averaged from 2% lower to 21% higher than controls for males and from 28% lower to 8% higher than controls for females. As a general guide, these results confirm that differences of + 20% are within the range of normal variability in this laboratory for groups of 10-12 rats/sex/dose level and, therefore, are not biologically significant.

For the overall 60-minute test session, there were no compound-related differences in session motor or locomotor activity for males or females at any dietary level (motor and locomotor activity results are summarized below). For low-dose females, there was one occasion on which measures of motor and locomotor (week 8 - decreased 25% and 29%, respectively) activities were slightly beyond the standard (+20%) range of normal variability. These differences from control are considered incidental and unrelated to treatment, since they were small and only occurred at the lowest dietary level in one sex

Table 5.7.4.-3: Summary Session Motor Activity Results (Percent Difference from Control)

MALES					
Nominal Dose	**Pre-treatment**	**Week 2**	**Week 4**	**Week 8**	**Week 13**
500 ppm	**- 2**	**- 13**	**- 10**	**+ 8**	**- 0.3**
2 000 ppm	**+ 19**	**+ 4**	**- 5**	**- 5**	**- 10**
6 000 ppm	**+ 4**	**+ 2**	**- 9**	**- 4**	**+ 2**
FEMALES					
Nominal Dose	**Pre-treatment**	**Week 2**	**Week 4**	**Week 8**	**Week 13**
500 ppm	**- 26**	**- 14**	**- 9**	**- 25**	**- 15**
2 000 ppm	**- 12**	**- 11**	**+ 9**	**- 6**	**+ 12**
6 000 ppm	**+ 4**	**- 14**	**- 3**	**- 12**	**- 5**

Percent greater (+) or less (-) than concurrent control for n=11 for low-dose females and 12 for remaining animals. Summary session motor activity was not statistically different from control ($p \leq 0.05$; ANOVA) at any time for any dietary level.

Table 5.7.1.-4 Summary Session Locomotor Activity Results (Percent Difference from Control)

MALES					
Nominal Dose	**Pre-treatment**	**Week 2**	**Week 4**	**Week 8**	**Week 13**
500 ppm	**+ 1**	**- 10**	**- 11**	**+ 9**	**- 5**
2 000 ppm	**+ 21**	**+ 6**	**- 7**	**- 3**	**- 9**
6 000 ppm	**- 2**	**+ 1**	**- 12**	**- 2**	**+ 4**
FEMALES					
Nominal Dose	**Pre-treatment**	**Week 2**	**Week 4**	**Week 8**	**Week 13**
500 ppm	**- 28**	**- 20**	**- 8**	**- 29**	**- 15**
2 000 ppm	**- 12**	**- 16**	**+ 11**	**- 6**	**+ 8**
6 000 ppm	**+ 8**	**- 15**	**- 2**	**- 19**	**- 12**

Percent greater (+) or less (-) than concurrent control for n=11 for low-dose females and 12 for remaining animals. Summary session locomotor activity was not statistically different from control ($p \leq 0.05$; ANOVA) at any time for any dietary level.

Motor and Locomotor activity data were also analyzed for differences at each 10-minute interval of each test session. Measures of motor and locomotor activity were not statistically different from controls at any dietary level on any test occasion in either sex.Habituation was not affected by treatment with BYH 18636 any dose level.

E. OPHTHALMOLOGY

All ophthalmologic findings were considered incidental and unrelated to exposure to BYH 18636.

F. SACRIFICE AND PATHOLOGY

1. Gross pathology

There were no compound-related gross lesions evident at terminal sacrifice in males or females at any dietary level.

2. Terminal body and brain weights

There were no treatment-related differences in terminal organ weight or fixed organ weight (absolute and relative) at any dietary level in either sex. Relative kidney weight for low-dose females was significantly lower than controls. This difference from control was considered not to be related to treatment because it occurred in only one sex and did not occur at higher dietary levels.

Table 5.7.4.-5: Absolute and Relative Brain Weights

Weights (g)	**Dose Level PPM (mg/kg bw/day)**			
	Control	**500 (♂ 33.1) (♀ 42.4)**	**2000 (♂ 137) (♀ 171)**	**6000 (♂ 411) (♀ 527)**
Males				
Body Wt. [a]	451.2±22.9	445.3±52.6	460.1±57.2	443.7±28.3
Brain Wt.[b]	1.952±0.064	1.934±0.110	1.959±0.075	1.921±0.060
Brain/Body Wt.[b]	0.437±0.018	0.443±0.064	0.432±0.048	0.432±0.008
Kidney Wt.[b]	3.275±0.478	3.357±0.427	3.290±0.256	3.223±0.423
Kidney/Body Wt.[b]	0.718±0.089	0.751±0.063	0.717±0.052	0.728±0.071

Females				
Body Wt.[a]	238.6±15.8	233.0±8.6	233.4±8.8	229.7±15.8
Brain Wt.[b]	1.840±0.051	1.831±0.043	1.796±0.091	1.794±0.053
Brain/Body Wt.[b]	0.754±0.029	0.788±0.029	0.762±0.061	0.792±0.065
Kidney Wt.[b]	1.917±0.247	1.733±0.115	1.865±0.080	1.887±0.189
Kidney/Body Wt.[b]	0.820±0.043	**0.741*±0.026**	0.809±0.055	0.813±0.049

Data were extracted from Table OW1K-Sum, pages 525-526. Values represent mean ± s.d
[a] N= n=11 for low-dose females and 12 for remaining animals. ** Statistically different (p ≤ 0.05) from the control.*
[b] N= n=5 for low-dose females and 6 for remaining animals.

3. Neuropathology

There were no compound-related microscopic lesions in the high-dose males or females. Therefore, tissues from animals that received a lower dose of BYH 18636 were not examined.

III. CONCLUSION

In conclusion, there was no evidence of toxicity or neurotoxicity at any dietary level and no compound-related gross or microscopic lesions at the highest dietary level of 6000 ppm. For both sexes, there were no treatment-related effects on mortality, clinical observations, FOB, body weight and weight gain, food consumption, motor activity, ophthalmology, brain weight or kidney weight. These results establish a NOAEL of 411 mg/kg/day in males and 527 mg/kg/day in females.

(Gilmore R.G., 2006)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: Totally reliable The study is fully compliant with OECD 424. The animals in this study stated in the study report to be approximately eight weeks old on Study Day 0, and were not 'at least 9 weeks' as stated in the Notifier's summary. The age of the animals is therefore compliant with that specified in the OECD guideline (see comments for the acute neurotoxicity study (Gilmore & Hoss, 2006), above). It is noted that there was no indication of neurotoxicity in any of the standard toxicity studies (those required under Directive 91/414/EEC), including the neurotoxicity assessment in the 90-day toxicity study (McElligott, 2003) or in the acute neurotoxicity study (Gilmore, 2006). A study of neurotoxicity is therefore not a specific data requirement for this compound according to EU legislation, based on the absence of findings in the other studies and the mode of action of the compound. No treatment-related findings were apparent in this study, however the top dose level is considered to be sufficiently high based on the results of the 90-day toxicity study in the rat, in which treatment-related mortality was apparent at the top dose level of 7000 ppm (equivalent to mean achieved dietary intakes of 439 and 543 mg/kg bw/d in males and females respectively).

Conclusions	No evidence of neurotoxicity or neuropathology was seen under the conditions of this study. A NOAEL of 6000 ppm (equivalent to mean achieved dietary intakes of 411 and 527 mg/kg bw/d in males and females respectively) can be determined, in the absence of any treatment-related findings at the top dose level. The Notifier has proposed the same NOAELs for this study.

Postnatal developmental neurotoxicity (IIA 5.7.5)

As BYH 18636 administration in rodents did not induce any neurotoxicological effect, a postnatal developmental neurotoxicity study is not triggered.

B.6.8 Further toxicological studies (IIA 5.8)

B.6.8.1 Toxicity studies on relevant metabolites (IIA 5.8)

- **BYH 18636-carboxylic acid**

In vitro genotoxicity - Bacterial assay for gene mutation

Report:	**KIIA 5.8/01, Wirnitzer U., 2004 (MRID # 47070139)**
Title:	BYH 18636-carboxylic acid, Salmonella/microsome test, plate incorporation and preincubation method
Report No & Document No	AT01522A M-092854-02-2
Guidelines:	OECD 471 (1997); EEC Directive 2000/32/EC Method B13/14 (2000); EPA Health Effects Test Guideline (OPPTS 870.5100; 1998)
GLP	Yes (certified laboratory)

Executive summary:
In this *in vitro* assessment of the mutagenic potential of BYH 18636-carboxylic acid (Batch GSE29091-6-1, 98.9% of purity), histidine dependent auxotrophic mutants of Salmonella typhimurium, strains TA 1535, TA 1537, TA 98, TA 100 and TA 102 were exposed to BYH 18636-carboxylic acid up to 5000 µg/plate, diluted in dimethyl sulphoxide (DMSO). For each bacterial strain and dose level, triplicate plates were used in both the presence and absence of an Aroclor 1254-induced rat liver metabolic activation system (S9 mix). DMSO was also used as a negative control. Specific positive controls were used for each strain. After 48 hours of incubation at 37°C, the numbers of revertant colonies were scored using an automated colony counter. Another assay testing a pre-incubation for 20 minutes at 37°C was also performed at doses from 16 to 5000 µg/plate.

Doses up to 1581 µg/plate did not cause any bacteriotoxic effects. At 5000 µg/plate, BYH 18636-carboxylic acid had only a weak, strain-specific bacteriotoxic effect. This dose could be used for assessment.

BYH 18636-carboxylic acid did not cause any significant increase in the number of revertant colonies in either the presence or absence of metabolic activation.

All the positive control compounds produced expected increases in the number of revertant colonies, thereby demonstrating the sensitivity of the assay and the efficacy of the S9 mix.

Therefore, BYH 18636-carboxylic acid was non-mutagenic with or without S9 mix in the plate incorporation as well as in the pre-incubation modification of the Salmonella/microsome test.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636-carboxylic acid
Description: Fine, white powder
Lot/Batch: GSE29091-6-1
Purity: 98.9% (March 2004)
CAS: No CAS entry yet
Stability of test compound: Stable for the duration of the study

2. Control materials:
Negative: Culture medium
Solvent: DMSO
Positive: Non activated
Sodium azide (Serva) for TA 1535 at 10 µg/plate,
Nitrofurantoin (Sigma) for TA 100 at 0.2 µg/plate,
4-Nitro-1,2-phenylene diamine (Merck-Schuchardt) for TA 1537 at 10 µg/plate and TA 98 at 0.5 µg/plate,
Mitomycin C (Fluka) for TA 102 at 0.2 µg/plate only in plate incorporation trials,
Cumene hydroperoxide (Sigma) for TA 102 in pre-incubation trials only at 50 µg/plate,
2-Aminoanthracene (Aldrich) for the activating effect of the S9 mix in all strains at 3 µg/plate.

3. Test organisms:
Species: Salmonella typhimurium LT2 mutants
Strain: Histidine-auxotrophic strains TA 1535, TA 100, TA 1537, TA 98 and TA 102
Source: Strains obtained from Prof. Bruce Ames in 1997 and stored in the laboratory since then.

4. Test compound concentrations:
Plate incorporation assay: For all strains with or without S9 mix: 16, 50, 158, 500, 1581 and 5000 µg/plate
Pre-incubation assay: For all strains with or without S9 mix: 16, 50, 158, 500, 1581 and 5000 µg/plate.

B. STUDY DESIGN AND METHODS:

The in life phase of the study was performed between July 28 to August 13, 2004 at Bayer Healthcare AG.
The *Salmonella*/microsome test is a screening method which detects point mutations caused by chemical agents *in vitro*. Auxotrophic mutants of *Salmonella typhimurium* are used to demonstrate this effect. For this purpose, the rate of reversion to prototrophy is evaluated in negative control and treated groups.

1. Plate incorporation assay:
DMSO (0.1 ml) containing BYH 18636-carboxylic acid or controls were added to glass vessels with 0.1 ml of bacterial cultures grown overnight, 0.5 ml of S9 mix or buffer and 2 ml of soft agar. The mixture was placed in a waterbath at 45°C for 30 seconds, shaken and overlaid onto Petri dishes containing solid agar. After 48 hours of incubation at 37°C, the numbers of revertant colonies were scored using an automated colony counter. Three plates were used, both with and without S9 mix, for each strain and dose. The doses for the first trial were routinely determined on the basis of a standard protocol with a maximum dose of 5000 µg/plate and at least 5 additional

doses. If less than three doses were used for assessment, at least two repeats were performed.

2. Pre-incubation assay:
An independent repeat was performed as pre-incubation of the previously described mixture in a water bath at 37°C for 20 minutes. At the end of the preincubation period, 2 ml of molten soft agar were added to the tubes, the content mixed and plated onto Petri dishes with solid agar. After 48 hours of incubation at 37°C, the numbers of revertant colonies were also scored using an automated colony counter.

3. Assessment criteria:
A reproducible and dose-related increase in mutant colonies of at least one strain was considered to be positive. For TA 1535, TA 100 and TA 98, this increase should be about twice that of negative controls, whereas for TA 1537, at least a threefold increase should be reached. For TA 102 an increase of about 100 mutants should be reached. Otherwise, the result was considered as negative.

II. RESULTS AND DISCUSSION

There was no indication of a bacteriotoxic effect of BYH 18636-carboxylic acid at doses of up to and including 1581 µg/plate. The total bacterial counts consistently produced results comparable to the negative controls, or differed only insignificantly. No inhibition of growth was noted as well. 5000 µg/plate had only a weak, strain-specific bacteriotoxic effect. Therefore this dose could be used for assessment purposes.
None of the five strains concerned showed in the plate incorporation test a dose-related and biologically relevant increase in mutant colonies over those of the negative controls. This applied both to the tests with and without S9 mix and was confirmed by the results of the pre-incubation trials.
The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, mitomycin C, cumene hydroperoxide and 2-aminoanthracene increased mutant colonies to well over those of the negative controls, and thus demonstrated the system's sensitivity and the activity of the S9 mix.

III. CONCLUSION

No indication of mutagenic effects of BYH 18636-carboxylic acid could be found in any of the *Salmonella typhimurium* strains used in the assay.

(Wirnitzer U., 2004)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: totally reliable The study (T6073259) is fully compliant with OECD 471. The efficacy of the metabolic activation system (male Sprague-Dawley rat liver S9 fraction) was additionally demonstrated using reference mutagens. Exposure to the test material at concentrations of up to and including the limit concentration of 5000 µg/plate in the absence or presence of metabolic activation did not result in an increase in the number of revertant colonies of any bacterial strain. The results of the original (plate incorporation) assay were confirmed in an independently repeated (pre-incubation) assay. Appropriate positive control compounds confirmed the sensitivity of the assay.
Conclusions	No evidence of mutagenicity was seen under the conditions of this study.

In vitro genotoxicity - Test for clastogenicity in mammalian cells

Report:	**KIIA 5.8/02, Herbold B., 2005 (MRID # 47070147)**
Title:	BYH 18636-carboxylic acid, *In vitro* chromosome aberration test with Chinese Hamster V79 cells
Report No & Document No	AT01980 M-250256-02-2
Guidelines:	OECD 473 (1997); EEC Directive 2000/32/EC Method B10 (2000); EPA Health Effects Test Guideline (OPPTS 870.5375; 1998)
GLP	Yes (certified laboratory)

Executive summary:
In this *in vitro* assessment of the clastogenic potential of BYH 18636-carboxylic acid (batch GSE29091-6-1, 98.5% of purity), Chinese Hamster V79 cells were exposed to BYH 18636-carboxylic acid at 300, 600 and 1200 µg/ml, diluted in dimethyl sulphoxide (DMSO). For each dose level, duplicate cultures were used in both the presence and absence of an Aroclor 1254-induced rat liver metabolic activation system (S9 mix). DMSO was also used as a negative control. Mitomycin C, which produces crosslinks in the DNA, and cyclophosphamide, which induces chromosomal damage after metabolic activation, were used as positive controls, diluted in Hanks' balanced salt solution. After 4 hours treatment, the medium was changed and the cells were harvested 14 hours later. A second harvest was performed 30 hours after the start of the study at 1200 µg/ml. In a second experiment, the cells were exposed to BYH 18636 at 300, 600 and 1200 µg/plate or controls without S9 mix for 18 hours and harvested at the end of the treatment period. Colcemid was added to each flask two hours prior to harvest to arrest the cells in a metaphase-like stage of mitosis.

With and without S9 mix no cytotoxic effects were observed. Precipitation in the medium did not occur. However, BYH 18636-carboxylic acid was tested up to its limits of solubility in the vehicle.

None of the cultures treated with BYH 18636-carboxylic acid in the presence and in the absence of S9 mix showed biologically relevantly increased numbers of aberrant metaphases.

The positive controls mitomycin C and cyclophosphamide induced clastogenic effects and demonstrated the sensitivity of the test system and the activity of the used S9 mix.

BYH 18636-carboxylic acid was considered not to be clastogenic for mammalian cells *in vitro*.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:		BYH 18636-carboxylic acid
Description:		fine, white powder
Lot/Batch:		GSE29091-6-1
Purity:		98.5% (August 26, 2004)
CAS:		No CAS entry yet
Stability of test compound:		Stable for the duration of the study
2. Control materials:	**Negative:**	Culture medium (Eagle's minimal essential medium)
	Solvent:	DMSO for BYH 18636-carboxylic acid and Hanks's balanced salt solution for positive controls (Seromed)
	Positive:	Mitomycin C (Fluka, batch 454188/2 44903116) without S9 mix at 0.1 µg/ml. Cyclophosphamide (Endoxan 100 mg injection vials of dry substance, Baxter Oncology GmbH) with S9 mix at 2 µg/ml.

3. Test organisms:

Cell line: Chinese hamster V79 lung cells

Source: Cells obtained from Dr Utesch, Merk AG, Darmstadt in 1993, stored in the laboratory since then.

4. Test compound concentrations:

BYH 18636 was used in each trial at 300, 600 and 1200 µg/ml (4 hrs -/+S9; 18 hrs –S9).

B. STUDY DESIGN AND METHODS:

The experimental phase of the study was performed from September 30, 2004 to March 15, 2005 at Bayer Healthcare AG.
The *in vitro* cytogenetic test is a mutagenicity test system for the detection of chromosome aberrations in cultured mammalian cells. The test is designed to detect structural aberrations (chromatid and chromosome aberrations) in cells at their first post-treatment mitosis.

1. Determination of cytotoxicity:

Duplicate cultures were exposed in a pre-test to BYH 18636-carboxylic acid at concentrations ranging from 1 to 1100 µg/ml with or without S9 mix for 4 hours. In addition, cells were exposed without S9 mix for 18 hours to concentrations ranging from 1 to 1200 µg/ml. The mitotic index was determined for all cultures. The number of mitotic cells among a total of 1000 cells per culture was determined using a light microscope at a magnification of about 630. All cells which were not in interphase were defined as mitotic.
In the main study, cultures with a total incubation period of 8 hours were additionally and exclusively used to determine the cytotoxicity of BYH 18636-carboxylic acid at concentrations ranging from 300 to 1200 µg/ml.

Concentrations of up to 300 µg/ml BYH 18636-carboxylic acid did not change the pH in the medium of the pre-test. At higher concentrations pH was reduced to 6.7 at 1200 µg/ml of BYH 18636-carboxylic acid. The osmolality in the medium of the pre-test was not changed by concentrations of up to 1200 µg/ml of BYH 18636-carboxylic acid.

2. Treatment protocol:

Chinese hamster V79 cells were passaged on the day prior to treatment. Approximately $1x10^6$ cells were seeded in 20 ml of medium per 75 m^2 flasks and incubated at 37°C in a CO_2-incubator (5% CO_2). Unless reported otherwise, the cells were grown in Eagle's minimal essential medium containing 10% foetal calf serum. Immediately before treatment, the medium was removed from the cultures. For the trials without S9 mix, 20 ml of medium containing 2% foetal calf serum and 0.2 ml of test substance solution were added to each flask. For the trials with S9 mix, 19 ml of medium containing 2% foetal calf serum, 1 ml of S9 mix and 0.2 ml of test substance solution were added to each flask. The cells were incubated for 4 hours at 37°C. After 4 hours of treatment, the medium was removed, the cells were washed with PBS and 20 ml of fresh medium containing 10% foetal calf serum was added to the flasks. The flasks were placed in a CO_2-incubator for the remaining incubation time. In the cultures treated for 18 hours, the medium was not removed.

To each flask, 0.2 ml of Colcemid-solution (40 µg/ml) was added 2 hours prior to the end of the incubation period to arrest the cells in a metaphase-like stage of mitosis(c-metaphase).

Positive controls and solvent controls (0.2 ml of solvent per culture) were set up in parallel and handled as described for BYH 18636-carboxylic acid-treated cultures. Untreated controls and solvent controls were used as negative controls.

3. Chromosome preparations:

After the removal of the medium from each flask, the cells were trypsinized, suspended in medium

and centrifuged for approximately 5 minutes at 700 rpm. The supernatant was removed and 1 to 2 ml of a hypotonic solution (0.4% KCl; 37°C) was added to each tube. Within 4 minutes, the volume was brought to 6 ml with additional hypotonic solution and cells were resuspended. The cells were centrifuged again and fixed with cold (4°C) fixative (ethanol/acetic acid 3:1) for 20 minutes at room temperature. Cells were pelleted and resuspended in fixative as before and centrifuged again. The pelleted cells were resuspended in a small volume of fixative and the suspension was dropped onto clean slides. The slides were allowed to dry for at least 2 hours. Thereafter, they were submerged in pure methanol for 3 minutes and stained for 15-20 minutes in 3% Giemsa solution. Slides were rinsed twice in water and once in acetone and were then kept in xylene for about 30 minutes. The slides were allowed to dry completely and covered. At least two slides were generated per culture.

4. Evaluation criteria:

Coded slides were evaluated using a light microscope at a magnification of about 1000. Chromosomes of approximately 200 metaphases per concentration, 100 metaphases from each of two parallel cultures, were examined. Only metaphases containing the modal chromosome number (22) were analyzed unless exchanges were detected. The following aberrations were recorded: gaps (an achromatic lesion within a chromatid arm without dislocation of the chromatid end), break (a discontinuity of one chromatid with dislocation of the chromatid end), fragment (part of chromosome without centromere), deletion (result of a break with the terminal chromatid part of the chromosome missing within the metaphase under assessment), exchange (exchange of chromatid-parts between different chromosomes or within the same chromosome), multiple aberration (when five or more structural changes occur within one metaphase). Observed polyploidy metaphases were recorded but not used for assessment.

5. Assessment criteria:

An assay was acceptable, if there was a biologically relevant increase in chromosome aberrations induced by positive controls and if the numbers of aberrations for the negative controls were in the expected range.

An increased incidence of gaps of both types without a concomitant increase of other aberration types was considered not to be an indication of a clastogenic effect.

A test was considered positive, if there was a relevant and statistically significant increase in the aberration rate.

A test was considered negative, if there was no such increase at any time interval or if there were statistically significant values, which were, however, within the range of historical negative controls.

A test was considered equivocal, if there was an increase above the range of historical negative controls which was statistically significant but not considered relevant, or if an increase occurred, which was considered relevant, but which was not statistically significant.

6. Statistics:

The statistical analysis was performed by pair-wise comparison of BYH 18636-carboxylic acid-treated and positive control groups to the respective solvent control group.

The mitotic index was statistically analyzed (provided that it was reduced compared to the mean of the corresponding solvent control) using the one-sided Chi^2-test.

The numbers of metaphases with aberrations (including and excluding gaps) and of metaphases with exchanges were compared (provided that these data superceded the respective solvent control). The one-side Chi^2-test was used for the statistical evaluation.

A difference was considered to be significant, if the probability of error was below 5%.

II. RESULTS AND DISCUSSION

A. Mitotic and survival indices:

In experiments with or without S9 mix, the mitotic indices in the treated cultures when compared to

the solvent control, were not relevantly reduced. The cultures treated with positive controls (mitomycin C without S9 mix and cyclophosphamide with S9 mix) showed a reduction in the mitosis rate.
In experiments with or without S9 mix, the survival indices in the treated cultures when compared to the solvent control, were not relevantly reduced. The cultures treated with positive controls (mitomycin C without S9 mix and cyclophosphamide with S9 mix) showed a reduction in the survival rate.

B. Chromosome aberrations:

In experiments without S9 mix, no biologically relevant and statistically significant increases in the numbers of metaphases with aberrations were detected after 4 hours of treatment and total culture times of 18 or 30 hours. The same was true for a treatment period and a culture time of 18 hours. The treatment with the positive control mitomycin C resulted in a clear and statistically significant increase of metaphases with aberrations and demonstrated the sensitivity of the test system.

In experiments with S9 mix, no biologically relevant increases in numbers of metaphases with aberrations was detected after total culture times of 18 or 30 hours. Due to the lack of any correlation to concentration the isolated observed statistical significance for the intermediate S9-activated concentration of the 4-hour treatment was considered to be a result by chance and not indicative for a clastogenic potential of BYH 18636-carboxylic acid.
The positive control cyclophosphamide induced statistically significant and biologically relevant increases in metaphases with aberrations and demonstrated the sensitivity of the test system and the activity of the used S9 mix.

III. CONCLUSION

None of the cultures treated with BYH 18636-carboxylic acid up to 1200 µg/ml in the absence or presence of S9 mix showed biologically relevant increases in numbers of metaphases with aberrations. The positive controls mitomycin C and cyclophosphamide induced clear clastogenic effects and demonstrated the sensitivity of the test system.

Based on the results of this test, BYH 18636-carboxylic acid was considered not to be clastogenic for mammalian cells *in vitro*.

(Herbold B., 2005)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: Totally reliable The study (T 8073260) is fully compliant with OECD 473 (1997) The test material was not found to induce structural or numerical chromosomal aberrations under the conditions of this study in the absence or presence of metabolic activation (male Sprague-Dawley rat liver S9 fraction). A slight (but statistically significant) increase in the proportion of metaphases with structural chromosomal aberrations was seen (in one replicate) at an intermediate concentration of the test material in the first assay, however this was not reproduced in the second assay. Findings are therefore not considered to be of biological significance. Appropriate positive control compounds confirmed the sensitivity of the assay.

No evidence of cytotoxicity was seen at the highest concentration of the test material, however this concentration was limited by the solubility of the test material in the vehicle (DMSO).

Clastogenicity study with thiencarbazone-methyl carboxylic acid

Culture		Metaphases with aberrations excluding gaps (%)					
		DMSO	300 µg/ml	600 µg/ml	1200 µg/ml	MMC	CPA
4h	-S9	3.5	5.0	5.5	5.0	51.0**	-
	+S9	3.0	6.0	8.0*	4.0	-	46.0**
18h	-S9	4.0	5.0	7.0	8.0	76.5**	-

Conclusions: No evidence of clastogenicity was seen under the conditions of this study. Thiencarbazone-methyl carboxylic acid was not found to induce structural or numerical chromosomal aberrations.

In vitro genotoxicity - Test for gene mutation in mammalian cells

Report:	**KIIA 5.8/03, Herbold B., 2005 (MRID # 47070143)**
Title:	BYH 18636-carboxylic acid, V79/HPRT-test *in vitro* for the detection of induced forward mutations
Report No & Document No	AT02038 M-251094-01-2
Guidelines:	OECD 476 (1997); EEC Directive 2000/32/EC Method B17 (2000); EPA Health Effects Test Guideline (OPPTS 870.5300; 1998)
GLP	Yes (certified laboratory)

Executive summary:

The purpose of the study was to assess the point mutagenic potential of BYH 18636-carboxylic acid (batch GSE29091-6-1, 98.5 % of purity) at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in V79 cells.

For dose selection, a preliminary cytotoxicity test was conducted with and without an Aroclor 1254-induced rat liver metabolic activation system (S9 mix) using concentrations of BYH 18636-carboxylic acid ranging from 18.8 to 1200 µg/ml. Although BYH 18636-carboxylic acid was tested up to and above the solubility of BYH 18636-carboxylic acid in the vehicle, no cytotoxic effects were observed. BYH 18636-carboxylic acid was tested in the first mutation experiment and the following independent repeats at concentrations ranging from 75 to 1200 µg/ml.

Exponentially growing V79 cells were plated in 20 ml culture medium in two 250 ml flasks. After attachment, cells were exposed with or without S9 mix to vehicle alone or to a range of concentrations of the test substance for 5 hours. Thereafter, each cell monolayer was washed with PBS, trypsinized and replated into 3 Petri dishes. Per culture one flask and 3 Petri dishes were used. These dishes were incubated for 6 to 8 days to allow colony development and to determine the cytotoxicity associated with each test substance directly after treatment (survival to treatment).

Cells ($3x10^5$ cells/ml) in 250 ml flasks were incubated to permit growth and expression of induced mutations. Cells were subcultured (= count 1, normally after 3 days) by reseeding cells in 250 ml flasks. At the end of the expression period (=count 2, normally a total of 6 days), cultures were reseeded in Petri dishes without hypoxanthine but containing 6-TG for selection of mutants. In addition, 200 cells per dish were seeded in 5 ml culture medium to determine the absolute cloning efficiency for each concentration. After incubation for 6 to 8 days, the colonies were fixed, stained with Giemsa and counted to determine the number of 6-TG resistant colonies in the mutation assay dishes and the number of colonies in the cloning efficiency dishes.

With and without S9 mix BYH 18636-carboxylic acid induced no biologically relevant decreases in survival to treatment and no decreases in relative population growth were observed. With and without

S9 mix there was no biologically relevant increase in mutant frequency above that of the vehicle controls. Ethyl methanesulfonate and dimethyl benzanthracene induced clear mutagenic effects demonstrating the sensitivity of the test system and the activity of the S9 mix.

Based on these results, BYH 18636-carboxylic acid was considered to be non-mutagenic in the V79/HPRT forward mutation assay, both with and without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636-carboxylic acid
Description: white powder
Lot/Batch: GSE29091-6-1
Purity: 98.5% (August, 2004)
CAS: No CAS entry yet
Stability of test compound: Stable for the duration of the study

2. Control materials:
Negative: Culture medium [Eagle's minimal essential medium supplemented with 1% L-glutamine, 1% MEM-vitamins, 1% MEM NEAA, 1% penicillin/streptomycin and 10% foetal calf serum (FCS)]
Solvent: DMSO for BYH 18636-carboxylic acid and Dimethylbenzanthracene not exceeding 1% (v/v) in the culture medium. No solvent needed for ethyl methanesulfonate as it is a liquid.
Positive: Ethyl methanesulfonate (EMS), a directly alkylating agent, used at a final concentration of 900 µg/ml in non-activation trials.
Dimethylbenzanthracene (DMBA), promutagen requiring a metabolic activation, used at a final concentration of 20 µg/ml for trials with S9 mix.

3. Test organisms:
Cell line: Chinese hamster V79 lung cells
Source: Cells obtained from Prof. G. Speit, University of Ulm, Germany. These cells have since been recloned to maintain karyotypic stability. They have a modal chromosome number of 22 and a rapid population doubling time (10 to 14 hours)
Culture condition: Incubation performed at 37°C in a humidified atmosphere with about 5% CO_2.

4. Test compound concentrations:
BYH 18636-carboxylic acid was used at 18.8, 37.5, 75, 150, 300, 600 and 1200 µg/ml -/+ in the clonal cytotoxicity assay and at 75, 150, 300, 450, 600, 900 and 1200 µg/ml in the mutagenic assays (2 Trials –S9; 2 Trials +S9).

5. Metabolic activation:
The S9 fraction was isolated from the livers of Aroclor 1254 induced male Sprague Dawley rats. The preparation dated from February 4, 2003 (protein content from 26.4 mg/ml) and was kept frozen at -80°C. The batch was tested for contamination and cytotoxicity prior to use in the first study. Cofactors were freshly dissolved in sodium phosphate buffer

(150 mM, pH 7.4)

B. STUDY DESIGN AND METHODS:

The experimental phase of the study was performed November 23, 2004 to February 24, 2005 at Bayer Healthcare AG.

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

1. Determination of cytotoxicity:

Exponentially growing V79 cells were plated in 20 ml culture medium in a 250 ml flask ($4x10^6$ cells per flasks). For each concentration, one culture was available. After attachment (16 to 24 hours later), cells were exposed without S9 mix to vehicle alone or to a range of concentrations of the test substance for 5 hours in 20 ml medium containing 2% FCS. In experiments with metabolic activation 1 ml of medium was replaced by 1 ml of S9 mix. Thereafter, each cell monolayer was washed with PBS, trypsinized and replated in 5 ml culture medium at a density of 200 cells into 3 Petri dishes (diameter of 60 mm). These dishes were incubated for 6 to 8 days to allow colony development. Thereafter, colonies were fixed with 95% methanol, stained with Giemsa (Merk; stock solution diluted 1:5 with deionized water) and counted automatically using an Artek counter, when there was no interference by precipitation on the plates or colouration of the plates. Cytotoxicity was expressed by comparison of colonies in treated cultures versus vehicle control cultures (relative cloning efficiency).

