Addendum 1

to the Draft Assessment Report

of 20 August 2002

(relating to Volume 4)

Tritosulfuron

confidential

26. August 2003

Rapporteur Member State: Germany

Confidential information available at RMS.

Addendum 2

to the Draft Assessment Report

20 August 2002

(relating to Volume 4)

Tritosulfuron

confidential

05 September 2003

Rapporteur Member State: Germany

Confidential information available at RMS.

Addendum 3

to the Draft Assessment Report

of 20 August 2002

(relating to Volume 1 + 3)

Tritosulfuron

04 February 2005

Rapporteur Member State: Germany

Contents

Introduction 1

VOLUME 1: 2

2 Reasoned statement of the overall conclusions 2
2.3 Impact on human and animal health 2
2.3.1 Effects having relevance to human and animal health arising from exposure to the active substance or to impurities contained in the active substance or to their transformation products 2
2.3.1.9 Further toxicological studies 2
2.8 Appendices 11
2.8.2 Appendix II: Specific terms and abbreviations 11
2.8.3 Appendix III: Listing of endpoints 11
2.8.3.3 Appendix III.3: Chapter 3 (impact on human and animal health) 11

VOLUME 3: 16

B.5 Methods of analysis 16

B.5.2 Analytical methods (residue) for plants, plant products, foodstuffs of plant and animal origin, feedingstuffs (Annex IIA 4.2.1; Annex IIIA 5.2) 16
B.5.2.1 Plant material 16
B.5.6 References relied on 17

B.6 Toxicology and Metabolism 18

B.6.8 Further toxicological studies (Annex IIA 5.8) 18
B.6.8.1 Toxicity studies of metabolites 18
B.6.8.1.1 635M02 18
B.6.8.1.3 635M01 45
B.6.8.2 Supplementary studies - AMTT (635M04) 48
B.6.8.2.9 *In vitro* chromosome aberration assay with AMTT 48
B.6.12 Dermal absorption (Annex IIIA 7.3) 51
B.6.14 Exposure data (Annex IIIA 7.2) 51
B.6.14.4 Exposure data: active substance tritosulfuron 52
B.6.14.4.1 Operator exposure 52
B.6.14.4.2 Worker exposure 53
B.6.14.4.3 Bystander exposure 54
B.6.14.5 Exposure data: AMTT 54
B.6.14.5.1 Operator exposure 56
B.6.14.5.2 Worker exposure 59
B.6.14.5.3 Bystander exposure 60
B.6.14.6 Overall conclusion 61
B.6.15 References relied on 66

B.7 Residue data 68

B.7.1 Metabolism, distribution and expression of residues in plants (Annex IIA 6.1; Annex IIIA 8.1) 68
B.7.1.4 Test system 68
B.7.1.5 Findings 69
B.7.1.6 Conclusion 72
B.7.1.7 Comparison of the plant metabolism studies 78
B.7.3 Definition of the residue 81

B.7.3.1 Plants 81
B.7.17 References relied on 82

B.8 Environmental fate and behaviour 83

B.8.1 Route and rate of degradation in soil (Annex IIA 7.1.1; Annex IIIA 9.1.1) 83
B.8.1.2 Rate of degradation 83
B.8.1.2.1 Laboratory studies 83
B.8.2 Adsorption, desorption and mobility in soil (Annex IIA 7.1.2 and 7.1.3; Annex IIIA 9.1.2) 88
B.8.2.2 Kinetic evaluation of the adsorption behaviour and estimation of effective relevant sorption values of BAS 635 H and its metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5 in soils 100
B.8.2.2.1 Estimation of sorption parameters from lysimeter studies 101
B.8.2.2.2 Kinetic sorption parameters of the laboratory sorption studies 109
B.8.2.2.3 Effective sorption of BAS 635 H in field studies 124
B.8.2.3 Evaluation of the sorption of BAS 635 H - tritosulfuron and metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5 considering the dependency of sorption to soil parameters and sorption kinetics 136
B.8.2.3.1 Relations between the adsorption of BAS 635 H and metabolites BH 635-2, BH 635 3, BH 635-4 and BH 635-5 and soil properties 137
B.8.2.3.2 Evaluation of the kinetic sorption behaviour of BAS 635 H and of metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5 147
B.8.2.3.3 Overall conclusions 148
B.8.2.4 Non-identified radioactivity in lysimeter leachates of lysimeters treated with BAS 635 H 149
B.8.3 Predicted environmental concentrations in soil (PECs) (Annex IIIA 9.1.3) 151
B.8.3.1 Calculation of PECsoil for tritosulfuron and metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5 using worst case half-lives 151
B.8.3.2 Predicted long term concentrations in soil of the soil metabolites of tritosulfuron (635M01, 635M02, 635M03, 635M04) (Dressel, 2003a) 154
B.8.6 Predicted environmental concentrations in surface water and in ground water (PECsw, PECgw) (Annex IIIA 9.2.1, 9.2.3) 161
B.8.6.4 Calculation of predicted environmental concentrations for BAS 635 H and metabolites BH 635-2, BH 635-4 and BH 635-5 in static surface waters (PECsw) and sediment (PECsed) after drift entry (Dressel, 2002a; Dressel, 2002b; Dressel, 2003c, Dressel, 2003d) 161
B.8.6.4.1 Method of calculation 162
B.8.6.4.2 Results 167
B.8.6.5 Calculation of predicted environmental concentrations in groundwater (PECgw) of BAS 635 H and its metabolites for UK under special considering of sorption dependencies 170
B.8.6.6 Evaluation of the soil degradation, soil adsorption and leaching potential of BAS 635 H and its metabolites 635M01, 635M02, 635M03, and 635M04 (Dressel, 2003b; Dressel, 2004) 172
B.8.10 References relied on 182

B.9 Ecotoxicology 186

B.9.2 Effects on aquatic organisms (Annex IIA 8.2; Annex IIIA 10.2) 186
B.9.2.1 Toxicity data 186
B.9.2.1.1 Aquatic plants 186
B.9.2.2 Risk assessment 187

B.9.5 Effects on other terrestrial vertebrates (Annex IIIA 10.3) 188
B.9.5.2 Risk assessment for wild mammals..... 188
B.9.8 Effects on earthworms (Annex IIA 8.4; Annex IIIA 10.6.1) 194
B.9.8.3 Sublethal effects (Annex IIA 8.4.2; Annex IIIA 10.6.1.2)..... 194
B.9.8.4 Acute and long-term risk assessment 200
B.9.8.4.1 Acute risk 200
B.9.8.4.2 Long-term risk..... 201
B.9.10 Effects on soil non-target micro-organisms (Annex IIA 8.5; Annex IIIA 10.7)..... 202
B.9.10.4 Laboratory testing..... 202
B.9.10.5 Carbon mineralisation risk assessment..... 208
B.9.13 References relied on 209

Introduction

With regard to the conclusions drawn at ECCO-Meeting 136 and the studies submitted by the notifier after the DAR had been completed, the following main issues are addressed in this Addendum:

Annex IIA, point 5.8: Additional toxicological studies with TBSA (metabolite 635M02)

Acute oral toxicity study in rats
28-day repeated dose toxicity study in rats (range finding study)
28-day repeated dose toxicity study in rats (main study)
1-generation toxicity study (range finding study)
1-generation toxicity study (main study)
Re-evaluation of the *in vitro* chromosome aberration assay in V79 cells
Statement on the toxicological relevance of 635M02 (TBSA)

Annex IIA, point 5.8: Additional toxicological studies with AMTT (metabolite 635M04)

In vitro chromosome aberration assay with Reg. No. 231700 (metabolite of BAS 635 H) in V79 cells

Annex IIA, point 5.8: Additional toxicological studies with metabolite 635M01

Reg. No. 335 184 (metabolite of BAS 635 H) - Subacute toxicity study in Wistar rats administration in the diet for 4 weeks
Statement on the toxicological relevance of 635M01

Annex IIA, point 5.1 (Volume 3, B.6.12): Dermal absorption

At ECCO 136 the following was concluded: Based on the *in vivo* rat study and *in vitro* data in rat and human skin 1 and 2 % absorption factors were set for the concentrate and in use dilutions respectively.

Annex IIIA, point 7.2: Exposure data

Estimation of the systemic exposure (absorbed doses) to tritosulfuron for operator, worker and bystander on the basis of the dermal absorption rates proposed by the ECCO-Meeting 136 (concentrate: 1 %; in-use dilution: 2 %) and the respective risk assessment taking into account a systemic AOEL of 0.15 mg/kg bw/d.
Operator, worker and bystander risk assessment of possible exposure to AMTT resulting from field application of tritosulfuron-containing products.

VOLUME 1:

2 Reasoned statement of the overall conclusions

2.3 Impact on human and animal health

2.3.1 Effects having relevance to human and animal health arising from exposure to the active substance or to impurities contained in the active substance or to their transformation products

2.3.1.9 Further toxicological studies

635M02 (Reg. No. 292 564; BH 635-2, TBSA) is a soil metabolite and was detected in the rat metabolism study (< 1 % in urine/feces). In the first data package submitted, it was tested in three mutagenicity assays as well as in two acute oral tests. When it became obvious that this metabolite exceeds the groundwater threshold of 0.1 µg/L by leaching the toxicological relevance had to be addressed in greater detail.
In an additional acute oral toxicity study in rats, TBSA of high purity (99.9 %) was tested in order to rule out the possibility that the acute oral toxicity seen in a previous study with a less pure batch of the test material was due to the presence of an impurity [see Wiemann C. and Hellwig J. 1999(a); BASF RegDoc# 1999/10099].
In an additional 28-day toxicity study no NOAEL could be derived and - beside others - effects on the female reproductive organs were observed. Therefore, a second 28-day study conducted with lower doses of TBSA was submitted.
The objective of the 1-generation studies was to determine the possible adverse effects of TBSA on the integrity and performance of the male and female reproductive systems since effects were noted in the first 28-day study.
For an overview of the study results see table below.

The metabolite TBSA was found to be not mutagenic in the *Salmonella typhimurium/ Escherichia coli* reverse mutation assay and not mutagenic *in vitro* in the CHO/HPRT mutation assay.
Based mainly on comments of the UK, at ECCO 136 it was concluded that 635M02 may have some chromosome-damaging (clastogenic) potential under *in vitro* conditions in V79 cells with S-9 mix. Therefore in the endpoint list the *in vitro* chromosome aberration assay was considered positive. The re-evaluation of the study led to the conclusion that 635M02 has no clastogenic potential. The justification is given in the assessment of the study results (see Vol. 3).
Three acute oral studies were conducted in total. One study resulted in an oral LD_{50} of 1000 mg/kg bw, one in an oral LD_{50} of > 2000 mg/kg bw. The new study was conducted with the test substance of high purity (99.9 %). The acute oral LD_{50} was found to be greater than 300 mg/kg and less than 2000 mg/kg body weight in rats. The median lethal dose lies between 500 and 1000 mg/kg bw. Thus, it can be considered that the metabolite 635M02 is of higher oral toxicity than the parent compound tritosulfuron (N12).
In addition, two 28-day repeated dose toxicity study in rats were submitted after the completion of the DAR. In the first study effects on the body weight (decreases), in the functional observation battery (reduced rearing and overall motor activity, tremor), on hematological (decreased red blood cell parameters) and clinico-chemical examinations

(increased γ–glutamyltransferase activities, increased bilirubin and cholesterol levels), on urinalysis (increased number of degenerated renal tubular epithelial cells and transitional epithelial cells as well as higher number of granular casts and epithelial cell casts) and on organ weights (higher kidney, liver and spleen weights and lower ovary and uterine weights) were noted. The histopathological examinations showed that the ovaries of high-dose group females had a significantly decreased number of corpora lutea. The differential ovarian follicle count (DOFC) indicated that the number of clearly discernible antral follicles was reduced to 60 % of the control value at 5000 ppm. A treatment-related altered hormonal function of unknown etiology and pathogenesis could also not be excluded for the increased number of females with slight or moderate atrophy of the uterus (mucosa and musculature) in the mid and high dose groups. In the spleen, increased incidences of hemosiderin deposition and congested blood vessels were observed in mid and/or high dose groups. A NOAEL could not be derived from this study, based on effects on red blood cells, organ weights and ovaries at the lowest dose of 200 ppm tested (corresponding to daily intakes of 18.6 and 20.0 mg/kg bw/d for males and females, respectively).

Therefore, a subsequent 28-day oral toxicity study was conducted using three lower dose levels (0, 50, 100 and 150 ppm, corresponding to approximately 5.0, 10.0 and 14.7 mg/kg bw/d; see BASF RegDoc# 2003/1004049). In this study an increased α2u-globulin accumulation in the kidneys of male rats were noted in all dose-groups. This finding was considered to represent a sex and species specific effect, without relevance to human males. Thus, the NOAEL was set at 150 ppm (14.7 mg/kg bw/d).