2. Treatment protocol without metabolic activation:

Exponentially growing V79 cells were plated in 20 ml culture medium in two 250 ml flasks per concentration ($4x10^6$ cells per flask) including all control groups. After attachment (16 to 24 hours later), cells were exposed to vehicle alone or to a range of concentrations of the test substance for 5 hours in 20 ml culture medium with reduced serum content (2%). Thereafter, cell monolayers were washed with PBS, trypsinized and replated in 20 ml culture medium using $1.5x10^6$ cells per 250 ml flask and in 5 ml culture medium using 200 cells per Petri dish (diameter of 60 mm). One flask and 3 Petri dishes were used per culture. These dishes were incubated for 6 to 8 days to allow colony development and to determine the cytotoxicity associated with each test substance directly after treatment (survival to treatment).

Cells in 250 ml flasks were incubated to permit growth and expression of induced mutations. Cells were subcultured (= count 1, normally after 3 days) by reseeding $1.5x10^6$ cells into 20 ml of medium in 250 ml flasks. At the end of the expression period (=count 2, normally a total of 6 days), cultures were reseeded in Petri dishes (diameter of 100 mm) at $3x10^5$ cells per dish (8 dishes per culture) in 20 ml culture medium without hypoxanthine but containing 10 g/ml 6-TG for selection of mutants. In addition, 200 cells per dish (diameter of 60 mm, 3 dishes per culture) were seeded in 5 ml culture medium to determine the absolute cloning efficiency for each concentration. After incubation for 6 to 8 days, the colonies were fixed, stained with Giemsa and counted to determine the number of 6-TG resistant colonies in the mutation assay dishes and the number of colonies in the cloning efficiency dishes.

Two trials were performed.

3. Treatment protocol with metabolic activation:

The activation assay was performed independantly. The procedure was identical to the non-activation assay except for the addition of S9 mix. In these experiments 19 ml instead of 20 ml culture medium

and additonally 1 ml of S9 mix were added to the flasks for the treatment period, resulting in a concentration of 5% S9 mix in the cultures. The number of 6-TG resistant mutants and viability were determined as in the non-activation assay.

Two trials were performed.

4. Parameters assessed:

The parameter "survival to treatment" in % was determined on the basis of the following calculation:

$$\frac{\text{Mean number of colonies (treated cultures) x 100}}{\text{Mean number of colonies (vehicle control cultures)}}$$

The "absolute population growth" was calculated using the following formula:

Absolute population growth (for each culture) = cell count 1 x cell count 2

The parameter "relative population growth" shows the cumulative growth of the treated cell populations, relative to the vehicle control.

$$\frac{\text{Absolute population growth treated culture x 100}}{\text{Absolute population growth of corresponding vehicle control culture}}$$

The ability of cells to form colonies at the time of mutant selection is measured by the parameter "absolute cloning efficiency". It is expressed in %.

$$\frac{\text{Mean number of colonies per dish x 100}}{200}$$

The "mutant frequency" is calculated for each group by dividing the total number of mutant colonies by the number of cells seeded (usually 8-10 plates at $3x10^5$ cells per plate), corrected for the absolute cloning efficiency. The mutant frequency is expressed as 6-TG resistant mutants par 10^6 clonable cells.

$$\frac{\text{Total number of mutant colonies x 100}}{\text{Number of evaluated dishes x } 3 \text{ x } 10^5 \text{ x C.E.}}$$

5. Acceptance criteria:

- The average cloning efficiency of the negative and vehicle controls should be at least 50%.
- The average of mutant frequency of the vehicle controls should not exceed 25 x 10^{-6} cells.
- The mutant frequency of the two cultures of the vehicle and /or the negative control should differ only to an acceptable extent. As a rule of thumb, the difference of mutant frequencies should not be greater than 5 x 10^{-6}.
- The positive control should induce an average mutant frequency of at least three times that of the vehicle control.
- If not limited by the solubility of the test substance in the vehicle the highest concentration should induce cytotoxicity of about 80 to 90% or should be a concentration where precipitation occurs in the medium. The survival at the lowest concentration should be in the range of the negative control.
- For the calculation of an acceptable mutant frequency at least 5 dishes per culture should be available and relative survival to treatment, relative population growth and absolute cloning efficiency should be 10% or greater.

However, these criteria may be overruled by good scientific judgement.

6. Assessment criteria:

- Mutant frequencies was only used for assessment, if at least 5 dishes per culture were available and relative survival to treatment, relative population growth and absolute cloning efficiency were 10% or greater.
- A trial was considered positive if a concentration-related and in parallel cultures reproducible increase in mutant frequencies was observed. To be relevant, the increase in mutant frequencies should be at least two to three times that of the highest negative or vehicle control value observed in the respective trial. If this result was repoduced in a second trial, the test substance was considered to be mutagenic.
- Despite these criteria, a positive result was only considered relevant, if no significant change in osmolality compared to the vehicle control was observed. Otherwise, unphysiological culture conditions may be the reason for the positive result.
- A test substance was judged as equivocal if there was no strictly concentration related increase in mutation frequencies but if one or more concentrations induced a reproducible and biologically relevant increase in mutant frequencies in all trials.
- An assay was considered negative if no reproducible and relevant increases of mutant frequencies were observed.

However, these criteria may be overruled by good scientific judgement.

7. Statistical analysis:

The statistical analysis relied on the mutant frequencies which were submitted to a weighted analysis of variance as well as to a weighted recursive regression, both with Poisson derived weights.

The two mutant frequency values obtained per group were, although somewhat related, considered as independent measurements thus increasing the power of the statistical tests applied. Since the protocol of the HPRT assay required at least two independent trials, the overall analysis without respectively with activation was the most important one for classifying substances into mutagens and non-mutagens. However, separate analyses were run for each trial in order to examine the consistency of the results.

All acceptable groups were included in the weighted analysis of variance followed by pairwise comparisons to the vehicle control on a nominal significance level of $\alpha = 0.05$ using the Dunnett test. The regression analysis part was performed on the basis of the actual concentrations thereby omitting the positive, negative and vehicle controls. If there was a significant concentration related increase of the mutant frequency ($\alpha = 0.05$) in the main analysis the highest concentration was dropped and the analysis repeated. This procedure was repeated until $p > 0.05$. In that way eliminated concentrations were flagged correspondingly.

II. RESULTS AND DISCUSSION

1. General remarks:

In the absence and in the presence of S9 mix Chinese hamster V79 cells were exposed to BYH 18636-carboxylic acid at concentrations of up to and including 1200 µg/ml. With and without S9 mix no substance precipitation occurred in the medium

The means of the absolute cloning efficiency for the vehicle controls in the mutation experiments were 51.2% and 60.6% in the experiments without activation, and 48.6% and 65.7% in the experiemnts with activation.These results demonstrated good cloning conditions for the experiments.

2. Mutation assay without metabolic activation:

Two trials were performed. The mutant frequencies of the negative controls (means of 0.9 x 10^{-6} and 10.2 x 10^{-6}, respectively) and of the vehicle controls (means of 0.35 x 10^{-6} and 9.9 x 10^{-6}, respectively) were all within the normal range. The positive control EMS induced clear mutagenic and statistically significant effects in all trials (means of 332.3 x 10^{-6} and 233.6 x 10^{-6}, respectively).

For BYH 18636-carboxylic acid treated cultures concentration-related decreases were observed neither in relative survival to treatment nor in relative population growth. Relevant BYH 18636-carboxylic acid induced increases in mutant frequencies could not be found (ranging from 0.0 x 10^{-6} to 4.4 x 10^{-6} in the first trial, and from 3.7 x 10^{-6} to 19.2 x 10^{-6} in the second trial). In addition, the overall statistical analysis revealed no statistically significant increases. Therefore, BYH 18636 was evaluated as non-mutagenic in the non activation trial.

3. Mutation assay with metabolic activation:

Two trials were performed with S9 mix. The mutant frequencies of the negative controls (means of 8.3 x 10^{-6} and 3.0 x 10^{-6}, respectively) and of the vehicle controls (means of 4.2 x 10^{-6} and 2.6 x 10^{-6}, respectively) were all within the normal range. The positive control DMBA induced clear mutagenic and statistically significant effects in all trials (means of 59.2 x 10^{-6} and 43.7 x 10^{-6}, respectively).

The BYH 18636-carboxylic acid treated cultures showed no concentration-related decreases neither in relative survival to treatment nor in relative population growth. Relevant BYH 18636-carboxylic acid induced increases in mutant frequencies could not be found (ranging from 1.6 x 10^{-6} to 7.4 x 10^{-6} in the first trial and from 0.0 x 10^{-6} to 5.0 x 10^{-6} in the second trial). In addition, the overall statistical analysis revealed no statistically significant increases. Therefore, BYH 18636 was evaluated as non-mutagenic in the activation trial.

III. CONCLUSION

No cytotoxic effects were induced by BYH 18636-carboxylic acid tested in the V79/HPRT- test in concentrations ranging from 75 to 1200 µg/ml with and without S9. However, BYH 18636 was tested up to its limit of solubility in the vehicle. BYH 18636-carboxylic acid induced no biologically relevant increases in mutant frequencies.
The positive controls EMS and DMBA had a marked mutagenic effect.
Based on these results, BYH 18636-carboxylic acid was considered not to be mutagenic in the V79/HPRT forward mutation assay.

(Herbold B., 2005)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: totally reliable The study (T9073261) is compliant with OECD guideline 476 (1997) and is considered to be acceptable; but see the detailed comments about the general acceptability of this type of study at IIA 5.4.3 (Herbold, 2005). Exposure to thiencarbazone-methyl in the absence and presence of a metabolic activation system (male Sprague-Dawley rat liver S9 fraction) did not induce any statistically or biologically significant increase in the mutation frequency. Appropriate positive control compounds confirmed the sensitivity of the assay No evidence of cytotoxicity was seen at the highest concentration of the test material, however this concentration was limited by the solubility of the test material in the vehicle (DMSO).

Conclusions	No evidence of mutagenicity was seen under the conditions of this study.

Acute oral toxicity

Report:	**KIIA 5.8/04, Schüngel M.; 2006 (MRID # 47070121)**
Title:	BYH 18636-carboxylic acid (AE 1394083), Acute toxicity in the rat after oral administration
Report No & Document No	AT02902 M-269981-01-2
Guidelines:	OECD 423 (2001); EEC Directive 67/548 Annex V – Method B.1.tris (2004); EPA Health Effects test Guidelines (OPPTS 870.1100) (1998)
GLP	Yes (certified laboratory)

Executive Summary
In an acute oral toxicity study using a stepwise procedure, two groups of three fasted, young adult female Wistar rats (HsdCpb: Wu) were given successively a single oral dose of BYH 18636-carboxylic acid (batch GSE29091-9-1, 98.7% purity) in 2% Cremophor EL of 2 000 mg/kg bw and were observed for 14 days.
The dose of 2 000 mg/kg bw was tolerated by both groups without mortalities, clinical signs, effects on weight gain or gross pathological findings.

According to OECD guideline 423, the LD_{50} cut-off of BYH 18636-carboxylic acid was ≥ 5 000 mg /kg bw (category 5 / unclassified of the global harmonized classification system).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636-carboxylic acid
- **Description:** White solid
- **Lot/Batch:** GSE29091-9-1
- **Purity:** 98.7% (December, 2005)
- **CAS:** No CAS entry yet
- **Stability of test compound:** Stable at 5 and 200 mg/ml at room temperature for at least 2 hours

2. Vehicle and /or positive control: 2% Cremophor EL in demineralized water

3. Test animals:
- **Species**: Rat
- **Strain:** HsdCpb:Wu
- **Age:** 10 to 12 weeks approximately
- **Weight at dosing:** 164 to 176 g
- **Source:** Harlan/Winkelmann GmbH, 33178 Borchen, Germany
- **Acclimation period:** At least 5 days
- **Diet:** Provimi Kliba 3883.0.15 maus/Ratte Haltung, Kaiseraugst Switzerland, *ad libitum*
- **Water:** Tap water, *ad libitum*
- **Housing:** Animals were group caged conventionally in polycarbonate cages on low dust wood granulate bedding

Environmental conditions –
- **Temperature:** 22 ± 2°C
- **Humidity:** 55 ± 5%

Air changes: Approximately 10 changes per hour
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS:

1. In life dates: February 02 to 22, 2006 performed at Bayer HealthCare AG, Germany

2. Animal assignment and treatment

The substance was tested using a stepwise procedure, each step using three female rats. The animals were assigned to their groups by randomization based on evenly distributed chance numbers. Following an overnight fast (16 to 24 hours), each group received a single dose of 2 000 mg/kg of BYH 18636-carboxylic acid (98.7% purity) by gavage. The test substance was administered in demineralized water with 2% Cremophor EL at a volume of 10 ml/kg bw. Clinical signs and mortality rates were determined several times on the day of administration and subsequently at least once daily for an observation period of at least 14 days. Body weights were recorded on days 1, 8 and 15. On day 15, surviving animals were sacrificed and all animals were necropsied and examined for gross pathological changes.

Table IIA 5.8-1 Doses, mortality / animals treated

Dose (mg/kg bw)	Females
2 000 (1st)	0/3
2 000 (2nd)	0/3

3. Statistics

The data did not warrant statistical analysis.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortalities occurred at 2 000 mg/kg bw, the only dose tested.

The oral LD_{50} cut-off was ≥ 5 000 mg /kg bw according to OECD guideline 423.

B. CLINICAL OBSERVATIONS

No clinical signs were observed.

C. BODY WEIGHT

There was no toxicological effect on body weight or body weight gain.

D. NECROPSY

No abnormalities were observed at gross necropsy.

III. CONCLUSION

The oral LD_{50} cut off of BYH 18636-carboxylic acid was ≥ 5 000 mg/kg bw according OECD Guideline 423. BYH 18636-carboxylic acid does not warrant classification as being toxic or harmful on the basis of its acute oral toxicity.

(Schüngel M. 2006)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: Totally reliable The study (T3076352) is fully compliant with OECD guideline 423 (2001). No treatment-related findings were apparent in this study following a single (limit) dose of 2000 mg/kg bw.
Conclusions	The acute oral LD_{50} of thiencarbazone-methyl carboxylic acid in female rats was found to be >2000 mg/kg bw under the conditions of this study. The test material is not classified for acute oral toxicity according to current EC criteria. EPA: For Toxicity Category/Classification EPA Toxicity Category III – LD_{50} > 2000 mg/kg - would be assigned.

Oral 90-day toxicity in the rat

Report:	**KIIA 5.8/05, Odin-Feurtet M, 2007 (MRID # 47070128)**
Title:	BYH 18636-carboxylic acid, 90-day toxicity study in the rat by dietary administration
Report No & Document No	SA 06035; M-282943-01-2
Guidelines:	OECD 408 (1998); EEC Directive 2001/59/EC Annex V – Method B.26. (2001); EPA Health Effects Test Guideline (OPPTS 870.3100; 1998); M.A.F.F. in Japan notification 12 Nousan N°8147 (2000) guidelines.
GLP	Yes (certified laboratory)

Executive summary:

BYH 18636-carboxylic acid, a soil metabolite of the herbicide BYH 18636, (batch number GSE29091-9-1: a white solid, 98.7% purity) was administered continuously via dietary administration to separate groups of Wistar rats (10/sex/group) at concentrations of 400, 2000 and 15000 ppm (corresponding to 25.3, 127, and 972 mg/kg/day for males and 30.4, 152, 1170 mg/kg/day for the females) for at least 90 days. A similarly constituted group of 10 males and 10 females received untreated diet and acted as a control.

Clinical signs were recorded daily, body weight and food consumption were measured weekly. A detailed physical examination was performed once during the acclimatization phase and weekly throughout the study. All surviving animals were subjected to a neurotoxicity assessment (motor activity, open field observations, sensory reactivity and grip strength) during Weeks 11 to 12 of the study. Ophthalmological examinations were performed on all animals during the acclimatization phase and on all animals of the control and high dose groups during Week 13. Urine samples were collected overnight during the week before necropsy from all animals. Before necropsy a blood sample was collected from the retro-orbital venous plexus of each animal for haematology and clinical chemistry investigations. All animals were necropsied, selected organs weighed and a range of tissues were taken, fixed and examined microscopically.

There were no treatment-related mortalities during the course of the study. There were no treatment-related findings for any of the parameters assessed for either sex at any of the dose levels tested.

The No Observed Effect Level (NOEL) in this study was 15000 ppm, equating to 972 and 1170 mg/kg/day in males and females, respectively.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636-carboxylic acid
Description: White solid
Lot/Batch: GSE29091-9-1
Purity: 98.7% (December, 2005)
CAS: No CAS entry yet
Stability of test compound: Stable in rodent diet at 50 and 20 000 ppm over a 106-day period at ambient temperature

2. Vehicle and /or positive control: None

3. Test animals:
Species: Rat
Strain: Wistar Rj: WI (IOPS HAN)
Age: 6 weeks approximately
Weight at dosing: 192 to 215 g for the males – 158 to 183 g for the females
Source: R. Janvier, Le Genest St Isle, France
Acclimation period: 6 days
Diet: Certified rodent powdered and irradiated diet A04CP1-10 from S.A.F.E. (Scientific Animal Food and Engineering, Augy, France), *ad libitum*
Water: Tap water, *ad libitum*
Housing: Animals were housed five per sex per cage in suspended stainless steel wire mesh cages.
Environmental conditions –
Temperature: 22 ± 2°C
Humidity: 55 ± 15%
Air changes: Approximately 10 to 15 changes per hour
Photoperiod: Alternating 12-hour light and dark cycles (7 am- 7 pm)

B. STUDY DESIGN:

1. In life dates: March 08 to June 15, 2006 performed at Bayer CropScience, Sophia Antipolis, France

2. Animal assignment and treatment

There were 10 animals of each sex per dose group. Animals were assigned to dose groups using a randomization by weight. BYH 18636-carboxylic acid was administered in the diet for at least 90 days to Wistar rats at the following doses – 0, 400, 2 000 and 15 000 ppm (equating approximately to 25.3, 127, and 972 mg/kg/day in males and 30.4, 152, 1170 mg/kg/day in females). A negative control group received plain diet. Animal housing and husbandry were in accordance with the regulations of the Guide for the Care and Use of Laboratory Animals (Public Health Service, National Institute of Health, NIH publication N°86-23, revised 1985) and "Le Guide du Journal Officiel des Communautés Européennes L358, 18 Décembre 1986, N°86/609/CEE du 24 Novembre 1986".

3. Diet preparation and analysis

BYH 18636-carboxylic acid was ground to a fine powder before being incorporated into the diet by dry mixing to provide the required concentrations. There were two preparations of each concentration for the whole study. The stability was demonstrated before the start of the study. Diet samples of 50 and 20 000 ppm were found to be stable over a 106-day period at ambient temperature or over a 95/96-day freezing period followed by 11/10 days at ambient temperature. The homogeneity of the diet was verified on the first preparation at 400 and 15 000 ppm and was within a range of 93 to 96 % of the nominal concentrations. Concentration was checked for each concentration of the two dietary preparations and was within a range of 93 to 101% of the nominal concentrations.

Table IIA 5.8-2Study design

Test group	Concentration in diet (ppm)	Dose per animal (study averages)		Animals assigned	
		Male (mg/kg bw/day)	Female (mg/kg bw/day)	Male	Female
1	0	0	0	10	10
2	400	25.3	30.4	10	10
3	2 000	127	152	10	10
4	15 000	972	1170	10	10

Table IIA 5.8-3: Homogeneity and concentration of test material

	Nominal concentrations (ppm)	Analysed concentrations (ppm)	% of nominal concentration
Homogeneity (first preparation)	400	H1 382 H3 380 M1 378 M3 378 B1 385 B3 373	96 95 95 95 96 93
	15 000	H1 14148 H3 14231 M1 14261 M3 14010 B1 14134 B3 13919	94 95 95 93 94 93
Concentration (first preparation)	400 (first load)	379	95
	400 (second load)	379	95
	2 000 (first load)	1856	93
	2 000 (second load)	1917	96
	7 000 (first load)	14117	94
	7 000 (second load)	14170	94
Concentration (second preparation)	400 (first load)	394	99
	400 (second load)	388	97
	2 000 (first load)	1995	100
	2 000 (second load)	2009	100
	7 000 (first load)	15129	101
	7 000 (second load)	14829	99

H = surface; M = middle; B = bottom of the preparation. Three samples were taken at each level of the preparation (H, M, B); only two were analyzed.

4. Statistics

Data were analyzed by the Bartlett's test for homogeneity of variances. When the data were homogeneous, an ANOVA was performed followed by Dunnett's test on parameters showing a significant effect by ANOVA. When the data were not homogeneous even after transformation, a Kruskal-Wallis ANOVA was performed followed by the Dunn's test if the Kruskal-Wallis was significant. When one or more group variance(s) equaled 0, means were compared using non-parametric procedures. Group means were compared at the 5% and 1% levels of significance. Statistical analyses were carried out using Path/Tox System V4.2.2. (Module Enhanced Statistics).

C. METHODS:

1. Observations

The animals were observed twice daily for moribundity and mortality (once daily on weekends or public holidays). Observed clinical signs were recorded at least once daily for all animals and detailed physical examinations were performed once during the acclimatization phase and at least weekly during the treatment period. On study Weeks 11 to 12, a neurotoxicity assessment was performed for all animals by observers who were blind with respect to the dose level. Animals were tested individually for motor activity using an automated photocell recording apparatus designed to measure quantitatively spontaneous exploratory motor activity in a novel environment. Motor activity was recorded for 90 minutes with data being collected at regular intervals throughout the session. For open field observation, changes in gait, posture, as well as presence of clonic or tonic movements, stereotypic behaviour (e.g. excessive grooming, repetitive circling), bizarre behaviour (e.g. self-mutilation, walking backward) and other neurological-related changes were recorded for all surviving animals.
For sensor reactivity, the following reflexes and responses were recorded: pupillary reflex, surface righting reflex, corneal reflex, flexor reflex, auditory startle response and tail pinch response. The fore- and hindlimb grip strength of all animals were measured quantitatively using a grip strength apparatus equipped with one pull and one push strain gauge. The mean of three successive measurements was determined for both fore- and hindlimb grip strength.

2. Body weight

Body weights were recorded two times during the acclimatization phase, on the first day of test substance administration, then at weekly intervals throughout the treatment. Additionally, diet fasted animals were weighed at final sacrifice.

3. Food consumption and compound intake

Food consumption was recorded twice per week during the first 6 weeks (to avoid spillage), then weekly from week 7.
The weekly mean achieved dosage intake in mg/kg/day for each week and for Weeks 1 to 13 was calculated for each sex using the formula:

$$\text{Test substance intake (mg/kg/day)} = \frac{\text{Dose level (ppm)} \times \text{Group mean food consumption (g/day)}}{\text{Group mean body weight (g) at the end of the food consumption period}}$$

4. Ophthalmic examination

During the acclimatization period all animals were subjected to an ophthalmological examination. After instillation of an atropinic agent (Mydriaticum, Merk Sharp and Dohme) each eye was examined by means of an indirect ophthalmoscope. During Week 13, all surviving animals from

control and high dose groups were re-examined.

5. Clinical chemistry

On study days 92, 93 or 94, blood samples were taken from all surviving animals in all groups by puncture of the retro-orbital venous plexus. Animals were diet fasted overnight prior to bleeding and anaesthetized by inhalation of Isoflurane. Blood was collected on EDTA for haematology, on lithium heparin for plasma and clot activator for serum for clinical chemistry and on sodium citrate for coagulation parameters.
The following haematology parameters were assayed using an Advia 120 (Bayer Diagnostics, Puteaux, France): red blood cell count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, reticulocyte count, white blood cell count and differential count evaluation and platelet count. A blood smear was prepared and stained with Wright stain. It was examined when results of Advia 120 determinations were abnormal. Prothrombin time was assayed on an ACL 3000 (Instrumentation Laboratory, Paris, France).
Any significant change in the general appearance of the plasma and the serum was recorded. Total bilirubin, glucose, urea, creatinine, total cholesterol, triglycerides, chloride, sodium, potassium, calcium and inorganic phosphorus concentrations and aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma-glutamyltransferase activities were assayed on plasma samples, total protein and albumin concentrations were assayed on serum samples using an Hitachi 911 (Roche Diagnostics, Meylan, France). Globulin and albumin/globulin ratio values were calculated.

6. Urinalysis

Overnight urine samples were collected on study Days 87 or 88 from all animals in all groups. Food and water were not accessible during urine collection.
Any significant change in the general appearance of the urine was recorded. The urine volume was measured. pH was assayed using a Clinitek 200+ and Ames Multistix dipsticks (Bayer Diagnostics, Puteaux, France). Urinary refractive index was measured using a RFM320 refractometer (Bioblock Scientific, Illkirch, France).
The following semi-quantitative parameters were assayed using a Clinitek 200+ and Ames Multistix dipsticks: glucose, bilirubin, ketone bodies, occult blood, protein and urobilinogen. Microscopic examination of the urinary sediment was performed after centrifugation of the urine. The presence of red blood cells, white blood cells, epithelial cells, bacteria, casts and crystals was graded.

7. Sacrifice and pathology

On study days 92, 93 or 94, a complete necropsy was performed on all surviving animals. Animals were deeply anaesthetized by inhalation of Isoflurane, then exsanguinated before necropsy. All animals were fasted prior to scheduled sacrifice. All animals were necropsied. The necropsy included the examination of all major organs, tissues and body cavities. Macroscopic abnormalities were recorded, sampled and examined microscopically.
Adrenal gland, brain, epididymides, heart, kidney, liver, ovary, pituitary gland, prostate gland, spleen, testis, thymus, thyroid gland (with parathyroid gland) and uterus (including cervix) were weighed fresh at scheduled sacrifice only. Paired organs were weighed together.
The following organs or tissues were sampled: adrenal gland, aorta, articular surface (femoro-tibial), bone (sternum), bone marrow (sternum), brain, epididymis, oesophagus, exorbital (lachrymal) gland, eye and optic nerve, harderian gland, heart, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidney, larynx/pharynx, liver, lung, lymph nodes (submaxillary, mesenteric), mammary gland, nasal cavities, ovary, pancreas, pituitary gland, prostate gland, sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, submaxillary (salivary) gland, testis, thymus, thyroid gland (with parathyroid),

tongue, trachea, urinary bladder, uterus (with cervix), vagina.
A bone marrow smear was prepared from femur, stained with May-Grünwald Giemsa, but not examined. Tissues samples were fixed by immersion in neutral buffered 10% formalin with the exception of the eye, optic nerve, harderian gland, epididymis and testis that were fixed in Davidson's fixative. Histopathological examinations were performed on all tissues from all the animals in the control and high dose groups. The liver, kidney, lung and thyroid gland were examined in all animals in the study.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS:

1. Mortality

There were no treatment-related mortalities during the course of the study.
One female from the 2000 ppm group died before necropsy, just after blood sampling.

2. Clinical signs

There were no treatment-related clinical signs during the course of the study.

B. BODY WEIGHT AND BODY WEIGHT GAIN:

There was no treatment-related effect on mean body weight and body weight gain parameters in either sex at any of the dose levels tested.
The lower mean body weight gain of +0.3 g/day (statistically significant, $p<0.05$) observed in females from the 15000 ppm group between study Days 43 and 50 only, compared with a gain of +1.2 g/day in the corresponding controls was considered to be incidental.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

Mean food consumption was comparable with the controls in both sexes at all dose levels tested.
In females at 15000 ppm, mean food consumption was statistically significantly higher ($p<0.05$) than in the control group between Study Days 8 and 11. In isolation, this finding was considered to be incidental.

D. ACHIEVED DOSAGES:

The mean achieved dosage intake of BYH 18636-Carboxylic Acid per group was as follows:

Table IIA 5.8-4: Mean achieved dietary intake of BYH 18636-carboxylic acid (Weeks 1-13)

Diet concentration (ppm)	Males mg/kg/day	Females mg/kg/day
400	25.3	30.4
2000	127	152
15000	972	1170

E. OPHTHALMOSCOPIC EXAMINATION:

No treatment-related ocular abnormalities were observed at ophthalmoscopic examination.

F. NEUROTOXICITY ASSESSMENT

1. Exploratory locomotor activity

Overall mean exploratory locomotor activity was unaffected by the treatment at any dose level in either sex. A slight decrease in overall mean exploratory locomotor activity was noted at 15000

and 2000 ppm in males (-22%, not statistically significant), but it was considered not to be treatment-related, due to the limited magnitude of the change and inter-individual variation of this parameter.
The pattern of the locomotor activity over time was also similar to controls. The decrease in activity observed in high dose males during interval 16-30 minutes (-47%, $p<0.05$) was considered to be fortuitous as this apparent effect was observed in isolation for this interval only.

2. Open field observation
No changes were recorded during the open field observation at any dose level in either sex.

3. Sensory reactivity
All reflexes and responses evaluated were unaffected by the treatment at any dose level in either sex. The slightly increased incidence of weak tail pinch response in the high and intermediate dose males was considered not to be treatment-related, due to the limited magnitude of the change and inter-individual variation of this parameter. The other few changes noted in both sexes were observed in isolation and/or with no dose-relationship and were thus considered not to be treatment-related.

4. Grip strength
The fore- and hindlimb grip strength were unaffected by the treatment at any dose level in either sex.

G. BLOOD ANALYSIS:

1. Haematological findings
No treatment-related change was noted at any dose level for the parameters assayed. The statistically significant difference noted in platelet count (+13%, $p<0.05$) in males at 15000 ppm was slight and considered to be incidental in view of the variation of the individual values.

2. Clinical chemistry findings
No treatment-related change was noted at any dose level for the parameters assayed.

H. URINALYSIS:

No treatment-related change was noted at any dose level for the parameters assayed.

I. SACRIFICE AND PATHOLOGY:

1. Organ weight
There was no change in mean terminal body weights and in mean organ weights in treated animals when compared to controls.

2. Gross Pathology
All findings were considered to be incidental and not treatment-related.

3. Microscopic Pathology
All findings were considered to be incidental and not treatment-related.

III. CONCLUSION

The No Observed Effect Level (NOEL) in this study was 15000 ppm, equating to 972 and 1170 mg/kg/day in males and females, respectively.

(Odin-Feurtet M., 2007)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: Totally reliable The study (SA06035) is fully compliant with OECD guideline 408 (1998). A discrepancy between the clinical chemistry analysers stated to have been used in the study report and the Notifier's summary is not considered to have affected the integrity of the study. Mean bodyweights of males administered 15000 ppm were marginally (but consistently) lower during the middle part of the study (Days 22-64) as a result of slightly reduced weight gain. Findings are not considered to be of toxicological significance due to the magnitude of change (<10% deviation from controls) and in the absence of statistical significance. Terminal bodyweights were comparable in all groups of males and a similar effect was not apparent in females. A slightly (but significantly) increased platelet count in top dose males [group mean values 1076, 17794, 1180, 1219 x10^9/l] is not considered to be of toxicological significance in isolation and in the absence of a clear dose-response relationship. The absence of any treatment-related effect on the urinary system even at the high dose level of 15000 ppm is notable and contrasts with the results of the 90-day study with thiencarbazone-methyl.
Conclusions	A NOAEL of 15000 ppm (equivalent to mean achieved dietary intakes of 972 and 1170 mg/kg bw/d in males and females respectively) can be determined for this study in the absence of any treatment-related findings of toxicological significance at the top dose level. The Notifier has proposed the same NOAEL for this study.

- **BYH 18636-sulfonamide**

***In vitro* genotoxicity - Bacterial assay for gene mutation**

Report:	**KIIA 5.8/06, Herbold B., 2006 (MRID # 47070208)**
Title:	BYH 18636-sulfonamide, Salmonella/microsome test, plate incorporation and preincubation method
Report No & Document No	AT03605 M-283583-01-2
Guidelines:	OECD 471 (1997); EEC Directive 2000/32/EC Method B13/14 (2000); EPA Health Effects Test Guideline (OPPTS 870.5100; 1998)
GLP	Yes (certified laboratory)

Executive summary:

In this *in vitro* assessment of the mutagenic potential of BYH 18636-sulfonamide (Batch CHZC007326, 99.0% of purity), histidine dependent auxotrophic mutants of Salmonella typhimurium, strains TA 1535, TA 1537, TA 98, TA 100 and TA 102 were exposed to BYH 18636-sulfonamide up to 5000 µg/plate, diluted in dimethyl sulphoxide (DMSO). An additional plate incorporation test with TA 1535 was conducted with doses up to and including 7000 µg per plate. For each bacterial strain and dose level, triplicate plates were used in both the presence and absence of an Aroclor 1254-induced rat liver metabolic activation system (S9 mix). DMSO was also used as a negative control. Specific positive controls were used for each strain. After 48 hours of incubation at 37°C, the numbers of revertant colonies were scored using an automated colony counter. Another assay testing a pre-

incubation for 20 minutes at 37°C was also performed at doses from 16 to 5000 µg/plate.

Doses up to and including 7000 µg/plate did not cause any bacteriotoxic effect. Total bacteria counts remained unchanged and no inhibition of growth was observed.

BYH 18636-sulfonamide did not cause any significant increase in the number of revertant colonies in either the presence or absence of metabolic activation.

All the positive control compounds produced expected increases in the number of revertant colonies, thereby demonstrating the sensitivity of the assay and the efficacy of the S9 mix.

Therefore, BYH 18636-sulfonamide was non-mutagenic with or without S9 mix in the plate incorporation as well as in the pre-incubation modification of the Salmonella/microsome test.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636-sulfonamide
Description: brown crystalline powder
Lot/Batch: CHZC007326
Purity: 99.0% (March 2006)
CAS: 317815-81-9
Stability of test compound: Stable at 0.01 to 50 mg/ml at room temperature for at least 4 hours

2. Control materials:
Negative: Culture medium
Solvent: DMSO
Positive: Non activated
Sodium azide (Serva) for TA 1535 at 10 µg/plate,
Nitrofurantoin (Sigma) for TA 100 at 0.2 µg/plate,
4-Nitro-1, 2-phenylene diamine (Merck-Schuchardt) for TA 1537 at 10 µg/plate and TA 98 at 0.5 µg/plate,
Mitomycin C (Fluka) for TA 102 at 0.2 µg/plate only in plate incorporation trials,
Cumene hydroperoxide (Sigma) for TA 102 in pre-incubation trials only at 50 µg/plate,
2-Aminoanthracene (Aldrich) for the activating effect of the S9 mix in all strains at 3 µg/plate.

3. Test organisms:
Species: Salmonella typhimurium LT2 mutants
Strain: Histidine-auxotrophic strains TA 1535, TA 100, TA 1537, TA 98 and TA 102
Source: Strains obtained from Prof. Bruce Ames in 1997 and stored in the laboratory since then.

4. Test compound concentrations:
Plate incorporation assay: For all strains with or without S9 mix: 16, 50, 158, 500, 1581 and 5000 µg/plate
For TA 1535 only wihtout S9 mix: 1000, 3000, 4000, 5000, 6000 and 7000 µg/plate.
Pre-incubation assay: For all strains with or without S9 mix: 16, 50, 158, 500, 1581 and 5000 µg/plate.

B. STUDY DESIGN AND METHODS :

The in life phase of the study was performed between November 21 to December 12, 2006 at Bayer Healthcare AG PH-GDD Toxicology).
The *Salmonella*/microsome test is a screening method which detects point mutations caused by chemical agents *in vitro*. Auxotrophic mutants of *Salmonella typhimurium* are used to demonstrate this effect. For this purpose, the rate of reversion to prototrophy is evaluated in negative control and treated groups.

1. Plate incorporation assay:
0.1 ml of DMSO containing BYH 18636-sulfonamide or controls were added to glass vessels with 0.1 ml of bacterial cultures grown overnight, 0.5 ml of S9 mix or buffer and 2 ml of soft agar. The mixture was placed in a waterbath at 45°C for 30 seconds, shaken and overlaid onto Petri dishes containing solid agar. After 48 hours of incubation at 37°C, the numbers of revertant colonies were scored using an automated colony counter. Three plates were used, both with and without S9 mix, for each strain and dose. The doses for the first trial were routinely determined on the basis of a standard protocol with a maximum dose of 5000 µg/plate and at least 5 additional doses. If less than three doses were used for assessment, at least two repeats were performed.

2. Pre-incubation assay:
An independent repeat was performed as pre-incubation of the previously described mixture in a water bath at 37°C for 20 minutes. At the end of the pre-incubation period, 2 ml of molten soft agar were added to the tubes, the content mixed and plated onto Petri dishes with solid agar. After 48 hours of incubation at 37°C, the numbers of revertant colonies were also scored using an automated colony counter.

3. Assessment criteria:
A reproducible and dose-related increase in mutant colonies of at least one strain was considered to be positive. For TA 1535, TA 100 and TA 98, this increase should be about twice that of negative controls, whereas for TA 1537, at least a threefold increase should be reached. For TA 102 an increase of about 100 mutants should be reached. Otherwise, the result was considered as negative.

II. RESULTS AND DISCUSSION

There was no indication of a bacteriotoxic effect of BYH 18636-sulfonamide at doses of up to and including 7000 µg per plate. The total bacteria counts consistently produced results comparable to the negative controls, or differed only insignificantly. No inhibition of growth was noted as well.

For the strain TA 1535, an increase in mutant counts of over double those of negative control was observed. However, despite the use of higher doses in the plate incorporation repeat the increase for Salmonella typhimurium TA 1535 could neither be confirmed in the plate incorporation trial nor could it be confirmed in the pre-incubation trial and is therefore to be regarded as a random result.
None of the other strains concerned showed in the plate incorporation test a dose-related or biologically relevant increase in mutant counts over those of the negative controls. This applied both to the tests with and without S9 mix and was confirmed by the results of the preincubation trials.

III. CONCLUSION

The Salmonella/microsome test, employing doses of up to 7000 µg per plate, showed BYH 18636-sulfonamide not to produce bacteriotoxic effects.
Evaluation of individual dose groups, with respect to relevant assessment parameters (dose effect, reproducibility) revealed no biologically relevant variations from the respective negative controls.
In spite of the low doses used, positive controls increased the mutant counts to well over those of the negative controls, and thus demonstrated the system's high sensitivity. Despite this sensitivity, no indications of mutagenic effects of BYH 18636-sulfonamide could be found at assessable doses of up

to 7000 µg per plate in any of the Salmonella typhimurium strains used. BYH 18636-sulfonamide has to be regarded as non-mutagenic.

(Herbold B., 2006)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable The study (T5076444) is fully compliant with OECD 471 (1997). The efficacy of the metabolic activation system (male Sprague-Dawley rat liver S9 fraction) was additionally demonstrated using reference mutagens. Exposure to the test material at concentrations of up and including to the limit concentration of 5000 µg/plate in the absence or presence of metabolic activation did not result in a reproducible increase in the number of revertant colonies of any bacterial strain. A slight increase in the number of revertant colonies of TA 1535 seen at the highest concentration of the test material in the absence of metabolic activation was not reproduced in a second (confirmatory) plate incorporation assay at this concentration or at concentrations up to 7000 µg/plate, and is therefore not considered to be of biological relevance. No increase in the number of revertant colonies of any strain was seen in an independently repeated (pre-incubation) assay using concentrations of up to 5000 µg/plate. Appropriate positive control compounds confirmed the sensitivity of the assay. Bacterial mutagenicity study with thiencarbazone-methyl sulphonamide

Concentration (µg/plate)	Mean revertant colonies (strain TA 1535)					
	Experiment 1		Experiment 2		Experiment 3	
	-S9	+S9	-S9	+S9	-S9	+S9
Vehicle control	22	10	20	-	14	18
16	25	9	-	-	16	14
50	26	10	-	-	15	14
158	25	9	-	-	14	10
500	30	11	-	-	14	11
1000	-	-	17	-	-	-
1581	31	8	-	-	13	10
3000	-	-	20	-	-	-
4000	-	-	20	-	-	-
5000	50	7	24	-	18	10
6000	-	-	23	-	-	-
7000	-	-	23	-	-	-
Positive control	693	129	575	-	782	248

Conclusions	No evidence of mutagenicity was seen under the conditions of this study. A slight increase in the number of revertant colonies seen in strain TA 1535 in the first (plate incorporation assay) at the highest concentration of 5000 µg/plate in the absence of metabolic activation was not reproducible at concentrations of up to 7000 µg/plate or at the same concentration in a pre-incubation assay.

Acute oral toxicity

Report:	**KIIA 5.8/07, Schüngel M.; 2006 (MRID # 47070210)**
Title:	BYH 18636-sulfonamide, Acute toxicity in the rat after oral administration
Report No &	AT03210
Document No	M-277277-01-2

Guidelines:	OECD 423 (2001); EEC Directive 67/548 Annex V – Method B.1.tris (2004); EPA Health Effects test Guidelines (OPPTS 870.1100) (1998)
GLP	Yes (certified laboratory)

Executive Summary
In an acute oral toxicity study using a stepwise procedure, two groups of three fasted, young adult female Wistar rats (HsdCpb: Wu) were given successively a single oral dose of BYH 18636-sulfonamide (batch CHZC007326, 99.0% purity) in 2% Cremophor EL of 2 000 mg/kg bw and were observed for 14 days.
The dose of 2 000 mg/kg bw was tolerated by both groups without mortalities, clinical signs, effects on weight gain or gross pathological findings.

According to OECD guideline 423, the LD_{50} cut-off of BYH 18636-sulfonamide was ≥ 5 000 mg /kg bw (category 5 / unclassified of the global harmonized classification system).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636-sulfonamide

Description:	light brown solid
Lot/Batch:	CHZC007326
Purity:	99.0% (March 2006)
CAS:	317815-81-9
Stability of test compound:	Stable at 5 and 200 mg/ml at room temperature for at least 2 hours

2. Vehicle and /or positive control: 2% Cremophor EL in demineralized water

3. Test animals:

Species:	Rat
Strain:	HsdCpb: Wu
Age:	10 to 12 weeks approximately
Weight at dosing:	160 to 175 g
Source:	Harlan/Winkelmann GmbH, 33178 Borchen, Germany
Acclimation period:	At least 5 days
Diet:	Provimi Kliba 3883.0.15 Maus/Ratte Haltung, Kaiseraugst Switzerland, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were group caged conventionally in polycarbonate cages on low dust wood granulate bedding

Environmental conditions –

Temperature:	22 ± 2°C
Humidity:	55 ± 5%
Air changes:	Approximately 10 changes per hour
Photoperiod :	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS:

1. In life dates: April 26 to May 17, 2006 performed at Bayer HealthCare AG, Germany

2. Animal assignment and treatment

The substance was tested using a stepwise procedure, each step using three female rats. The animals were assigned to their groups by randomization based on evenly distributed chance numbers. Following an overnight fast (16 to 24 hours), each group received a single dose of 2 000 mg/kg of BYH 18636-sulfonamide (99.0% purity) by gavage. The test substance was administered in demineralized water with 2% Cremophor EL at a volume of 10 ml/kg bw. Clinical signs and mortality rates were determined several times on the day of administration and subsequently at least once daily for an observation period of at least 14 days. Body weights were recorded on days 1, 8 and 15. On day 15, surviving animals were sacrificed and all animals were necropsied and examined for gross pathological changes.

Table IIA 5.8-5 Doses, mortality / animals treated

Dose (mg/kg bw)	Females
2 000 (1st)	0/3
2 000 (2nd)	0/3

3. Statistics

The data did not warrant statistical analysis.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortalities occurred at 2 000 mg/kg bw, the only dose tested.

The oral LD_{50} cut-off was ≥ 5 000 mg /kg bw according to OECD guideline 423.

B. CLINICAL OBSERVATIONS

No clinical signs were observed.

C. BODY WEIGHT

There was no toxicological effect on body weight or body weight gain.

D. NECROPSY

No abnormalities were observed at gross necropsy.

III. CONCLUSION

The oral LD_{50} cut off of BYH 18636-sulfonamide was ≥ 5 000 mg/kg bw. BYH 18636-sulfonamide does not warrant classification as being toxic or harmful on the basis of its acute oral toxicity.

(Schüngel M., 2006)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY

Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: Totally reliable The study (T8076771) is fully compliant with OECD 423 (2001) No treatment-related findings were seen following the administration of a single (limit) dose of 2000 mg/kg bw.
Conclusions	The acute oral LD_{50} of thiencarbazone-methyl-sulphonamide in female rats was found to be >2000 mg/kg bw under the conditions of this study. The test material is therefore not classified for acute oral toxicity according to current EC criteria. EPA: For Toxicity Category/Classification EPA Toxicity Category III – LD_{50} > 2000 mg/kg - would be assigned.

Oral 28-day toxicity in the rat

Report:	**KIIA 5.8/08, Rascle J-B., 2007 (MRID # 47070212)**
Title:	BYH 18636-sulfonamide, 28-day toxicity study in the rat by dietary administration
Report No & Document No	SA 06084; M-282938-01-2
Guidelines:	OECD 407 (1995); EEC Directive 96/54/EC Annex V – Method B.7. (1996);
GLP	Yes (certified laboratory)

Executive summary:
BYH 18636-sulfonamide (batch number CHZC007326: a light brown solid, 99.0% purity), a synthesis intermediate of BYH 18636, was administered continuously via the diet to groups of Wistar rats (10/sex/group) for at least 28 days at concentrations of 500, 5000 and 10000 ppm. A similarly constituted group received untreated diet and acted as a control. Animals were observed daily for mortality and clinical signs. Physical examinations were performed weekly. Body weight and food consumption were recorded once weekly. Ophthalmological examinations were performed once on all animals during the acclimatization phase and on control and high dose group animals at the end of the study. All surviving animals were subjected to neurotoxicity assessment (motor activity, open field observations, sensory reactivity and grip strength) during Week 4 of the study. Urine investigations were performed during the last week of the study. Haematology and clinical chemistry investigations were performed at the end of the study. All animals were necropsied, selected organs weighed and a range of tissues were taken, fixed and examined microscopically.

There were no mortalities or treatment-related findings recorded during the course of the study in either sex in any dose level.
The No Observed Effect Level (NOEL) in this study was 10000 ppm, the highest dose level tested, in both sexes, corresponding to 800 mg/kg/day in males and 917 mg/kg/day in females.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	BYH 18636-sulfonamide
Description:	light brown solid
Lot/Batch:	CHZC007326
Purity:	99.0% (March, 2006)
CAS:	317815-81-9

Stability of test compound: Stable in rodent diet at 100 and 20 000 ppm over a 108-day period at ambient temperature or over a period of 97-day frozen storage followed by 11 days at room temperature

2. Vehicle and /or positive control: None

3. Test animals:

Species:	Rat
Strain:	Wistar Rj: WI (IOPS HAN)
Age:	6 weeks approximately
Weight at dosing:	186 to 258 g for the males – 147 to 175 g for the females
Source:	R. Janvier, Le Genest St Isle, France
Acclimation period:	6 days
Diet:	Certified rodent powdered and irradiated diet A04CP1-10 from S.A.F.E. (Scientific Animal Food and Engineering, Augy, France), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were housed individually in suspended stainless steel wire mesh cages.
Environmental conditions –	
Temperature:	22 ± 2°C
Humidity:	55 ± 15%
Air changes:	Approximately 10 to 15 changes per hour
Photoperiod :	Alternating 12-hour light and dark cycles (7 am- 7 pm)

B. STUDY DESIGN:

1. In life dates: May 17 to June 30, 2006 performed at Bayer CropScience, Sophia Antipolis, France

2. Animal assignment and treatment

There were 5 animals of each sex per dose group. Animals were assigned to dose groups using a randomization procedure based on body weight. BYH 18636-sulfonamide was administered in the diet for at least 28 days to Wistar rats at the following doses – 0, 500, 5 000 and 10 000 ppm (equating approximately to 40.4, 399, and 800 mg/kg/day in males and 47.3, 460, 917 mg/kg/day in females). A negative control group received plain diet. Animal housing and husbandry were in accordance with the regulations of the Guide for the Care and Use of Laboratory Animals (Public Health Service, National Institute of Health, NIH publication N°86-23, revised 1985) and "Le Guide du Journal Officiel des Communautés Européennes L358, 18 Décembre 1986, N°86/609/CEE du 24 Novembre 1986".

3. Diet preparation and analysis

BYH 18636-sulfonamide was ground to a fine powder before being incorporated into the diet by dry mixing to provide the required concentrations. There was one preparation of each concentration for the whole study. The stability was demonstrated during the course of the study. Diet samples of 100 and 20 000 ppm were found to be stable over a 108-day period at ambient temperature or over a 97-day freezing period followed by 11 days at ambient temperature. The homogeneity of the diet was verified at 500 and 10 000 ppm and was within a range of 94 to 99 % of the nominal concentrations. Concentration was checked for each concentration and was within a range of 94 to 97% of the nominal concentrations.

Table IIA 5.8-6 Study design

Test group	Concentration in diet (ppm)	Dose per animal (study averages)		Animals assigned	
		Male (mg/kg bw/day)	Female (mg/kg bw/day)	Male	Female
1	0	0	0	5	5
2	500	40.4	47.3	5	5
3	5 000	399	460	5	5
4	10 000	800	917	5	5

Table IIA 5.8-7: Homogeneity and concentration of test material

	Nominal concentrations (ppm)	Analysed concentrations (ppm)	% of nominal concentration
Homogeneity	500	H1 490 H3 497 M1 482 M3 493 B1 468 B3 488	98 99 96 99 94 98
	10 000	H1 9694 H3 9574 M1 9877 M3 9730 B1 9654 B3 9765	97 96 99 97 97 98
Concentration	500	486	97
	5000	4708	94
	10000	9716	97
Stability	100	H1 97 H3 91 B1 92 B3 96	97 91 92 96
	20000	H1 19359 M2 19517 B3 19559	97 98 98
Stability after 97 days frozen storage and 11 days at room temperature	100	102	102
	20000	19849	99
Stability after 108 days at room temperature	100	104	104
	20000	19863	99

H = surface; M = middle; B = bottom of the preparation. Three samples 1, 2 and 3 were taken at each level of the preparation (H, M, B); only two were analyzed.

4. Statistics

Data were analyzed by the Bartlett's test for homogeneity of variances. When the data were homogeneous, an ANOVA was performed followed by Dunnett's test on parameters showing a significant effect by ANOVA. When the data were not homogeneous even after transformation, a Kruskal-Wallis ANOVA was performed followed by the Dunn's test if the Kruskal-Wallis was significant. When one or more group variance(s) equaled 0, means were compared using non-parametric procedures. Group means were compared at the 5% and 1% levels of significance. Statistical analyses were carried out using Path/Tox System V4.2.2. (Module Enhanced Statistics).

C. METHODS:

3. Observations

The animals were observed twice daily for moribundity and mortality (once daily on weekends or public holidays). Observed clinical signs were recorded at least once daily for all animals and detailed physical examinations were performed at least weekly during the treatment period. On study Week 4, a neurotoxicity assessment was performed for all animals by observers who were blind with respect to the dose level. Animals were tested individually for motor activity using an automated photocell recording apparatus designed to measure quantitatively spontaneous exploratory motor activity in a novel environment. Motor activity was recorded for 90 minutes with data being collected at 15-minute intervals throughout the session. For open field observation, changes in gait, posture, as well as presence of clonic or tonic movements, stereotypic behaviour (e.g. excessive grooming, repetitive circling), bizarre behaviour (e.g. self-mutilation, walking backward) and other neurological-related changes were recorded for all surviving animals.
For sensor reactivity, the following reflexes and responses were recorded: pupillary reflex, surface righting reflex, corneal reflex, flexor reflex, auditory startle response and tail pinch response. The fore- and hindlimb grip strength of all animals were measured quantitatively using a grip strength apparatus equipped with one pull and one push strain gauge. The mean of three successive measurements was determined for both fore- and hindlimb grip strength.

2. Body weight

Body weights were recorded once during the acclimatization phase, on the first day of test substance administration, then at weekly intervals throughout the treatment. Additionally, diet fasted animals were weighed at final sacrifice.

3. Food consumption and compound intake

Food consumption was recorded once per week during the treatment period.
The weekly mean achieved dosage intake in mg/kg/day for each week and for Weeks 1 to 4 was calculated for each sex using the formula:

$$\text{Test substance intake (mg/kg/day)} = \frac{\text{Dose level (ppm)} \times \text{Group mean food consumption (g/day)}}{\text{Group mean body weight (g) at the end of the food consumption period}}$$

4. Ophthalmic examination

During the acclimatization period all animals were subjected to an ophthalmological examination. After instillation of an atropinic agent (Mydriaticum, Merk Sharp and Dohme) each eye was examined by means of an indirect ophthalmoscope. During Week 4, all surviving animals from control and high dose groups were re-examined.

5. Clinical chemistry

On study days 30 or 31, blood samples were taken from all animals in all groups by puncture of the retro-orbital venous plexus. Animals were diet fasted overnight prior to bleeding and anaesthetized by inhalation of Isoflurane. Blood was collected on EDTA for haematology, on lithium heparin for plasma and clot activator for serum for clinical chemistry and on sodium citrate for coagulation parameters.
The following haematology parameters were assayed using an Advia 1650 (Bayer Diagnostics, Puteaux, France): red blood cell count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, reticulocyte count, white blood cell count and differential count evaluation and platelet count. A blood smear was prepared and stained with Wright stain. It was examined when results of Advia 1650

determinations were abnormal. Prothrombin time was assayed on an ACL 3000 (Instrumentation Laboratory, Paris, France).
Any significant change in the general appearance of the plasma and the serum was recorded. Total bilirubin, glucose, urea, creatinine, total cholesterol, triglycerides, chloride, sodium, potassium, calcium and inorganic phosphorus concentrations and aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma-glutamyltransferase activities were assayed on plasma samples, total protein and albumin concentrations were assayed on serum samples using an Hitachi 911 (Roche Diagnostics, Meylan, France). Globulin and albumin/globulin ratio values were calculated.

6. Urinalysis

Overnight urine samples were collected on study Day 27 from all animals in all groups. Food and water were not accessible during urine collection.
Any significant change in the general appearance of the urine was recorded. The urine volume was measured. pH was assayed using a Clinitek 200+ and Ames Multistix dipsticks (Bayer Diagnostics, Puteaux, France). Urinary refractive index was measured using a RFM320 refractometer (Bioblock Scientific, Illkirch, France).
The following semi-quantitative parameters were assayed using a Clinitek 200+ and Ames Multistix dipsticks: glucose, bilirubin, ketone bodies, occult blood, protein and urobilinogen. Microscopic examination of the urinary sediment was performed after centrifugation of the urine. The presence of red blood cells, white blood cells, epithelial cells, bacteria, casts and crystals was graded.

7. Sacrifice and pathology

On study days 30 or 31, a complete necropsy was performed on all animals. Animals were deeply anaesthetized by inhalation of Isoflurane, then exsanguinated before necropsy. All animals were fasted prior to scheduled sacrifice. All animals were necropsied. The necropsy included the examination of all major organs, tissues and body cavities. Macroscopic abnormalities were recorded, sampled and examined microscopically.
Adrenal gland, brain, epididymides, heart, kidney, liver, ovary, pituitary gland, prostate gland, spleen, testis, thymus, thyroid gland (with parathyroid gland) and uterus (including cervix) were weighed fresh at scheduled sacrifice only. Paired organs were weighed together.
The following organs or tissues were sampled : adrenal gland, aorta, articular surface (femoro-tibial), bone (sternum), bone marrow (sternum), brain, epididymis, oesophagus, exorbital (lachrymal) gland, eye and optic nerve, harderian gland, heart, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidney, larynx/pharynx, liver, lung, lymph nodes (submaxillary, mesenteric), mammary gland, nasal cavities, ovary, pancreas, pituitary gland, prostate gland, sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, submaxillary (salivary) gland, testis, thymus, thyroid gland (with parathyroid), tongue, trachea, urinary bladder, uterus (with cervix), vagina.
A bone marrow smear was prepared from femur, stained with May-Grünwald Giemsa, but not examined. Tissues samples were fixed by immersion in neutral buffered 10% formalin with the exception of the eye, optic nerve, harderian gland, epididymis and testis that were fixed in Davidson's fixative. Histopathological examinations were performed on all tissues from all the animals in the control and high dose groups. The liver, kidney, lung thyroid gland and macroscopic findings were examined in all animals in the study.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS:

1. Mortality

There were no mortalities during the course of the study.

2. Clinical signs

There were no treatment-related clinical signs during the course of the study.

B. BODY WEIGHT AND BODY WEIGHT GAIN:

Body weight parameters were unaffected by treatment throughout the study in either sex at any dose level tested.
At 10000 ppm, mean absolute body weight gain was slightly reduced at each weekly interval in males only, when compared to the controls (between 3 and 9% reduction). However, this reduction was attributable to one male animal showing a lower body weight gain compared to the rest of the group. When the body weight parameters from this animal were excluded from the mean calculations, the body weight parameters in males were unaffected by treatment at 10000 ppm.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

Food consumption parameters were unaffected by treatment in either sex at any dose level during the course of the study

D. ACHIEVED DOSAGES:

The mean achieved dosage intake of BYH 18636-sulfonamide per group was as follows:

Table IIA 5.8-8: Mean achieved dietary intake of BYH 18636-sulfonamide (Weeks 1-4)

Diet concentration (ppm)	Males mg/kg/day	Females mg/kg/day
500	40.4	47.3
5000	399	460
10000	800	917

E. OPHTHALMOSCOPIC EXAMINATION:

No treatment-related ocular abnormalities were observed at ophthalmoscopic examination.

F. NEUROTOXICITY ASSESSMENT

1. Exploratory locomotor activity

No treatment-related changes were recorded in overall mean exploratory locomotor activity at any dose level in either sex. The decrease noted in isolation in the low and high dose male groups during the interval 61 to 75 minutes was observed with no dose-relationship and was thus considered not to be treatment-related. The decrease in overall mean exploratory locomotor activity (-51%, $p<0.01$) and during the first recorded interval (-30%, $p<0.05$) at 5000 ppm in females was considered to be fortuitous and was attributable to the particularly high mean value recorded in the control group.

2. Open field observations

No treatment-related changes were recorded during the open field observation at any dose level in either sex. The only change noted in gait score in females treated at 5000 ppm, was observed in isolation with no dose-relationship and was thus considered not to be treatment-related.

3. Sensory reactivity

All reflexes and responses evaluated were unaffected by the treatment at any dose level in either sex. The few changes noted in both sexes were considered to reflect inter-individual variations and/or were observed with no dose-relationship and were thus considered not to be treatment-related.

4. Grip strength

The fore- and hindlimb grip strength were unaffected by the treatment at any dose level in either sex. The slight changes in hindlimb grip strength noted in the low and mid dose groups were considered to reflect inter-individual variations and not to be treatment-related effects.

G. BLOOD ANALYSIS:

1. Haematological findings

No treatment-related change was noted at any dose level for the parameters assayed.

2. Clinical chemistry findings

No treatment-related change was noted at any dose level for the parameters assayed.

H. URINALYSIS:

No treatment-related change was noted at any dose level for the parameters assayed.

I. SACRIFICE AND PATHOLOGY:

1. Organ weight

There was no change in mean terminal body weights and in mean organ weights in treated animals when compared to controls.

2. Gross Pathology

All findings were considered to be incidental and not treatment-related.

3. Microscopic Pathology

All findings were considered to be incidental and not treatment-related.

III. CONCLUSION

The No Observed Effect Level (NOEL) in this study was 10000 ppm, equating to 800 and 917 mg/kg/day in males and females, respectively.

(Rascle J-B., 2007)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY

Name of authority: Pesticides Safety Directorate, UK

Reviewer's comments:

Reliability rating: Totally reliable

The study (SA 06084) is fully compliant with OECD 407 (1995).

A discrepancy between the clinical chemistry analysers stated to have been used in the study report and the Notifier's summary is not considered to affect the integrity of the study.

Mean bodyweights of top dose males were slightly (but not statistically significantly) lower than controls throughout the study period as a consequence of reduced weight gain. Findings are not considered to be of toxicological significance due to the magnitude of change (<10% compared to controls).

Absolute organ weights in top dose males were generally lower than controls, reflecting the slightly lower mean terminal bodyweight in this group. Organ weights relative to bodyweight and brain weight were comparable in all groups.

Mean thymus weight (relative to bodyweight) was slightly (but statistically significantly) lower in females at the highest dose level. Thymus weight relative to brain weight was not significantly lower than controls; this finding is not considered to be of clear toxicological significance due to the magnitude of change, in the absence of a consistent response and in the absence of histopathological correlates. The absence of urinary tract findings in this study is notable.

28-day rat study with thiencarbazone-methyl sulphonamide: thymus weight

Mean thymus weight		Dose level (ppm)			
		0	500	50000	10000
Absolute (g)	M	0.757 ±0.230	0.810 ±0.174	0.748 ±0.172	0.720 ±0.205
	F	0.607 ±0.058	0.556 ±0.013	0.564 ±0.081	0.526 ±0.047
Relative to bodyweight (%)	M	0.2065 ±0.0443	0.2203 ±0.0284	0.2089 ±0.0465	0.2081 ±0.0313
	F	0.2897 ±0.0179	0.2656 ±0.0152	0.2696 ±0.0228	0.2483* ±0.0219
Relative to brain weight (%)	M	36.36 ±10.81	40.00 ±6.40	36.04 ±7.15	35.03 9.17±
	F	31.78 ±3.18	29.71 ±0.72	31.09 ±4.06	27.61 ±2.92

Conclusions:

A NOAEL of 10000 ppm (equivalent to mean achieved dietary intakes of 800 and 917 mg/kg bw/d in males and females respectively) can be determined for this study in the absence of any treatment-related findings of toxicological significance at the top dose level.

The Notifier proposes the same NOAEL for this study.

- **BYH 18636-N-desmethyl**

In vitro genotoxicity - Bacterial assay for gene mutation

Report:	**KIIA 5.8/09, Herbold B., 2006 (MRID # 47070138)**
Title:	BYH 18636-N-desmethyl, Salmonella/microsome test, plate incorporation and preincubation method
Report No & Document No	AT03497 M-283429-01-2
Guidelines:	OECD 471 (1997); EEC Directive 2000/32/EC Method B13/14 (2000); EPA Health Effects Test Guideline (OPPTS 870.5100; 1998)
GLP	Yes (certified laboratory)

Executive summary:
In this *in vitro* assessment of the mutagenic potential of BYH 18636-N-desmethyl (Batch KATH 4779-3-12, 98.9% of purity), histidine dependent auxotrophic mutants of Salmonella typhimurium, strains TA 1535, TA 1537, TA 98, TA 100 and TA 102 were exposed to BYH 18636-N-desmethyl up to 5000 µg/plate, diluted in dimethyl sulphoxide (DMSO). For each bacterial strain and dose level, triplicate plates were used in both the presence and absence of an Aroclor 1254-induced rat liver metabolic activation system (S9 mix). DMSO was also used as a negative control. Specific positive controls were used for each strain. After 48 hours of incubation at 37°C, the numbers of revertant colonies were scored using an automated colony counter. Another assay testing a pre-incubation for 20 minutes at 37°C was also performed at doses from 16 to 5000 µg/plate.

Doses up to and including 500 µg/plate did not cause any bacteriotoxic effects. At higher concentrations, BYH 18636-N-desmethyl had only a weak, strain-specific bacteriotoxic effect in the plate-incorporation trial. Due to the weakness of this effect this range could be used for assessment purposes. No bacteriotoxic effects were observed in the pre-incubation trial.

BYH 18636-N-desmethyl did not cause any significant increase in the number of revertant colonies in either the presence or absence of metabolic activation.

All the positive control compounds produced expected increases in the number of revertant colonies, thereby demonstrating the sensitivity of the assay and the efficacy of the S9 mix.

Therefore, BYH 18636-N-desmethyl was non-mutagenic with or without S9 mix in the plate incorporation as well as in the pre-incubation modification of the Salmonella/microsome test.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:		BYH 18636-N-desmethyl
Description:		Fine, white powder
Lot/Batch:		KATH 4779-3-12
Purity:		98.9% (August, 2006))
CAS:		No CAS entry yet
Stability of test compound:		Stable at 0.01 mg/ml to 130 mg/ml for at least four hours at room temperature
2. Control materials:	**Negative:**	Culture medium
	Solvent:	DMSO
	Positive:	Non activated Sodium azide (Serva) for TA 1535 at 10 µg/plate, Nitrofurantoin (Sigma) for TA 100 at 0.2 µg/plate, 4-Nitro-1,2-phenylene diamine (Merck-Schuchardt) for TA 1537 at 10 µg/plate and TA 98 at 0.5 µg/plate, Mitomycin C (Fluka) for TA 102 at 0.2 µg/plate only in

plate incorporation trials,
Cumene hydroperoxide (Sigma) for TA 102 in pre-incubation trials only at 50 µg/plate,
2-Aminoanthracene (Aldrich) for the activating effect of the S9 mix in all strains at 3 µg/plate.