To possibly exclude effects of 635M02 on fertility and reproduction two 1-generation studies were conducted with similar dose levels (0, 200 and 400 ppm; corresponding to approximately 12.8/16.2 and 25.3/32.4 mg/kg bw/d in males/females). In the first study, the male/female fertility index was slightly reduced in the high dose of 400 ppm (70 % versus 90 % and 100 % in the control and low dose), with 3/10 non-pregnant females in the 400 ppm group. One female of the 400 ppm group showed a severely reduced number of corpora lutea and a massive follicle degeneration in both ovaries. No further effect on female reproduction, delivery and litter/pup data was noted.

Cholesterol concentrations were increased in males and females at 400 ppm. High dose females showed lower magnesium levels. The mean absolute and relative liver, kidney and spleen weights were significantly increased in high dose F_0 parental males, when compared with controls. In high dose F_0 parental females, the relative spleen weight was significantly increased.

It cannot be concluded with certainty that the impaired fertility in high dose animals (one pair in the control versus 3 pairs in the high dose group) was not substance-related. A fertility index of 70 % is below the historical control data for this laboratory. In addition, the findings in the ovary of a single high dose female (severely reduced number of corpora lutea, massive degeneration of follicles) may indicate a high sensitivity of single animals. Moreover, effects on the ovaries and uterus were also noted in the 28-day repeated dose study at concentrations of ≥ 200 ppm (20 mg/kg bw/d).

Under the conditions of this study the NOAEL for reproductive toxicity is 200 ppm (corresponding to approximately 12.8 mg/kg bw/d and 16.2 mg/kg bw/d in males and females respectively). The NOAEL for general toxicity is below 200 ppm based on lower body weight and organ weight changes at this dose level.

In the second 1-generation study, one female of the high dose group (400 ppm) was fertile (proved by 6 implantation sites in utero at terminal sacrifice) but did not deliver pups. Historical control data were not provided for this endpoint. There was no further substance-related effect on female reproduction, delivery and litter data. The DOFC revealed no changes in the number of primordial and/or growing follicles between the control and treated groups.
The F1 pups body weights and body weight gains were minimally lower in the 400 ppm group at all investigation points, although without statistical significance and with values lying mainly within the historical control values. In the presence of maternal toxicity at this dose level, these changes were not considered to represent a reproductive adverse effect.
Under the conditions of this study the NOAEL for reproductive toxicity is 200 ppm (corresponding to approximately 17.6 mg/kg bw/d and 17.3 mg/kg bw/d in males and females respectively) based on postimplantation loss noted in one female at the next higher dose of 400 ppm (corresponding to approximately 35.0 mg/kg bw/d and 33.1 mg/kg bw/d in males and females respectively).
The NOAEL for general toxicity is below 200 ppm based on lower body weight and clinico-chemical findings (higher white blood cell count in males, higher cholesterol concentrations in males and females, lower magnesium concentrations in females) at this dose level.

Table 2.3-9: Summary of toxicity studies of metabolite 635M02 (Reg.-No. 292 564)

Study/strains/species	Test material/conditions	Results
Acute oral toxicity in rats Wistar rats (Chbb:THOM (SPF)) 5 male / 5 female 500, 2000 and 5000 mg/kg bw	Batch No. 00831-201, purity: 98.2 %, Test substance preparation in 0.5 % Tylose CB 30.000 in aqua bidest	LD_{50}: 1000 mg/kg bw Mortality at ≥ 2000 mg/kg bw (delayed after 3 to 4 days), main signs of toxicity: dyspnea, apathy, excitation, abdominal and lateral position, staggering, ataxia, atonia, paresis, narcotic-like state, pain and corneal reflex absent, tremor, spastic gait, erythema, exsiccosis, salivation, lacrimation, discoloured urine, squatting posture and red clammy snout and eyelid.
Acute oral toxicity of TBSA in rats Wistar rats (Chbb:THOM (SPF)) 3 male / 3 female 200 and 2000 mg/kg bw	Batch No. 26778/99; 26778/101, Purity: > 98.5 %, Test substance preparation in olive oil DAB 10	LD_{50}: > 2000 mg/kg bw No mortality Main signs of toxicity: dyspnea, apathy, staggering, exsiccosis, red discoloured urine until day 5 post dosing
Acute oral toxicity of TBSA in rats Wistar rats (CrlGlxBrlHan:WI) 3 x 3 female 2000 and 300 mg/kg bw	Batch no: 2059-029, purity: 99.9 % Test substance preparation in 0.5 % CMC-solution (cleaned sodium carboxymethyl-cellulose) in double distilled water	Median lethal dose: around 500 mg/kg bw All animals at 2000 mg/kg bw were found dead on study day 3 No mortality at 300 mg/kg bw. Main signs of toxicity: dyspnoea, abdominal or lateral position, staggering, smeared fur, lacrimation and red smeared fur in the anogenital area

Study/strains/species	Test material/conditions	Results
Salmonella typhimurium/ *Escherichia coli* reverse mutation assay *S. typhimurium* strains TA 100, TA 1535, TA 1537, TA 98 and *E. coli* strain WP2 uvrA	Batch No. 00831-201, Purity: 98.2 %.	Not mutagenic
Gene mutation assay in Chinese hamster ovary (CHO) V79 cells *in vitro* (V79 / HPRT)	Batch No. 00831-201, Purity: 98.2 %.	Not mutagenic
In vitro chromosome aberration assay in V79 cells	Batch No. 00831-201, Purity: 98.2 %	Not mutagenic
28-day oral toxicity study in rats Wistar rats (CrlGlxBrlHan:WI) 5 males / 5 females 0; 200; 1000 and 5000 ppm (18.6/20.0, 90.7/96.1, 417.3/414.2 mg/kg bw/d for males and females)	Batch no. 25887 Fass 2; purity: 98.4 %.	No NOAEL LOAEL 200 ppm: ↑ liver wt.,↑ kidney wt. (males),↓ RBC (f), α_{2u}–globulin accumulation (male kidneys), hyperplastic ovarian stroma cells 1000 ppm: ↓ bw (females), ↓ overall motor activity, ↓ RBC, ↑ bilirubin, ↑ cholesterol, cells and casts in urine sediment (males), ↑ liver wt., ↑ kidney wt. (males), ↑ spleen wt., α_{2u}–globulin accumulation (male kidneys), hepatocellular hypertrophy (males), hyperplastic ovarian stroma cells, atrophy of the uterus (mucosa and musculature), hemosiderin deposition (spleen) 5000 ppm: tremor, piloerection from day 14 onwards, ↓ bw, ↓ rearing, ↓ overall motor activity, ↓ RBC, ↑ γ–glutamyltransferase activities, ↑ bilirubin, ↑ cholesterol, cells and casts in urine sediment (males), ↑ liver wt., ↑ kidney wt. (males), ↓ ovary and uterus wt., ↑ spleen wt., α_{2u}–globulin accumulation (male kidneys), chronic progressive nephropathy (male), hepatocellular hypertrophy, ↓ number of corpora lutea (ovary), hyperplastic ovarian stroma cells, atrophy of the uterus (mucosa and musculature), hemosiderin deposition (spleen)
28-day oral toxicity study in rats Wistar rats (CrlGlxBrlHan:WI) 5 male / 5 female 0, 50, 100 and 150 ppm (4.8/5.0; 9.0/10.0; 13.9/14.7 mg/kg bw/d in males and females)	Batch no. 25887 Fass 2; purity: 98.4 %.	NOAEL: 150 ppm All dose groups: $\alpha_{2\mu}$-globulin accumulation in the kidney (males)
1-generation study in rats (range finding) Wistar rats (CrlGlxBrlHan:WI) 10 male / 10 female 5 male / 5 female as satellite groups 0; 200 and 400 ppm (12.9/16.2; 25.3/32.4 mg/kg bw/d in males and females) premating phase: 4 weeks	Batch no. 25887 Fass 2; purity: 98.4 %	NOAEL (reproduction): 200 ppm NOAEL (general toxicity): ≤ 200 ppm 200 ppm: ↓ bw (f), ↑ liver wt.(f) 400 ppm: ↓ bw (f), ↓ male/female fertility index, severely reduced number of corpora lutea/ massive follicle degeneration in the ovaries (1 female), ↑ cholesterol, ↓ magnesium (f), ↑ kidney wt. (m); ↑ spleen wt., ↑ liver wt., ↑ thyroid wt. (f)
1-generation study in rats (main study) Wistar rats (CrlGlxBrlHan:WI) 10 male / 10 female	Batch no. 25887 Fass 2; purity: 98.4 %.	NOAEL (reproduction): 200 ppm NOAEL (general toxicity): ≤ 200 ppm

Study/strains/species	Test material/conditions	Results
5 male / 5 female as satellite groups 0; 200 and 400 ppm (17.7/20.7; 35.0/40.9 mg/kg bw/d in males and females) premating phase: 10 weeks		200 ppm: ↓ bw (f), ↑ WBC (m), ↑ cholesterol, ↓ magnesium (f) 400 ppm: ↓ bw (f), postimplantation loss (1 female), ↑ WBC (m), ↓ RBC, Hb, ,HCT, ↑ Reticulocytes, ↑ cholesterol, ↓ magnesium (f), ↑ kidney wt. (m), ↑ liver wt., ↑ spleen wt.

635M03 (Reg. No. 335 182; BH 635-3) is a soil metabolite. It was detected in the rat metabolism study as a transient metabolite. It was tested in three mutagenicity assays as well as in an acute oral test and a 90-day feeding study. 635M03 was found to be not mutagenic in the *Salmonella typhimurium*/*Escherichia coli* reverse mutation assay, not mutagenic *in vitro* in the CHO/HPRT mutation assay and not chromosome-damaging (clastogenic) under *in vitro* conditions in V79 cells. An acute oral toxicity study revealed an LD_{50} of > 5000 mg/kg bw. The NOAEL in a 90-day dietary rat study was 15000 ppm (equal to 1187 mg/kg bw).

Table 2.3-10: Summary of toxicity studies of metabolite 635M03 (Reg.-No. 335 182)

Study/strains/species	Test material/conditions	Results
Acute oral toxicity in rats Wistar rats (Chbb:THOM (SPF))	Batch No. 00831-274, Purity: 98.7 %; Batch 01185-044, purity 99.4 %.	LD_{50}: >5000 mg/kg bw
Subchronic toxicity study in Wistar rats [CRL:WI(GLX/BRL/HAN)IGS BR]. Administration in the diet for 3 months	Batch No. 01185-269, Purity: 99.2 %.	No substance related effects NOAEL: 15000 ppm (1187 mg/kg bw/d)
Salmonella typhimurium/Escherichia coli reverse mutation assay *S. typhimurium* strains TA 100, TA 1535, TA 1537, TA 98 and *E. coli* strain WP2 uvrA	Batch No. 00831-274, Purity: 98.7 %.	Not mutagenic
Gene mutation assay in Chinese hamster V79 cells *in vitro* (V79 / HPRT)	Batch No. 01185-085, Purity: 99.8 %.	Not mutagenic
In vitro chromosome aberration assay in Chinese hamster V79 cells	Batch No. 01185-085, Purity: 99.8 %.	Not mutagenic

635M01 (Reg. No. 335 184; BH 635-4) is a soil metabolite. It was detected in the rat metabolism study. It was tested in three mutagenicity assays as well as in an acute oral test. It was found to be not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay, not mutagenic *in vitro* in the CHO/HPRT mutation assay and not chromosome-damaging (clastogenic) under *in vitro* conditions in V79 cells. An acute oral toxicity study revealed an LD_{50} of > 5000 mg/kg bw. A 28-day dietary toxicity study in rats revealed a NOAEL of 3900 ppm, the highest dose tested. There were no signs of toxicity.

Table 2.3-11: Summary of toxicity studies of metabolite 635M01 (Reg.-No. 335 184)

Study/strains/species	Test material/conditions	Results
Acute oral toxicity in Wistar rats (Chbb:THOM (SPF))	Batch No. 01185-088, Purity: 97.0 %	LD_{50}: > 5000 mg/kg bw
Salmonella typhimurium/Escherichia coli reverse mutation assay *S. typhimurium* strains TA 100, TA 1535, TA 1537, TA 98 and *E. coli* strain WP2 uvrA	Batch No. 00831-277, Purity: 97.9 %	Not mutagenic
Gene mutation assay in Chinese hamster V79 cells *in vitro* (V79 / HPRT) Chinese hamster ovary (CHO) cells	Batch No. 01185-088, Purity: 97.0 %	Not mutagenic
In vitro chromosome aberration assay in V79 cells (cells derived Chinese hamster)	Batch No. 01185-088, Purity: 97.0 %	Not mutagenic
28-d oral (diet) toxicity study in Wistar rats (CrlGlxBrlHan:WI) 10 male / 10 female 0; 430; 1300 and 3900 ppm (corresponding to approximately 38, 115 and 344 mg/kg bw/d)	Batch no. 2059-011, purity: 96.4 %.	NOAEL: 3900 ppm No signs of toxicity

635M17 (Reg. No. 373 906) is a plant metabolite. It was detected in the rat metabolism study in minor quantities. It was tested in three mutagenicity assays as well as in an acute oral test. It was found to be not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay, not mutagenic *in vitro* in the CHO/HPRT mutation assay and not chromosome-damaging (clastogenic) *in vivo*. There were no indications of any impairment of chromosome distribution in the course of mitosis.
An acute oral toxicity study revealed an LD_{50} of > 2000 mg/kg bw.

Table 2.3-12: Summary of toxicity studies of metabolite 635M17 (Reg.-No. 373 906)

Study/strains/species	Test material/ conditions	Results
Acute oral toxicity study in Wistar rats Crl: WI(GLX/BRL/HAN)IGS BR (SPF)	Batch No. 01742-22, Purity: 98.3 %.	LD_{50}: >2000 mg/kg bw
Salmonella typhimurium/Escherichia coli reverse mutation assay (standard plate test and preincubation test) *S. typhimurium* strains TA 100, TA 1535, TA 1537, TA 98 and *E. coli* strain WP2 uvrA	Batch No. 01742-22, Purity: 98.3 %.	Not mutagenic
In vitro gene mutation test in Chinese hamster ovary (CHO) cells (HPRT Locus Assay)	Batch No. 01742-22, Purity: 98.3 %.	Not mutagenic
Cytogenetic study *in vivo* in the mouse micronucleus test after two intraperitoneal administrations (NMRI mice)	Batch No. 01742-22, Purity: 98.3 %.	Not mutagenic

AMTT (635M04) was an impurity in the batch no. N24 (2.45 %). It is also a metabolite in the rat, soil and water. A separate metabolism study was conducted with AMTT, it was tested in an acute oral test, as well as in four mutagenicity tests. Furthermore, in order to prove that the effects seen in the 2-generation study using tritosulfuron batch no. N24 was due to high AMTT content, it was tested in a pre/postnatal toxicity study. In order to understand the mechanism by which AMTT exerts its effects it was subjected to two additional studies: a subchronic toxicity study with estrus cycle determination as well as hormone analysis and determination of the binding capacity to the estrogen receptor.
AMTT does not accumulate in rats, but is effectively excreted. The major metabolite AHTT is generated by demethylation and is detected as different tautomeric structures. The oral LD_{50} was found to be > 200 < 2000 mg/kg bw. Estrus cycle determination, hormone analysis as well as PCNA resp. BrdU and TUNEL–stain analysis of mammary glands and a density

calculation of estrogen (Eα)– and progesterone receptors in uterus and vagina revealed no treatment-related changes in a subchronic toxicity study.
AMTT is not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay, not mutagenic *in vitro* in the CHO/HPRT mutation assay and in a chromosomal aberration test in V79 cells and did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the mouse and therefore is considered to be non-mutagenic in this micronucleus assay. Altogether, AMTT has no genotoxic potential under the conditions of the *in vitro* and *in vivo* studies conducted.
The oral application of AMTT induced severe maternal and developmental toxicity at 20 mg/kg bw/day and at 50 mg/kg bw/day in a pre/postnatal screening study. Therefore, AMTT might be responsible for the effects observed in the 2-generation study with tritosulfuron containing high levels of AMTT, with respect to pup mortality. In the presence of endogenous estrogens, the bonding capacity of tritosulfuron and AMTT to the estrogen receptor is regarded as extremely low. A biological effect of the substances, the activation of the receptor-mediated gene expression, is extremely unlikely.
Based on the 2-generation study in rats conducted with batch no. N24 with a calculated level of AMTT of 0.06 mg/kg bw/d and applying a safety factor of 500, the following reference values were derived for AMTT:

ADI:	0.0001 mg/kg bw
AOEL (syst.):	0.0001 mg/kg bw/d
ARfD:	0.0001 mg/kg bw

Table 2.3-13: Summary of supplementary studies with AMTT (635M04) (CAS-Nr. 5311-05-07)

Study/strains/species	Test material/ conditions	Results
Study of the biokinetics and metabolism in Wistar rats Chbb:THOM (SPF)	^{14}C-AMTT; Batch No. 687-1008, chemical purity > 98 %, radiochemical purity: > 95 %.	Rapid exceretion, major metabolite: AHTT
Study on the acute oral toxicity of AMTT in Wistar rats Chbb:THOM (SPF)	Batch No. 27 939/16, purity: 92.3 % - 94.2 %.	LD_{50}: > 200 < 2000 mg/kg bw
Subchronic toxicity study with estrus cycle determination and hormone analysis in female Wistar rats (Chbb:THOM (SPF)) Administration in the diet up to 32 weeks 0, 40, 120 ppm	Batch No. 01185-097, purity: 99.9 %	LOAEL: 40 ppm (3.6 mg/kg bw/d): Estrus cycle determination, hormone analysis, PCNA resp. BrdU and TUNEL–stain analysis of mammary glands and a density calculation of estrogen (Eα)– and progesterone receptors in uterus and vagina revealed no treatment-related changes.
Ames *Salmonella* / mammalian-microsome mutagenicity test and *Escherichia coli* / mammalian microsome reverse mutation assay (standard plate test and preincubation test) *S. typhimurium* strains TA 100, TA 1535, TA 1537, TA 98 and *E. coli* strain WP2 uvrA	Batch No. 27 939/16, purity: 92.3 % - 94.2 %.	Not mutagenic
Gene mutation assay in Chinese hamster ovary (CHO) V79 cells *in vitro* (V79/HPRT) with AMTT	Batch No. 01185-097, purity: 99.9 %.	Not mutagenic
Micronucleus assay in bone marrow cells of the mouse (NMRI) after a single intraperitoneal administration	Batch No. 27939-141 CP031929, purity: 99.8 %.	Not mutagenic
In vitro chromosome aberration assay in V79 cells	Batch No. 00831-237, purity: 99.9 %.	No chromosomal aberrations, suppression of mitotic activity
AMTT and BisSH - Pre-/ postnatal screening toxicity study in Wistar rats [Chbb: THOM (SPF)] – Oral administration (gavage)	Batch No. CP031929, purity: 99.8 %, BisSH; batch No. CP 031930, purity: 99.7 %	Severe maternal and developmental toxicity at 20 mg/kg bw/d and at 50 mg/kg bw/d AMTT
Study of a possible bond of AMTT and tritosulfuron to the estrogen receptor in the cytosol from the endometrial RUCA-I adenocarcinoma cell line	Endometrial RUCA-I-adenocarcinoma cell line of the rats	Extremely low bonding capacity of tritosulfuron and AMTT to the estrogen receptor in the presence of endogenous estrogens

Dermal absorption

The *in vivo* dermal absorption of tritosulfuron in rats is approximately 3 % or less depending on the duration of exposure and concentration. The initial rate of absorption through rat epidermal membranes was at least 2.27 fold greater relative to human epidermal membranes. Taking the *in vivo* and *in vivo* dermal penetration data together, they support the use of a dermal penetration figure of 1 % for the concentrate and 2 % for the in-use dilution in the operator exposure calculations.

Exposure data

BAS 635 00 H is intended as a post emergence herbicide in cereals. The maximum application rate is 0.07 kg product/ha and therefore 0.050 kg as/ha (WG: 714 g tritosulfuron/kg product). The intended use is in a tank-mix with surfactants. Hydrolytical cleavage of tritosulfuron in the spray tank results in increased concentrations of AMTT and hence leads to higher exposures to AMTT.

At the ECCO 136 meeting in contrast to the monograph the systemic AOEL for tritosulfuron was derived to be 0.15 mg/kg bw/d and the dermal absorption rates to be 1 % for the concentrate and 2 % for the spray dilution of the product BAS 635 00 H. Therefore a re-assessment is given for the active ingredient tritosulfuron.

On the basis of the data from laboratory experiments and an outdoor study a specific risk assessment regarding the possible exposure to the metabolite AMTT resulting from field application of tritosulfuron-containing products was prepared.

The results show that under the given conditions the operator and worker exposure for the active ingredient tritosulfuron is below the respective systemic AOEL even if no personal protection is used. In result of the risk assessment for the metabolite AMTT, PPE is needed for the operator to stay below the systemic $AOEL_{AMTT}$ (German model). Calculating with the modified UK POEM the $AOEL_{AMTT}$ is exceeded (103.3 %) even if gloves are worn during mixing/loading and application (however in this model it is not possible to consider a protective garment in the calculation).

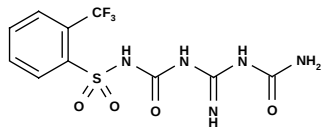
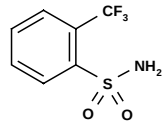
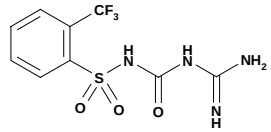
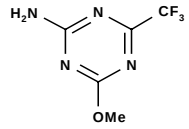
With regard to the active ingredient tritosulfuron as well as to the metabolite AMTT it is concluded that for BAS 635 00 H the intended use is acceptable if PPE is worn by the operator which is in line with the notifier´s view. Although the exposure calculation for both the worker and the bystander results in an acceptable level considering the possible classification/labelling of AMTT [T; R 40-48/22-61(64)] the product should be handled carefully.

2.8 Appendices

2.8.2 Appendix II: Specific terms and abbreviations

Different names were used for the metabolites of tritosulfuron (BAS 635 H) in the additional studies submitted by the notifier and assessed in this addendum. For the readers' convenience, an overview of names, structural formulae and occurrences of the metabolites referred to in this addendum has been extracted from Volume 1 of the monograph and is presented as Table 2.8.2-1.

Table 2.8.2-1: List of metabolites of tritosulfuron discussed in Addendum 3 (excerpt from Volume 1 of the monograph)

Code	Structure	Chemical Name [CAS]/or IUPAC Name	Trivial Name, Codes used	Found in matrix
635M01		1-(carbamoylamidino)-3-(2-trifluoromethyl-benzenesulfonyl) urea	335 184 (BH 635-4)	rat, maize, rotat. crops, soil, water, sediment
635M02		2-trifluoromethyl-benzenesulfonamide	TBSA, 292 564 (BH 635-2)	rat, goat, hen, rotat. crops, soil, water, sediment
635M03		1-amidino-3-(2-trifluoromethyl-benzenesulfonyl) urea	335 182 (BH 635-3)	rotat. crops, soil, water, sediment
635M04		2-amino-4-methoxy-6-(trifluoromethyl)-1,3,5-triazine	AMTT, 231 700 (BH 635-5)	rat, goat, hen, rotat. crops, soil, water

2.8.3 Appendix III: Listing of endpoints

2.8.3.3 Appendix III.3: Chapter 3 (impact on human and animal health)

Note: Figures in parenthesis indicate the batch number of tritosulfuron used in the respective study (N24 contained 2.4 % AMTT, other batches contained AMTT ranging from 0.006 – 2.45 % w/w)

Absorption, distribution, excretion and metabolism in mammals (Annex IIA, point 5.1)

Rate and extent of absorption	Rapid (T_{max}0.5h) and complete (≥90 % based on urinary and bile (10-27 %) excretion over 48 h) - rats 50 and 500 mg/kg bw
Distribution	Widely distributed
Potential for accumulation	No potential for accumulation
Rate and extent of excretion	Rapid (approx. 80 % via urine and 12 % via feces over 48 h)
Metabolism in animals	Limited (hydroxylation at the 4-position of the phenyl ring followed by conjugation; cleavage of the triazine ring and degradation to sulfonamide and sulfonate)
Toxicologically significant compounds (animals, plants and environment)	Parent compound and metabolites (especially AMTT/AHTT and 635 M02

Acute toxicity (Annex IIA, point 5.2)

Rat LD_{50} oral	(N12)	4700 mg/kg bw
Rat LD_{50} dermal	(N12)	> 2000 mg/kg bw
Rat LC_{50} inhalation	(N12)	> 5.4 mg/L air (dust aerosol, 4 h, MMAD 9.2 µm)
Skin irritation	(N12)	Not irritating
Eye irritation	(N12)	Not irritating
Skin sensitisation (test method used and result)	(N12)	Sensitising (M&K test) **R43**

Short term toxicity (Annex IIA, point 5.3)

Target / critical effect	Liver, kidney/centrilobular hypertrophy, nephropathy (rat and dog). Urinary bladder and kidney (mouse)
Lowest relevant oral overall NOAEL/NOEL*	12-month, dog (N24): 200 ppm (6 mg/kg bw/d) [calculated level of AMTT: 0.15 mg/kg bw/day]
Lowest relevant dermal NOAEL/NOEL*	28-day (20 exposure), rat (N24): 1000 mg/kg bw , no systemic effects, local skin effects were present
Lowest relevant inhalation NOAEL / NOEL	No data-not necessary

Genotoxicity* (Annex IIA, point 5.4)

(N24)	No evidence of genotoxic potential *in vivo* and *in vitro* (supported by a new *in vitro* cytogenetics assay with AMTT; see point 5.8).