3. Test organisms:

Species: Salmonella typhimurium LT2 mutants

Strain: Histidine-auxotrophic strains TA 1535, TA 100, TA 1537, TA 98 and TA 102

Source: Strains obtained from Prof. Bruce Ames in 1997 and stored in the laboratory since then.

4. Test compound concentrations:

Plate incorporation assay: For all strains with or without S9 mix : 16, 50, 158, 500, 1581 and 5000 µg/plate

Pre-incubation assay: For all strains with or without S9 mix : 16, 50, 158, 500, 1581 and 5000 µg/plate.

B. STUDY DESIGN AND METHODS:

The in life phase of the study was performed between November 10 to 16, 2006 at Bayer Healthcare AG (PH-GDD Toxicology).
The *Salmonella*/microsome test is a screening method which detects point mutations caused by chemical agents *in vitro*. Auxotrophic mutants of *Salmonella typhimurium* are used to demonstrate this effect. For this purpose, the rate of reversion to prototrophy is evaluated in negative control and treated groups.

1. Plate incorporation assay:
0.1 ml of DMSO containing BYH 18636-N-desmethyl or controls were added to glass vessels with 0.1 ml of bacterial cultures grown overnight, 0.5 ml of S9 mix or buffer and 2 ml of soft agar. The mixture was placed in a waterbath at 45°C for 30 seconds, shaken and overlaid onto Petri dishes containing solid agar. After 48 hours of incubation at 37°C, the numbers of revertant colonies were scored using an automated colony counter. Three plates were used, both with and without S9 mix, for each strain and dose. The doses for the first trial were routinely determined on the basis of a standard protocol with a maximum dose of 5000 µg/plate and at least 5 additional doses. If less than three doses were used for assessment, at least two repeats were performed.

2. Pre-incubation assay:
An independent repeat was performed as pre-incubation of the previously described mixture in a water bath at 37°C for 20 minutes. At the end of the pre-incubation period, 2 ml of molten soft agar were added to the tubes, the content mixed and plated onto Petri dishes with solid agar. After 48 hours of incubation at 37°C, the numbers of revertant colonies were also scored using an automated colony counter.

3. Assessment criteria:
A reproducible and dose-related increase in mutant colonies of at least one strain was considered to be positive. For TA 1535, TA 100 and TA 98, this increase should be about twice that of negative controls, whereas for TA 1537, at least a threefold increase should be reached. For TA 102 an increase of about 100 mutants should be reached. Otherwise, the result was considered as negative.

II. RESULTS AND DISCUSSION

There was no indication of a bacteriotoxic effect of BYH 18636 N-desmethyl at doses of up to and including 500 µg per plate. The total bacteria counts consistently produced results comparable to the negative controls, or differed only insignificantly. No inhibition of growth was noted as well. Higher

doses had only a weak, strain-specific bacteriotoxic effect in the plate incorporation trial. Therefore they could nevertheless be used for assessment. No bacteriotoxic effects were observed in the pre-incubation trial.

None of the five strains concerned showed a dose related and biologically relevant increase in mutant counts over those of the negative controls in the plate incorporation test. This applied both to the tests with and without S9 mix and was confirmed by the results of the pre-incubation trials.

The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, mitomycin C, cumene hydroperoxide and 2-aminoanthracene increased mutant counts to well over those of the negative controls, and thus demonstrated the system's sensitivity and the activity of the S9 mix.

III. CONCLUSION

No indication of mutagenic effects of BYH 18636-N-desmethyl were found in any of the *Salmonella typhimurium* strains used in the assay.

(Herbold B., 2006)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: reliable with restrictions. The study (T1076880) is fully compliant with OECD 471. The efficacy of the metabolic activation system (male Sprague-Dawley rat liver S9 fraction) was additionally demonstrated using reference mutagens. Exposure to the test material at concentrations of up to and including the limit concentration of 5000 µg/plate in the absence or presence of metabolic activation did not result in an increase in the number of revertant colonies of any bacterial strain. A discrepancy in reporting is noted: the highest concentration is stated to be 500 µg/plate in the Notifier's summary. This figure originates from the summary section of the study report, however it can be confirmed that the highest concentration of the test material used in this study was 5000 µg/plate. The results of the original (plate incorporation) assay were confirmed in an independently repeated (pre-incubation) assay. Appropriate positive control compounds confirmed the sensitivity of the assay.
Conclusions	No evidence of mutagenicity was seen under the conditions of this study, However, the response of the positive control compounds with strain TA 102 shows a limited sensitivity of the strain.

In vitro genotoxicity - Test for clastogenicity in mammalian cells

Report:	**KIIA 5.8/10, Nern M., 2007 (MRID # 47070146)**
Title:	BYH 18636-N-desmethyl, *In vitro* chromosome aberration test with Chinese Hamster V79 cells
Report No & Document No	AT03678 M-284861-01-2
Guidelines:	OECD 473 (1997); EEC Directive 2000/32/EC Method B10 (2000); EPA Health Effects Test Guideline (OPPTS 870.5375; 1998)
GLP	Yes (certified laboratory)

Executive summary:
In this *in vitro* assessment of the clastogenic potential of BYH 18636-N-desmethyl (batch KATH 4779-3-12, 98.9% of purity), Chinese Hamster V79 cells were exposed to BYH 18636-N-desmethyl at 325, 650 and 1300 µg/ml, diluted in dimethyl sulphoxide (DMSO). For each dose level, duplicate cultures were used in both the presence and absence of an Aroclor 1254-induced rat liver metabolic activation system (S9 mix). DMSO was also used as a negative control. Mitomycin C, which produces crosslinks in the DNA, and cyclophosphamide, which induces chromosomal damage after metabolic activation, were used as positive controls, diluted in Hanks' balanced salt solution. After 4 hours treatment, the medium was changed and the cells were harvested 14 hours later. A second harvest was performed 30 hours after the start of the study at 1300 µg/ml. In a second experiment, the cells were exposed to BYH 18636 at 325, 650 and 1300 µg/plate or controls without S9 mix for 18 hours and harvested at the end of the treatment period. Colcemid was added to each flask two hours prior to harvest to arrest the cells in a metaphase-like stage of mitosis.

Without S9 mix no cytotoxic effects were observed. With S9 mix cytotoxic effects were observed at 1300 µg/ml. Precipitation in the medium did not occur.

None of the cultures treated with BYH 18636-N-desmethyl in the presence or in the absence of S9 mix showed biologically relevantly increased numbers of aberrant metaphases.

The positive controls mitomycin C and cyclophosphamide induced clastogenic effects and demonstrated the sensitivity of the test system and the activity of the used S9 mix.

BYH 18636-N-desmethyl was considered not to be clastogenic for mammalian cells *in vitro*.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:		BYH 18636-N-desmethyl
Description:		fine, white powder
Lot/Batch:		KATH 4779-3-12
Purity:		98.9% (August 22, 2006)
CAS:		No CAS entry yet
Stability of test compound:		Stable at 0.01 mg/ml to 130 mg/ml at room temperature for at least 4 hours.
2. Control materials:	**Negative:**	Culture medium (Eagle's minimal essential medium)
	Solvent:	DMSO for BYH 18636-N-desmethyl and Hanks's balanced salt solution for positive controls (Seromed)
	Positive:	Mitomycin C (Fluka, batch 1289272 12006009) without S9 mix at 0.1 µg/ml for a treatment period of 4 hours and 0.03

µg/ml for a treatment period of 18 hours
Cyclophosphamide (Endoxan 100 mg injection vials of dry substance, Baxter Oncology GmbH) with S9 mix at 2 µg/ml.

3. Test organisms:

Cell line: Chinese hamster V79 lung cells

Source: Cells obtained from Dr Utesch, Merk AG, Darmstadt in 1993, stored in the laboratory since then.

4. Test compound concentrations:

BYH 18636-N-desmethyl was used in each trial at 325, 650 and 1300 µg/ml (4hrs -/+S9; 18 hrs –S9).

B. STUDY DESIGN AND METHODS:

The experimental phase of the study was performed from November 14, 2006 to January 9, 2007 at Bayer Healthcare AG.
The *in vitro* cytogenetic test is a mutagenicity test system for the detection of chromosome aberrations in cultured mammalian cells. The test is designed to detect structural aberrations (chromatid and chromosome aberrations) in cells at their first post-treatment mitosis.

1. Determination of cytotoxicity:

Duplicate cultures were exposed in a pre-test to BYH 18636-N-desmethyl at concentrations ranging from 1 to 1300 µg/ml with or without S9 mix for 4 hours. In addition, cells were exposed without S9 mix for 18 hours to concentrations ranging from 1 to 1300 µg/ml. The mitotic index was determined for all cultures. The number of mitotic cells among a total of 1000 cells per culture was determined using a light microscope at a magnification of about 630. All cells which were not in interphase were defined as mitotic.
In the main study, cultures with a total incubation period of 8 hours were additionally and exclusively used to determine the cytotoxicity of BYH 18636-N-desmethyl at concentrations ranging from 325 to 1300 µg/ml.

In the pre-test with BYH 18636 N-desmethyl the pH in the medium was reduced from 7.40 in the solvent control to 7.10 (300µg/ml), 6.89 (600µg/ml) and to 6.50 (1300µg/ml). The osmolality in the medium of the pre-test was not changed by concentrations of up to 1300 µg/ml BYH 18636 N-desmethyl.

2. Treatment protocol:

Chinese hamster V79 cells were passaged on the day prior to treatment. Approximately $1x10^6$ cells were seeded in 20 ml of medium per 75 m^2 flasks and incubated at 37°C in a CO_2-incubator (5% CO_2). Unless reported otherwise, the cells were grown in Eagle's minimal essential medium containing 10% foetal calf serum. Immediately before treatment, the medium was removed from the cultures. For the trials without S9 mix, 20 ml of medium containing 2% foetal calf serum and 0.2 ml of test substance solution were added to each flask. For the trials with S9 mix, 19 ml of medium containing 2% foetal calf serum, 1 ml of S9 mix and 0.2 ml of test substance solution were added to each flask. The cells were incubated for 4 hours at 37°C. After 4 hours of treatment, the medium was removed, the cells were washed with PBS and 20 ml of fresh medium containing 10% foetal calf serum was added to the flasks. The flasks were placed in a CO_2-incubator for the remaining incubation time. In the cultures treated for 18 hours, the medium was not removed.

0.2 ml of Colcemid-solution (40 µg/ml) was added to each flask 2 hours prior to the end of the incubation period to arrest the cells in a metaphase-like stage of mitosis(c-metaphase).

Positive controls and solvent controls (0.2 ml of solvent per culture) were set up in parallel and handled as described for BYH 18636-N-desmethyl-treated cultures. Untreated controls and solvent controls were used as negative controls.

3. Chromosome preparations :

After the removal of the medium from each flask, the cells were trypsinized, suspended in medium and centrifuged for approximately 5 minutes at 700 rpm. The supernatant was removed and 1 to 2 ml of a hypotonic solution (0.4% KCl; 37°C) was added to each tube. Within 4 minutes, the volume was brought to 6 ml with additional hypotonic solution and cells were resuspended. The cells were centrifuged again and fixed with cold (4°C) fixative (ethanol/acetic acid 3:1) for 20 minutes at room temperature. Cells were pelleted and resuspended in fixative as before and centrifuged again. The pelleted cells were resuspended in a small volume of fixative and the suspension was dropped onto clean slides. The slides were allowed to dry for at least 2 hours. Thereafter, they were submerged in pure methanol for 3 minutes and stained for 15-20 minutes in 3% Giemsa solution. Slides were rinsed twice in water and once in acetone and were then kept in xylene for about 30 minutes. The slides were allowed to dry completely and covered. At least two slides were generated per culture.

4. Evaluation criteria :

Coded slides were evaluated using a light microscope at a magnification of about 1000. Chromosomes of approximately 200 metaphases per concentration, 100 metaphases from each of two parallel cultures, were examined. Only metaphases containing the modal chromosome number (22) were analyzed unless exchanges were detected. The following aberrations were recorded : gaps (an achromatic lesion within a chromatid arm without dislocation of the chromatid end), break (a discontinuity of one chromatid with dislocation of the chromatid end), fragment (part of chromosome without centromere), deletion (result of a break with the terminal chromatid part of the chromosome missing within the metaphase under assessment), exchange (exchange of chromatid-parts between different chromosomes or within the same chromosome), multiple aberration (when five or more structural changes occur within one metaphase). Observed polyploidy metaphases were recorded but not used for assessment.

5. Assessment criteria :

An assay was acceptable, if there was a biologically relevant increase in chromosome aberrations induced by positive controls and if the numbers of aberrations for the negative controls were in the expected range.

An increased incidence of gaps of both types without a concomitant increase of other aberration types was considered not to be an indication of a clastogenic effect.

A test was considered positive, if there was a relevant and statistically significant increase in the aberration rate.

A test was considered negative, if there was no such increase at any time interval or if there were statistically significant values, which were, however, within the range of historical negative controls.

A test was considered equivocal, if there was an increase above the range of historical negative controls which was statistically significant but not considered relevant, or if an increase occurred, which was considered relevant, but which was not statistically significant.

6. Statistics :

The statistical analysis was performed by pair-wise comparison of BYH 18636-carboxylic acid-treated and positive control groups to the respective solvent control group.

The mitotic index was statistically analyzed (provided that it was reduced compared to the mean of the corresponding solvent control) using the one-sided Chi^2-test.

The numbers of metaphases with aberrations (including and excluding gaps) and of metaphases with exchanges were compared (provided that these data superceded the respective solvent control). The one-side Chi^2-test was used for the statistical evaluation.

A difference was considered to be significant, if the probability of error was below 5%.

II. RESULTS AND DISCUSSION

A. Mitotic and survival indices:

Mitotic index without S9 mix:
In comparison to the solvent control, the mitotic indices in the treated cultures were not relevantly reduced. The cultures treated with mitomycin C showed only in the 4 hours treatment a reduction in mitosis rate.
Mitotic index with S9 mix:
In comparison to the solvent control, the treated cultures showed a relevant reduction of the mitosis rate at 1300 µg/ml. The positive control cyclophosphamide also reduced the mitosis rate.

Survival index without S9 mix:
In comparison to the solvent control, the survival indices in the treated cultures were not relevantly reduced. The cultures treated with mitomycin C showed a reduction in survival rate.
Survival index with S9 mix:
In comparison to the solvent control, the treated cultures showed no relevant reduction of the survival rate. The positive control cyclophosphamide reduced the survival rate.

B. Chromosome aberrations:

BYH 18636 N-desmethyl without S9 mix:
No biologically relevant and statistically significant increases of numbers of metaphases with aberrations were detected after 4 hours treatment and total culture times of 18 or 30 hours. The same was true for a treatment period and total culture time of 18 hours. The treatment with the positive control mitomycin C resulted in a clear and statistically significant increase of metaphases with aberrations and demonstrated the sensitivity of the test system.

BYH 18636 N-desmethyl with S9 mix:
No biologically relevant and statistically significant increases of numbers of metaphases with aberrations were detected after 4 hours treatment and total culture times of 18 or 30 hours. The positive control cyclophosphamide induced statistically significant and biologically relevant increases of metaphases with aberrations and demonstrated the sensitivity of the test system and the activity of the used S9 mix.

III. CONCLUSION

None of these cultures treated with BYH 18636 N-desmethyl in the absence or presence of S9 mix showed statistically significant or biologically relevant increases of numbers of metaphases with aberrations. The positive controls mitomycin C and cyclophosphamide induced clear clastogenic effects and demonstrated the sensitivity of the test system and in the case of cyclophosphamide the activity of the used S9 mix.
Based on the results of this test, BYH 18636 N-desmethyl was considered not to be clastogenic for mammalian cells *in vitro*.

(Nern M., 2007)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: Totally reliable The study (T9076880) is fully compliant with OECD 473 (1997).

The test material was not found to induce structural or numerical chromosomal aberrations under the conditions of this study in the absence or presence of metabolic activation (male Sprague-Dawley rat liver S9 fraction). A slight (and statistically non-significant) increase in the proportion of metaphases with structural chromosomal aberrations was seen at an intermediate concentration of the test material in the first assay, however this was not reproduced in confirmatory assays and is therefore not considered to be of biological significance. Appropriate positive control compounds confirmed the sensitivity of the assay.

Only slight evidence of cytotoxicity was seen at the highest concentration of the test material, however this concentration was limited by the solubility of the test material in the vehicle (DMSO).

Thiencarbazone-methyl N-desmethyl: clastogenicity study

Culture		Metaphases with aberrations excluding gaps (%)					
		DMSO	325 µg/ml	650 µg/ml	1300 µg/ml	MMC	CPA
4h/18h	-S9	3.5	4.0	7.0	4.5	78.0**	-
	+S9	5.0	3.0	5.5	4.5	-	53.0**
4h/30h	-S9	7.0	-	-	4.5	-	-
	+S9	2.0	-	-	1.5	-	-
18h/18h	-S9	6.0	4.0	3.5	5.0	44.5**	-

Conclusions No evidence of clastogenicity was seen under the conditions of this study.

In vitro genotoxicity - Test for gene mutation in mammalian cells

Report:	**KIIA 5.8/11, Herbold B., 2007 (MRID # 47070142)**
Title:	BYH 18636-N-desmethyl, V79/HPRT-test *in vitro* for the detection of induced forward mutations
Report No & Document No	AT03687 M-284868-01-2
Guidelines:	OECD 476 (1997); EEC Directive 2000/32/EC Method B17 (2000); EPA Health Effects Test Guideline (OPPTS 870.5300; 1998)
GLP	Yes (certified laboratory)

Executive summary:

The purpose of the study was to assess the point mutagenic potential of BYH 18636-N-desmethyl (batch KATH 4779-3-12, 98.9 % of purity) at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in V79 cells.

For dose selection, a preliminary cytotoxicity test was conducted with and without an Aroclor 1254-induced rat liver metabolic activation system (S9 mix) using concentrations of BYH 18636-N-desmethyl ranging from 7.8 to 1300 µg/ml. Although BYH 18636-N-desmethyl was tested up to its limit of solubility in the vehicle, no decreases in the absolute cloning efficiency were observed at 1312 mg/ml with or without S9 mix. BYH 18636-N-desmethyl was tested in the first mutation experiment and the following independent repeats at concentrations ranging from 41 to 1312 µg/ml.

Exponentially growing V79 cells were plated in 20 ml culture medium in two 75 cm^2 flasks. After attachment, cells were exposed with or without S9 mix to vehicle alone or to a range of concentrations of the test substance for 5 hours. Thereafter, each cell monolayer was washed with PBS, trypsinized and replated into 3 Petri dishes. Per culture one flask and 3 Petri dishes were used. These dishes were incubated for 6 to 8 days to allow colony development and to determine the cytotoxicity associated with each test substance directly after treatment (survival to treatment).

Cells ($3x10^5$ cells/ml) in 75 cm^2 flasks were incubated to permit growth and expression of induced mutations. Cells were subcultured (= count 1, normally after 3 days) by reseeding cells in 75 cm^2 flasks. At the end of the expression period (=count 2, normally a total of 6 days), cultures were

reseeded in Petri dishes without hypoxanthine but containing 6-TG for selection of mutants. In addition, 200 cells per dish were seeded in 5 ml culture medium to determine the absolute cloning efficiency for each concentration. After incubation for 6 to 8 days, the colonies were fixed, stained with Giemsa and counted to determine the number of 6-TG resistant colonies in the mutation assay dishes and the number of colonies in the cloning efficiency dishes.

Without and with S9 mix there was no biologically relevant increase in mutant frequency above that of the vehicle controls. Ethyl methanesulfonate and Dimethylbenzanthracene induced clear mutagenic effects and demonstrated the sensitivity of the test system and the activity of the S9 mix.

Based on these results, BYH 18636 N-desmethyl was considered to be non-mutagenic in the V79/HPRT Forward Mutation Assay, both with and without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636-N-desmethyl
Description: white powder
Lot/Batch: KATH 4779-3-12
Purity: 98.9% (August 21, 2006)
CAS: No CAS entry yet
Stability of test compound: Stable at 0.01 mg/ml to 130 mg/ml for at least four hours at room temperature

2. Control materials:
Negative: Culture medium [Eagle's minimal essential medium supplemented with 1% L-glutamine, 1% MEM-vitamins, 1% MEM NEAA, 1% penicillin/streptomycin and 10% foetal calf serum (FCS)]
Solvent: DMSO for BYH 18636-N-desmethyl and Dimethylbenzanthracene not exceeding 1% (v/v) in the culture medium. No solvent needed for ethyl methanesulfonate as it is a liquid.
Positive: Ethyl methanesulfonate (EMS), a directly alkylating agent, used at a final concentration of 900 µg/ml in non-activation trials.
Dimethylbenzanthracene (DMBA), promutagen requiring a metabolic activation, used at a final concentration of 20 µg/ml for trials with S9 mix.

3. Test organisms:
Cell line: Chinese hamster V79 lung cells
Source: Cells obtained from Prof. G. Speit, University of Ulm, Germany. These cells have since been recloned to maintain karyotypic stability. They have a modal chromosome number of 22 and a rapid population doubling time (10 to 14 hours)
Culture condition: Incubation performed at 37°C in a humidified atmosphere with about 5% CO_2.

4. Test compound concentrations:
BYH 18636-N-desmethyl was used at 7.8, 15.6, 31.3, 62.5, 125, 250, 500, 1000 and 1300 µg/ml -/+ in the clonal cytotoxicity assay and at 41, 82, 164, 328, 656 and 1312 µg/ml in the mutagenic assays (3 Trials –S9; 2 Trials +S9).

5. Metabolic activation:

The S9 fraction was isolated from the livers of Aroclor 1254 induced male Sprague Dawley rats. The preparation dated from September 13, 2005 (protein content from 24.0 mg/ml) and was kept frozen at -80°C. The batch was tested for contamination and cytotoxicity prior to use in the first study. Cofactors were freshly dissolved in sodium phosphate buffer (150 mM, pH 7.4)

B. STUDY DESIGN AND METHODS:

The experimental phase of the study was performed from October 13 to December 7, 2006 at Bayer Healthcare AG.

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

1. Determination of cytotoxicity:

Exponentially growing V79 cells were plated in 20 ml culture medium in a 75 cm^2 flask ($4x10^6$ cells per flasks). For each concentration, one culture was available. After attachment (16 to 24 hours later), cells were exposed without S9 mix to vehicle alone or to a range of concentrations of the test substance for 5 hours in 20 ml medium containing 2% FCS. In experiments with metabolic activation 1 ml of medium was replaced by 1 ml of S9 mix. Thereafter, each cell monolayer was washed with PBS, trypsinized and replated in 5 ml culture medium at a density of 200 cells into 3 Petri dishes (diameter of 60 mm). These dishes were incubated for 6 to 8 days to allow colony development. Thereafter, colonies were fixed with 95% methanol, stained with Giemsa (Merk; stock solution diluted 1:5 with deionized water) and counted automatically using an Artek counter, when there was no interference by precipitation on the plates or colouration of the plates. Cytotoxicity was expressed by comparison of colonies in treated cultures versus vehicle control cultures (relative cloning efficiency).

2. Treatment protocol without metabolic activation:

Exponentially growing V79 cells were plated in 20 ml culture medium in two 75 cm^2 flasks per concentration ($4x10^6$ cells per flask) including all control groups. After attachment (16 to 24 hours later), cells were exposed to vehicle alone or to a range of concentrations of the test substance for 5 hours in 20 ml culture medium with reduced serum content (2%). Thereafter, cell monolayers were washed with PBS, trypsinized and replated in 20 ml culture medium using $1.5x10^6$ cells per 75 cm^2 flask and in 5 ml culture medium using 200 cells per Petri dish (diameter of 60 mm). One flask and 3 Petri dishes were used per culture. These dishes were incubated for 6 to 8 days to allow colony development and to determine the cytotoxicity associated with each test substance directly after treatment (survival to treatment).

Cells in 75 cm^2 flasks were incubated to permit growth and expression of induced mutations. Cells were subcultured (= count 1, normally after 3 days) by reseeding $1.5x10^6$ cells into 20 ml of medium in 250 ml flasks. At the end of the expression period (=count 2, normally a total of 6 days), cultures were reseeded in Petri dishes (diameter of 100 mm) at $3x10^5$ cells per dish (8 dishes per culture) in 20 ml culture medium without hypoxanthine but containing 10 µg/ml 6-TG for selection of mutants. In addition, 200 cells per dish (diameter of 60 mm, 3 dishes per culture) were seeded in 5 ml culture medium to determine the absolute cloning efficiency for each concentration. After incubation for 6 to 8 days, the colonies were fixed, stained with Giemsa and

counted to determine the number of 6-TG resistant colonies in the mutation assay dishes and the number of colonies in the cloning efficiency dishes.

Three trials were performed.

3. Treatment protocol with metabolic activation:

The activation assay was performed independantly. The procedure was identical to the non-activation assay except for the addition of S9 mix. In these experiments 19 ml instead of 20 ml culture medium and additonally 1 ml of S9 mix were added to the flasks for the treatment period, resulting in a concentration of 5% S9 mix in the cultures. The number of 6-TG resistant mutants and viability were determined as in the non-activation assay.

Two trials were performed.

4. Parameters assessed:

The parameter "survival to treatment" in % was determined on the basis of the following calculation:

$$\frac{\text{Mean number of colonies (treated cultures)} \times 100}{\text{Mean number of colonies (vehicle control cultures)}}$$

The "absolute population growth" was calculated using the following formula:

Absolute population growth (for each culture) = cell count 1 x cell count 2

The parameter "relative population growth" shows the cumulative growth of the treated cell populations, relative to the vehicle control.

$$\frac{\text{Absolute population growth treated culture} \times 100}{\text{Absolute population growth of corresponding vehicle control culture}}$$

The ability of cells to form colonies at the time of mutant selection is measured by the parameter "absolute cloning efficiency". It is expressed in %.

$$\frac{\text{Mean number of colonies per dish} \times 100}{200}$$

The "mutant frequency" is calculated for each group by dividing the total number of mutant colonies by the number of cells seeded (usually 8-10 plates at $3x10^5$ cells per plate), corrected for the absolute cloning efficiency. The mutant frequency is expressed as 6-TG resistant mutants par 10^6 clonable cells.

$$\frac{\text{Total number of mutant colonies} \times 100}{\text{Number of evaluated dishes} \times 3 \times 10^5 \times \text{C.E.}}$$

5. Acceptance criteria:

- The average cloning efficiency of the negative and vehicle controls should be at least 50%.
- The average of mutant frequency of the vehicle controls should not exceed 25×10^{-6} cells.
- The mutant frequency of the two cultures of the vehicle and /or the negative control should differ only to an acceptable extent. As a rule of thumb, the difference of mutant frequencies

should not be greater than 5 x 10^{-6}.

- The positive control should induce an average mutant frequency of at least three times that of the vehicle control.
- If not limited by the solubility of the test substance in the vehicle the highest concentration should induce cytotoxicity of about 80 to 90% or should be a concentration where precipitation occurs in the medium. The survival at the lowest concentration should be in the range of the negative control.
- For the calculation of an acceptable mutant frequency at least 5 dishes per culture should be available and relative survival to treatment, relative population growth and absolute cloning efficiency should be 10% or greater.

However, these criteria may be overruled by good scientific judgement.

6. Assessment criteria:

- Mutant frequency was only used for assessment, if at least 5 dishes per culture were available and relative survival to treatment, relative population growth and absolute cloning efficiency were 10% or greater.
- A trial was considered positive if a concentration-related and in parallel cultures reproducible increase in mutant frequencies was observed. To be relevant, the increase in mutant frequencies should be at least two to three times that of the highest negative or vehicle control value observed in the respective trial. If this result was repoduced in a second trial, the test substance was considered to be mutagenic.
- Despite these criteria, a positive result was only considered relevant, if no significant change in osmolality compared to the vehicle control was observed. Otherwise, unphysiological culture conditions may be the reason for the positive result.
- A test substance was judged as equivocal if there was no strictly concentration related increase in mutation frequencies but if one or more concentrations induced a reproducible and biologically relevant increase in mutant frequencies in all trials.
- An assay was considered negative if no reproducible and relevant increases of mutant frequencies were observed.

However, these criteria may be overruled by good scientific judgement.

7. Statistical analysis:

The statistical analysis relied on the mutant frequencies which were submitted to a weighted analysis of variance as well as to a weighted recursive regression, both with Poisson derived weights.

The two mutant frequency values obtained per group were, although somewhat related, considered as independent measurements thus increasing the power of the statistical tests applied. Since the protocol of the HPRT assay required at least two independent trials, the overall analysis without respectively with activation was the most important one for classifying substances into mutagens and non-mutagens. However, separate analyses were run for each trial in order to examine the consistency of the results.

All acceptable groups were included in the weighted analysis of variance followed by pairwise comparisons to the vehicle control on a nominal significance level of $\alpha = 0.05$ using the Dunnett test. The regression analysis part was performed on the basis of the actual concentrations thereby omitting the positive, negative and vehicle controls. If there was a significant concentration related increase of the mutant frequency ($\alpha = 0.05$) in the main analysis the highest concentration was dropped and the analysis repeated. This procedure was repeated until $p > 0.05$. In that way eliminated concentrations were flagged correspondingly.

II. RESULTS AND DISCUSSION

1. General remarks:

In the absence and in the presence of S9 mix Chinese hamster V79 cells were exposed to BYH 18636-N-desmethyl at concentrations of up to and including 1312 µg/ml, limited by the solubility of BYH 18636-N-desmethyl in DMSO. Without and with S9 mix no substance precipitation occurred in the medium However, the pH indicator in the medium changed its colour at 656 µg/ml and above. Nevertheless, all tested concentration could be used for assessment.

The means of the absolute cloning efficiency for the vehicle controls in the mutation experiments were 69.9%, 76.2% and 83.3% in the experiments without activation. In experiments with metabolic activation 74.6% and 76.1% were observed. These results demonstrate good cloning conditions for the experiments.

2. Mutation assay without metabolic activation:

Three trials were performed. The mutant frequencies of the negative controls (means of 1.5 x 10^{-6}, 2.5 x 10^{-6} and 3.7 x 10^{-6}, respectively) and of the vehicle controls (means of 2.9 x 10^{-6}, 0.9 x 10^{-6} and 2.1 x 10^{-6}, respectively) were all within the normal range. The positive control EMS induced clear mutagenic and statistically significant effects in all trials (means of 451.2 x 10^{-6}, 277.6 x 10^{-6} and 523.4 x 10^{-6} respectively).
For BYH 18636-N-desmethyl treated cultures no cytotoxic effects of 80% to 90% were induced. However, BYH 18636-N-desmethyl was tested up to its limits of solubility in the vehicle.

Relevant BYH 18636-N-desmethyl induced increases in mutant frequencies could not be found (ranging from 0.6 x 10^{-6} to 5.2 x 10^{-6} in the first trial, from 0.0 x 10^{-6} to 4.1 x 10^{-6} in the second trial and from 1.2 x 10^{-6} to 7.2 x 10^{-6} in the third trial). In addition, the overall statistical analysis revealed no statistically significant increases. Therefore, BYH 18636-N-desmethyl was evaluated as non-mutagenic in the non-activation trial.

3. Mutation assay with metabolic activation:

Two trials were performed with S9 mix. The mutant frequencies of the negative controls (means of 1.2 x 10^{-6} and 0.5 x 10^{-6}, respectively) and of the vehicle controls (means of 5.7 x 10^{-6} and 1.3 x 10^{-6}, respectively) were all within the normal range. The positive control DMBA induced clear mutagenic and statistically significant effects in all trials (means of 49.4 x 10^{-6} and 45.4 x 10^{-6}, respectively).

For BYH 18636-N-desmethyl treated cultures no cytotoxic effects of 80% to 90% were induced. However, BYH 18636-N-desmethyl was tested up to its limits of solubility in the vehicle. Relevant BYH 18636-N-desmethyl induced increases in mutant frequencies could not be found (ranging from 0.0 x 10^{-6} to 4.0 x 10^{-6} in the first trial and from 0.0 x 10^{-6} to 3.0 x 10^{-6} in the second trial). In addition, the overall statistical analysis revealed no statistically significant increases. Therefore, BYH 18636-N-desmethyl was evaluated as non-mutagenic in the activation trial.