Long term toxicity and carcinogenicity (Annex IIA, point 5.5)

Target / critical effect	Kidney (interstitial nephritis), Liver (pericholangitis)
Lowest relevant NOAEL / NOEL*	2-yr, rat (N24): 100 ppm (5 mg/kg bw/d) [calculated level of AMTT: 0.123 mg/kg bw/Day] 2-year rat (N34, 42, 53, 59): 1000 ppm (48 mg/kg bw/d) 18-month mouse: LOAEL 250 ppm (36 mg/kg bw/day)
Carcinogenicity*	Mammary gland tumours in female rats at 1000 ppm (N24). NOAEL (tumours 250 ppm, 16 mg/kg bw/day) **R 40**

Reproductive toxicity (Annex IIA, point 5.6)

Reproduction target / critical effect*	Pup mortality in absence of parental toxicity
Lowest relevant reproductive NOAEL / NOEL*	2-gen. rat: 25 ppm (N24) (2.4 mg/kg bw/d) [calculated level of AMTT: 0.06 mg/kg bw/d] **R61** 2-gen. rat: 600 ppm (N34) (40 mg/kg bw/d) [calculated level of AMTT: 0.01 mg/kg bw/d]
Developmental target / critical effect	2-gen. rat: cleft palate in 2 F1a and in 1 F1b pups, agenesia of kidney in F2 pups at 3500/2100 ppm (N24) Developmental rat (N12): Hydrourethers, renal pelves dilatation Developmental rabbbit (N14): accessory $13t^h$ rib(s) (rabbits).
Lowest relevant developmental NOAEL / NOEL	120 mg/kg bw/d (rat)

Neurotoxicity / Delayed neurotoxicity (Annex IIA, point 5.7)

Acute oral and 90-day neurotoxicity*	No signs of neurotoxicity in the presence of general toxicity (rat) NOAEL acute neurotoxicity (N24): 2000 mg/kg, NOAEL 90-day neurotoxicity (N24) 3500 ppm (243 mg/kg bw/d) (NOAEL general toxicity, 90-d, rat: 100 ppm (7 mg/kg bw/d)
Developmental neurotoxicity	No developmental neurotoxicity (N59)
Lowest relevant NOAEL for neurotoxicity*	90-d, rat: 3500 ppm (243 mg/kg bw/d)

Other toxicological studies (Annex IIA, point 5.8)

Supplementary studies with metabolites:

635M01:
LD_{50} oral rat: > 5000 mg/kg bw;
Ames test, CHO-HPRT test, *in vitro* chromosome aberration test: negative,
28-day oral rat: No signs of toxicity; NOAEL: 3900 ppm (344 mg/kg bw/d)
Conclusion: No toxicological relevance.
635M02:
LD_{50} oral rat: 1000 mg/kg bw (purity 98.2 %);
LD_{50} oral rat: > 2000 mg/kg bw (purity >98.5 %);
Median lethal dose: approx. 500 mg/kg bw (purity 99.9 %),
Ames test, CHO-HPRT test, *in vitro* chromosome aberration test: negative
Two 28-d studies, rat (purity 98.4 %): NOAEL 150 ppm (14 mg/kg bw/d); effects on kidney, liver, spleen, uterus and ovaries.

R 48/22

Two 1-gen. Studies, rat (purity 98.4 %): NOAEL (reproduction): 200 ppm (approx. 13 mg/kg bw/d); NOAEL (general toxicity): ≤ 200 ppm,
Main effects 1. study at 400 ppm: reduced number of corpora lutea/massive follicle degeneration in the ovaries (1 female), ↓ male/female fertility index (3/10 pairs)
Main effects 2. Study at 400 ppm: postimplantation loss (1 female).
Conclusion: No toxicological relevance (according to EU Guidance document on relevant metabolites in groundwater).
635M03:
LD_{50} oral rat: > 5000 mg/kg bw;
subchronic study in rats: no effects;
Ames test, CHO-HPRT test, *in vitro* chromosome aberration test: negative
Conclusion: No toxicological relevance.
635M017:
LD_{50} oral rat: > 2000 mg/kg bw;
Ames test, CHO-HPRT test, in vivo mouse micronucleus test: negative
Conclusion: No toxicological relevance.
Supplementary studies with AMTT (635M04):
Toxicokinetic, rats: rapid excretion, major metabolite AHTT (635M11);
LD_{50} oral rat: > 200 < 2000 mg/kg bw (ulcer in glandular stomach);
no changes in estrus cycle and hormone analysis parameters;
Ames test, CHO-HPRT test, mouse micronucleus test: negative;
developmental toxicity < 20 mg/kg bw/d; low bonding capacity of tritosulfuron and AMTT to the estrogen receptor in the presence of endogenous estrogens;
In vitro chromosome aberration assay in V79 cells: negative for chromosomal aberration, suppression of the mitotic activity
Conclusion: Toxicologically relevant

Medical data (Annex IIA, point 5.9)

Limited data (new compound); no human health problems reported

Summary (Annex IIA, point 5.10) Proposal

The following reference values relate to tritosulfuron containing ≤ 0.02 % AMTT.

	Value	Study	Safety factor
ADI	0.06 mg/kg bw	12-mo dog	100
AOEL systemic	0.15 mg/kg bw/d	90-day dog	100
ARfD	Not allocated	Not necessary	

The following reference values relate to AMTT

ADI	0.0001 mg/kg bw	2-gen., rat	500
AOEL systemic	0.0001 mg/kg bw/d	2-gen., rat	500
ARfD	0.0001 mg/kg bw	2-gen., rat	500

Dermal absorption (Annex IIIA, point 7.3)

1 % (concentrate), 2 % (in-use dilution) based on *in vitro* (rat/human) and *in vivo* (rat) studies conducted using the commercial formulation

Acceptable exposure scenarios (including method of calculation)

Operator	Use acceptable (German model, with PPE)
Workers	Use acceptable
Bystanders	Use acceptable

Suggested classification and labelling of tritosulfuron:

Classification and proposed labelling (Annex IIA, point 10)

with regard to toxicological data	Tritosulfuron (≤ 0.02 % AMTT): Xi, R43 AMTT: T, R40, R 48/22, R61 (R64)

* study/studies conducted with batch no. N24 (2.45 % AMTT)
** study/studies conducted with AMTT

VOLUME 3:

B.5 Methods of analysis

B.5.2 Analytical methods (residue) for plants, plant products, foodstuffs of plant and animal origin, feedingstuffs (Annex IIA 4.2.1; Annex IIIA 5.2)

B.5.2.1 Plant material

Report: Bross M., Mackenroth C., (2003): Validation of the analytical method 405/1: Determination of BAS 635 H(LAB 271 272) in plant matrices, BASF AG, unpublished, BASF DocID 2003/1001356, Date 2003-04-01, MET2003-330

GLP: yes

Guideline: SANCO/825/00 rev. 6 (20.06.00), Commission Directive 96/46/EC

Acceptability: The method is considered to be acceptable.

Materials and Methods
Test material: commodities with high water content and fruits with high acid content

Principle of method: For the determination of tritosulfuron in lettuce, oranges and maize grain the method no. 405/1 is used as described in the DAR. Final determination is performed by HPLC-UV(260 nm) and by Diode-Array-Detection (quantification at 260 nm).

Findings
The validated limit of quantification is 0.01 mg/kg for lettuce, oranges and maize grain. Acceptable chromatograms from samples and blank materials, appropriate calibration graphs, individual recovery data and information on the precision of the method are presented. For the detection with DAD no spectra are given.
In maize grain the recovery of one sample at fortification level 0.1 mg/kg was very low (38 %). The reason could not be found but seemed to be caused by a not provable mistake and not due to the method.

Table B.5.2-1: Validation data for analytical method for the determination of residues in plant material

Reference	Matrix	Detection method	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of Analysis
Bross, Mackenroth (2003)	Lettuce	HPLC-UV	0.01	80.4	5.6	5
			0.1	86.2	1.6	5
	Orange		0.01	69.1	6.0	5
			0.1	83.2	5.0	4
	Lettuce	HPLC-DAD	0.01	98.8	3.9	5
			0.1	91.9	3.5	5
	Orange		0.01	79.4	1.6	5
			0.1	86.3	4.5	4
	Maize grain		0.01	82.1	8.3	5
			0.1	73.6	29.1	5

Conclusion
The method is suitable for the determination of tritosulfuron in commodities with high water content and in fruits with high acid content down to a limit of quantification of 0.01 mg/kg. For the proposed confirmatory method by DAD no spectra are presented.

B.5.6 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
AIIA-4.2.1	Bross, M. and Mackenroth, C.	2003	Validation of the analytical method 405/1: Determination of BAS 635 H(LAB 271 272) in plant matrices BASF DocID 2003/1001356 GLP, unpublished MET2003-330	Y	BAS

B.6 Toxicology and Metabolism

B.6.8 Further toxicological studies (Annex IIA 5.8)

B.6.8.1 Toxicity studies of metabolites

B.6.8.1.1 635M02

635M02 (Reg. No. 292 564; BH 635-2, TBSA) is a soil metabolite and was detected in the rat metabolism study (< 1 % in urine/feces). In the first data package submitted, it was tested in three mutagenicity assays as well as in two acute oral tests. When it became obvious that this metabolite exceeds the groundwater threshold of 0.1 µg/L by leaching the toxicological relevance had to be addressed in greater detail.
In an additional acute oral toxicity study in rats, TBSA of high purity (99.9 %) was tested in order to rule out the possibility that the acute oral toxicity seen in a previous study with a less pure batch of the test material was due to the presence of an impurity [see Wiemann C. and Hellwig J. 1999(a); BASF RegDoc# 1999/10099].
In an additional 28-day toxicity study no NOAEL could be derived and - beside others - effects on the female reproductive organs were observed. Therefore, a second 28-day study conducted with lower doses of TBSA was submitted.
The objective of the 1-generation studies was to determine the possible adverse effects of TBSA on the integrity and performance of the male and female reproductive systems since effects were noted in the first 28-day study.
For an overview of the study results see table below.

The metabolite TBSA was found to be not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay and not mutagenic *in vitro* in the CHO/HPRT mutation assay.
Based mainly on comments of the UK, at ECCO 136 it was concluded that 635M02 may have some chromosome-damaging (clastogenic) potential under *in vitro* conditions in V79 cells with S-9 mix. Therefore in the endpoint list the *in vitro* chromosome aberration assay was considered positive. The re-evaluation of the study led to the conclusion that 635M02 has no clastogenic potential. The justification is given in the assessment of the study results (see Vol. 3).
Three acute oral studies were conducted in total. One study resulted in an oral LD_{50} of 1000 mg/kg bw, one in an oral LD_{50} of > 2000 mg/kg bw. The new study was conducted with the test substance of high purity (99.9 %). The acute oral LD_{50} was found to be greater than 300 mg/kg and less than 2000 mg/kg body weight in rats. The median lethal dose lies between 500 and 1000 mg/kg bw. Thus, it can be considered that the metabolite 635M02 is of higher oral toxicity than the parent compound tritosulfuron (N12).
In addition, two 28-day repeated dose toxicity study in rats were submitted after the completion of the DAR. In the first study effects on the body weight (decreases), in the functional observation battery (reduced rearing and overall motor activity, tremor), on hematological (decreased red blood cell parameters) and clinico-chemical examinations (increased γ–glutamyltransferase activities, increased bilirubin and cholesterol levels), on urinalysis (increased number of degenerated renal tubular epithelial cells and transitional epithelial cells as well as higher number of granular casts and epithelial cell casts) and on organ weights (higher kidney, liver and spleen weights and lower ovary and uterine weights) were noted. The histopathological examinations showed that the ovaries of high-dose group

females had a significantly decreased number of corpora lutea. The differential ovarian follicle count (DOFC) indicated that the number of clearly discernible antral follicles was reduced to 60 % of the control value at 5000 ppm. A treatment-related altered hormonal function of unknown etiology and pathogenesis could also not be excluded for the increased number of females with slight or moderate atrophy of the uterus (mucosa and musculature) in the mid and high dose groups. In the spleen, increased incidences of hemosiderin deposition and congested blood vessels were observed in mid and/or high dose groups. A NOAEL could not be derived from this study, based on effects on red blood cells, organ weights and ovaries at the lowest dose of 200 ppm tested (corresponding to daily intakes of 18.6 and 20.0 mg/kg bw/d for males and females, respectively).
Therefore, a subsequent 28-day oral toxicity study was conducted using three lower dose levels (0, 50, 100 and 150 ppm, corresponding to approximately 5.0, 10.0 and 14.7 mg/kg bw/d; see BASF RegDoc# 2003/1004049). In this study an increased α2u-globulin accumulation in the kidneys of male rats were noted in all dose-groups. This finding was considered to represent a sex and species specific effect, without relevance to human males. Thus, the NOAEL was set at 150 ppm (14.7 mg/kg bw/d).
To possibly exclude effects of 635M02 on fertility and reproduction two 1-generation studies were conducted with similar dose levels (0, 200 and 400 ppm; corresponding to approximately 12.8/16.2 and 25.3/32.4 mg/kg bw/d in males/females). In the first study, the male/female fertility index was slightly reduced in the high dose of 400 ppm (70 % versus 90 % and 100 % in the control and low dose), with 3/10 non-pregnant females in the 400 ppm group. One female of the 400 ppm group showed a severely reduced number of corpora lutea and a massive follicle degeneration in both ovaries. No further effect on female reproduction, delivery and litter/pup data was noted.
Cholesterol concentrations were increased in males and females at 400 ppm. High dose females showed lower magnesium levels. The mean absolute and relative liver, kidney and spleen weights were significantly increased in high dose F_0 parental males, when compared with controls. In high dose F_0 parental females, the relative spleen weight was significantly increased.
It cannot be concluded with certainty that the impaired fertility in high dose animals (one pair in the control versus 3 pairs in the high dose group) was not substance-related. A fertility index of 70 % is below the historical control data for this laboratory. In addition, the findings in the ovary of a single high dose female (severely reduced number of corpora lutea, massive degeneration of follicles) may indicate a high sensitivity of single animals. Moreover, effects on the ovaries and uterus were also noted in the 28-day repeated dose study at concentrations of ≥ 200 ppm (20 mg/kg bw/d).
Under the conditions of this study the NOAEL for reproductive toxicity is 200 ppm (corresponding to approximately 12.8 mg/kg bw/d and 16.2 mg/kg bw/d in males and females respectively). The NOAEL for general toxicity is below 200 ppm based on lower body weight and organ weight changes at this dose level.
In the second 1-generation study, one female of the high dose group (400 ppm) was fertile (proved by 6 implantation sites in utero at terminal sacrifice) but did not deliver pups. Historical control data were not provided for this endpoint. There was no further substance-related effect on female reproduction, delivery and litter data. The DOFC revealed no changes in the number of primordial and/or growing follicles between the control and treated groups.
The F1 pups body weights and body weight gains were minimally lower in the 400 ppm group at all investigation points, although without statistical significance and with values lying mainly within the historical control values. In the presence of maternal toxicity at this dose level, these changes were not considered to reperesent a reproductive adverse effect.
Under the conditions of this study the NOAEL for reproductive toxicity is 200 ppm (corresponding to approximately 17.6 mg/kg bw/d and 17.3 mg/kg bw/d in males and females

respectively) based on postimplantation loss noted in one female at the next higher dose of 400 ppm (corresponding to approximately 35.0 mg/kg bw/d and 33.1 mg/kg bw/d in males and females respectively).
The NOAEL for general toxicity is below 200 ppm based on lower body weight and clinico-chemical findings (higher white blood cell count in males, higher cholesterol concentrations in males and females, lower magnesium concentrations in females) at this dose level.

Table B.6.8-1: Summary of toxicity studies of metabolite 635M02 (Reg.-No. 292 564)

Study/strains/species	Test material/conditions	Results
Acute oral toxicity in rats Wistar rats (Chbb:THOM (SPF)) 5 male / 5 female 500, 2000 and 5000 mg/kg bw	Batch No. 00831-201, purity: 98.2 %, Test substance preparation in 0.5 % Tylose CB 30.000 in aqua bidest	LD_{50}: 1000 mg/kg bw Mortality at ≥ 2000 mg/kg bw (delayed after 3 to 4 days), main signs of toxicity: dyspnea, apathy, excitation, abdominal and lateral position, staggering, ataxia, atonia, paresis, narcotic-like state, pain and corneal reflex absent, tremor, spastic gait, erythema, exsiccosis, salivation, lacrimation, discoloured urine, squatting posture and red clammy snout and eyelid.
Acute oral toxicity of TBSA in rats Wistar rats (Chbb:THOM (SPF)) 3 male / 3 female 200 and 2000 mg/kg bw	Batch No. 26778/99; 26778/101, Purity: > 98.5 %, Test substance preparation in olive oil DAB 10	LD_{50}: > 2000 mg/kg bw No mortality Main signs of toxicity: dyspnea, apathy, staggering, exsiccosis, red discoloured urine until day 5 post dosing
Acute oral toxicity of TBSA in rats Wistar rats (CrlGlxBrlHan:WI) 3 x 3 female 2000 and 300 mg/kg bw	Batch no: 2059-029, purity: 99.9 % Test substance preparation in 0.5 % CMC-solution (cleaned sodium carboxymethylcellu-lose) in double distilled water	Median lethal dose: around 500 mg/kg bw All animals at 2000 mg/kg bw were found dead on study day 3 No mortality at 300 mg/kg bw. Main signs of toxicity: dyspnoea, abdominal or lateral position, staggering, smeared fur, lacrimation and red smeared fur in the anogenital area
Salmonella typhimurium/ *Escherichia coli* reverse mutation assay *S. typhimurium* strains TA 100, TA 1535, TA 1537, TA 98 and *E. coli* strain WP2 uvrA	Batch No. 00831-201, Purity: 98.2 %.	Not mutagenic
Gene mutation assay in Chinese hamster ovary (CHO) V79 cells *in vitro* (V79 / HPRT)	Batch No. 00831-201, Purity: 98.2 %.	Not mutagenic
In vitro chromosome aberration assay in V79 cells	Batch No. 00831-201, Purity: 98.2 %	Not mutagenic
28-day oral toxicity study in rats Wistar rats (CrlGlxBrlHan:WI) 5 males / 5 females 0; 200; 1000 and 5000 ppm (18.6/20.0, 90.7/96.1, 417.3/414.2 mg/kg bw/d for males and females)	Batch no. 25887 Fass 2; purity: 98.4 %.	No NOAEL LOAEL 200 ppm: ↑ liver wt.,↑ kidney wt. (males),↓ RBC (f), α_{2u}–globulin accumulation (male kidneys), hyperplastic ovarian stroma cells 1000 ppm: ↓ bw (females), ↓ overall motor activity, ↓ RBC, ↑ bilirubin, ↑ cholesterol, cells and casts in urine sediment (males), ↑ liver wt., ↑ kidney wt. (males), ↑ spleen wt., α_{2u}–globulin accumulation (male kidneys), hepatocellular hypertrophy (males), hyperplastic ovarian stroma cells, atrophy of the uterus (mucosa and

Study/strains/species	Test material/conditions	Results
		musculature), hemosiderin deposition (spleen) 5000 ppm: tremor, piloerection from day 14 onwards, ↓ bw, ↓ rearing, ↓ overall motor activity, ↓ RBC, ↑ γ–glutamyltransferase activities, ↑ bilirubin, ↑ cholesterol, cells and casts in urine sediment (males), ↑ liver wt., ↑ kidney wt. (males), ↓ ovary and uterus wt., ↑ spleen wt., α_{2u}–globulin accumulation (male kidneys), chronic progressive nephropathy (male), hepatocellular hypertrophy, ↓ number of corpora lutea (ovary), hyperplastic ovarian stroma cells, atrophy of the uterus (mucosa and musculature), hemosiderin deposition (spleen)
28-day oral toxicity study in rats Wistar rats (CrlGlxBrlHan:WI) 5 male / 5 female 0, 50, 100 and 150 ppm (4.8/5.0; 9.0/10.0; 13.9/14.7 mg/kg bw/d in males and females)	Batch no. 25887 Fass 2; purity: 98.4 %.	NOAEL: 150 ppm All dose groups: $\alpha_{2\mu}$-globulin accumulation in the kidney (males)
1-generation study in rats (range finding) Wistar rats (CrlGlxBrlHan:WI) 10 male / 10 female 5 male / 5 female as satellite groups 0; 200 and 400 ppm (12.9/16.2; 25.3/32.4 mg/kg bw/d in males and females) premating phase: 4 weeks	Batch no. 25887 Fass 2; purity: 98.4 %	NOAEL (reproduction): 200 ppm NOAEL (general toxicity): ≤ 200 ppm 200 ppm: ↓ bw (f), ↑ liver wt.(f) 400 ppm: ↓ bw (f), ↓ male/female fertility index, severely reduced number of corpora lutea/ massive follicle degeneration in the ovaries (1 female), ↑ cholesterol, ↓ magnesium (f), ↑ kidney wt. (m); ↑ spleen wt., ↑ liver wt., ↑ thyroid wt. (f)
1-generation study in rats (main study) Wistar rats (CrlGlxBrlHan:WI) 10 male / 10 female 5 male / 5 female as satellite groups 0; 200 and 400 ppm (17.7/20.7; 35.0/40.9 mg/kg bw/d in males and females) premating phase: 10 weeks	Batch no. 25887 Fass 2; purity: 98.4 %.	NOAEL (reproduction): 200 ppm NOAEL (general toxicity): ≤ 200 ppm 200 ppm: ↓ bw (f), ↑ WBC (m), ↑ cholesterol, ↓ magnesium (f) 400 ppm: ↓ bw (f), postimplantation loss (1 female), ↑ WBC (m), ↓ RBC, Hb, ,HCT, ↑ Reticulocytes, ↑ cholesterol, ↓ magnesium (f), ↑ kidney wt. (m), ↑ liver wt., ↑ spleen wt.

Statement on the toxicological relevance of 635M02 (TBSA)

The acute oral toxicity of TBSA is approximately 4 times higher than the parent compound tritosulfuron (TBSA: LD_{50} approximately 1000 mg/kg bw versus tritosulfuron: LD_{50} 4700 mg/kg bw). The results of the toxicity studies (28-day repeat-dose and 1-generation studies) in rats mainly indicated the ovaries (decreased number of corpora lutea, stromal hyperplasia) and the uterus (atrophy of mucosa and musculature) as target organs. Repeated high doses of TBSA (5000 ppm corresponding to approximately 400 mg/kg bw/d) affected all female rats, whereas at lower doses (400 ppm corresponding to approximately 30 mg/kg bw/d) only single females were affected. Effects on the uterus (atrophy of mucosa and musculature as noted in the 28-day study at ≥ 1000 ppm, 96.1 mg/kg bw/d) were not reported in the 1-generation studies but may be responsible for the intrauterine total litter loss noted in one female at 400 ppm.

The following is therefore proposed in accordance with the latest classification and labelling guidance under Directive 67/548/EEC (i.e. in the 18th ATP published as Directive 93/21/EEC):

TBSA

Hazard symbol:	Xn
Indication of danger:	Harmful
Risk phrase:	R 22 Harmful if swallowed. R 48/22 Harmful: Danger of serious damage to health by prolonged exposure if swallowed.

In conclusion, according to the criteria given in the EU guidance document on relevant metabolites in groundwater, the metabolite has not to be considered as toxicologically relevant. However, because the acute oral toxicity of TBSA suggests a moderate toxicity that is markedly higher than exhibited by the active ingredient and the effects observed mainly in the female reproductive organs (ovaries and uterus, decreased fertility) after repeated dose, a groundwater concentration of 0.75 µg/L must not be exceeded.

B.6.8.1.1.6 Acute oral toxicity study in rats

Report: Gamer A.O., Leibold E., 2003
TBSA - Acute oral toxicity study in rats
BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep.
Unpublished, BASF RegDoc# 2003/1021650

GLP: Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 423, 96/54/EC, EPA / OPPTS 870.1100

Deviations: None, which can be considered to have influenced the integrity of the study.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Reg. No. 292 564 (*syn.* TBSA); batch: 2059-029: purity: 99.9 %
Test animals: Female Wistar rats (CrlGlxBrlHan:WI), Source: Charles River Deutschland GmbH, Sulzfeld.
In this acute oral toxicity study in rats, TBSA of high purity (99.9 % pure) was tested in order to rule out the possibility that the acute oral toxicity seen in a previous study with a less pure batch of the test material was due to the presence of an impurity [see Wiemann C. and Hellwig J., 1999(a); BASF RegDoc# 1999/10099].
Single doses of the test material was administered by gavage to groups of three fasted female Wistar rats as a preparation in 0.5 % CMC-solution (cleaned sodium carboxymethylcellulose) in doubly distilled water. The application volume administered was 10 mL/kg body weight. The first group of three females received a dose of 2000 mg/kg bw. Based on the findings in this first group, two further groups of three animals were sequentially treated with a dose of 300 mg/kg bw. Animals were examined for mortality for at least 14 days. Clinical signs and

symptoms were recorded several times on the day of administration, and thereafter at least once each workday for the individual animals throughout the observation period. Individual body weights were determined shortly before administration (day 0), weekly thereafter and at the end of the study. Additionally, at body weights were recorded on the day of death in animals that died starting with study day 1.