III. CONCLUSION

BYH 18636 N-desmethyl induced no biologically relevant increases in mutant frequencies. The positive controls EMS and DMBA had a marked mutagenic effect, as was seen by a biologically relevant increase in mutant frequencies as compared to the corresponding negative controls and demonstrated the sensitivity of the test system and the activity of the used S9 mix.

Despite this sensitivity, neither without nor with S9 mix indications of mutagenic effects of BYH 18636 N-desmethyl were found, in the V79/HPRT forward mutation assay.

(Herbold B., 2007)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: totally reliable The study (T0076881) is compliant with OECD guideline 476 (1997) and is considered to be acceptable; see the detailed comments about the general acceptability of this type of study at IIA 5.4.3 (Herbold, 2005). Exposure to the test material in the absence and presence of a metabolic activation system (male Sprague-Dawley rat liver S9 fraction) did not induce any statistically or biologically significant increase in the mutation frequency. Appropriate positive control compounds confirmed the sensitivity of the assay No evidence of cytotoxicity was seen at the highest concentration of the test material, however this concentration was limited by the solubility of thiencarbazone-methyl in the vehicle (DMSO).
Conclusions	No evidence of mutagenicity was seen under the conditions of this study.

Acute oral toxicity

Report:	**KIIA 5.8/12, Schüngel M.; 2007 (MRID # 47070120)**
Title:	BYH 18636-N-desmethyl, Acute toxicity in the rat after oral administration
Report No &	AT03550
Document No	M-283442-01-2
Guidelines:	OECD 423 (2001); EEC Directive 67/548 Annex V – Method B.1.tris (2004); EPA Health Effects test Guidelines (OPPTS 870.1100) (1998)
GLP	Yes (certified laboratory)

Executive Summary

In an acute oral toxicity study using a stepwise procedure, two groups of three fasted, young adult female Wistar rats (HsdCpb: Wu) were given successively a single oral dose of BYH 18636-N-desmethyl (batch KATH 4779-3-12, 98.9% purity) in 2% Cremophor EL of 2 000 mg/kg bw and were observed for 14 days.
The dose of 2 000 mg/kg bw was tolerated by both groups without mortalities, clinical signs, effects on weight gain or gross pathological findings.

According to OECD guideline 423, the LD_{50} cut-off of BYH 18636-N-desmethyl was ≥ 5 000 mg /kg bw (category 5 / unclassified according to the global harmonized classification system).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	BYH 18636-N-desmethyl
Description:	Off-white solid
Lot/Batch:	KATH 4776-3-12
Purity:	98.9% (August 21, 2006)
CAS:	No CAS entry yet
Stability of test compound:	Stable at 5 and 200 mg/ml at room temperature for at least 2 hours

2. Vehicle and /or positive control: 2% Cremophor EL in demineralized water

3. Test animals:

Species:	Rat
Strain:	HsdCpb: Wu
Age:	10 to 12 weeks approximately
Weight at dosing:	167 to 182 g
Source:	Harlan/Winkelmann GmbH, 33178 Borchen, Germany
Acclimation period:	At least 5 days
Diet:	Provimi Kliba 3883.0.15 Maus/Ratte Haltung, Kaiseraugst Switzerland, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were group caged conventionally in polycarbonate cages on low dust wood granulate bedding

Environmental conditions –

Temperature:	22 ± 2°C
Humidity:	55 ± 5%
Air changes:	Approximately 10 changes per hour
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS:

1. In life dates: September 27 to October 19, 2006 performed at Bayer HealthCare AG, Germany

2. Animal assignment and treatment

The substance was tested using a stepwise procedure, each step using three female rats. The animals were assigned to their groups by randomization based on evenly distributed chance numbers. Following an overnight fast (16 to 24 hours), each group received a single dose of 2 000 mg/kg of BYH 18636-N-desmethyl (98.9% purity) by gavage. The test substance was administered in demineralized water with 2% Cremophor EL at a volume of 10 ml/kg bw. Clinical signs and mortality rates were determined several times on the day of administration and subsequently at least once daily for an observation period of at least 14 days. Body weights were recorded on days 1, 8 and 15. On day 15, surviving animals were sacrificed and all animals were necropsied and examined for gross pathological changes.

Table IIA 5.8-9 Doses, mortality / animals treated

Dose (mg/kg bw)	Females
2 000 (1st)	0/3
2 000 (2nd)	0/3

3. Statistics

The data did not warrant statistical analysis.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortalities occurred at 2 000 mg/kg bw, the only dose tested.

The oral LD_{50} cut-off was ≥ 5 000 mg /kg bw according to OECD guideline 423.

B. CLINICAL OBSERVATIONS

No clinical signs were observed.

C. BODY WEIGHT

There was no toxicological effect on body weight or body weight gain.

D. NECROPSY

No abnormalities were observed at gross necropsy.

III. CONCLUSION

The oral LD_{50} cut off of BYH 18636-N-desmethyl was ≥ 5 000 mg/kg bw. BYH 18636-N-desmethyl does not warrant classification as being toxic or harmful on the basis of its acute oral toxicity.

(Schüngel M. 2007)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: Totally reliable The study (T1077241) is fully compliant with OECD 423 (2001) No treatment-related findings were observed following gavage with a single (limit) dose of 2000 mg/kg bw.
Conclusions	The acute oral LD_{50} of thiencarbazone-methyl desmethyl in female rats was found to be >2000 mg/kg bw under the conditions of this study. The test material is therefore not classified for acute oral toxicity according to current EC criteria. EPA: For Toxicity Category/Classification EPA Toxicity Category III – LD_{50} > 2000 mg/kg - would be assigned.

Oral 28-day toxicity in the rat

Report:	**KIIA 5.8/13, Rascle J-B., 2007 (MRID # 47070125)**
Title:	BYH 18636-N-desmethyl (AE 1417257), 28-day toxicity study in the rat by dietary administration
Report No & Document No	SA 06247; M-284133-01-2
Guidelines:	OECD 407 (1995); EEC Directive 96/54/EC Annex V – Method B.7. (1996); EPA Health Effects test Guidelines (OPPTS 870.3050) (2000)
GLP	Yes (certified laboratory)

Executive summary:
BYH 18636-N-Desmethyl (product code AE 1417257), a plant metabolite of the herbicide BYH 18636, (batch number KATH 4779-3-12: an off white solid, 98.9% purity) was administered continuously via the diet to groups of Wistar rats (10/sex/group) for at least 28 days at concentrations of 60, 120, 1200 and 12000 ppm. A similarly constituted group received untreated diet and acted as a control group. Animals were observed daily for mortality and clinical signs. Physical examinations were performed weekly. Body weight and food consumption were recorded once weekly. Ophthalmological examinations were performed once on all animals during the acclimatization phase and on control and high dose group animals at the end of the study. Haematology, clinical chemistry and urine parameters were determined at the end of the study. All animals were necropsied, selected organs weighed and a range of tissues were taken, fixed and examined microscopically.

There were no mortalities or treatment-related findings recorded during the course of the study in either sex at any dose level.

In conclusion, the No Observed Effect Level (N.O.E.L.) in this study was 12000 ppm, the highest dose level tested, in both sexes, corresponding to 1045 mg/kg/day in males and 1133 mg/kg/day in females.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BYH 18636-N-desmethyl
- **Description:** off white solid
- **Lot/Batch:** KATH 4779-3-12
- **Purity:** 98.9% (August, 2006)
- **CAS:** No CAS entry yet
- **Stability of test compound:** Stable in rodent diet at 60 and 12 000 ppm over a 26-day period at ambient temperature or over a period of 16-day frozen storage followed by 10 days at room temperature

2. Vehicle and /or positive control: None

3. Test animals:
- **Species**: Rat
- **Strain:** Wistar Rj: WI (IOPS HAN)
- **Age:** 6 weeks approximately
- **Weight at dosing:** 167 to 207 g for the males – 150 to 185 g for the females
- **Source:** R. Janvier, Le Genest St Isle, France
- **Acclimation period:** 6 days
- **Diet:** Certified rodent powdered and irradiated diet A04CP1-10 from S.A.F.E. (Scientific Animal Food and Engineering,

Augy, France), *ad libitum*

Water: Tap water, *ad libitum*

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

Environmental conditions –

Temperature: 22 ± 2°C

Humidity: 55 ± 15%

Air changes: Approximately 10 to 15 changes per hour

Photoperiod: Alternating 12-hour light and dark cycles (7 am- 7 pm)

B. STUDY DESIGN:

1. In life dates: September 11 to October 20, 2006 performed at Bayer CropScience, Sophia Antipolis, France

2. Animal assignment and treatment

There were 10 animals of each sex per dose group. Animals were assigned to dose groups using a randomization procedure based on body weight. BYH 18636-N-desmethyl was administered in the diet for at least 28 days to Wistar rats at the following doses – 0, 60, 120, 1 200 and 12 000 ppm (equating approximately to 5.2, 10.3, 106, and 1045 mg/kg/day in males and 5.5, 11.1, 116, 1133 mg/kg/day in females). A negative control group received plain diet. Animal housing and husbandry were in accordance with the regulations of the Guide for the Care and Use of Laboratory Animals (Public Health Service, National Institute of Health, NIH publication N°86-23, revised 1985) and "Le Guide du Journal Officiel des Communautés Européennes L358, 18 Décembre 1986, N°86/609/CEE du 24 Novembre 1986".

3. Diet preparation and analysis

BYH 18636-N-desmethyl was ground to a fine powder before being incorporated into the diet by dry mixing to provide the required concentrations. There were two preparations of each concentration for the whole study. The stability was demonstrated during the course of the study. Diet samples of 60, 120 and 12 000 ppm were found to be stable over a 26-day period at ambient temperature or over a 16-day freezing period followed by 10 days at ambient temperature. The homogeneity of the diet was verified at 60 and 12 000 ppm and was within a range of 88 to 101 % of the nominal concentrations. Concentration was checked for each concentration and was within a range of 88 to 101 % of the nominal concentrations.

Table IIA 5.8-10 Study design

Test group	Concentration in diet (ppm)	Dose per animal (study averages)		Animals assigned	
		Male (mg/kg bw/day)	Female (mg/kg bw/day)	Male	Female
1	0	0	0	10	10
2	60	5.24	5.5	10	10
3	120	10.3	11.1	10	10
4	1 200	106	116	10	10
54	12 000	1045	1133	10	10

Table IIA 5.8-11: Homogeneity and concentration of test material

	Nominal concentrations	Analysed	% of nominal

	(ppm)	concentrations (ppm)	concentration
Homogeneity	60	H1 53 H3 53.3 M1 53.7 M3 52.9 B1 53.8 B3 53.0	88 89 90 88 90 88
	12 000	H1 12072 H3 11893 M1 12128 M3 11784 B1 11662 B3 11978	101 99 101 98 97 100
Concentration (First formulation)	60	53.3	89
	120	105	88
	1200	1159	97
	12000	11920	99
Concentration (Second formulation)	60	60.3	101
	120	114	95
	1200	1168	97
	12000	11517	96
Stability after 16 days frozen storage and 10 days at room temperature	60	56	93
	120	107	89
	12000	11942	100
Stability after 26 days at room temperature	60	56.9	95
	120	108	90
	12000	11973	100

H = surface; M = middle; B = bottom of the preparation. Three samples 1,2,3 were taken from each level of the preparation (H, M, B); only two were analyzed.

4. Statistics

Data were analyzed by the Bartlett's test for homogeneity of variances. When the data were homogeneous, an ANOVA was performed followed by Dunnett's test on parameters showing a significant effect by ANOVA. When the data were not homogeneous even after transformation, a Kruskal-Wallis ANOVA was performed followed by the Dunn's test if the Kruskal-Wallis was significant. When one or more group variance(s) equaled 0, means were compared using non-parametric procedures. Group means were compared at the 5% and 1% levels of significance. Statistical analyses were carried out using Path/Tox System V4.2.2. (Module Enhanced Statistics).

C. METHODS:

1. Observations

The animals were observed twice daily for moribundity and mortality (once daily on weekends or public holidays). Observed clinical signs were recorded at least once daily for all animals and detailed physical examinations were performed at least weekly during the treatment period.

2. Body weight

Body weights were recorded once during the acclimatization phase, on the first day of test substance administration, then at weekly intervals throughout the treatment. Additionally, diet fasted animals were weighed at final sacrifice.

3. Food consumption and compound intake

Food consumption was recorded once per week during the treatment period.
The weekly mean achieved dosage intake in mg/kg/day for each week and for Weeks 1 to 4 was calculated for each sex using the formula:

$$\text{Test substance intake (mg/kg/day)} = \frac{\text{Dose level (ppm)} \times \text{Group mean food consumption (g/day)}}{\text{Group mean body weight (g) at the end of the food consumption period}}$$

4. Ophthalmic examination

During the acclimatization period all animals were subjected to an ophthalmological examination. After instillation of an atropinic agent (Mydriaticum, Merk Sharp and Dohme) each eye was examined by means of an indirect ophthalmoscope. During Week 4, all surviving animals from control and high dose groups were re-examined.

5. Clinical chemistry

On study days 30, 31 or 32, blood samples were taken from all animals in all groups by puncture of the retro-orbital venous plexus. Animals were diet fasted overnight prior to bleeding and anaesthetized by inhalation of Isoflurane. Blood was collected on EDTA for haematology, on lithium heparin for plasma and clot activator for serum for clinical chemistry and on sodium citrate for coagulation parameters.
The following haematology parameters were assayed using an Advia 120 (Bayer Diagnostics, Puteaux, France): red blood cell count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, reticulocyte count, white blood cell count and differential count evaluation and platelet count. A blood smear was prepared and stained with May-Grünwald-Giemsa. It was examined when results of Advia 120 determinations were abnormal. Prothrombin time was assayed on an ACL 3000 (Instrumentation Laboratory, Paris, France).
Any significant change in the general appearance of the plasma and the serum was recorded. Total bilirubin, glucose, urea, creatinine, total cholesterol, triglycerides, chloride, sodium, potassium, calcium and inorganic phosphorus concentrations and aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma-glutamyltransferase activities were assayed on plasma samples, total protein and albumin concentrations were assayed on serum samples using an Advia 1650 (Bayer Diagnostics, Puteaux, France). Globulin and albumin/globulin ratio values were calculated.

6. Urinalysis

Overnight urine samples were collected on study Days 23, 24 or 25 from all animals in all groups. Food and water were not accessible during urine collection.
Any significant change in the general appearance of the urine was recorded. The urine volume was measured. pH was assayed using a Clinitek 200+ and Multistix dipsticks (Bayer Diagnostics, Puteaux, France). Urinary refractive index was measured using a RFM320 refractometer (Bioblock Scientific, Illkirch, France).
The following semi-quantitative parameters were assayed using a Clinitek 200+ and Multistix dipsticks: glucose, bilirubin, ketone bodies, occult blood, protein and urobilinogen. Microscopic examination of the urinary sediment was performed after centrifugation of the urine. The presence of red blood cells, white blood cells, epithelial cells, bacteria, casts and crystals was graded.

7. Sacrifice and pathology

On study days 30, 31 or 32, a complete necropsy was performed on all animals. Animals were deeply anaesthetized by inhalation of Isoflurane, then exsanguinated before necropsy. All animals

were fasted prior to scheduled sacrifice. All animals were necropsied. The necropsy included the examination of all major organs, tissues and body cavities. Macroscopic abnormalities were recorded, sampled and examined microscopically.
Adrenal gland, brain, epididymides, heart, kidney, liver, ovary, pituitary gland, prostate gland, spleen, testis, thymus, thyroid gland (with parathyroid gland) and uterus (including cervix) were weighed fresh at scheduled sacrifice only. Paired organs were weighed together.
The following organs or tissues were sampled : adrenal gland, aorta, articular surface (femoro-tibial), bone (sternum), bone marrow (sternum), brain, epididymis, oesophagus, exorbital (lachrymal) gland, eye and optic nerve, harderian gland, heart, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidney, larynx/pharynx, liver, lung, lymph nodes (submaxillary, mesenteric), mammary gland, nasal cavities, ovary, pancreas, pituitary gland, prostate gland, sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, submaxillary (salivary) gland, testis, thymus, thyroid gland (with parathyroid), tongue, trachea, urinary bladder, uterus (with cervix), vagina.
A bone marrow smear was prepared from femur, stained with May-Grünwald Giemsa, but not examined. Tissues samples were fixed by immersion in neutral buffered 10% formalin with the exception of the eye, optic nerve, harderian gland, epididymis and testis that were fixed in Davidson's fixative. Histopathological examinations were performed on all tissues from all the animals in the control and high dose groups. The liver, kidney, lung thyroid gland, parathyroid gland and macroscopic findings were examined in all animals in the study.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS:

1. Mortality

There were no mortalities during the course of the study.

2. Clinical signs

There were no treatment-related clinical signs during the course of the study.

B. BODY WEIGHT AND BODY WEIGHT GAIN:

Body weight parameters were unaffected by treatment throughout the study in either sex at any dose level tested.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

Food consumption parameters were unaffected by treatment in either sex at any dose level during the course of the study

D. ACHIEVED DOSAGES:

The mean achieved dosage intake of BYH 18636-N-desmethyl per group was as follows:

Table IIA 5.8-12: Mean achieved dietary intake of BYH 18636-N-desmethyl (Weeks 1-4)

Diet concentration (ppm)	Males mg/kg/day	Females mg/kg/day
60	5.2	5.5

120	10.3	11.1
1200	106	116
12000	1045	1133

E. OPHTHALMOSCOPIC EXAMINATION:

No treatment-related ocular abnormalities were observed at ophthalmoscopic examination.

F. BLOOD ANALYSIS:

1. Haematological findings

No treatment-related change was noted at any dose level for the parameters assayed.

2. Clinical chemistry findings

No treatment-related change was noted at any dose level for the parameters assayed.

G. URINALYSIS:

No treatment-related change was noted at any dose level for the parameters assayed.

H. SACRIFICE AND PATHOLOGY:

1. Organ weight

There was no change in mean terminal body weights and in mean organ weights in treated animals when compared to controls.

2. Gross Pathology

All findings were considered to be incidental and not treatment-related.

3. Microscopic Pathology

All findings were considered to be incidental and not treatment-related.

III. CONCLUSION

The No Observed Effect Level (NOEL) in this study was 12000 ppm, equating to 1045 and 1133 mg/kg/day in males and females, respectively.

(Rascle J-B., 2007)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: Totally reliable The study is fully compliant with OECD 407 (1995) No treatment-related findings were observed in this study. A single isolated observation of chromodacryorrhoea in one male at the top dose level of 12000 ppm is not considered to be related to treatment.

	Simple diffuse hyperplasia of the urinary bladder urothelium associated with mononuclear cell infiltration (both graded as minimal) was observed in one female at 12000 ppm. A number of renal findings (basophilic tubules, cortical mineralisation, hyperplasia of the pelvic epithelium and mononuclear cell infiltration) were also observed in this animal. Similar urinary bladder findings were not observed in any other dose group, however similar renal findings were observed in a number of animals in all groups with no apparent relationship to treatment. Findings in the bladder are similar to those seen in studies with thiencarbazone-methyl, however in the case of thiencarbazone-methyl N-desmethyl it is notable that there is apparently no association with urolithiasis. Findings in the bladder at the top dose level are therefore not considered to be clearly treatment-related, and in any case would only represent a minimal effect of debatable toxicological significance. In 12000 ppm females, mean absolute and relative uterus weights were slightly (but not statistically significantly) higher compared to controls. Findings correlate macroscopically with an increased incidence of dilated uterine horns (2,0,0,3,5) and histopathologically with observations of and increased incidence of pro-oestrus (2,0,0,2,5). Analysis of individual uterus weights confirms the association of higher uterus weight with macroscopic and histopathological findings.
Conclusions	A NOAEL of 12000 ppm (equivalent to mean achieved dietary intakes of 1045 and 1133 mg/kg bw/d in males and females respectively) in the absence of any treatment-related findings at the top dose level. The Notifier has proposed the same NOAEL.

B.6.9 Medical data and information (IIA 5.9)

B.6.9.1 Report on medical surveillance on manufacturing plant personnel (IIA 5.9.1)

Medical surveillance, not directly related to exposures, did not reveal any unwanted effects in the workers, nor have there been any medical complaints regarding the product.

B.6.9.2 Report on clinical cases and poisoning incidents (IIA 5.9.2)

No poisoning incidents or clinical cases have been seen.

B.6.9.3 Observations on general population exposure & epidemiological studies (IIA 5.9.3)

There has been no exposure of the general public so far.

B.6.9.4 Clinical signs and symptoms of poisoning and details of clinical tests (IIA 5.9.4)

There are no experiences with human poisoning, animal experiments did not show any symptoms except slight eye irritation.

B.6.9.5 First aid measures (IIA 5.9.5)

Remove patient from exposure/terminate exposure.
Thorough skin decontamination with copious amounts of water and soap.
Flushing of the eyes with lukewarm water for 15 minutes.
Induction of vomiting is not indicated.

B.6.9.6 Therapeutic regimes

Gastric lavage is not required in regard of the very low toxicity of the compound. However, the application of activated charcoal and sodium sulphate is always advisable in significant ingestions. As there is no antidote, treatment has to be symptomatic and supportive.

B.6.9.7 Expected effects & duration of poisoning as a function of exposure (IIA 5.9.6)

There are no experiences with human poisoning, animal experiments did not show any symptoms except slight eye irritation.

B.6.9.8 Expected poisoning effects in relation to time of treatment initiation

There are no experiences with human poisoning, animal experiments did not show any symptoms except slight eye irritation.

B.6.9.9 Dermal penetration (IIIA 7.6)

A dermal penetration study with the plant protection product has been conducted and is reported in Annex IIIAI point 7.6.

B.6.10 Other/special studies (IIA 5.10)

Report:	**KIIA 5.10/1, Iyengar S., Schumacher G., Kaune A., Lasserre-Bigot D., Ecker U., 2007**
Title:	The Non Relevance of the BYH 18636-carboxylic acid (Thiencarbazone-methyl-carboxylic acid)
Report No & Document No	M-285847-01-2
Guidelines:	EU DG SANCO/221/2000-rev. 10, 25th February 2003
GLP	Not applicable

Executive summary:
The environmental fate of BYH 18636 and its metabolites have been fully elucidated. The potential mobility of the metabolites formed in soil has been assessed using the recognised models with input values derived from experimental data under laboratory and field conditions.
The potential relevance of the environmental metabolite for regulatory purposes in the EU has been investigated. The only metabolite for which non relevance testing was triggered was the BYH 18636-carboxylic acid as the PEC groundwater concentrations were above 0.1 µg/L. As PEC groundwater PEARL and FOCUS-PELMO for this metabolite exceeded 0.75 µg/L for some scenarios, a dietary risk assessment was conducted.

Summary of non relevance of the metabolite BYH 18636-carboxylic acid

• BYH 18636-carboxylic acid has no biological efficacy.

• BYH 18636-carboxylic acid is not genotoxic.

• BYH 18636-carboxylic acid has a very low acute toxicity to the rats (no mortalities at 2000 mg/kg/d).

• Repeated dietary exposure of BYH 18636-carboxylic acid to rat for 90-days confirmed the low toxicity potential of this compound (NOEL 1000 mg/kg/day).

• Conducting a risk assessment for the potential presence of BYH 18636-carboxylic acid in groundwater showed an extremely low percent of the ADI consumed for adults (0.0009%) and for infants (0.002 %).

• BYH 18636-carboxylic acid present in soil were not eco-toxicologically relevant either in the terrestrial compartment nor in the aquatic environment after run-off or drainage. From the above information it is clear that BYH 18636-carboxylic acid does not meet the criteria provided for relevant metabolites.

Hence, BYH 18636-carboxylic acid is a non relevant metabolite.

It can be concluded that even under the worst case conditions the use of BYH 18636 in Europe is safe for use in corn.

- **Toxicological endpoints in rodents for ecotoxicological wild mammals risk assessment purposes (text taken from Applicant's dossier)**

Use of mammalian toxicity study results in the eocotoxicological assessment (Annex III 10.3. and Document N) was based on recommendations given in EU document SANCO/4145/2000 (final rev6, 2002). The selection of endpoints is also relevant for the USA or Canada.

The toxicity profile of BYH 18636 to the rat and other laboratory animals indicates that the compound would present little if any risk to wild mammal populations.

In acute exposure of rat, no mortality or other sign of toxicity was observed at the regulatory top dose of 2000 mg a.s./kg bw. This suggests that the LD50 would in fact be much higher than 2000 mg a.s./kg bw. Therefore the acute oral study results can be expressed in terms of the LD50 cut-off of 5000 mg a.s./kg bw.

Typical effects of high doses of BYH 18636 administered over a prolonged period to the rat and other laboratory animals are histopathological findings in kidneys and urinary bladder, secondary to stone formation in the urinary tract. These stones are mainly formed of parent compound. Results from the 90-day study suggest reversibility after termination of exposure.
Such effects are considered not to be relevant for the ecotoxicological assessment (see below).
Effects on reproductive parameters were not observed up to the top dose levels used in any of the species tested.

With regards to the selection of endpoints for the long-term assessment, it is considered that effects on populations will not occur if the survival rate, reproduction rate and development of individuals are not affected. Therefore, in principle, only endpoints in toxicity tests which are related to these key factors of population dynamics are ecotoxicologically relevant.
If not indicated otherwise by the overall toxicological data available, an endpoint relating to overall reproductive success should be selected to define the long term endpoint for wild mammals. These would be effects on the reproduction rate, the survival or growth rate of the offspring, or behavioural parameters in adults or young.

In the rat multigeneration study, emaciation was observed in the high dose treatment level (10000 ppm), and one F1 male died as result of treatment. No such effects were observed in parents or offspring at the medium dose level, 2500 ppm. At this level, the only effects observed were dilatations and stones in the ureter of one F0 female. No other effect was observed in any other animal at this treatment level and below, and the survival, growth, development and reproduction of all animals was not impaired at this treatment level over the entire 2-generation study.
For the ecotoxicological assessment of BYH 18636, the LOAEL of this rat multigeneration study can therefore be established at 10000 ppm, defining the NOAEL at 2500 ppm (245 mg a.s./kg bw/day).

In the developmental toxicity studies in rat and rabbit, effects of ecotoxicological relevance were only observed at the top dose. In rat, treatment related effects on body weight in dams and pups were only observed at 1000 mg a.s./kg bw/day.
In rabbits, treatment related maternal effects (one mortality, body weight effects) and subsequently in pup weight were only observed at 500 mg a.s./kg bw/d.
These effects were observed after gavage exposure which is of low relevance to long-term exposure of wild mammals to BYH 18636 in the field, and the LOELs from the developmental toxicity studies were well above the NOAEL at 245 mg a.s./kg bw/d as derived from the rat multigeneration study.

Therefore it is proposed to establish the long-term ecotoxicological endpoint for wild mammals at 2500 ppm (245 mg a.s./kg bw/day).

Furthermore, based on the toxicological profile obtained in rodents, there was no indication in any study that BYH 18636 might be an endocrine disruptor.

B.6.11(a) Acute toxicity, irritancy and skin sensitisation of the Plant Protection Product – SC450 (IIIA 7.1)

B.6.11.1(a) Acute oral toxicity (IIIA 7.1)

BYH 18636 + AE 0001789 SC 450 also called BYH 18636 + AE 0001789 SC 225 + 225 g/L or thiencarbazone-methyl + cyprosulfamide SC 225 + 225 g/L is an suspension concentrate formulation (SC) containing BYH 18636 (thiencarbazone-methyl) at 225 g/L and AE 0001789 (cyprosulfamide) at 225 g/L.

IIIA1 7.1.1 Acute oral toxicity

Report:	**KIIIA1 7.1.1/01; Schüngel M., 2006**
Title:	BYH 18636 + AE 0001789 SC 225 + 225 g/L - Acute toxicity in the rat after oral administration
Report No & Document No	AT03052, T0076610 M-273331-01-2
Guidelines:	**OECD Guidelines N° 423 (2001)** **EEC Directive 67/548/EEC Annex V, B1.tris (in its current version)** **EPA (OPPTS 870.1100)**
Deviation(s):	The test compound is a product known to be stable and homogenous in both undiluted and in ready-to-use formulation with water. Therefore, analytical determinations of stability and homogeneity of the aqueous formulations were not performed. The deviation does not limit the assessment of the results.
GLP	**Yes**

I. Materials and methods

A. Materials

1. Test material:	BYH 18636 + AE 0001789 SC 225 + 225 g/L
Specification no.:	102000013579
Description:	White milky liquid
Lot/Batch no.:	2005-003981
Content:	BYH 18636: 225 g/L, AE 0001789: 225 g/L (Nominal values) BYH 18636: 215 g/L, AE 0001789: 217 g/L (Certified by analysis)
Stability of test compound:	Guaranteed for study duration; expiry date: 16th Dec. 2006
2. Vehicle and/or positive control:	Tap water
3. Test animals	
Species:	Rat, females
Strain:	Wistar (Hsd CpPb:WU)
Age:	10-12 weeks approximately
Weight at dosing:	164 g – 185 g
Source:	Harlan/Winkelmann GmbH, Borchen, Germany
Acclimatization period:	At least 5 days
Diet:	Provimi Kliba 3883.0.15; Kaiseraugst, Switzerland
Water:	Tap water *ad libitum*

Housing:	The animals were group caged conventionally in polycarbonate cages on low dust wood granulate bedding (J. Rettenmaier & Söhne, 73479 Ellwangen-Holzmühle, Germany). The cages of the animals were placed on racks. The wood granulate was randomly checked for contaminants at regular intervals and the results have been stored at the Department for Laboratory Animal Services, Bayer HealthCare AG, Wuppertal, Germany. Room temperature: 22 ± 2°C; Air humidity: 55 ± 5%; Ventilation: approx. 10 changes per hour; Light/ Dark cycle : 12 hour rhythm

B. Study design and methods

1. Animal assignment and treatment

Dose:	2000 mg/kg bw
Application route:	Oral by gavage
Application volume:	10 mL/kg bw
Fasting time:	For administration, food was withheld from the animals for approximately 16 - 24 h before administration of the test compound, and they were fed again approximately 2-4 h after administration
Group size:	3 rats/dose group
Post-treatment observation period:	14 days
Observations:	Mortality, clinical signs, body weight, gross necropsy
In life dates:	9th March 2006 to 29th March 2006

The test compound was formulated in tap water; and the test material was administered per os in a single dose (2000 mg/kg) by gavage to 3 fasted female Wistar rats. As no mortality occurred, three additional animals were treated with the same dose.

II. Results and discussion

A. Mortality

Mortality was not observed at 2000 mg/kg bw.

Table 7.1.1-1 Doses, mortality / animals treated

Dose (mg/kg bw)	Toxicological results*			Occurrence of signs	Time of death	Mortality (%)
Females						
2000 1st	0	0	3	--	--	0
2000 2nd	0	0	3	--	--	0
acute oral LD_{50}**: ≥ 5000 mg/kg bw						

* 1st number = number of dead animals; 2nd number = number of animals with signs;
3rd number = number of animals used; ** according to the principles of OECD Guideline 423

B. Clinical observations

No clinical signs were observed.

C. Body weight

Body weight and body-weight gain were not affected by the treatment.

D. Necropsy

No particular gross pathological changes were observed in animals sacrificed at the end of the study period.

III. Conclusion

According to the OECD Guideline 423 the acute oral LD_{50} cut-off of BYH 18636 + AE 0001789 SC 225 + 225 g/L formulation in rats is ≥ 5000 mg/kg and as a result is a category 5 product (i.e. unclassified according to the Globally Harmonised Classification System).

According to the EC classification criteria (2001/59/EC Directive), the formulation BYH 18636 + AE 0001789 SC 225 + 225 g/L is labelled as follows:

Symbols of danger : None
Risk phrases : None

According to EPA guidelines, the LD_{50} for this formulation is greater than 2000 mg/kg, which is consistent with the criteria for EPA Toxicity Category III.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: totally reliable The study (T0077610) is fully compliant with OECD 423 (2001) Reduced weight gain seen in one rat is possibly related to treatment: no additional treatment-related findings were observed in this study.
Conclusions	The acute oral LD50 of the test material in female rats was found to be >2000 mg/kg bw under the conditions of this study. The product is not classified for acute oral toxicity according to current EC criteria.