Findings:
All animals of the 2000 mg/kg administration group were found dead on study day 3.
No mortality occurred in the 300 mg/kg bw administration groups.
Clinical observation in the 2000 mg/kg bw administration group revealed impaired and poor general state, dyspnoea, abdominal or lateral position, staggering, smeared fur, lacrimation and red smeared fur in the anogenital area. Findings were observed from hour 4 until including study day 2 after administration.
Clinical observation in one 300 mg/kg bw administration group revealed impaired general state, dyspnoea and staggering. Findings were observed from hour 4 until including hour 5 after administration.
No clinical signs and findings were observed in the second 300 mg/kg administration group.
The mean body weights of one 300 mg/kg administration group increased during the first post exposure observation week but did not adequately increase during the second week. This effect is observed at times in the rat strain used, because in the required age range the female animals have already reached the phase of slow growth.
The mean body weights of the second 300 mg/kg administration group increased throughout the study period.
During necropsy the animals that died showed many black erosions/ulcers in the glandular stomach and red diffuse discoloration of the large and small intestine.
No macroscopic pathologic abnormalities were noted in the animals examined at the end of the observation period.

Discussion:
According to OECD Guideline No. 423 the starting dose was 2000 mg/kg bw. All animals died. The next step was 300 mg/kg bw, instead of the suggested dose of 200 mg/kg bw. None of the six animals died. The testing was stopped. According to Annex 1c of the guideline the median lethal dose is around 500 mg/kg bw. Thus, the metabolite 635 M02 is more toxic than the parent compound tritosulfuron. The results of this study confirm the results of the previously conducted study (Wiemann C., Hellwig J., 1999; BASF RegDoc# 1999/10099) where all animals of the 5000 mg/kg bw dose group died within 4 days after application. Nine animals (4 males and 5 females) of the 2000 mg/kg bw dose group died within 3 days after application. A delayed mortality (6 days after application) was observed in 1 female rat of the 500 mg/kg bw dose group.

Conclusion:
Under the conditions of this study the median lethal dose of Reg. No. 292 564 after oral administration was found to be greater than 300 mg/kg and less than 2000 mg/kg body weight in rats. The median lethal dose lies between 500 and 1000 mg/kg bw.

B.6.8.1.1.7 Range finding study: 28-day subacute study with 635 M02 (TBSA)

Report: Kaspers U. et al., 2003
BSA - Subacute toxicity study in Wistar rats administration in the diet for 4 weeks
BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep., Unpublished,
BASF RegDoc# 2003/1004048

GLP: Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 96/54, OECD 407

Deviations: None, which can be considered to have influenced the integrity of the study.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Reg. No. 292 564 (syn. TBSA, 635 M02); batch no. 25887 Fass 2; purity: 98.4 %.
Test animals: Males and female Wistar rats, CrlGlxBrlHan:WI; supplied by Charles River, Sulzfeld, Germany, age: 32-34 days.

Reg. No. 292 564 was administered to groups of 5 male and 5 female Wistar rats in the diet for four weeks at doses of 0; 200; 1000 and 5000 ppm, corresponding to 18.6/20.0, 90.7/96.1, 417.3/414.2 mg/kg bw/d for males and females respectively.
Food consumption and body weight were determined weekly. The animals were examined for clinical signs of toxicity or mortality at least once a day. Detailed clinical examinations were conducted prior to the start of the administration period and weekly thereafter. A functional observational battery (FOB) and measurement of motor activity were performed towards the end of administration. The FOB included evaluation of home cage observations, Open Field examinations, sensorimotor tests/reflexes, quantitative parameters (feces, urine, rearing, grip strength, landing foot splay).
Clinicochemical, hematological examinations and urinalyses were carried out towards the end of the administration period. Finally, all animals were assessed by gross pathology including the determination of organ weights (liver, kidneys, adrenal glands, testes, epididymides, ovaries, uterus, spleen, brain, heart and thymus), followed by histopathological examinations.

Findings:
Sufficient stability over the study period and homogenous distribution of the test substance in the diet was demonstrated. Concentration control analyses showed that actual concentrations in the diet were within a range of 94.2 – 101.4 % of the nominal concentrations.

Mortality
No animals died during the study.

Clinical examinations
One male animal administered 5000 ppm showed slight tremor from day 14 onwards and piloerection from day 5 onwards. In addition, one male animal of this dose group showed piloerection from day 5 to day 13. In four high dose females tremor as well as in three high

dose females piloerection was observed at several days of the study. These observations were assessed as substance-related.

Table B.6.8.1-1: Treatment-related clinical observations

Sex	Males				Females			
Dietary concentration (ppm)	0	200	1000	5000	0	200	1000	5000
Clinical observations								
Tremor, slight or moderate	0	0	0	1 (20 %)	0	0	0	4 (80 %)
Observed how many times	0	0	0	12	0	0	0	50
Mean onset (day)				15				20
Piloerection	0	0	0	2 (40 %)	0	0	0	3 (60 %)
Observed on x days	0	0	0	28				21
Mean onset (day)				5				7

Food consumption and body weight data
Food consumption was significantly decreased in males and females at 5000 ppm throughout the entire study with a maximum of –33.0 % and –43.2 % at the beginning of the study (day 7). This effect was assessed as related to the test compound. Body weight as well as body weight change was statistically significantly reduced in males and females at 5000 ppm from day 7 until the end of the study. In male animals the decreased body weight reached a maximum of –23.5 % on day 28, whereas the highest decrease measured in female rats was -21.5 % on day 21.

Food efficiency
In high-dose group males food efficiency was decreased over the whole study period, statistically significant on day 7, 21, and 28. In females, food efficiency was statistically significantly reduced at 5000 ppm on day 7, only.

Functional observation battery and motor activity assessment
The following treatment related findings were observed:

Reduced rearing: at 5000 ppm statistically significant effect in both sexes (males: -77.8 %, females: -90.7 %), slightly reduced rearing without statistical significance in females at 1000 ppm.

Slight to moderate tremor (home cage or open field observations): at 5000 ppm in all females and in three of five males. Some of the involved animals also showed an impairment of coordination with unsteady or shuffling gait.

Reduced overall motor activity: at 5000 ppm statistically significant effect in males. In females, motor activity was also slightly reduced at 1000 and 5000 ppm when compared to control and low dose level, although without statistical significance (except at interval 5 at 1000 ppm).

No substance-related effects were observed in the sensorimotor/reflex tests at any dose level.

Hematology
At the end of the study statistically significantly decreased red blood cell counts, hemoglobin concentrations and hematocrit values were found in the peripheral blood of the mid dose males and the low and mid dose females. When compared to control values, red blood cell parameters were decreased in high dose animals, although without statistical significance.
Mean corpuscular volume (MCV) was increased in the high dose animals of both sexes and in the mid dose females. Reticulocyte counts were increased in the mid and high dose rats of

either sex. Since most of these changes occurred not dose-related, they were considered only as a minor trend towards lower red blood cell values.

Table B.6.8.1-2: Hematological findings

Test parameter		Dose level (ppm)							
		0		200		1000		5000	
RBC [10^{12}/L]	M	7.87	(100 %)	7.66	(97 %)	7.12**	(90 %)	7.38	(94 %)
	F	7.87	(100 %)	7.04*	(89 %)	6.88*	(87 %)	7.28	(93 %)
Hb [mmol/L]	M	9.3	(100 %)	9.1	(98 %)	8.6**	(92 %)	9.2	(99 %)
	F	9.3	(100 %)	8.6*	(92 %)	8.3*	(89 %)	8.8	(95 %)
Hct [ratio]	M	0.441	(100 %)	0.425	(96 %)	0.404**	(92 %)	0.435	(99 %)
	F	0.430	(100 %)	0.394*	(92 %)	0.388*	(90 %)	0.417	(97 %)
MCV [fl]	M	56.1	(100 %)	55.5	(99 %)	56.8	(101 %)	58.9*	(105 %)
	F	54.6	(100 %)	55.9	(102 %)	56.6*	(104 %)	57.3*	(105 %)
Reticulocytes [%]	M	25±5	(100 %)	29±8	(116 %)	47±6	(188 %)	35±7	(140 %)
	F	21±7	(100 %)	24±6	(114 %)	62±12	(295 %)	43±6	(205 %)

Statistics: * = p< 0.05; ** = p< 0.01 (Kruskal-Wallis + Wilcoxon-test, 2-sided)

Clinicochemistry

Serum enzyme examinations revealed increased γ–glutamyltransferase activities in the high dose females.

Total bilirubin levels were increased in the serum of the mid and high dose animals of either sex. In males of the high dose group increased urea and decreased glucose levels were noted. In the mid and high dose females cholesterol concentrations were increased. All other changes were considered not to be treatment-related.

Table B.6.8.1-3: Clinicochemical findings

Test parameter		Dose level (ppm)							
		0		200		1,000		5,000	
γ GT [nkat/L]	M	0	(100 %)	0	–	0	–	3	–
	F	1	(100 %)	0	(0 %)	0	(0 %)	40*	(4000 %)
Total bilirubin [µmol/L]	M	2.05	(100 %)	2.28	(111 %)	2.56*	(125 %)	3.30*	(161 %)
	F	2.68	(100 %)	2.54	(95 %)	3.69	(138 %)	4.30**	(160 %)
Urea [mmol/L]	M	5.27	(100 %)	5.62	(107 %)	5.86	(111 %)	7.23**	(137 %)
	F	8.61	(100 %)	6.43	(75 %)	7.42	(86 %)	8.00	(93 %)
Glucose [mmol/L]	M	5.73	(100 %)	5.80	(101 %)	5.44	(95 %)	4.14*	(72 %)
	F	4.76	(100 %)	6.12*	(129 %)	4.95	(104 %)	3.90	(82 %)
Inorganic phosphate [mmol/L]	M	2.69	(100 %)	2.63	(98 %)	2.54	(94 %)	2.66	(99 %)
	F	2.96	(100 %)	2.07*	(70 %)	2.17*	(73 %)	2.64	(89 %)
Creatinine [µmol/L]	M	51.1	(100 %)	48.2	(94 %)	47.4	(93 %)	51.8	(101 %)
	F	60.5	(100 %)	52.4	(87 %)	50.2	(83 %)	47.6*	(79 %)
Cholesterol [mmol/L]	M	1.55	(100 %)	1.76	(114 %)	2.66	(172 %)	2.10	(135 %)
	F	1.21	(100 %)	1.35	(112 %)	1.95**	(161 %)	2.48**	(205 %)

Statistics: * = p< 0.05; ** = p< 0.01 (Kruskal-Wallis + Wilcoxon-test, 2-sided)

Urinalyses

Blood was found in the urine of males with a dose-related increase, reaching statistical significance in the high dose.

Males and females of the high dose group produced slightly increased amounts of urine with decreased specific gravity.

Microscopic examination of the urine sediments revealed increased numbers of degenerated renal tubular epithelial cells and transitional epithelial cells as well as higher numbers of granular casts and epithelial cell casts from the low dose males onwards. Leucocytes were recorded in the urine of high dose animals of either sex.

Table B.6.8.1-4: Urinalyses

Test parameter			Dose level (ppm)			
			0	200	1000	5000
Urine volume [mL]	M		3.5	3.1	3.6	4.3
	F		1.5	1.7	2.1	2.4
Specific gravity	M		1060	1088	1077	1058
	F		1092	1098	1081	1062
Urinary blood 1 = 10 ery/µL 2 = 50 ery/µL	M	<=1	5	3	2	0**
		>=2	0	2	3	5
	F	<=1	4	5	5	5
		>=2	1	0	0	0
Renal tubular 1 = few 2 = many	M	<=1	5	5	2	2
		>=2	0	0	3	3
	F	<=1	5	5	5	5
		>=2	0	0	0	0
Transitional epithelial cells 1 = few 2 = many	M	<=1	5	3	1*	0**
		>=2	0	2	4	5
	F	<=1	5	5	5	5
		>=2	0	0	0	0
Casts 1 = few 2 = many	M	<=1	5	3	1*	0**
		>=2	0	2	4	5
	F	<=1	5	5	5	5
		>=2	0	0	0	0
Leucocytes	M	<=1	5	5	5	3
		>=2	0	0	0	2
	F	<=1	5	5	4	4
		>=2	0	0	1	1

Statistics: * = p< 0.05; ** = p< 0.01 (Fisher's Exact-test, 2-sided)

Terminal body weight and organ weight changes

The terminal body weight was decreased in females of the mid dose group and in either sex at the high dose group.

Absolute and relative liver weight was dose-related increased in either sex from the low dose onwards. Likewise, in males the relative kidney weights were increased from the low dose onwards. In high dose females the weight of the ovaries and the uterus was decreased. Spleen weight was increased at 1000 ppm and above in both sexes.

All other changes in absolute and/or relative weights were considered to be a consequence of decreased terminal body weight.

Table B.6.8.1-5: Organ weight changes

Test parameter		Dose level (ppm)							
		0		200		1000		5000	
Terminal body wt [g]	M	251.88	(100 %)	252.72	(100 %)	254.8	(101 %)	193.36**	(77 %)
	F	155.02	(100 %)	158.4	(102 %)	148.86	(96 %)	122.02**	(79 %)
Abs. brain wt	M	1.86	(100 %)	1.888	(102 %)	1.838	(99 %)	1.792	(96 %)
	F	1.754	(100 %)	1.706	(97 %)	1.742	(99 %)	1.616*	(92 %)
Rel. brain wt [%]	M	0.739	(100 %)	0.747	(101 %)	0.723	(98 %)	0.928**	(126 %)
	F	1.137	(100 %)	1.078	(95 %)	1.171	(103 %)	1.325**	(117 %)
Abs. liver wt [g]	M	7.632	(100 %)	8.288	(109 %)	8.954	(117 %)	8.576	(112 %)
	F	4.556	(100 %)	4.968	(109 %)	5.054	(111 %)	5.25	(115 %)
Rel. liver wt [%]	M	3.026	(100 %)	3.279	(108 %)	3.509**	(116 %)	4.423**	(146 %)
	F	2.936	(100 %)	3.131	(107 %)	3.392*	(116 %)	4.302**	(147 %)
Abs. kidney wt [g]	M	1.96	(100 %)	2.182**	(111 %)	2.366*	(121 %)	1.94	(99 %)
	F	1.336	(100 %)	1.362	(102 %)	1.284	(96 %)	1.034**	(77 %)
Rel. kidney wt [%]	M	0.778	(100 %)	0.864**	(111 %)	0.921	(118 %)	1.004**	(129 %)
	F	0.864	(100 %)	0.86	(100 %)	0.864	(100 %)	0.849	(98 %)
Abs. spleen wt [g]	M	0.532	(100 %)	0.578	(109 %)	0.662*	(124 %)	0.700**	(132 %)
	F	0.348	(100 %)	0.42	(121 %)	0.444	(128 %)	0.416	(120 %)
Rel. spleen wt [%]	M	0.211	(100 %)	0.228	(108 %)	0.261	(124 %)	0.363**	(172 %)
	F	0.223	(100 %)	0.265	(119 %)	0.297**	(133 %)	0.341**	(153 %)
Abs. thymus wt [mg]	M	482.8	(100 %)	447	(93 %)	551.2	(114 %)	342	(71 %)
	F	405.4	(100 %)	410.8	(101 %)	368.4	(91 %)	268.0*	(66 %)
Rel. thymus wt. [%]	M	0.193	(100 %)	0.176	(91 %)	0.217	(112 %)	0.177	(92 %)
	F	0.258	(100 %)	0.259	(100 %)	0.246	(95 %)	0.22	(85 %)
Abs. adrenal wt [mg]	M	58.4	(100 %)	61.4	(105 %)	64.2	(110 %)	57.2	(98 %)
	F	70	(100 %)	71.6	(102 %)	68.6	(98 %)	57.0*	(81 %)
Rel. adrenal wt [%]	M	0.023	(100 %)	0.024	(104 %)	0.025	(109 %)	0.03**	(130 %)
	F	0.045	(100 %)	0.045	(100 %)	0.046	(102 %)	0.047	(104 %)
Abs. heart wt [g]	M	0.866	(100 %)	0.91	(105 %)	0.866	(100 %)	0.668**	(77 %)
	F	0.606	(100 %)	0.634	(105 %)	0.57	(94 %)	0.454**	(75 %)
Rel. heart wt [%]	M	0.344	(100 %)	0.36	(105 %)	0.34	(99 %)	0.345	(100 %)
	F	0.392	(100 %)	0.4	(102 %)	0.383	(98 %)	0.372	(95 %)
Abs. testes wt [g]	M	3.102	(100 %)	2.182	(70 %)	2.366	(76 %)	1.94	(63 %)
Rel. testes wt [%]	M	1.233	(100 %)	1.2	(97 %)	1.159	(94 %)	1.643**	(133 %)
Abs. epididymides wt [g]	M	0.682	(100 %)	0.694	(102 %)	0.662	(97 %)	0.634	(93 %)
Rel. epididymides wt [%]	M	0.272	(100 %)	0.275	(101 %)	0.261	(96 %)	0.327**	(120 %)
Abs. ovary wt [mg]	F	76.8	(100 %)	89.4	(116 %)	77.8	(101 %)	41.0**	(53 %)
Rel. ovary wt [%]	F	0.049	(100 %)	0.056	(114 %)	0.052	(106 %)	0.034**	(69 %)
Abs. uterus wt [g]	F	0.438	(100 %)	0.440	(100 %)	0.398	(91 %)	0.250	(57 %)
Rel. uterus wt [%]	F	0.288	(100 %)	0.277	(96 %)	0.266	(92 %)	0.207	(72 %)

Statistics: * = p< 0.05; ** = p< 0.01 (Kruskal-Wallis + Wilcoxon-test, 2-sided)

Gross necropsy

There were no gross lesions noted during necropsy.

Histopathology

α_{2u}–globulin accumulation in the kidneys of male rats was increased in severity with increasing dose groups. Chronic progressive nephropathy was noted in high dose males.

In the liver, minimal-slight liver cell hypertrophy was observed in males at 1000 and at 5000 ppm in either sex.

Histopathological examination of the ovaries of high-dose group females revealed a significantly decreased number of corpora lutea (mean of about 12 CL in the high dose group as compared to a mean of about 23 in the control group), which corresponded to decreased mean absolute and relative ovary weights in this group. A DOFC was performed, which showed that the numbers of primordial and growing follicles as well as the number of the combined primordial plus growing follicles were marginally lower in the animals of the high dose group (-3 %, -12 % or -4 %, respectively) as compared to the control group. The DOFC also indicated that the number of clearly discernible antral follicles was reduced to 60 % of the control value at 5000 ppm. However, this was not significant, and as only one cut level of both ovaries per animal was evaluated, the evidence of this observation was limited – this the more as the number of antral follicles with clearly visible ovum was considerably higher in the low (260 %) and mid dose groups (280 %) than in the control group. In addition, the ovarian stroma cells were hyperplastic in all dose groups, thus, a no adverse effect level for this finding was not obtained. Etiology and pathogenesis of the ovarian weight changes and microscopic findings were not clearly understood.
A treatment-related altered hormonal function of unknown etiology and pathogenesis could also not be excluded for the increased number of females with slight or moderate atrophy of the uterus (mucosa and musculature) in the mid and high dose groups.
In the spleen, increased incidences of hemosiderin deposition and congested blood vessels were observed associated with significantly increased mean absolute (males) and relative spleen weights (males and females) in mid and/or high dose groups. Etiology and pathogenesis of the congested blood vessels remained unresolved. For the latter finding, a clear no adverse effect level was established for both sexes in the low dose group, whereas increased iron pigment accumulation was noted down to the low dose group in females, thus with lack of a no adverse effect level for female rats.

Table B.6.8.1-6: Histopathology findings

	Males				Females			
Dose level (ppm)	0	200	1000	5000	0	200	1000	5000
Liver								
Hypertrophy, central	0/5	0/5	1/5	5/5	0/5	0/5	0/5	5/5
Minimal	0	0	1	2	0	0	0	4
Slight	0	0	0	3	0	0	0	1
Kidney								
Alpha-2µ accumulation,	5/5	5/5	5/5	5/5	0/5	N.D.	N.D.	0/5
Minimal-slight	5	3	2	0	0			0
Moderate-severe	0	2	3	5	0			0
Chronic nephropathy	0/5	0/5	1/5	5/5	2/5	N.D.	N.D.	0/5
Minimal	0	0	0	0	2			0
Moderate	0	0	1	5	0			0
Spleen								
Congested vessels	0/5	0/5	5/5	5/5	0/5	0/5	4/5	5/5
Hemosiderin deposit	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Minimal-slight	5	5	3	1	5	4	0	0
Moderate-severe	0	0	2	4	0	1	5	5
Hematopoiesis, slight	0/5	0/5	1/5	1/5	0/5	0/5	1/5	0/5
Ovaries								
Hyperplasia, stroma					0/5	3/5	5/5	5/5
Minimal					0	3	0	0
Slight					0	0	5	1
Moderate					0	0	0	4
1-20 corpora lutea					1	1	0	5
21-40 corpora lutea					4	4	5	0
Thyroid gland								
Hypertrophy/hyperplasia, slight	0/5	0/5	1/5	1/5	0/5	N.D.	N.D.	0/5
Uterus								
Atrophy					1/5	1/5	3/5	4/5
Slight					1	1	3	0
Severe					0	0	0	4

Conclusion:
In this 28-day oral feed study with Reg. No. 292 564 in Wistar rats, a NOAEL could not be derived, based on lower RBC, effects on organ weights and on ovaries at the lowest dose of 200 ppm tested (corresponding to daily intakes of 18.6 and 20.0 mg/kg bw/d for males and females, respectively). Therefore, a subsequent 28-day oral toxicity study was conducted using three lower dose levels (BASF RegDoc# 2003/1004049).

B.6.8.1.1.8 Second 28-day subacute study with 635 M02 (TBSA)

Report: Kaspers U. et al., 2003
TBSA - Repeated dose oral toxicity study in Wistar rats administration in the diet for 4 weeks.
BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep., Unpublished, BASF RegDoc# 2003/1004049

GLP: Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 96/54, OECD 407

Deviations: In this supplementary study, the methods applied generally complied to OECD 407 requirements. Based on the findings of the previous 4-week investigation with Reg. No. 292 564, the following deviations applied in this supplementary study:
An examination of the sensory reactivity to stimuli of different types was not conducted at the end of the study period. Moreover, assessments of grip strength and motor activity, as well as FOB investigations were not performed.
In addition to guideline requirements, organ weights were determined for ovaries and uterus.
Histopathology was confined to assessment of all gross lesions, spleen, kidneys and ovaries in all control and test groups.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Reg. No. 292 564 (syn. TBSA); batch no. 25887 Fass 2; purity: 98.4 %.
Test animals: Males and female Wistar rats, CrlGlxBrlHan:WI; supplied by Charles River, Sulzfeld, Germany, age: 32-34 days.
This study was performed as a supplementary study to a former sub-acut toxicity study in Wistar rats (BASF RegDoc# 2003/1004048, dose levels**:** 0, 200, 1,000 and 5,000 ppm) to obtain a clear "no observed adverse effect level" (NOAEL) of the test substance.
Reg. No. 292 564 was administered to groups of 5 male and 5 female Wistar rats at dietary concentrations of 0, 50, 100 and 150 ppm, corresponding to 4.8 / 5.0; 9.0 / 10.0; 13.9 / 14.7 mg/kg bw/d in males and females.
Food consumption and body weight were determined weekly. The animals were examined for signs of toxicity or mortality at least once a day. Detailed clinical examinations in an open field were conducted prior to the start of the administration period and weekly thereafter. Clinicochemical, hematological examinations and urinalyses were performed towards the end of the administration period. Finally, animals were assessed by gross pathology, followed by histopathological examinations.

Findings:
Sufficient stability over the study period and homogenous distribution of the test substance in the diet was demonstrated. Concentration control analyses showed that actual concentrations in the diet were within a range of 99.4–107.7 % of the nominal concentrations.
No treatment-related findings were evident from clinical observations, assessment of food consumption, water consumption, body weight data, food efficiency, clinicochemistry, hematology or urinalysis.

Organ weight
In female rats, the mean relative weight of the kidneys was significantly increased in the high dose group (+12.8 %). This was possibly incidental, as no such weight changes were recorded in males. Anyhow, histopathological examinations on the female kidneys were not performed to exclude a treatment-related effect.
The other mean relative weight parameters did not show significant differences when compared with the control group.

Gross necropsy and histopathology
No substance-related effects were revealed upon gross necropsy of the animals under study.
Treatment-related microscopic findings were detected in the kidneys of male rats. They consisted of a slightly increased $\alpha_{2\mu}$-globulin accumulation in the epithelia (and occasionally in the tubular lumen) of the proximal tubules of the renal cortex of treated males as compared to the control animals. Detection of $\alpha_{2\mu}$-globulin was based on Mallory's stain. The graded severity ranged from minimal (grade 1) to moderate (grade 3) in treated animals. In the control group, the amount of $\alpha_{2\mu}$-globulin accumulation was minimal or slight, whereas in the low, mid and high dose groups, more animals with higher grades of severity were affected with $\alpha_{2\mu}$-globulin accumulation in a kind of dose related fashion:

Table B.6.8.1-7 Kidney findings in male rats

	Control	50 ppm	100 ppm	150 ppm
$\alpha_{2\mu}$-globulin accumulation	5/5	5/5	5/5	5/5
grade 1 (minimal)	3/5	1/5	2/5	0/5
grade 2 (slight)	2/5	3/5	1/5	2/5
grade 3 (moderate)	0/5	1/5	2/5	3/5
Mean grade	1.4	2.0	2.0	2.6

The $\alpha_{2\mu}$-globulin accumulation did not cause any other microscopic findings in the kidneys. The only other microscopic findings in the kidneys were a unilateral small cortical scar, associated with a cortical cyst in a high dose male rat (No. 17). Both findings were regarded to have developed spontaneously and unrelated to treatment or $\alpha_{2\mu}$-globulin accumulation.
In the spleen of female rats, hemosiderin deposition was noted in all animals of the control and treatment groups, giving no indication of a treatment-related change:

Table B.6.8.1-8: Spleen findings in female rats

	Control	50 ppm	100 ppm	150 ppm
Hemosiderin deposition	5/5	5/5	5/5	5/5
grade 1 (minimal)	4/5	4/5	3/5	3/5
grade 2 (slight)	1/5	1/5	2/5	2/5
Mean grade	1.2	1.2	1.4	1.4

In the ovaries, no microscopic findings were noted up to 150 ppm, the highest dose tested.