B.6.11.2(a) Acute percutaneous (dermal) toxicity (IIIA 7.1.2)

Report: **KIIIA1 7.1.2/01; Schuengel M., 2006**

Title:	BYH 18636 + AE 0001789 SC 225 + 225 g/L - Acute toxicity in the rat after dermal administration
Report No & Document No	AT03050 , T1076611 M-279823-01-2
Guidelines:	**OECD Guidelines N° 402 (1987)** **EEC Directive 67/548/EEC Annex V, B.3 (1967)** **EPA (OPPTS 870.1200 – 712-C-98-192, August 1998)**
GLP	**Yes**

I. Materials and methods

A. Materials

1. Test material:	BYH 18636 + AE 0001789 SC 225 + 225 g/L
Specification no.:	102000013579
Description:	White milky liquid
Lot/Batch no.:	2005-003981
Content:	BYH 18636: 225 g/L, AE 0001789: 225 g/L (Nominal values) BYH 18636: 215 g/L, AE 0001789: 217 g/L (Certified by analysis)
Stability of test compound:	Guaranteed for study duration; expiry date: 16th Dec 2006
2. Vehicle and/or positive control:	None
3. Test animals	
Species:	Rat, males and females
Strain:	Wistar (Hsd Cpb:WU)
Age:	9 – 13 weeks approximately
Weight at dosing:	Males: 238 g – 263 g Females: 206 g – 225 g
Source:	Harlan/Winkelmann GmbH, Borchen, Germany
Acclimation period:	At least 5 days
Diet:	Provimi Kliba 3883.0.15; Kaiseraugst, Switzerland
Water:	Tap water *ad libitum*
Housing:	The animals were caged individually in polycarbonate cages on low dust wood granulate bedding (J. Rettenmaier & Söhne, 73479 Ellwangen-Holzmühle, Germany). The cages of the animals were placed on racks. The wood granulate was randomly checked for contaminants at regular intervals and the results have been stored at the Department for Laboratory Animal Services, Bayer HealthCare AG, Wuppertal, Germany. Room temperature: 22 ± 2°C; Air humidity: 55 ± 5%; Ventilation: approx. 10 changes per hour; Light/Dark cycle: 12 hour rhythm.

B. Study design and methods

1. Animal assignment and treatment

One day before the start of the treatment the back and flanks of 5 male and 5 female Wistar rats were shorn. They received a single dermal dose of 2000 mg/kg bw of the pure liquid test compound applied semi-occlusively. After an exposure time of 24 hours, the dressings were removed and the treated area was rinsed with tepid water using soap and gently patting the area dry.

Dose (mg/kg bw)		Surface area (cm²)	Range of doses (mg/cm²)
males	2000	10.5	45.3-50.1
females	2000	10.5	39.2-42.9

Application route:	Dermal, semi-occlusive dressing
Duration:	24 hours
Group size:	5 rats/sex/group
Post-treatment observation period:	14 days
Observations:	Mortality, clinical signs, skin effects, body weight, gross necropsy
In life dates:	6th March 2006 to 23rd March 2006

II. Results and discussion

A. Mortality

Mortality was not observed at 2000 mg/kg bw.

Table 7.1.2-1 Doses, mortality / animals treated

Dose (mg/kg bw)	Toxicological results*			Duration of signs	Time of death	Mortality [%]
Males						
2000	0	0	5	--	--	0
Females						
2000	0	0	5	--	--	0
acute dermal LD_{50}: > 2000 mg/kg bw						

* 1st number = number of dead animals:; 2nd number = number of animals with signs;
3rd number = number of animals in the group

B. Clinical observations

No clinical signs were observed.

C. Body weight

Body weight and body weight gain was not affected by treatment in males or females.

D. Necropsy

No particular gross pathological changes were observed in animals sacrificed at the end of the study period.

III. Conclusion

The dermal LD50 of the formulation BYH 18636 + AE 0001789 SC 225 + 225 g/L was greater than 2000 mg/kg bw for rats.

According to the EC classification criteria (2001/59/EC Directive), the formulation BYH 18636 + AE 0001789 SC 225 + 225 g/L is labelled as follows:

Symbols of danger	:	None
Risk phrases	:	None

The dermal LD_{50} of BYH 18636 + AE 0001789 SC 225 + 225 g/L is greater than 2000 mg/kg body weight for both male and female rats, which is consistent with the criteria for EPA Tox Category III.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: totally reliable The study (T1076611) is fully compliant with OECD 402 (1987) No treatment-related findings were observed in this study.
Conclusions	The acute dermal LD50 of the test material the rat was found to be >2000 mg/kg bw under the conditions of this study. The product is not classified for acute dermal toxicity according to current EC criteria.

B.6.11.3(a) Acute inhalation toxicity to rats (IIIA 7.1.3)

Report:	**KIIIA1 7.1.3/01; Pauluhn J., 2006**
Title:	BYH 18636 + AE 0001789 SC 225 + 225 g/L - Acute inhalation toxicity in rats
Report No &:	AT03250, T0076809
Document No:	M-277825-01-2
Guidelines:	**OECD Guidelines N° 403 (12 May 1981)** **EEC Directive 92/69/EEC Annex V – Method B2 (1992)** **EPA: OPPTS 870.1300 (1998)** **MAFF, notification 12 Nousan-8147 (2000)**
GLP	**Yes**

I. Materials and methods

1. Test material:	BYH 18636 + AE 0001789 SC 225 + 225 g/L
Specification no.:	102000013579
Description:	White milky liquid
Lot/Batch no.:	2006-004132
Content:	BYH 18636: 225 g/L, AE 0001789: 225 g/L (Nominal values) BYH 18636: 220 g/L, AE 0001789: 215 g/L (Certified by analysis)

Stability of test compound:	Guaranteed for study duration; expiry date: 8th May 2007
2. Vehicle and/or positive control:	The test article was aerosolised as aqueous solution.

3. Test animals

Species:	Rat, males and females
Strain:	SPF Wistar (Hsd Cpb:WU)
Age:	Approximately two months
Weight at dosing:	Males: 182 g – 190 g Females: 173 g – 193 g
Source:	Harlan/Winkelmann GmbH, Borchen, Germany
Acclimation period:	At least 5 days
Diet:	Provimi Kliba 3883 9441 pellets, Kaiseraugst, Switzerland
Water:	tap water *ad libitum*
Housing:	During the acclimation and study periods, the animals were housed singly in conventional Makrolon® Type III cages (based on A. Spiegel and R. Gönnert, Zschr. Versuchstierkunde, 1, 38 (1961) and G. Meister, Zschr. Versuchstierkunde, 7, 144-153 (1965)). Cages were changed twice a week while unconsumed feed and water bottles were changed once per week. The legal requirements for housing experimental animals (Directive 86/609 EEC) were followed. Bedding consisted of type BK8/15 low-dust wood granulate from Ssniff, Soest/Westfalen, Germany. The wood granulate was randomly checked for harmful constituents at the request of the Laboratory Animal Services, Bayer Healthcare AG. Room temperature: 22 ± 2°C; Air humidity: 40-60%; Ventilation: approx. 10 changes per hour; Light/Dark cycle: 12 hour rhythm.

B. Study design and methods

1. Animal assignment and treatment

One group of 10 Wistar rats (5 animals/sex) was exposed to mean aerosol concentration of 2.207 mg/L for up to 4 hours using nose only exposure system. Attempts were made so that liquid aerosol generated was respirable to rats. The test item was aerosolised undiluted.

Dose:	0 – 2.207 mg/L air (maximum technically attainable concentration)
Application route:	Inhalation (nose-only exposure)
Duration:	4 hours
Group size:	5 rats/dose/sex
Post-treatment observation period:	14 days
Observations:	Mortality, clinical signs, body weights, rectal temperature, reflex measurements, gross necropsy
In life dates:	3rd March 2006 to 17th July 2006

2. Generation of the test atmosphere / chamber description

Generation and characterization of chamber atmosphere

Target concentration (mg/L)	Nominal concentration (mg/L)	Mean achieved concentration (mg/L)	Mean mass Aerodynamic Diameter (µm)	Geometric standard deviation (µm)	Respirable fraction (% < 3 µm)
5.000	17.982	2.207	3.53	2.49	43.1

II. Results and discussion

A. Mortality

Mortality was not observed at 2.207 mg/L air.

Table 7.1.3-1 Doses, mortality / animals treated

Actual concentration (mg/L air)	Toxicological results*			Duration of signs	Time of death	Mortality [%]
Males						
0	0	0	5	--	--	0
2.207	0	0	5	--	--	0
Females						
0	0	0	5	--	--	0
2.207	0	0	5	--	--	0
acute inhalative LC_{50}: > 2.207 mg/L air						

* 1st number = number of dead animals; 2nd number = number of animals with signs; 3rd number = number of animals exposed

B. Clinical observations

All rats tolerated the exposure without specific signs.
Reflex measurements: no exposed rats exhibited changes in reflexes.
Rectal temperature: no significant changes.

C. Body weight

Body weights: no significant changes.

D. Necropsy

Effects on organs: necropsy findings were unremarkable in rats sacrificed at the end of the observation period.

III. Conclusion

The inhalation LC_{50} of the formulation BYH 18636 + AE 0001789 SC 225 + 225 g/L for male and female Wistar rats was higher than 2.207 mg/L, the highest attainable concentration.

According to the EC classification criteria (2001/59/EC Directive), the formulation BYH 18636 + AE 0001789 SC 225 + 225 g/L is labelled as follows:

Symbols of danger : None
Risk phrases : None

The acute inhalation LC_{50} of the test substance for males and females is greater than 2.207 mg/L, which is consistent with the criteria for EPA Tox Category IV.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: Totally reliable The study (T0076809) is fully compliant with OECD 403 (1987) The Notifier's summary states that no significant effects were observed on rectal temperature, however the mean rectal temperature of the exposed group of males (36.9°C) was statistically significantly ($p<0.01$) lower than that of control males (38.2°C). The mean rectal temperature of the exposed group of females was slightly (36.9°C) but not statistically significantly lower than the controls (38.5°C). The finding in males is considered to be treatment-related and indicates that the test material may be a mild respiratory irritant. The Notifier's summary states that no significant effects were observed on bodyweight, however the mean bodyweights of the exposed group of males were consistently lower than controls throughout the study period. Mean terminal bodyweights of exposed males (260.4 g) were lower than controls (299.8 g), and total weight gain by this group (76.0 g) was also lower than controls (99.8 g). Findings are considered to be treatment-related. No further effects of treatment were observed.
Conclusions	The acute inhalation LC50 of the test material in the rat was found to be >2.207 mg/l, stated to be the maximum concentration technically achievable under the conditions of the study. The product is therefore not classified for acute inhalation toxicity according to current EC criteria. The slight (but significant) reduction in body temperature seen in treated males indicates that the product is a mild respiratory irritant, however classification of the product as a respiratory irritant is not considered to be appropriate in the absence of any correlating clinical signs.

B.6.11.4(a) Skin irritation (IIIA 7.1.4)

Report:	**KIIIA 7.1.4/01, Schuengel M.; 2006**
Title:	BYH 18636 + AE 0001789 SC 225 + 225 g/L – Acute skin irritation/corrosion on rabbits
Report No &	AT03000, T 6076553
Document No	M-272076-01-2
Guidelines:	**OECD: No. 404, 24th April 2002** **EEC Directive 67/548 Annex V – Method B.4 (1967) in its current version** **EPA OPPTS 870.2500 United States EPA 712-C-98-196 (1998)**
GLP	**Yes**

I. Materials and methods

A. Materials

1. Test material:	BYH 18636 + AE 0001789 SC 225 + 225 g/L
Article no.:	102000013579
Description:	White milky liquid
Lot/Batch no:	2005-003981
Content:	BYH 18636: 225 g/L, AE 0001789: 225 g/L (Nominal values) BYH 18636: 215 g/L, AE 0001789: 217 g/L (Certified by analysis)
Stability of test compound:	Guaranteed for study duration; expiry date: 16th Dec 2006
2. Vehicle and/or positive control:	None
3. Test animals	
Species:	Rabbit, females
Strain:	Albino (Crl:KBL(NZW)BR)
Age:	Young adult animals
Weight at dosing:	2.4 kg – 2.9 kg
Source:	Charles River, Kißlegg, Germany
Acclimation period:	At least 5 days
Diet:	Ssniff K-Z, Soest, Germany
Water:	Tap water *ad libitum*
Housing:	The animals were housed individually in cage units Metall/Noryl by EBECO. Excrement trays below the cages contained low dust wood granulate bedding (J. Rettenmaier & Söhne, 73479 Ellwangen-Holzmühle, Germany). The wood granulate was changed at least twice weekly. The animals were regularly transferred to clean cages. The animal room had a standardized climate: Room temperature: 20 ± 3°C; Air humidity: 50 ± 25%; Light/ Dark cycle: 12 hour rhythm.

B. Study design and methods

1. Animal assignment and treatment

Doses of 0.5 mL of the undiluted test item were placed on a dry gauze pad which was then applied to the clipped, intact skin of three rabbits for 4 hours. The treatment site was observed shortly after the end of the exposure period then daily for up to 72h.

Dose:	0.5 mL pure liquid test substance/animal
Application route:	Dermal (semi occlusive procedure)
Duration:	4 hours
Group size:	3 rabbits
Observations:	Clinical signs, skin effects, body weight (at beginning of study)
In life dates:	4th April 2006 to 7th April 2006

II. Results and discussion

A. Findings

Under the present test conditions the following findings were noted:
There were no systemic intolerance reactions. A transcient erythema was noted in 2/3 rabbits 1 hour post application. No other reactions were noted.

Table 7.1.4-1 Summary of irritant effects (Scores)

Rabbit number and sex	E or O	Scores Time after application						Mean score 24-48-72h	Response	Reversibility (days)
		1h	24h	48h	72h	D7	D14			
1 F	E	1	0	0	0	-	-	0.0	(-)	1*
	O	0	0	0	0	-	-	0.0	(-)	NA
2 F	E	1	0	0	0	-	-	0.0	(-)	1*
	O	0	0	0	0	-	-	0.0	(-)	NA
3 F	E	0	0	0	0	-	-	0.0	(-)	NA
	O	0	0	0	0	-	-	0.0	(-)	NA

E = erythema, O = oedema
No positive response (-): mean score 24-48-72h < 2 Positive response (+): mean score 24-48-72h ≥ 2
NA = not applicable, - examination not performed
*: in respect of the result 1h post application

III. Conclusion

It was concluded that BYH 18636 + AE 0001789 SC 225 + 225 g/L was not irritating to the rabbit skin.

According to the criteria for classification defined in the Directive 2001/59/EC, the formulation BYH 18636 + AE 0001789 SC 225 + 225 g/L is labelled as follows:

Symbols of danger : None
Risk phrases : None

According to classification criteria, BYH 18636 + AE 0001789 SC 225 + 225 g/L is not a dermal irritant, which is consistent with the criteria for EPA Tox Category IV.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: Totally reliable The study (T6076553) is fully compliant with OECD 404 (2002)
Conclusions	The test material was found to be a minimal skin irritant under the conditions of this study and is not classified as a skin irritant according to current EC criteria.

B.6.11.5(a) Eye Irritation (IIIA 7.1.5)

Report:	**KIIIA 7.1.5/01, Schuengel M., 2006**
Title:	BYH 18636 + AE 0001789 SC 225 + 225 g/L – Acute eye irritation in rabbits
Report No &	AT03001, T8076555
Document No	M-272093-01-2
Guidelines:	**OECD: No. 405, 24th April 2002**
	EEC Directive 67/548 Annex V – Method B.5 (1967)
	EPA OPPTS 870.2400, United States EPA 712-C-98-195 (1998)
GLP	**Yes**

I. Materials and methods

A. Materials

1. Test material:	BYH 18636 + AE 0001789 SC 225 + 225 g/L
Specification no.:	102000013579
Description:	White milky liquid
Lot/Batch no:	2005-003981
Content:	BYH 18636: 225 g/L, AE 0001789: 225 g/L (Nominal values) BYH 18636: 215 g/L, AE 0001789: 217 g/L (Certified by analysis)
Stability of test compound:	Guaranteed for study duration; expiry date: 16th Dec 2006
2. Vehicle and/or positive control:	None
3. Test animals	
Species:	Rabbit, females
Strain:	Albino (Crl:KBL(NZW)BR)
Age:	Young adult animals
Weight at dosing:	2.1 kg – 2.2 kg
Source:	Charles River, Kißlegg, Germany
Acclimation period:	At least 5 days
Diet:	Ssniff K-Z, Soest, Germany
Water:	Tap water *ad libitum*
Housing:	The animals were housed individually in cage units Metall/Noryl by EBECO. Excrement trays below the cages contained low dust wood granulate bedding (J. Rettenmaier & Söhne, 73479 Ellwangen-Holzmühle, Germany). The wood granulate was changed at least twice weekly. The animal room had a standardized climate: Room temperature: 20 ± 3°C; Air humidity: 50 ± 25%; Light/Dark cycle: 12 hour rhythm.

B. Study design and methods

1. Animal assignment and treatment

A single dose of 0.1 mL of the undiluted test item was instilled under the lower lid of the left eye of one rabbit. The right eye was untreated and served as the control. The eyes were not rinsed after administration of the test item. Since the test item was not severely irritant on the first animal, it was then evaluated in two other animals. Ocular reactions were observed approximately 1 hours, 24, 48 and 72 hours after instillation and then daily until the end of the observation period.

Dose:	0.1 mL pure liquid test substance/animal
Application route:	Instillation into the conjunctival sac of one eye. The eye was not rinsed for at least 24 hours following instillation.
Group size:	3 rabbits
Observations:	Clinical signs, eye effects, body weight (at beginning of study)
In life dates:	11th April 2006 to 14th April 2006

II. Results and discussion

A. Findings

Under the present test conditions the following findings were noted:

The individual findings of the treated eyes at the various observation times are summarized in the table below. The control eyes did not show any abnormal findings and are not listed in the Table 7.1.5-1. Animal 3, 1 hour after application, the test compound adhered to conjunctivae.

Table 7.1.5-1 Summary of Irritant Effects (Scores)

Animal N° Sex	Parameter	1h	24h	48h	72h	Index* 24-48-72h	Reversible (days)
1 F	Chemosis	0	0	0	0	0.0 (-)	NA
	Conjunctival redness	1	1	0	0	0.3 (-)	2
	Iris lesions	0	0	0	0	0.0 (-)	NA
	Corneal opacity	0	0	0	0	0.0 (-)	NA
2 F	Chemosis	0	0	0	0	0.0 (-)	NA
	Conjunctival redness	2	1	0	0	0.3 (-)	2
	Iris lesions	0	0	0	0	0.0 (-)	NA
	Corneal opacity	0	0	0	0	0.0 (-)	NA
3 F	Chemosis	0	0	0	0	0.0 (-)	NA
	Conjunctival redness	2	1	0	0	0.3 (-)	2
	Iris lesions	0	0	0	0	0.0 (-)	NA
	Corneal opacity	0	0	0	0	0.0 (-)	NA

* = Mean of scores on 24-48-72h instillation

Response: corneal opacity: mean scores <2 = (-), ≥2<3 = (+), ≥3 = (++)
Iritis: mean scores <1 = (-), ≥1<2 = (+), =2 = (++)
Conjunctival redness: mean scores <2.5 = (-), ≥2.5 = +
Conjunctival oedema: mean scores <2 = (-), ≥ 2 = +
NA = not applicable

III. Conclusion

It was concluded that BYH 18636 + AE 0001789 SC 225 + 225 g/L was not irritating to the rabbit eye.

According to the criteria for classification defined in the Directive 2001/59/EC, the formulation is labelled as follows:

Symbols of danger : None
Risk phrases : None

According to OECD classification criteria, BYH 18636 + AE 0001789 SC 225 + 225 g/L is not irritating to eyes. Based on the EPA guidelines, the formulation is in EPA Tox Category III.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: Totally reliable The study (T8076555) is fully compliant with OECD 405 (2002)
Conclusions	The test material was found to be a slight eye irritant under the conditions of this study and is not classified as an eye irritant according to current EC criteria.

B.6.11.6(a) Skin sensitization (IIIA 7.1.6)

Report: **KIIIA1 7.1.6/01; Repetto – Larsay, M., 2006**

Title: BYH 18636 + AE 0001789 SC 450 - Evaluation of potential dermal sensitization in the local lymph node assay in the mouse.

Report No.: SA 06028

Doc. No.: M-279626-01-2

Guidelines: **OECD Guideline 429 (2002)**

GLP **Yes**

I. Materials and methods

A. Materials

1. Test material: BYH 18636 + AE 0001789 SC 225 + 225 g/L

Specification no.: 102000013579

Description: White milky liquid

Lot/Batch no: 2005-003981

Content: BYH 18636: 225 g/L, AE 0001789: 225 g/L (Nominal values)
BYH 18636: 215 g/L, AE 0001789: 217 g/L (Certified by analysis)

Stability of test compound: Guaranteed for study duration; expiry date: 16th Dec 2006

2. Vehicle and/or positive control: Vehicle: Water containing 1% Pluronic Acid was selected to ensure compatibility with the test substance and maximum wetting of the mouse ears with the maximum possibility of skin penetration of the various formulation ingredients.

A positive control group received 0.25% p-benzoquinone in 25% BYH 18636 + AE 0001789 SC 450 and 75% Pluronic acid at 1% in water. The positive control was spiked in the formulation to ensure that under the conditions of this assay, the study demonstrated appropriate sensitivity with the positive control.

3. Test animals

Species: Mice, females

Strain: CBA/J

Age:	At least 8 weeks old
Source:	R. Janvier, le Genest St Isle, France
Acclimation period:	At least 5 days
Diet:	Certified rodent pellet diet: AO4C-10, S.A.F.E. (Scientific Animal Food and Engineering, Route de Saint Bris, Augy, France)
Water:	Tap water *ad libitum*
Housing:	During the study period the animals were individually housed in suspended, stainless steel, wire mesh cages. Room temperature: 20-24°C Humidity: 40 - 70 %; Light/dark cycle: Twelve hours rhythm Air exchange rate: 10-15 times per hour.

B. Study design and methods

1. Animal assignment and treatment

Twenty-four female CBA/J mice were allocated to 6 groups of four animals each:

- Four groups received the test substance at a concentration of 10, 25, 50 or 100% in vehicle (aqueous pluronic acid at 1%),
- A positive control group received 0.25% p-benzoquinone in 25% BYH 18636 + AE 0001789 SC 450 and 75% Pluronic acid at 1% in water. The positive control was spiked in the formulation to ensure that under the conditions of this assay, the study demonstrated appropriate sensitivity with the positive control.
- A control group received the vehicle, 1% pluronic acid in water.

Observations:	Mortality, clinical signs, skin effects, body weight (at beginning and termination of study)
In life dates:	28th Feb 2006 to 9th March 2006

The test substance, positive control or the vehicle were applied on external surfaces of each ear (50 µL/animal) for three consecutive days (Days 0, 1 and 2) at the appropriate concentrations. On Day 5, the cell proliferation in the local lymph nodes was measured by incorporation of tritiated thymidine and the obtained values were used to calculate proliferation indices.

II. Results and discussion

A. Findings

Mortality and clinical signs - No mortality and no clinical signs were observed during the study. No cutaneous reactions were observed at the treated site for the negative control, positive control or BYH 18636 + AE 0001789 SC 450 treated groups.

Body weights - No significant body weight changes were observed during the study either in the control or in the treated groups.

The proliferation index values of the test substance were 1.08, 0.98, 0.65 and 1.33 at treatment concentrations of 10, 25, 50 and 100% respectively.
The proliferation index value of the positive control was 7.9 at treatment concentration of 0.25% of p-Benzoquinone.

GROUP	TEST SUBSTANCE(S)	# OF ANIMALS	CONCENTRATION % DAYS 0-2	DPM/NODE	STIMULATION INDEX (SI)
1	Vehicle control	4	0	535	
2	BYH 18636 + AE 0001789 SC 450	4	10	577	1.08
3		4	25	525	0.98
4		4	50	346	0.65
5		4	100	711	1.33
6	p-Benzoquinone	4	0.25	4253	7.9

Negative lympho-proliferative responses (SI<3) were noted for BYH 18636 + AE 0001789 SC 450 at all concentrations tested. In the positive control group given p-Benzoquinone, a SI value >3 was noted. This positive response to p-Benzoquinone demonstrates the validity of this assay under the current condition using the specific test formulation.

III. Conclusion

As no stimulation index value was over 3 for treated group and as no dose-related effect was noticed, BYH 18636 + AE 0001789 SC 450 was found to be not a sensitizing formulation in the Local Lymph Node Assay.

According to the commission directive 2001/59/EC, the test substance BYH 18636 + AE 0001789 SC 450 is labelled as follows:

Symbols of danger : None
Risk phrases : None

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: Totally reliable The study (SA 06028) is fully compliant with OECD 429 (2002)

Conclusions	No evidence of skin sensitisation was seen under the conditions of this study. The product is not classified as a skin sensitiser accruing to current EC criteria. EPA: The 2003 OPPTS harmonized guidelines state the "LLNA is the preferred method, where applicable." The "where applicable" correlates to the performance parameters in the appendix — the 1999 ICCVAM report. In 1999, ICCVAM validated the method using 209 "single compound compounds" but did not validate the assay for mixtures. The appendix clearly states the LLNA should not be used for metals, aqueous solutions, and mixtures. In January 2008, ICCVAM updated the validation report on LLNA regarding mixtures, metals and aqueous solutions. ICCVAM findings are that when compared to the guinea pig it has a false negative rate of 50%, a false positive rate of 44%, and accuracy rate of 53% in mixtures. Due to these limitations, EPA questioned whether the negative result found in this study is correct. EPA decided to perform a weight of the evidence approach for this joint review chemical by obtaining information on each component (inerts ingredient) in this mixture. After reviewing the components sensitization potential, EPA determined that most were non-sensitizers and other components were assumed to be below the sensitizing threshold. Therefore, to avoid further animal testing EPA will classify the study as "supplemental" and recommend label language as a non-sensitizer for SC 450[BYH 18636 + AE 0001789 SC 450].

B.6.11.7(a) Supplementary studies for combinations of plant protection products (IIIA1 7.1.7)

None.

B.6.11.8(a) Short-term toxicity studies (IIIA1 7.2)

Not required by Directive 91/414/EEC, EPA or PMRA.

B.6.12(a) Dermal absorption 'SC 450' (IIIA 7.3)

The extent of dermal absorption of thiencarbazone-methyl and cyprosulfamide formulated as thiencarbazone-methyl & cyprosulfamide SC 450 was investigated *in vitro* on human and rat skin. A summary of the studies and conclusion on dermal absorption is given below.

Summary and conclusion on dermal absorption of thiencarbazone-methyl

In vivo data

No *in vivo* data available.

In vitro data

For thiencarbazone-methyl a dermal absorption of 2.5 % is considered to be appropriate to calculate for Europe systemic exposure from dermal contamination with the in-use spray concentration. For the concentrate, the comparative *in vitro* dermal absorption study resulted in a dermal absorption value of 0.3 %.

Summary and conclusion on dermal absorption of cyprosulfamide

In vivo data

No *in vivo* data available.

In vitro data

For cyprosulfamide a dermal absorption of 1.5 % is considered to be appropriate to calculate for Europe systemic exposure from dermal contamination with the in-use spray concentration. For the concentrate, the comparative *in vitro* dermal absorption study resulted in a dermal absorption value of 0.9 %.

B.6.12.1(a) Comparative dermal absorption, *in vitro* using rat and human skin (IIIA1 7.6.2)

a) Dermal absorption of thiencarbazone-methyl, *in vitro*

Report:	**KIIIA1 7.6.2/01, Capt. A., 2006 (MRID # 47070202)**
Title:	[^{14}C]-BYH 18636 in SC450 formulation: Comparative *in vitro* dermal absorption study using human and rat skin
Report No &	SA 06151
Document No	M-277416-01-2
Guidelines:	**OECD Guideline for the Testing of Chemicals; Skin Absorption: In Vitro Method. Guideline 428 (April 2004)**
GLP	**Yes (certified laboratory)**

I. Material and methods

In a 2006 GLP study, the potential dermal penetration of [^{14}C]-BYH 18636 formulated as SC 450 (450 g a.s./L) was investigated at two concentrations corresponding to the neat product and a representative spray dilution (nominal values: 225 mg a.s./mL and 0.27 mg a.s./mL, respectively; applied at a rate of 10 µL/cm²) to excised human and rat dermatomed skin. Human skin was obtained from seven different donors obtained from a recognised Tissue Bank. Rat skin was obtained from ten different male Wistar rats.

The skin samples were placed in flow-through diffusion cells (modified Franz cell; exposure area of 1 cm² skin). The receptor fluid was Eagles medium supplemented with 5% bovine serum albumine and gentamycin (50 mg/L, pH 7.3). The integrity of the selected skin samples was assessed by measuring the trans-epidermal water loss (TEWL) from the stratum corneum (only skin with a TEWL <40 g/m²/h was used).

Samples of the receptor fluid were collected at 1-hour intervals for the duration of the experiment (24 hours). At 8 hours post-application, the skin was swabbed with 1% v/v Tween 80 in PBS (phosphate buffer saline) until no radioactivity was detected on the swabs. At 24 hours post-application the treated skin cell was swabbed again prior to tape-stripping. Tape stripping with Monaderm adhesive tape was performed until a “shiny” appearance of the epidermis indicated that the stratum corneum had been removed. The tape-strips were collected as well as the remaining skin was taken for analysis.

II. Results and discussion

An overview of the results is given in Table 7.6.2-1

Table 7.6.2-1: Summary of the in vitro dermal penetration through human and rat epidermal membranes; low and high dose

SC 450	[14C]-BYH 18636			
	Low dose (nom. 0.27 g/L) Mean (n = 5)		**High dose (nom. 225 g/L) Mean (n = 5)**	
	Human	Rat	Human	Rat
SURFACE COMPARTMENT				
Surface dose (Tape strips 1 & 2)	1.06	1.67	0.70	0.21
Skin swabs at 8 hrs	88.20	80.75	93.06	93.20
Skin swabs at 24 hrs	0.71	1.03	0.10	0.01
Dose remaining in the doner chamber	0.03	0.13	0.05	0.15
Total % non-absorbed	**90.01**	**83.59**	**93.91**	**93.57**
SKIN COMPARTMENT				
Skin a	1.23	1.12	0.14	0.07
Stratum corneum b	0.88	3.23	0.20	0.46
Total % at dose site	**2.11**	**4.35**	**0.34**	**0.53**
RECEPTOR COMPARTMENT				
Receptor fluid (0 to 24 hrs)	0.39	2.32	<0.005	0.01
Receptor fluid terminal	<0.005	0.04	<0.005	<0.005
Receptor chamber	<0.005	<0.005	<0.005	<0.005
Total % directly absorbed	**0.39**	**2.36**	**<0.005**	**0.01**
TOTAL ABSORBABLE				
Total % directly absorbed	0.39	2.36	<0.005	0.01
Total % at dose site	2.11	4.35	0.34	0.53
Total % potentially absorbable c	2.50	6.71	0.34	0.54
Total % recovery d	**92.51**	**90.29**	**94.25**	**94.11**

n: number of skin cells used for calculation
[a]: skin after tape stripping procedure
[b]: tape-strips excluding numbers 1 & 2 which were considered to be non-absorbed dose
[c]: sum of "Totals % directly absorbed" and "Total % at dose site"
[d]: calculated by the DEBRA 5.2a software

The mean total recoveries of radioactivity were 93 % dose for the human skin and 90 % dose for the rat skin samples for low dose application. For high dosing the mean total recoveries were > 94 % dose for human skin and rat skin, respectively.

The data demonstrated that the majority of the radioactivity was removed by the swabbing procedure performed at 8 hours post application and by the surface tape-strips. Lower levels of radioactivity were recovered from the dose site for human and rat skin; mean total percentage of the applied radioactivity recovered from the dose site was 2.1 % for human skin and 4.4 % for rat skin (low dose) and 0.3% for human skin and 0.5% for rat skin (high dose).

The mean total percentage of the applied radioactivity found to be directly absorbed was 0.4 % dose for the human skin and 2.4 % dose for the rat skin in case of low dosage and <0.005% dose and 0.01% dose for human and rat skin, respectively in case of high dosage.

The radioactivity counted in the skin compartment (skin and stratum corneum) could be considered to be potentially absorbable. The percentage of [^{14}C]-BYH 18636 considered to be potentially absorbable over a period of 24 hours was 2.5 % and 6.7 % for the human and rat skin, respectively, for the diluted

product. In case of the neat product potentially 0.3 % and 0.5 % dose could be absorbable for human skin and rat skin, respectively.

III. Conclusion

These data show that the potentially dermal absorbable fraction for the neat formulation of thiencarbazone-methyl, formulated as a soluble concentrate, is fairly low and in a comparable range for both human and rat skin. Rat skin revealed a higher penetration by a factor of 1.6 than human skin. For the diluted formulation a more pronounced difference exists. Again, taking into account the potentially absorbable fraction, a factor of approx. 2.7 was found.

For estimating systemic exposure the following figures are taken:
0.3 % for the concentrate and 2.5 % for the spray solution.

(Capt. A., 2006)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: Totally reliable The study is fully compliant with OECD 428 (2004). The dermal absorption values proposed by the Notifier are agreed, however the derivation of these values is considered to be conservative, as discussed below. The exclusion of the first two tape strips from calculation of the dermal absorption values is considered to be acceptable is this material is likely to be lost by desquamation. The Notifier proposes that the total skin residue (with the exception of the first two tape strips) is included in the dermal absorption vales. Current EU guidance (SANCO/222/2000) states that this material may be discounted when 'serial non-detects' are observed. For human skin membranes, serial non-detects are apparent for the product concentrate (no radioactivity detected in the receptor fluid at any time point) and for the diluted product (21-24 hours). It would therefore seem unlikely that the skin residue would be systemically available. Results for rat skin indicate greater absorption (0.5 and 6.7% for the concentrate and dilution respectively), however these are not used for calculation of the dermal absorption values in the absence of an *in vivo* study.
Conclusions	In line with current EU guidance, dermal absorption values for thiencarbazone-methyl from the 'SC 450' product of 0.3% for the concentrate and 2.5% for the dilution can be calculated, based on the values from human skin membranes *in vitro*.

b) Dermal absorption of cyprosulfamide, *in vitro*

Report:	**KIIIA1 7.6.2/02, Capt. A. 2006**
Title:	[^{14}C]-AE 0001789 in SC450 formulation: Comparative *in vitro* dermal absorption study using human and rat skin
Report No & Document No	SA 06152 M-279026-01-2
Guidelines:	**OECD Guideline for the Testing of Chemicals; Skin Absorption: In Vitro Method. Guideline 428 (April 2004)**
GLP	**Yes (certified laboratory)**

I. Material and methods

In a 2006 GLP study, the potential dermal penetration of [^{14}C]-AE 0001789 formulated as SC 450 (450 g a.s./L) was investigated at two concentrations corresponding to the neat product and a representative spray dilution (nominal values: 225 mg a.s./mL and 0.27 mg a.s./mL, respectively; applied at a rate of 10 µL/cm²) to excised human and rat dermatomed skin. Human skin was obtained from six different donors obtained from a recognised Tissue Bank. Rat skin was obtained from seven different male Wistar rats.

The skin samples were placed in flow-through diffusion cells (modified Franz cell; exposure area of 1 cm² skin). The receptor fluid was Eagles medium supplemented with 5% bovine serum albumine and gentamycin (50 mg/L, pH 7.3). The integrity of the selected skin samples was assessed by

measuring the trans-epidermal water loss (TEWL) from the stratum corneum (only skin with a TEWL <40 g/m²/h was used).

Samples of the receptor fluid were collected at 1-hour intervals for the duration of the experiment (24 hours). At 8 hours post-application, the skin was swabbed with 1% v/v Tween 80 in PBS (phosphate buffer saline) until no radioactivity was detected on the swabs. At 24 hours post-application the treated skin cell was swabbed again prior to tape-stripping. Tape stripping with Monaderm adhesive tape was performed until a "shiny" appearance of the epidermis indicated that the stratum corneum had been removed. The tape-strips were collected as well as the remaining skin was taken for analysis.

II. Results and discussion

A summary of the study results is given in Table 7.6.2-2.

Table 7.6.2-2: Summary of the in vitro dermal penetration through human and rat epidermal membranes; low and high dose

SC 450	[14C]-AE 0001789			
	Low dose (nom. 0.27 g/L)		High dose (nom. 225 g/L)	
	Human Mean (n = 6)	Rat Mean (n = 5)	Human Mean (n = 5)	Rat Mean (n = 6)
SURFACE COMPARTMENT				
Surface dose (Tape strips 1 & 2)	0.40	4.08	0.74	1.39
Skin swabs at 8 hrs	94.01	87.58	94.70	97.40
Skin swabs at 24 hrs	0.05	0.35	0.10	0.04
Dose remaining in the donor chamber	0.25	0.07	0.90	0.27
Total % non-absorbed	**94.71**	**92.08**	**96.43**	**99.11**
SKIN COMPARTMENT				
Skin a	1.03	0.93	0.20	0.34
Stratum corneum b	0.38	2.75	0.66	1.37
Total % at dose site	**1.41**	**3.68**	**0.85**	**1.70**
RECEPTOR COMPARTMENT				
Receptor fluid (0 to 24 hrs)	0.08	0.42	<0.005	0.06
Receptor fluid terminal	<0.005	0.01	<0.005	<0.005
Receptor chamber	<0.005	<0.005	<0.005	<0.005
Total % directly absorbed	**0.08**	**0.43**	**<0.005**	**0.06**
TOTAL ABSORBABLE				
Total % directly absorbed	0.08	0.43	<0.005	0.06
Total % at dose site	1.41	3.68	0.85	1.70
Total % potentially absorbable c	1.50	4.11	0.85	1.76
Total % recovery d	**96.21**	**96.19**	**97.29**	**100.87**

n: number of skin cells used for calculation
[a]: skin after tape stripping procedure
[b]: tape-strips excluding numbers 1 & 2 which were considered to be non-absorbed dose
[c]: sum of "Totals % directly absorbed" and "Total % at dose site"
[d]: calculated by the DEBRA 5.2a software

The mean total recoveries of radioactivity were > 96 % dose for the human skin and rat skin samples for low dose application. For high dosing the mean total recoveries were > 97 % dose for human skin and rat skin, respectively.

The data demonstrated that the majority of the radioactivity was removed by the swabbing procedure performed at 8 hours post application and by the surface tape-strips. Lower levels of radioactivity were recovered from the dose site for human and rat skin; mean total percentage of the applied radioactivity recovered from the dose site was 1.4 % for human skin and 3.7 % for rat skin (low dose) and 0.9 % for human skin and 1.7 % for rat skin (high dose).
The mean total percentage of the applied radioactivity found to be directly absorbed was 0.08 % dose for the human skin and 0.4 % dose for the rat skin in case of low dosage and < 0.005 % dose and 0.06 % dose for human and rat skin, respectively in case of high dosage.

The radioactivity counted in the skin compartment (skin and stratum corneum) could be considered to be potentially absorbable. The percentage of [^{14}C]-AE 0001789 considered to be potentially absorbable over a period of 24 hours was 1.5 % and 4.1 % for the human and rat skin, respectively, for the diluted product. In case of the neat product potentially 0.9 % and 1.8 % dose could be absorbable for human skin and rat skin, respectively

III. Conclusion

The potentially dermal absorbable fraction for the neat formulation of cyprosulfamide, formulated as a soluble concentrate, is fairly low and in comparable range for both, human and rat skin. Rat skin revealed a penetration higher by a factor of 2.1 than human skin.
For the diluted formulation a more pronounced difference exists. Again, taking into account the potentially absorbable fraction a factor of approx. 2.7 was found.

For estimating systemic exposure the following figures are taken:
0.9 % for the concentrate and 1.5 % for the spray solution.

(Capt. A. 2006)

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable with no deviations from the study protocol. Good recovery data were obtained for all samples, with mean total recoveries of radioactivity in the range of 96.19% to 100.87% of the applied dose. Reported SD values were acceptable (no outliers) and data obtained from some of the cells was excluded only when skin was damaged during swabbing or a technical problem occurred during the collection of the receptor fluid. The radiolabelled test substance had a reported radiochemical purity >98% and an initial specific activity of 4.24 MBq/mg. The study (Capt, A. 2006, SA 06152) is fully compliant with OECD 428 (2004). The dilution tested in the study (0.27 mg AE 0001789/ml) is slightly lower than that specified on the GAP table for the 'SC 450' formulation (0.3 mg/ml), however, this difference is not considered to be significant. The lead cyprosulfamide formulation 'SC 480' has a cyprosulfamide content of 240 g/l for the undiluted product and 0.666 g/l for the spray solution. The dermal absorption values derived from this study are, therefore, likely to be a worst case. The solubility of AE 0001789 in the receptor fluid was demonstrated at a concentration equivalent to the maximum applied dose and was therefore shown to not limit the rate of diffusion. The exclusion of the first two tape strips from the calculation of the absorbed dose is acceptable because the stripped material reflects that likely to be lost by desquamation. The applicant has taken the precautionary approach of including the skin residue (with the exception of the first two tape strips) in the calculation of the absorbed dose although current EU guidance (SANCO/222/2000) states that the skin residue may be discounted when 'serial non-detects' are observed. For human skin membranes, serial non-detects were reported for the concentrate (no radioactivity was detected in the receptor fluid at any time point) and for the dilution (from 15 to 24 hours after exposure). Therefore, it is unlikely that the skin residue would be systemically available. The applicant's dermal absorption values for human skin of 0.85% (0.9%) for the concentrate and 1.5% for the dilution are considered to be appropriate. Results for rat skin show greater absorption than for human skin (1.76% for the concentrate and 4.11% for the dilution) however, in the absence of a rat *in vivo* study, these results are not used to calculate the dermal absorption values.
Conclusions	Based on current EU guidance, dermal absorption of [^{14}C]-AE 0001789 through human skin membranes *in vitro* over a period of 24 hours is considered to be 0.85% for the concentrate (rounded up to 0.9%) and 1.5% for the spray solution. The 'SC 450' formulation tested is considered to be sufficiently similar to the lead cyprosulfamide formulation 'SC 480' to allow the extrapolation of these dermal absorption values. The derivation of these values is based on a precautionary approach, as explained above.

B.6.12.2(a) Dermal absorption, *in vivo* in the rat (IIIA1 7.6.1)

There are no *in vivo* data available for both compounds.

Epidemiology (IIIA1 7.8)

There is no such EU data requirement; (the OECD) point concerned is not covered by or part of an EU point according to Council Directive 91/414/EEC. Hence, such studies were not performed

There is no data requirement in the US for such data. As thiencarbazone-methyl & cyprosulfamide SC450 is a new product, do such data is available from other sources.

Data on formulants (IIIA1 7.9)

Material safety data sheet for each formulant (IIIA1 7.9.1)

The information concerned is included in **Document H**.

Available toxicological data for each formulant (IIIA1 7.9.2)

The information concerned is included in **Document H**.

Domestic animal/livestock safety (IIIA1 7.10)

Such data are currently not required in the EU or the US. Hence, such studies were not performed.

Other/special studies (IIIA1 7.11)

No other special study was necessary or performed.

B.6.11(b) Acute toxicity, irritancy and skin sensitisation of the Plant Protection Product (SC 465) (IIIA 7)

BYH 18636 + AE 0001789 + IFT SC 465 (also called BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L or thiencarbazone-methyl + isoxaflutole + cyprosulfamide SC 90 + 225 + 150 g/L) is an suspension concentrate formulation (SC) containing BYH 18636 (thiencarbazone-methyl) at 90 g/L, isoxaflutole at 225 g/L and AE 0001789 (cyprosulfamide) at 150 g/L.

B.6.11.1(b) Acute oral toxicity (IIIA 7.1.1)

Report:	**KIIIA1 7.1.1/01; Schuengel M., 2006**
Title:	BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L - Acute toxicity in the rat after oral administration
Report No &	AT03049, T7076608
Document No	M-273329-01-2
Guidelines:	**OECD Guidelines N° 423 (2001).** **EEC Directive 67/548/EEC Annex V, B1.tris (1967) in its current version** **EPA (OPPTS 870.1100 – 712-C-98-190), August 1998**
Deviation(s):	The test compound is a product known to be stable and homogenous in both undiluted and in ready-to-use formulation with water. Therefore, analytical determinations of stability and homogeneity of the aqueous formulations were not performed. The deviation does not limit the assessment of the results.
GLP	**Yes**

I. Materials and methods

A. Materials

1. Test material:	BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L
Specification no.:	102000013459
Description:	White milky liquid
Lot/Batch no.:	2005-004304
Content:	BYH 18636: 90 g/L, Isoxaflutole 225 g/L, AE 0001789: 150 g/L (Nominal values) BYH 18636: 85.2 g/L, Isoxaflutole 215 g/L, AE 0001789: 142 g/L (Certified by analysis)
Stability of test compound:	Guaranteed for study duration; expiry date: 16th Dec. 2006
2. Vehicle and/or positive control:	Tap water
3. Test animals	
Species:	Rat, females
Strain:	Wistar (Hsd CpPb:Wu)
Age:	10-12 weeks approximately
Weight at dosing:	172 g – 194 g
Source:	Harlan/Winkelmann GmbH, Borchen, Germany
Acclimatization period:	At least 5 days

Diet: Provimi Kliba 3883.0.15; Kaiseraugst, Switzerland

Water: Tap water *ad libitum*

Housing: The animals were group caged conventionally in polycarbonate cages on low dust wood granulate bedding (J. Rettenmaier & Söhne, 73479 Ellwangen-Holzmühle, Germany). The cages of the animals were placed on racks. The wood granulate was randomly checked for contaminants at regular intervals and the results have been stored at the Department for Laboratory Animal Services, Bayer HealthCare AG, Wuppertal, Germany.
Room temperature: 22 ± 2°C; Air humidity: 55 ± 5%;
Ventilation: approx. 10 changes per hour;
Light/ Dark cycle : 12 hour rhythm

B. Study design and methods

1. Animal assignment and treatment

Dose: 2000 mg/kg bw

Application route: Oral by gavage

Application volume: 10 mL/kg bw

Fasting time: For administration, food was withheld from the animals for approximately 16 - 24 h before administration of the test compound, and they were fed again approximately 2-4 h after administration

Group size: 3 rats/dose group

Post-treatment observation period: 14 days

Observations: Mortality, clinical signs, body weight, gross necropsy

In life dates: 9th March 2006 to 29th March 2006

The test compound was formulated in tap water; and the test material was administered per os in a single dose (2000 mg/kg) by gavage to 3 fasted female Wistar rats. As no mortality occurred, three additional animals were treated with the same dose.

II. Results and discussion

A. Mortality

Mortality was not observed at 2000 mg/kg bw.

Table 7.1.1-1 Doses, mortality / animals treated

Dose (mg/kg bw)	Toxicological results*			Occurrence of signs	Time of death	Mortality (%)
Females						
2000 1st	**0**	**0**	**3**	--	--	**0**
2000 2nd	**0**	**0**	**3**	--	--	**0**
acute oral LD_{50}: ≥ 5000 mg/kg bw**						

*** 1st number = number of dead animals; 2nd number = number of animals with signs; 3rd number = number of animals used; ** according to the principles of OECD Guideline 423**

B. Clinical observations

No clinical signs were observed.

C. Body weight

Body weight and body-weight gain were not affected by the treatment.

D. Necropsy

No particular gross pathological changes were observed in animals sacrificed at the end of the study period.

III. Conclusion

According to the OECD Guideline 423 the acute oral LD_{50} cut-off of BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L formulation in rats is ≥ 5000 mg/kg and as a result is a category 5 product (i.e. unclassified according to the Globally Harmonised Classification System).

According to the EC classification criteria (2001/59/EC Directive), the formulation BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L is labelled as follows:

Symbols of danger : None
Risk phrases : None

According to EPA guidelines, the LD_{50} for this formulation is greater than 2000 mg/kg, which is consistent with the criteria for EPA Toxicity Category III.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: totally reliable The study (T7076608) is fully compliant with OECD 423 (2001) No treatment-related findings were observed in this study.
Conclusions	The acute oral LD50 of the test material in female rats was found to be >2000 mg/kg bw under the conditions of this study. The product is not classified for acute oral toxicity according to current EC criteria.

B.6.11.2(b) Acute percutaneous (dermal) toxicity (IIIA 7.1.2)

Report:	**KIIIA1 7.1.2/02; Schuengel M., 2006**
Title:	BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L - Acute toxicity in the rat after dermal administration
Report No & Document No	AT03067, T8076609 M-279655-01-2
Guidelines:	**OECD Guidelines N° 402 (1987)** **EEC Directive 67/548/EEC Annex V, B.3 (1967)** **EPA (OPPTS 870.1200 – 712-C-98-192, August 1998)**
GLP	**Yes**

I. Materials and methods

A. Materials

1. Test material:	BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L
Specification no.:	102000013459
Description:	White milky liquid
Lot/Batch no.:	2005-004304
Content:	BYH 18636: 90 g/L, Isoxaflutole 225 g/L, AE 0001789: 150 g/L (Nominal values) BYH 18636: 85.2 g/L, Isoxaflutole 215 g/L, AE 0001789: 142 g/L (Certified by analysis)
Stability of test compound:	Guaranteed for study duration; expiry date: 16th Dec 2006
2. Vehicle and/or positive control:	None
3. Test animals	
Species:	Rat, males and females
Strain:	Wistar (Hsd Cpb:WU)
Age:	9 – 13 weeks approximately
Weight at dosing:	Males: 241 g – 250 g Females: 213 g – 220 g
Source:	Harlan/Winkelmann GmbH, Borchen, Germany
Acclimation period:	At least 5 days
Diet:	Provimi Kliba 3883.0.15; Kaiseraugst, Switzerland
Water:	Tap water *ad libitum*
Housing:	The animals were caged individually in polycarbonate cages on low dust wood granulate bedding (J. Rettenmaier & Söhne, 73479 Ellwangen-Holzmühle, Germany). The cages of the animals were placed on racks. The wood granulate was randomly checked for contaminants at regular intervals and the results have been stored at the Department for Laboratory Animal Services, Bayer HealthCare AG, Wuppertal, Germany. Room temperature: 22 ± 2°C; Air humidity: 55 ± 5%; Ventilation: approx. 10 changes per hour; Light/Dark cycle: 12 hour rhythm.

B. Study design and methods

1. Animal assignment and treatment

One day before the start of the treatment the back and flanks of 5 male and 5 female Wistar rats were shorn. They received a single dermal dose of 2000 mg/kg bw of the pure liquid test compound applied semi-occlusively. After an exposure time of 24 hours, the dressings were removed and the treated area was rinsed with tepid water using soap and gently patting the area dry.

	Dose (mg/kg bw)		Surface area (cm²)	Range of doses (mg/cm²)
	males	**2000**	**12.0**	**40.2-41.7**
	females	**2000**	**12.0**	**35.5-36.7**

Application route:	Dermal, semi-occlusive dressing
Duration:	24 hours
Group size:	5 rats/sex/group
Post-treatment observation period:	14 days
Observations:	Mortality, clinical signs, skin effects, body weight, gross necropsy
In life dates:	9th March 2006 to 23rd March 2006

II. Results and discussion

A. Mortality

Mortality was not observed at 2000 mg/kg bw.

Table 7.1.2-1 Doses, mortality / animals treated

Dose (mg/kg bw)	Toxicological results*			Duration of signs	Time of death	Mortality [%]
Males						
2000	**0**	**0**	**5**	**--**	**--**	**0**
Females						
2000	**0**	**0**	**5**	**--**	**--**	**0**
acute dermal LD_{50}: > 2000 mg/kg bw						

*** 1st number = number of dead animals:; 2nd number = number of animals with signs; 3rd number = number of animals in the group**

B. Clinical observations

No clinical signs were observed.

C. Body weight

Body weight and body weight gain was not affected by treatment in males or females.

D. Necropsy

No particular gross pathological changes were observed in animals sacrificed at the end of the study period.

III. Conclusion

The dermal LD_{50} of the formulation BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L was greater than 2000 mg/kg bw for rats.

According to the EC classification criteria (2001/59/EC Directive), the formulation BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L is labelled as follows:

Symbols of danger : None
Risk phrases : None

The dermal LD_{50} of the formulation BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L is greater than 2000 mg/kg body weight for both male and female rats, which is consistent with the criteria for EPA Tox Category III.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: totally reliable The study (T8076609) is fully compliant with OECD 402 (1987) Minimal weight loss was seen in one female rat during the first week of the study, however all animals gained weight over the study period. No additional treatment-related findings were observed in this study.
Conclusions	The acute dermal LD50 of the test material in female rats was found to be >2000 mg/kg bw under the conditions of this study. The product is not classified for acute dermal toxicity according to current EC criteria.

B.6.11.3(b) Acute inhalation toxicity to rats (IIIA 7.1.3)

Report: **KIIIA1 7.1.3/01; Pauluhn J., 2006**
Title: BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L - Acute inhalation toxicity in the rat
Report No &: AT03363, T2076810
Document No: M-279825-01-2
Guidelines: **OECD Guidelines N° 403 (12 May 1981)**
EEC Directive 92/69/EEC Annex V – Method B2 (1992)
EPA: OPPTS 870.1300 (1998)
MAFF, notification 12 Nousan-8147 (2000)
GLP **Yes**

I. Materials and methods

1. Test material: BYH 18636 + Isoxaflutole + AE 0001789
SC 90 + 225 + 150 g/L

Specification no.: 102000013459

Description: White milky liquid

Lot/Batch no.: 2006-004041

Content: BYH 18636: 90 g/L, Isoxaflutole 225 g/L, AE 0001789: 150 g/L (Nominal values)
BYH 18636: 86.2 g/L, Isoxaflutole 214 g/L, AE 0001789: 144 g/L (Certified by analysis)

Stability of test compound: Guaranteed for study duration; expiry date: 8th May 2007

2. Vehicle and/or positive control: The test article was aerosolised as aqueous solution.

3. Test animals

Species: Rat, males and females

Strain: SPF Wistar (Hsd Cpb:WU)

Age: Approximately two months

Weight at dosing: Males: 186 g – 196 g
Females: 179 g – 188 g

Source: Harlan/Winkelmann GmbH, Borchen, Germany

Acclimation period: At least 5 days

Diet: Provimi Kliba 3883 9441 pellets, Kaiseraugst, Switzerland

Water: tap water *ad libitum*

Housing: During the acclimation and study periods, the animals were housed singly in conventional Makrolon® Type III cages (based on A. Spiegel and R. Gönnert, Zschr. Versuchstierkunde, 1, 38 (1961) and G. Meister, Zschr. Versuchstierkunde, 7, 144-153 (1965)). Cages were changed twice a week while unconsumed feed and water bottles were changed once per week. The legal requirements for housing experimental animals (Directive 86/609 EEC) were followed. Bedding consisted of type BK8/15 low-dust wood granulate from Ssniff, Soest/Westfalen, Germany. The wood granulate was randomly checked for harmful constituents at the request of the Laboratory Animal Services, Bayer Healthcare AG. Room temperature: 22 ± 2°C; Air humidity: 40-60%; Ventilation: approx. 10 changes per hour; Light/Dark cycle: 12 hour rhythm.

B. Study design and methods

1. Animal assignment and treatment

One group of 10 Wistar rats (5 animals/sex) was exposed to mean aerosol concentration of 2.607 mg/L for up to 4 hours using nose only exposure system. Attempts were made so that liquid aerosol generated was respirable to rats. The test item was aerosolised undiluted.

Dose: 0 – 2.607 mg/L air (maximum technically attainable concentration)

Application route:	Inhalation (nose-only exposure)
Duration:	4 hours
Group size:	5 rats/dose/sex
Post-treatment observation period:	14 days
Observations:	Mortality, clinical signs, body weights, rectal temperature, reflex measurements, gross necropsy
In life dates:	10th July 2006 to 24th July 2006

2. Generation of the test atmosphere / chamber description

Generation and characterization of chamber atmosphere

Target concentration (mg/L)	Nominal concentration (mg/L)	Mean achieved concentration (mg/L)	Mean mass Aerodynamic Diameter (µm)	Geometric standard deviation (µm)	Respirable fraction (% < 3 µm)
5.000	18.544	2.607	3.4	2.57	45

II. Results and discussion

A. Mortality

Mortality was not observed at 2.607 mg/L air.

Table 7.1.3-1 Doses, mortality / animals treated

Actual concentration (mg/L air)	Toxicological results*			Duration of signs	Time of death	Mortality [%]
Males						
0	0	0	5	--	--	0
2.607	0	0	5	--	--	0
Females						
0	0	0	5	--	--	0
2.607	0	0	5	--	--	0
acute inhalative LC_{50}: > 2.607 mg/L air						

* 1st number = number of dead animals; 2nd number = number of animals with signs; 3rd number = number of animals exposed

B. Clinical observations

All rats tolerated the exposure without specific signs.
Reflex measurements: no exposed rats exhibited changes in reflexes.
Rectal temperature: no significant changes.

C. Body weight

Body weights: no significant changes.

D. Necropsy

Effects on organs: necropsy findings were unremarkable in rats sacrificed at the end of the observation period.

III. Conclusion

The inhalation LC_{50} of the formulation BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L for male and female Wistar rats was higher than 2.607 mg/L, the highest attainable concentration.

According to the EC classification criteria (2001/59/EC Directive), the formulation BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L is labelled as follows:

Symbols of danger : None
Risk phrases : None

The acute inhalation LC_{50} of the test substance for males and females is greater than 2.607 mg/L, which is consistent with the criteria for EPA Tox Category IV.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable The study (T2073810) is fully compliant with OECD 403 (1987) The Notifier's summary states that no significant effects were observed on rectal temperature, however the mean rectal temperature of the exposed group of females (37.2°C) was statistically significantly ($p<0.05$) lower than that of control females (38.2°C). The mean rectal temperature of the exposed group of males (37.7°C) was comparable to the control males (38.2°C). Findings indicate that the test material may be a mild respiratory irritant, however the absence of clinical signs is notable.
Conclusions	The acute inhalation LC_{50} of the test material in the rat was found to be >2.607 mg/l, stated to be the maximum concentration technically achievable under the conditions of this study. The product is therefore not classified for acute inhalation toxicity according to current EC criteria. The slight (but statistically significant) reduction in body temperature seen in treated females indicates that the product may be a mild respiratory irritant, however classification of the product as a respiratory irritant is not considered to be appropriate in the absence of any correlating clinical signs.

B.6.11.4(b) Skin irritation (IIIA 7.1.4)

Report: **KIIIA1 7.1.4/01, Gmelin, C.; 2006**
Title: BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L – Acute skin irritation/corrosion on rabbits
Report No & Document No: AT03245, T9076583
M-278731-01-2
Guidelines: **OECD: No. 404, 24th April 2002**
EEC Directive 67/548 Annex V – Method B.4 (1967) in its current version
EPA OPPTS 870.2500 United States EPA 712-C-98-196 (1998)
GLP **Yes**

I. Materials and methods

A. Materials

1. Test material:	BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L
Article no.:	102000013459
Description:	White milky liquid
Lot/Batch no:	2005-004041
Content:	BYH 18636: 90 g/L, Isoxaflutole 225 g/L, AE 0001789: 150 g/L (Nominal values) BYH 18636: 86.2 g/L, Isoxaflutole 214 g/L, AE 0001789: 144 g/L (Certified by analysis)
Stability of test compound:	Guaranteed for study duration; expiry date: 8th May 2007
2. Vehicle and/or positive control:	None
3. Test animals	
Species:	Rabbit, females
Strain:	Albino (Crl:KBL(NZW)BR)
Age:	Young adult animals
Weight at dosing:	2.7 kg – 3.1 kg
Source:	Charles River, Kißlegg, Germany
Acclimation period:	At least 5 days
Diet:	Ssniff K-Z, Soest, Germany
Water:	Tap water *ad libitum*
Housing:	The animals were housed individually in cage units Metall/Noryl by EBECO. Excrement trays below the cages contained low dust wood granulate bedding (J. Rettenmaier & Söhne, 73479 Ellwangen-Holzmühle, Germany). The wood granulate was changed at least twice weekly. The animals were regularly transferred to clean cages. The animal room had a standardized climate: Room temperature: 20 ± 3°C; Air humidity: 50 ± 25%; Light/ Dark cycle: 12 hour rhythm.

B. Study design and methods

1. Animal assignment and treatment

Doses of 0.5 mL of the undiluted test item were placed on a dry gauze pad which was then applied to the clipped, intact skin of three rabbits for 4 hours. The treatment site was observed shortly after the end of the exposure period then daily for up to 72h.

Dose:	0.5 mL pure liquid test substance/animal
Application route:	Dermal (semi occlusive procedure)
Duration:	4 hours
Group size:	3 rabbits
Observations:	Clinical signs, skin effects, body weight (at beginning of study)
In life dates:	27th June 2006 to 30th June 2006

II. Results and discussion

A. Findings

Under the present test conditions the following findings were noted:
There were no systemic intolerance reactions.

Table 7.1.4-1 Summary of irritant effects (Scores)

Rabbit number and sex	E or O	Scores Time after application						Mean score 24-48-72h	Response	Reversibility (days)
		1h	24h	48h	72h	D7	D14			
1 F	E	0	0	0	0	-	-	0.0	(-)	NA
	O	0	0	0	0	-	-	**0.0**	(-)	NA
2 F	E	0	0	0	0	-	-	**0.0**	(-)	NA
	O	0	0	0	0	-	-	**0.0**	(-)	NA
3 F	E	0	0	0	0	-	-	**0.0**	(-)	**NA**
	O	0	0	0	0	-	-	**0.0**	(-)	**NA**

E = erythema, O = oedema
No positive response (-): mean score 24-48-72h < 2 Positive response (+): mean score 24-48-72h ≥ 2
NA = not applicable, - examination not performed
*: in respect of the result 1h post application

III. Conclusion

It was concluded that BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L was not irritating to the rabbit skin.

According to the criteria for classification defined in the Directive 2001/59/EC, the formulation BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L is labelled as follows:

Symbols of danger : None
Risk phrases : None

According to classification criteria, BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L is not a dermal irritant, which is consistent with the criteria for EPA Tox Category IV.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: Totally reliable The study (T9076583) is fully compliant with OECD 404 (2002) (Note that the Test Material: Lot/Batch no. used in this study was 2006-004041 not 2005-004041)
Conclusions	The test material was found to be non-irritating to skin under the conditions of this study. The product is therefore not classified according to current EC criteria.

B.6.11.5(b) Eye Irritation (IIIA 7.1.5)

Report: **KIIIA1 7.1.5/01, Gmelin, C., 2006**
Title: BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L – Acute eye irritation in rabbits
Report No & Document No: AT03244, T0076584 / M-277838-01-2
Guidelines: **OECD: No. 405, 24th April 2002**
EEC Directive 67/548 Annex V – Method B.5 (1967)
EPA OPPTS 870.2400, United States EPA 712-C-98-195 (1998)
GLP **Yes**

I. Materials and methods

A. Materials

1. Test material: BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L

Specification no.: 102000013459

Description: White milky liquid

Lot/Batch no: 2005-004041

Content: BYH 18636: 90 g/L, Isoxaflutole 225 g/L, AE 0001789: 150 g/L (Nominal values)
BYH 18636: 86.2 g/L, Isoxaflutole 214 g/L, AE 0001789: 144 g/L L (Certified by analysis)

Stability of test compound: Guaranteed for study duration; expiry date: 8th May 2007

2. Vehicle and/or positive control: None

3. Test animals

Species: Rabbit, females

Strain: Albino (Crl:KBL(NZW)BR)

Age: Young adult animals

Weight at dosing: 2.7 kg – 2.9 kg

Source: Charles River, Kißlegg, Germany

Acclimation period: At least 5 days

Diet: Ssniff K-Z, Soest, Germany

Water: Tap water *ad libitum*

Housing:	The animals were housed individually in cage units Metall/Noryl by EBECO. Excrement trays below the cages contained low dust wood granulate bedding (J. Rettenmaier & Söhne, 73479 Ellwangen-Holzmühle, Germany). The wood granulate was changed at least twice weekly. The animal room had a standardized climate: Room temperature: 20 ± 3°C; Air humidity: 50 ± 25%; Light/Dark cycle: 12 hour rhythm.

B. Study design and methods

1. Animal assignment and treatment

A single dose of 0.1 mL of the undiluted test item was instilled under the lower lid of the left eye of one rabbit. The right eye was untreated and served as the control. The eyes were not rinsed after administration of the test item. Since the test item was not severely irritant on the first animal, it was then evaluated in two other animals. Ocular reactions were observed approximately 1 hours, 24, 48 and 72 hours after instillation and then daily until the end of the observation period.

Dose:	0.1 mL pure liquid test substance/animal
Application route:	Instillation into the conjunctival sac of one eye. The eye was not rinsed for at least 24 hours following instillation.
Group size:	3 rabbits
Observations:	Clinical signs, eye effects, body weight (at beginning of study)
In life dates:	4th July 2006 to 7th July 2006

II. Results and discussion

A. Findings

Under the present test conditions the following findings were noted:

The individual findings of the treated eyes at the various observation times are summarized in the table below. The control eyes did not show any abnormal findings and are not listed in the Table 7.1.5-1. One hour after application, the test compound adhered to conjunctivae of animals 1 and 3.

Table 7.1.5-1 Summary of Irritant Effects (Scores)

Animal N° Sex	Parameter	1h	24h	48h	72h	Index* 24-48-72h	Reversible (days)
1 F	**Chemosis**	**0**	**0**	**0**	**0**	**0.0 (-)**	**NA**
	Conjunctival redness	**2**	**1**	**0**	**0**	**0.3 (-)**	**2**
	Iris lesions	**0**	**0**	**0**	**0**	**0.0 (-)**	**NA**
	Corneal opacity	**0**	**0**	**0**	**0**	**0.0 (-)**	**NA**
2 F	**Chemosis**	**0**	**0**	**0**	**0**	**0.0 (-)**	**NA**
	Conjunctival redness	**2**	**2**	**0**	**0**	**0.7 (-)**	**2**
	Iris lesions	**0**	**0**	**0**	**0**	**0.0 (-)**	**NA**
	Corneal opacity	**0**	**0**	**0**	**0**	**0.0 (-)**	**NA**
3 F	**Chemosis**	**1**	**0**	**0**	**0**	**0.0 (-)**	**NA**
	Conjunctival redness	**2**	**1**	**0**	**0**	**0.3 (-)**	**2**
	Iris lesions	**0**	**0**	**0**	**0**	**0.0 (-)**	**NA**
	Corneal opacity	**0**	**0**	**0**	**0**	**0.0 (-)**	**NA**

* = Mean of scores on 24-48-72h instillation

Response:
corneal opacity: mean scores <2 = (-), ≥2<3 = (+), ≥3 = (++)
Iritis: mean scores <1 = (-), ≥1<2 = (+), =2 = (++)
Conjunctival redness: mean scores <2.5 = (-), ≥2.5 = +
Conjunctival oedema: mean scores <2 = (-), ≥ 2 = +
NA = not applicable

III. Conclusion

It was concluded that BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 +150 g/L was not irritating to the rabbit eye.

According to the criteria for classification defined in the Directive 2001/59/EC, the formulation is labelled as follows:

Symbols of danger : None
Risk phrases : None

According to OECD classification criteria, BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 +150 g/L is not irritating to eyes. Based on the EPA guidelines, the formulation is in EPA Tox Category III.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: Totally reliable The study (T0076584) is fully compliant with OECD 405 (2002) (Note that the Test Material: Lot/Batch no. used in this study was 2006-004041 not 2005-004041)

Conclusions	The test material was found to be a slight eye irritant under the conditions of this study and is not classified as an eye irritant according to current EC criteria.

B.6.11.6(b) Skin sensitization (IIIA 7.1.6)

Report:	**KIIIA1 7.1.6/01 Repetto-Larsay, M., 2006**
Title:	BYH 18636 + IFT + AE 0001789 SC 90 + 225 + 150 g/L - Evaluation of potential dermal sensitization in the local lymph node assay in the mouse.
Report No.:	SA 06027
Doc. No.:	M-271415-01-2
Guidelines:	**O.E.C.D. Guideline 429 (2002)**
GLP	**Yes**

I. Materials and methods

A. Materials

1. Test material:	BYH 18636 + Isoxaflutole + AE 0001789 SC 90 + 225 + 150 g/L
Specification no.:	102000013459
Description:	White milky liquid
Lot/Batch no:	2005-004304
Content:	BYH 18636: 90 g/L, Isoxaflutole 225 g/L, AE 0001789: 150 g/L (Nominal values) BYH 18636: 85.2 g/L, Isoxaflutole 215 g/L, AE 0001789: 142 g/L L (Certified by analysis)
Stability of test compound:	Guaranteed for study duration; expiry date: 16th Dec 2006
2. Vehicle and/or positive control:	Vehicle: Water containing 1% Pluronic Acid was selected to ensure compatibility with the test substance and maximum wetting of the mouse ears with the maximum possibility of skin penetration of the various formulation ingredients. A positive control group received 0.25% p-benzoquinone in 25% BYH 18636 + Isoxaflutole + AE 0001789 SC 465 and 75% Pluronic acid at 1% in water. The positive control was spiked in the formulation to ensure that under the conditions of this assay, the study demonstrated appropriate sensitivity with the positive control.

3. Test animals

Species:	Mice, females
Strain:	CBA/J
Age:	At least 8 weeks old
Source:	R. Janvier, le Genest St Isle, France
Acclimation period:	At least 5 days

Diet:	Certified rodent pellet diet: AO4C-10, S.A.F.E. (Scientific Animal Food and Engineering, Route de Saint Bris, Augy, France)
Water:	Tap water *ad libitum*
Housing:	During the study period the animals were individually housed in suspended, stainless steel, wire mesh cages. Room temperature: 20-24°C Humidity: 40 - 70 %; Light/dark cycle: Twelve hours rhythm Air exchange rate: 10-15 times per hour.

B. Study design and methods

2. Animal assignment and treatment

Twenty-four female CBA/J mice were allocated to 6 groups of four animals each and these were treated as outlined below:

- Four groups received the test substance at a concentration of 10, 25, 50 or 100% in vehicle (aqueous pluronic acid at 1%);
- A positive control group received 0.25% p-benzoquinone in 50% BYH 18636 + IFT + AE 0001789 SC 90 + 225 + 150 g/L and 50% Pluronic acid at 1% in water. The positive control was spiked in the formulation to ensure that under the conditions of this assay, the study demonstrated appropriate sensitivity with the positive control;
- A control group received the vehicle, 1% pluronic acid in water.

Observations:	Mortality, clinical signs, skin effects, body weight (at beginning and termination of study)
In life dates:	24th March 2006 to 5th April 2006

The test substance, positive control or the vehicle were applied on external surfaces of each ear (25 µL/animal) for three consecutive days (Days 0, 1 and 2) at the appropriate concentrations. On Day 5, the cell proliferation in the local lymph nodes was measured by incorporation of tritiated thymidine and the obtained values were used to calculate proliferation indices.

II. Results and discussion

A. Findings

Mortality and clinical signs - No mortality and no clinical signs were observed during the study. No cutaneous reactions were observed at the treated site for the negative control, positive control or BYH 18636 + IFT + AE 0001789 SC 90 + 225 + 150 g/L treated groups.

Body weights - No significant body weight changes were observed during the study either in the control or in the treated groups.

The proliferation index values of the test substance were 1.6, 1.2, 1.5 and 2.3 at treatment concentrations of 10, 25, 50 and 100% respectively.

The proliferation index value of the positive control was 14.8 at treatment concentration of 0.25% of p-Benzoquinone

GROUP	TEST SUBSTANCE(S)	# OF ANIMALS	CONCENTRATION % DAYS 0-2	DPM/NODE	STIMULATION INDEX (SI)
1	**Vehicle control**	**4**	**0**	**394**	
2	**BYH 18636 + Isoxaflutole AE 0001789 SC 465**	**4**	**10**	**614**	**1.6**
3		**4**	**25**	**482**	**1.2**
4		**4**	**50**	**607**	**1.5**
5		**4**	**100**	**924**	**2.3**
6	**p-Benzoquinone**	**4**	**0.25**	**5826**	**14.8**

*** DPM = disintegrations per minute**
**** SI = DPM of treated group / DPM of control group**

Negative lympho-proliferative responses (SI<3) were noted for BYH 18636 + IFT + AE 0001789 SC 90 + 225 + 150 g/L at all concentrations tested. In the positive control group given p-Benzoquinone, a SI value >3 was noted. This positive response to p-Benzoquinone demonstrates the validity of this assay under the current condition using the specific test formulation.

III. Conclusion

As no stimulation index value was over 3 for the treated groups and as no dose-related effect was noticed, BYH 18636 + IFT + AE 0001789 SC 90 + 225 + 150 g/L was found to be not a sensitizing formulation in the Local Lymph Node Assay.

According to the commission directive 2001/59/EC, the test substance BYH 18636 + IFT + AE 0001789 SC 90 + 225 + 150 g/L is labeled as follows:

Symbols of danger : None
Risk phrases : None

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: Totally reliable The study (SA 06027) is fully compliant with OECD 429 (2002) Some evidence of weak sensitisation (stimulation index of 2.3) was seen at the highest concentration (100%) of the test material, however a concentration response is not apparent and the stimulation index is not sufficiently high (stimulation index of ≥3). The response to the positive control compound is slightly high, but is not considered to be excessive and therefore meets the requirements of the guideline.
Conclusions	Evidence of weak sensitisation was seen under the conditions of this study, however the findings are not of sufficient magnitude to trigger classification according to current EC criteria. EPA: The 2003 OPPTS harmonized guidelines state the "LLNA is the preferred method, where applicable." The "where applicable" correlates to the performance parameters in the appendix — the 1999 ICCVAM report. In 1999, ICCVAM validated the method using 209 "single compound compounds" but did not validate the assay for mixtures. The appendix clearly states the LLNA should not be used for metals, aqueous solutions, and mixtures. In January 2008, ICCVAM updated the validation report on LLNA regarding mixtures, metals and aqueous solutions. ICCVAM findings are that when compared to the guinea pig it has a false negative rate of 50%, a false positive rate of 44%, and accuracy rate of 53% in mixtures. Due to these limitations, EPA questioned whether the negative result found in this study is correct. EPA decided to perform a weight of the evidence approach for this joint review chemical by obtaining information on each component (inerts ingredient) in this mixture. After reviewing the components sensitization potential, EPA determined that most were non-sensitizers and other components were assumed to be below the sensitizing threshold. Therefore, to avoid further animal testing EPA will classify the study as "supplemental" and recommend label language as a non-sensitizer for SC 465[BYH 18636 + AE 0001789 + IFT SC 465].

B.6.11.7(b) Supplementary studies for combinations of plant protection products (IIIA1 7.1.7)

None.

B.6.11.8(b) Short-term toxicity studies (IIIA1 7.2)

Not required by Directive 91/414/EEC or by EPA.

B.6.12(b) Dermal absorption studies 'SC 465' (IIIA 7.3)

The extent of dermal absorption of thiencarbazone-methyl and cyprosulfamide formulated as a SC 450 formulation was investigated *in vitro* on human and rat skin, whereas for the active substance isoxaflutole an *in vivo* rat study is available. A summary of the studies and conclusion on dermal absorption is given below.

a) Summary and conclusion on dermal absorption of thiencarbazone-methyl

In vivo data
No in vivo data available.

In vitro data

For thiencarbazone-methyl a dermal absorption of 2.5 % is considered to be appropriate to calculate for Europe systemic exposure from dermal contamination with the in-use spray concentration. For the concentrate, the comparative *in vitro* dermal absorption study resulted in a dermal absorption value of 0.3 %.

b) Summary and conclusion on dermal absorption of isoxaflutole
In vivo data
There are not data for an isoxaflutole SC formulation available, but data on the active ingredient have been generated. Such data are considered more suitable than default values of 100% or 10%. Due to the limitation of the *in vivo* rat study i.e. 24 h sacrifice time, the skin direct and skin indirect (absorbed dose plus quantity present in the skin) are considered for risk assessment. The figures are for direct dermal absorption 0.2 and 4.5 % (for the concentrate and the dilution, respectively) and 2.3 and 16.4 % (for the concentrate and the dilution, respectively) for indirect dermal absorption.

In vitro data
No *in vitro* data available.

c) Summary and conclusion on dermal absorption of cyprosulfamide
In vivo data
No in vivo data available.

In vitro data
For cyprosulfamide a dermal absorption of 1.5 % is considered to be appropriate to calculate for Europe systemic exposure from dermal contamination with the in-use spray concentration. For the concentrate, the comparative *in vitro* dermal absorption study resulted in a dermal absorption value of 0.9 %.

B.6.12.1(b) Comparative dermal absorption, *in vitro* using rat and human skin (IIIA1 7.6.2)

a) Dermal absorption of thiencarbazone-methyl, *in vitro*

Report:	**KIIIA1 7.6.2/01, Capt. A. (2006)**
Title:	[^{14}C]-BYH 18636 in SC450 formulation: Comparative *in vitro* dermal absorption study using human and rat skin
Report No & Document No	SA 06151 M-277416-01-2
Guidelines:	OECD Guideline for the Testing of Chemicals; Skin Absorption: In Vitro Method. Guideline 428 (April 2004)
GLP	Yes (certified laboratory)

I Material and methods

In a 2006 GLP study, the potential dermal penetration of [^{14}C]-BYH 18636 formulated as SC 450 (450 g a.s./L) was investigated at two concentrations corresponding to the neat product and a representative spray dilution (nominal values: 225 mg a.s./mL and 0.27 mg a.s./mL, respectively; applied at a rate of 10 µl/cm²) to excised human and rat dermatomed skin. Human skin was obtained from seven different donors obtained from a recognised Tissue Bank. Rat skin was obtained from ten different male Wistar rats.

The skin samples were placed in flow-through diffusion cells (modified Franz cell; exposure area of 1 cm² skin). The receptor fluid was Eagles medium supplemented with 5% bovine serum albumine and gentamycin (50 mg/L, pH 7.3). The integrity of the selected skin samples was assessed by measuring the trans-epidermal water loss (TEWL) from the stratum corneum (only skin with a TEWL <40 g/m²/h was used).

Samples of the receptor fluid were collected at 1-hour intervals for the duration of the experiment (24 hrs). At 8 hours post-application, the skin was swabbed with 1 % v/v Tween 80 in PBS (phosphate buffer saline) until no radioactivity was detected on the swabs. At 24 hours post-application the treated skin cell was swabbed again prior to tape-stripping. Tape stripping with Monaderm adhesive tape was performed until a "shiny" appearance of the epidermis indicated that the stratum corneum had been removed. The tape-strips were collected as well as the remaining skin was taken for analysis.

II Results and discussion

An overview of the results is given in the following table.

Table 7.6.2-1: Summary of the in vitro dermal penetration through human and rat epidermal membranes; low and high dose

SC 450	[^{14}C]-BYH 18636			
	Low dose Mean (n = 5)		**High dose** Mean (n = 5)	
	Human	**Rat**	**Human**	**Rat**
SURFACE COMPARTMENT				
Surface dose (Tape strips 1 & 2)	1.06	1.67	0.70	0.21
Skin swabs at 8 hrs	88.20	80.75	93.06	93.20
Skin swabs at 24 hrs	0.71	1.03	0.10	0.01
Dose remaining in the doner chamber	0.03	0.13	0.05	0.15
Total % non-absorbed	**90.01**	**83.59**	**93.91**	**93.57**
SKIN COMPARTMENT				
Skin [a]	1.23	1.12	0.14	0.07
Stratum corneum [b]	0.88	3.23	0.20	0.46
Total % at dose site	**2.11**	**4.35**	**0.34**	**0.53**
RECEPTOR COMPARTMENT				
Receptor fluid (0 to 24 hrs)	0.39	2.32	<0.005	0.01
Receptor fluid terminal	<0.005	0.04	<0.005	<0.005
Receptor chamber	<0.005	<0.005	<0.005	<0.005
Total % directly absorbed	**0.39**	**2.36**	**<0.005**	**0.01**
TOTAL ABSORBABLE				
Total % directly absorbed	0.39	2.36	<0.005	0.01
Total % at dose site	2.11	4.35	0.34	0.53

Total % potentially absorbable [c]	**2.50**	**6.71**	**0.34**	**0.54**
Total % recovery [d]	**92.51**	**90.29**	**94.25**	**94.11**

n: number of skin cells used for calculation
[a]: skin after tape stripping procedure
[b]: tape-strips excluding numbers 1 & 2 which were considered to be non-absorbed dose
[c]: sum of "Totals % directly absorbed" and "Total % at dose site"
[d]: calculated by the DEBRA 5.2a software

The mean total recoveries of radioactivity were 93 % dose for the human skin and 90 % dose for the rat skin samples for low dose application. For high dosing the mean total recoveries were > 94 % dose for human skin and rat skin, respectively.

The data demonstrated that the majority of the radioactivity was removed by the swabbing procedure performed at 8 hours post application and by the surface tape-strips. Lower levels of radioactivity were recovered from the dose site for human and rat skin; mean total percentage of the applied radioactivity recovered from the dose site was 2.1 % for human skin and 4.4 % for rat skin (low dose) and 0.3 % for human skin and 0.5 % for rat skin (high dose).

The mean total percentage of the applied radioactivity found to be directly absorbed was 0.4 % dose for the human skin and 2.4 % dose for the rat skin in case of low dosage and <0.005 % dose and 0.01 % dose for human and rat skin, respectively in case of high dosage.
The radioactivity counted in the skin compartment (skin and stratum corneum) could be considered to be potentially absorbable. The percentage of [^{14}C]-BYH 18636 considered to be potentially absorbable over a period of 24 hours was 2.5 % and 6.7 % for the human and rat skin, respectively, for the diluted product. In case of the neat product potentially 0.3 % and 0.5 % dose could be absorbable for human skin and rat skin, respectively.

III Conclusion
These data show that the potentially dermal absorbable fraction for the neat formulation of thiencarbazone-methyl, formulated as a soluble concentrate, is fairly low and in comparable range for both, human and rat skin. Rat skin revealed a penetration higher by a factor of 1.6 than human skin. For the diluted formulation a more pronounced difference exists. Again, taking into account the potentially absorbable fraction a factor of approx. 2.7 was found.

For estimating systemic exposure the following figures are taken:
0.3 % for the concentrate and 2.5 % for the spray solution.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's comments	Reliability rating: Totally reliable The study is fully compliant with OECD 428 (2004). Adequate solubility of the test material in the receptor fluid was demonstrated. The dermal absorption values proposed by the Notifier are agreed, however the derivation of these values is considered to be conservative, as discussed below. The exclusion of the first two tape strips from calculation of the dermal absorption values is considered to be acceptable is this material is likely to be lost by desquamation.

	The Notifier proposes that the total skin residue (with the exception of the first two tape strips) is included in the dermal absorption vales. Current EU guidance (SANCO/222/2000) states that this material may be discounted when 'serial non-detects' are observed. For human skin membranes, serial non-detects are apparent for the product concentrate (no radioactivity detected in the receptor fluid at any time point) and for the diluted product (21-24 hours). It would therefore seem unlikely that the skin residue would be systemically available. Results for rat skin indicate greater absorption (0.5 and 6.7% for the concentrate and dilution respectively), however these are not used for calculation of the dermal absorption values in the absence of an *in vivo* study.
Conclusions	In line with current EU guidance, dermal absorption values for thiencarbazone-methyl from the 'SC 450' product of 0.3% for the concentrate and 2.5% for the dilution can be calculated, based on the values from human skin membranes *in vitro*. The 'SC 465' product is considered to be sufficiently comparable to the 'SC 450' product used in this study to allow the extrapolation of dermal absorption values. The derivation of these values is noted to be relatively conservative.

b) Dermal absorption of cyprosulfamide, *in vitro*

Report:	**KIIIA1 7.6.2/02, Capt. A. (2006)**
Title:	[^{14}C]-AE 0001789 in SC450 formulation: Comparative *in vitro* dermal absorption study using human and rat skin
Report No &	SA 06152
Document No	M-279026-01-2
Guidelines:	OECD Guideline for the Testing of Chemicals; Skin Absorption: In Vitro Method. Guideline 428 (April 2004)
GLP	Yes (certified laboratory)

I Material and methods

In a 2006 GLP study, the potential dermal penetration of [^{14}C]-AE 0001789 formulated as SC 450 (450 g a.s./L) was investigated at two concentrations corresponding to the neat product and a representative spray dilution (nominal values: 225 mg a.s./mL and 0.27 mg a.s./mL, respectively; applied at a rate of 10 µl/cm²) to excised human and rat dermatomed skin. Human skin was obtained from six different donors obtained from a recognised Tissue Bank. Rat skin was obtained from seven different male Wistar rats.

The skin samples were placed in flow-through diffusion cells (modified Franz cell; exposure area of 1 cm² skin). The receptor fluid was Eagles medium supplemented with 5% bovine serum albumine and gentamycin (50 mg/L, pH 7.3). The integrity of the selected skin samples was assessed by measuring the trans-epidermal water loss (TEWL) from the stratum corneum (only skin with a TEWL <40 g/m²/h was used).

Samples of the receptor fluid were collected at 1-hour intervals for the duration of the experiment (24 hrs). At 8 hours post-application, the skin was swabbed with 1% v/v Tween 80 in PBS (phosphate buffer saline) until no radioactivity was detected on the swabs. At 24 hours post-application the treated skin cell was swabbed again prior to tape-stripping. Tape stripping with Monaderm adhesive tape was performed until a "shiny" appearance of the epidermis indicated that the stratum corneum had been removed. The tape-strips were collected as well as the remaining skin was taken for analysis.

II Results and discussion

A summary of the study results is given in the following table.

Table 7.6.2-2: Summary of the in vitro dermal penetration through human and rat epidermal membranes; low and high dose

SC 450	[^{14}C]-AE 0001789			
	Low dose		**High dose**	
	Human Mean (n = 6)	**Rat** Mean (n = 5)	**Human** Mean (n = 5)	**Rat** Mean (n = 6)
SURFACE COMPARTMENT				
Surface dose (Tape strips 1 & 2)	0.40	4.08	0.74	1.39
Skin swabs at 8 hrs	94.01	87.58	94.70	97.40
Skin swabs at 24 hrs	0.05	0.35	0.10	0.04
Dose remaining in the donor chamber	0.25	0.07	0.90	0.27
Total % non-absorbed	**94.71**	**92.08**	**96.43**	**99.11**
SKIN COMPARTMENT				
Skin [a]	1.03	0.93	0.20	0.34
Stratum corneum [b]	0.38	2.75	0.66	1.37
Total % at dose site	**1.41**	**3.68**	**0.85**	**1.70**
RECEPTOR COMPARTMENT				
Receptor fluid (0 to 24 hrs)	0.08	0.42	<0.005	0.06
Receptor fluid terminal	<0.005	0.01	<0.005	<0.005
Receptor chamber	<0.005	<0.005	<0.005	<0.005
Total % directly absorbed	**0.08**	**0.43**	**<0.005**	**0.06**
TOTAL ABSORBABLE				
Total % directly absorbed	0.08	0.43	<0.005	0.06
Total % at dose site	1.41	3.68	0.85	1.70
Total % potentially absorbable [c]	**1.50**	**4.11**	**0.85**	**1.76**
Total % recovery [d]	**96.21**	**96.19**	**97.29**	**100.87**

n: number of skin cells used for calculation
[a]: skin after tape stripping procedure
[b]: tape-strips excluding numbers 1 & 2 which were considered to be non-absorbed dose
[c]: sum of "Totals % directly absorbed" and "Total % at dose site"
[d]: calculated by the DEBRA 5.2a software

The mean total recoveries of radioactivity were > 96 % dose for the human skin and rat skin samples for low dose application. For high dosing the mean total recoveries were > 97 % dose for human skin and rat skin, respectively.

The data demonstrated that the majority of the radioactivity was removed by the swabbing procedure performed at 8 hours post application and by the surface tape-strips. Lower levels of radioactivity were recovered from the dose site for human and rat skin; mean total percentage of the applied radioactivity recovered from the dose site was 1.4 % for human skin and 3.7 % for rat skin (low dose) and 0.9 % for human skin and 1.7 % for rat skin (high dose).

The mean total percentage of the applied radioactivity found to be directly absorbed was 0.08 % dose for the human skin and 0.4 % dose for the rat skin in case of low dosage and < 0.005 % dose and 0.06 % dose for human and rat skin, respectively in case of high dosage.

The radioactivity counted in the skin compartment (skin and stratum corneum) could be considered to be potentially absorbable. The percentage of [^{14}C]-AE 0001789 considered to be potentially absorbable over a period of 24 hours was 1.5 % and 4.1 % for the human and rat skin, respectively, for the diluted product. In case of the neat product potentially 0.9 % and 1.8 % dose could be absorbable for human skin and rat skin, respectively

III Conclusion

The potentially dermal absorbable fraction for the neat formulation of cyprosulfamide, formulated as a soluble concentrate, is fairly low and in comparable range for both, human and rat skin. Rat skin revealed a penetration higher by a factor of 2.1 than human skin.
For the diluted formulation a more pronounced difference exists. Again, taking into account the potentially absorbable fraction a factor of approx. 2.7 was found.

For estimating systemic exposure the following figures are taken:
0.9 % for the concentrate and 1.5 % for the spray solution.

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable with no deviations from the study protocol. Good recovery data were obtained for all samples, with mean total recoveries of radioactivity in the range of 96.19% to 100.87% of the applied dose. Reported SD values were acceptable (no outliers) and data obtained from some of the cells was excluded only when skin was damaged during swabbing or a technical problem occurred during the collection of the receptor fluid. The radiolabelled test substance had a reported radiochemical purity >98% and an initial specific activity of 4.24 MBq/mg. The study (Capt, A. 2006, SA 06152) is fully compliant with OECD 428 (2004). The dilution tested in the study (0.27 mg AE 0001789/ml) is slightly lower than that specified on the GAP table for the 'SC 450' formulation (0.3 mg/ml), however, this difference is not considered to be significant. The lead cyprosulfamide formulation 'SC 480' has a cyprosulfamide content of 240 g/l for the undiluted product and 0.666 g/l for the spray solution. The dermal absorption values derived from this study are, therefore, likely to be a worst case. The solubility of AE 0001789 in the receptor fluid was demonstrated at a concentration equivalent to the maximum applied dose and was therefore shown to not limit the rate of diffusion. The exclusion of the first two tape strips from the calculation of the absorbed dose is acceptable because the stripped material reflects that likely to be lost by desquamation. The applicant has taken the precautionary approach of including the skin residue (with the exception of the first two tape strips) in the calculation of the absorbed dose although current EU guidance (SANCO/222/2000) states that the skin residue may be discounted when 'serial non-detects' are observed. For human skin membranes, serial non-detects were reported for the concentrate (no radioactivity was detected in the receptor fluid at any time point) and for the dilution (from 15 to 24 hours after exposure). Therefore, it is unlikely that the skin residue would be systemically available. The applicant's dermal absorption values for human skin of 0.85% (0.9%) for the

	concentrate and 1.5% for the dilution are considered to be appropriate. Results for rat skin show greater absorption than for human skin (1.76% for the concentrate and 4.11% for the dilution) however, in the absence of a rat *in vivo* study, these results are not used to calculate the dermal absorption values.
Conclusions	Based on current EU guidance, dermal absorption of [^{14}C]-AE 0001789 through human skin membranes *in vitro* over a period of 24 hours is considered to be 0.85% for the concentrate (rounded up to 0.9%) and 1.5% for the spray solution. The 'SC 450' formulation tested is considered to be sufficiently similar to the lead cyprosulfamide formulation 'SC 480' to allow the extrapolation of these dermal absorption values. The derivation of these values is based on a precautionary approach, as explained above.

B.6.12.2(b) Dermal absorption, *in vivo* in the rat (IIIA1 7.6.1)

a) Dermal absorption of isoxaflutole, *in vivo*

Report:	**KIIIA1 7.6.1/01, Cheng T. (1996)**
Title:	Dermal absorption of [^{14}C]-Isoxaflutole in Male rats (preliminary and definitive phases).
Report No &	CHW 6224-225
Document No	M-165796-01-1
Dates of work:	Nov. 01 to Dec. 27, 1995
Guidelines:	Subdivision F, Series 85-3
GLP	Yes

I Materials and methods

The extent of absorption of isoxaflutole was studied following application of ^{14}C-isoxaflutole to the skin of male rats (approximately 12.5 cm^2) at dose levels of 0.011 mg/animal (0.865 µg/ cm^2), 0.092 mg/animal (7.32 µg/ cm^2) and 0.987 mg/animal (79.0 µg/ cm^2).

A total of 72 rats were dermally dosed at three dose levels (Groups 4, 5, and 6). The skin at the application site was washed just before sacrifice at 0.5, 1, 2, 4, 10 and 24 hours post dose, for 4 rats from each group. Samples of blood, urine and feces were collected throughout the test periods. The control animals (Group 3) received only the vehicle: 1.0% carboxymethylcellulose. For these animals, the skin at the application site was washed just before sacrifice at 24 hours post dose, and blood, urine and feces were collected at 24 hours post dose.

II Results and discussion

The amounts of radioactivity found in the blood, eliminated in the excreta, and retained in the carcass were considered as the direct dermal absorption of isoxaflutole by rats. Among the test groups, a small amount of radioactivity was dermally absorbed by the rats, with the majority of the absorbed radioactivity being retained in the carcass. Within the test groups, the highest direct absorption (radioactivity from blood, carcass, cage wash, cage wipe, urine and feces) was at the longest exposure time (24 hours after dose). It accounted for 4.49% (0.494 µg isoxaflutole equivalent), 0.92% (0.846 µg isoxaflutole equivalent) and 0.2% (0.1.97 µg isoxaflutole equivalent) for groups 4, 5 and 6, respectively. The indirect absorption (direct absorption and the amount of radioactivity detected on/in skin of the test site after skin wash) accounted for 16.4% (0.494 µg isoxaflutole equivalent), 7.23% (0.846 µg isoxaflutole equivalent) and 2.31% (0.1.97 µg isoxaflutole equivalent) for groups 4, 5 and 6,

respectively at the longest exposure time. The amount of dermal absorption of isoxaflutole was not proportional to the dose.

Table 7.6.1-1: Summary of direct dermal absorption of isoxaflutole

Sample Hour	Group 4 (0.011 mg/rat) Total % absorbed	Group 5 (0.092 mg/rat) Total % absorbed	Group 6 (0.987 mg/rat) Total % absorbed
0.5	**0.23**	**ND**	**ND**
1	**0.29**	**0.12**	**<0.005**
2	**0.91**	**0.14**	**<0.005**
4	**1.81**	**0.28**	**0.08**
10	**3.46**	**0.54**	**0.03**
24	**4.49**	**0.92**	**0.20**

ND: Not detectable

Table 7.6.1-2: Summary of indirect dermal absorption of isoxaflutole

Sample Hour	Group 4 (0.011 mg/rat) Total % absorbed	Group 5 (0.092 mg/rat) Total % absorbed	Group 6 (0.987 mg/rat) Total % absorbed
0.5	**3.04**	**2.01**	**1.14**
1	**4.38**	**1.98**	**1.38**
2	**8.45**	**1.25**	**0.92**
4	**10.6**	**3.17**	**1.43**
10	**15.4**	**4.09**	**2.50**
24	**16.4**	**7.23**	**2.31**

III Conclusion

Taking a conservative approach, the total amount of [^{14}C]-isoxaflutole absorbed was calculated as direct and indirect dermal absorption and were approximately 4.49% and 0.20% for direct absorption and 16.4 and 2.31% for indirect absorption at 24 hours post-application for the diluted and concentrate test substance groups, respectively.

Hence, the values of dermal penetration considered for the operator risk assessment are the following:

	undiluted	diluted
Direct absorption	0.2 %	4.5 %

Indirect absorption	2.3 %	16.4 %

EVALUATION, SUMMARY AND CONCLUSION BY REGULATORY AUTHORITY	
Name of authority	Pesticides Safety Directorate, UK
Reviewer's Comments	Reliability rating: Totally reliable
Conclusions	Animals were sacrificed following termination of exposure; no measurements of the excretion of radioactivity were therefore made after termination of exposure. Systemic availability of the radioactivity associated with the application site at termination therefore has to be assumed. Values for potential dermal absorption of isoxaflutole can therefore be calculated by adding the values for 'direct' and 'indirect' absorption. Dermal absorption values of 215, 8% and 2.5% are therefore calculated for the concentrations of 0.011, 0.092 and 0.987 mg/rat (calculated to be equivalent to approximately 8.3, 0.83 and 0.083 mg/l) respectively.

Epidemiology (IIIA1 7.8)

There is no such EU data requirement; (the OECD) point concerned is not covered by or part of an EU point according to Council Directive 91/414/EEC. Hence, such studies were not performed

Data on formulants (IIIA1 7.9)

Material safety data sheet for each formulant (IIIA1 7.9.1)

The information concerned is included in **Document H**.

Available toxicological data for each formulant (IIIA1 7.9.2)

The information concerned is included in **Document H**.

Domestic animal/livestock safety (IIIA1 7.10)

Such data are currently not required in the EU or the US. Hence, such studies were not performed.

Other/special studies (IIIA1 7.11)

No other special study was necessary or performed.