Discussion:
In this study no signs of toxicity were observed in female animals at the concentrations tested. Thus, the NOAEL for female rats can be established at 150 ppm (14.7 mg/kg bw/d). In male animals, an increased incidence of $\alpha_{2\mu}$-globulin accumulation in the kidney was found. This finding represents an unique feature of male rats that does not occur in any other mammalian species, especially not in human males. Since an increased incidence of $\alpha_{2\mu}$-globulin accumulation has no relevance for humans, consequently the NOAEL for male rats regarding human risk assessment is also 150 ppm (13.9 mg/kg bw/d).

Conclusion:
Under the conditions of this 4-week dietary study, the NOAEL was 150 ppm, equivalent to 13.9 mg/kg bw/d for male and 14.7 mg/kg bw/d for female rats.

B.6.8.1.1.9 First 1-generation study with 635 M02 (TBSA)

Report: Schneider S. et al., 2004
BSA - One-Generation Reproduction Toxicity Study in Wistar Rats Range finding study (with 4 weeks premating) Continuous Dietary Administration.
BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep., unpublished, BASF RegDoc# 2004/1017198

GLP: Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 415; OECD 416; OPTTS 870.3800

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Reg. No. 292 564 (syn. TBSA); batch no. 25887 Fass 2; purity: 98.4 %.
Test animals: Male and female Wistar rats, CrlGlxBrlHan:WI; supplied by Charles River, Sulzfeld, Germany.
The objective of this range finding study was to determine the possible adverse effects of Reg. No. 292 564 on the integrity and performance of the male and female reproductive systems, including gonadal function, mating behavior, conception, gestation, parturition, lactation and weaning, and on growth and development of offspring from one generation of Wistar rats continuously administered to the test substance in the diet. The study should also provide information about the effects of Reg. No. 292 564 on neonatal morbidity, mortality, possible target organs in the offspring and data on pre- and postnatal developmental toxicity.
Reg. No. 292 564 was administered to groups of 10 male and 10 female healthy young adult Wistar rats (CrlGlxBrlHan:Wi) via the diet at dose levels of 0; 200 and 400 ppm, respectively. In addition, satellite groups each consisting of 5 male and 5 female rats were treated at the same dose levels. The satellite animals were sacrificed after about four weeks. The main groups formed the F_0 parental generation. These animals were allowed to mate at least 33 days after the beginning of treatment to produce a litter (F_1). Mating pairs were from the same treatment group. Treatment of the parental animals continued throughout mating, gestation and the three-week lactation period until about 16 hours before terminal sacrifice. The F_1 pups were raised up until day 4 (culling) or 21 post partum (p.p.). Thereafter, the F_1 weanlings and the F_0 adult animals were sacrificed.
The health status of the F_0 rats and the satellite animals was checked each day, and the F_0 parental animals were examined for their mating and reproductive performance. Food consumption of the F_0 parents and satellite animals was determined regularly during premating (once weekly over a period of 7 days each), and during gestation (days 0-7, 7-14, 14-20) and lactation periods (days 1-4, 4-7, 7-14). In general, body weights of F_0 parents and satellite animals were determined once weekly (each time for a period of 7 days). However, F_0 females were weighed on days 0, 7, 14 and 20 of gestation and on days 1, 4, 7, 14 and 21 of lactation.
Blood samples were taken from 5 satellite animals per sex and group shortly before terminal sacrifice for clinical pathology examinations, which included haematology, clinicochemistry and determination of total triiodothyronine (T_3), total thyroxine (T_4) and thyroid stimulating hormone (TSH).

Sperm head counts and morphology were assessed in all control and high dose satellite males, while sperm motility was examined in the satellite males of all groups at scheduled sacrifice.
All F_1 pups were sexed on the day of birth and were weighed on the subsequent day as well as on day 4 after birth. Their viability was recorded. Standardised litters were weighed on days 4, 7, 14 and 21 post partum. All pups were examined macroscopically at necropsy (including weight determinations of brain, spleen and thymus in one pup/sex/litter).
In the F_0 animals and satellite animals of both genders organ weights (liver, kidneys, adrenal glands, testes, epididymides, cauda epididymis, prostate, seminal vesicle with coagulation gland, ovaries, uterus, spleen, brain, pituitary gland, thyroid gland with parathyroid gland) were determined. Histopathological examinations were performed in F_0 animals and satellite animals of both genders of selected organs (vagina, cervix uteri, uterus, ovaries, oviducts, testes, epididymides, seminal vesicle, coagulation glands, prostate, pituitary, adrenal gland, all gross lesions). Of the ovaries, a DOFC was performed.

Findings:
Sufficient stability over the study period and homogenous distribution of the test substance in the diet was demonstrated. Concentration control analyses showed that actual concentrations in the diet were within a range of 94.5–104.7 % of the nominal concentrations.

Test substance intake
The calculated test substance intakes are presented in the table below:

Table B.6.8.1-9: Test substance intake (mg/kg bw/d)

	200 ppm	400 ppm
F_0 males	12.9	25.3
F_0 females		
Premating	16.2	32.4
Gestation period	16.2	32.6
Lactation period	29.2	60.9
Satellite males	12.8	25.7
Satellite females	16.6	32.2

Body weights, food consumption, food efficiency, water consumption
Body weights gains of in the F_0 parental and satellite females were slightly reduced (about 10 % - 20 %, occasionally attaining statistical significance) compared to control group females at both dose levels tested 200 and 400 ppm.

Male reproduction data
Mating was confirmed for all F0 parental males, which were placed with females to generate F1 pups. Thus the mating index was 100 % in all groups.
The male fertility index was reduced in the 400 ppm group when compared to control and low dose.

Table B.6.8.1-10: Fertility indices of F0 males/females

	0 ppm	200 ppm	400 ppm
Concerning F1 litters	9/10 (90 %)	10/10 (100 %)	7/10 (70 %)

Sperm parameters
Sperm parameters were not influenced by the treatment and values obtained were comparable among control and treatment groups.

Female reproduction data

The female fertility index varied between 70 % and 100 % (see table above), with 3/10 non-pregnant females in the 400 ppm group. One out of these females (No. 121) showed macroscopically a reduced size of the ovaries, which correlates with the severely reduced number of corpora lutea and a massive follicle degeneration in the ovaries. Implantation was not affected by the test substance. The lower number of total implantation sites in the 400 ppm group is due to the fact that 3 females did not become pregnant.

There was no further substance-related effect on female reproduction, delivery and litter data. The viability index (pups surviving days 1 to 4) was 99 %, 97 % and 100 % in the control, 200 and 400 ppm group, the lactation index was 100 % in all groups.

Table B.6.8.1-11: Summary of female reproduction, delivery and litter data

	0 ppm	200 ppm	400 ppm
Females with liveborn (N) Gestation index (%)	9 100	10 100	7 100
With stillborn pups (N)	2	1	0
Pups delivered (mean)	12.2	10.2*	10.6
Liveborn (N) Live birth index (%)	104 95	101 99	74 100
Pups died (N)	0	1	0
pups dead day 1-4	1	3	0
pups dead days 15-21	0	0	0

Laboratory investigations (satellite animals)

There were no treatment-related changes in hematological parameters. Hormone examinations (T3, T4, TSH) revealed slightly higher values in high dose males, although without statistical significance.

Cholesterol concentrations were increased in males and females at 400 ppm. High dose females showed lower magnesium levels.

Table B.6.8.1-12: Laboratory investigations at day 34

	0 ppm	200 ppm	400 ppm
Males			
T3 (nmol/L)	1.25	1.23	1.40
T4 (nmol/L)	43.82	47.60	51.48
TSH (µg/L)	11.12	11.12	14.88
Cholesterol (mmol/L)	1.66	2.17	2.47*
Magnesium (mmol/L)	0.89	0.89	0.88
Females			
T3 (nmol/L)	1.28	1.47	1.38
T4 (nmol/L)	29.73	35.08	35.93
TSH (µg/L)	8.49	9.69	8.76
Cholesterol (mmol/L)	1.68	1.60	2.33*
Magnesium (mmol/L)	0.97	0.93	0.89*

* $p \leq 0.05$ Kruskal-Wallis + Wilcoxon-test

Organ weights

The mean absolute and relative kidney and spleen weights were significantly increased in high dose F_0 parental males, when compared with controls. In high dose F_0 parental females, the relative spleen weight was significantly increased. The increased kidney and spleen weights are related to the administration of the test substance. Moreover, the relative liver weight was significantly increased in high dose F_0 males. In the F_0 females, the relative liver

weights were significantly increased in both treatment groups (200 and 400 ppm). Although the increased liver weights did not show a dose-response relationship, a substance-related effect cannot be ruled out with certainty. The absolute and relative weights of the thyroid glands were significantly increased in high dose F_0 females. However, statistically significantly increased thyroid weights could not be reproduced in male or female animals from the satellite groups of this study, which had been administered the test substance for 4 weeks.
No substance-related weight changes occurred in the male and female satellite rats.

Histopathological examination
One female of the high dose group (F0-Generation) showed a reduced number of corpora lutea and degenerated follicles in the ovaries. None of the satellite animals showed a similar finding. A focal degeneration in the testes was noted in 4 high dose rats versus 1 in the control and none in the low dose (F0-Generation).

Differential ovarian follicle count (DOFC)
One high dose female (No. 121) of the F0 generation group showed a severely reduced number of corpora lutea in the ovaries. This finding correlates with the macroscopically observed reduced organ size. In addition, a massive degeneration of follicles was noted in these ovaries. In the DOFC only one primordial follicle and one growing follicle were counted in this female. The degeneration of follicles caused the infertility in this female. There were no further significant deviations between controls and animals of the high dose.

F1-generation pups/litters

The F1 pups body weight data were unaffected by the treatment.
The absolute and relative spleen weights were higher in male pups of the 400 ppm group although without statistical significance.

Table B.6.8.1-13: Pup organ weight to body weight data

	0 ppm	200 ppm	400 ppm
Body weight day 21 in grams (mean male and female pups)	46.8	44.6	46.2
Absolute spleen weight (grams) of male pups of female pups	 0.185 0.208	 0.187 0.190	 0.229 0.207
Relative spleen weight (organ to bw ratio) of male pups of female pups	 0.396 0.451	 0.403 0.418	 0.484 0.454

Pups necropsy observations
The few changes noted at necropsy were considered not related to the treatment with the test article.

Discussion:
It cannot be concluded with certainty that the impaired fertility in high dose animals (one pair in the control versus 3 pairs in the high dose group) was not substance-related. Historical control data from the laboratory on this endpoint (female fertiliy index) indicate 84 % as the minimum. Thus, 70 % are outside the historical data. In addition, the findings in the ovary of

a single high dose female (severely reduced number of corpora lutea, massive degeneration of follicles) may indicate a high sensitivity of single animals. Moreover, effects on the ovaries and uterus were also noted in the 28-day repeated dose study at concentrations of ≥ 200 ppm (20 mg/kg bw/d).

Conclusion:
Under the conditions of this study the NOAEL for reproductive toxicity is 200 ppm (corresponding to approximately 12.8 mg/kg bw/d and 16.2 mg/kg bw/d in males and females respectively) based on impaired fertility and effects on the ovaries noted at the next higher dose of 400 ppm (corresponding to approximately 25.3 mg/kg bw/d and 32.2 mg/kg bw/d in males and females respectively). The NOAEL for general toxicity is below 200 ppm based on lower body weight and organ weight changes at this dose level.

B.6.8.1.1.9 Second 1-generation study with 635 M02 (TBSA)

Report: Schneider S. et al., 2004
TBSA - One-generation reproduction toxicity study in Wistar rats range finding study (with 10 weeks premating) continuous dietary administration
BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep., unpublished
BASF RegDoc# 2004/1017197

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 415; OECD 416; OPTTS 870.3800

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Reg. No. 292 564 (syn. TBSA); batch no. 25887 Fass 2; purity: 98.4 %.
Test animals: Male and female Wistar rats, CrlGlxBrlHan:WI; supplied by Charles River, Sulzfeld, Germany.
In the following study, Reg. No. 292 564 (635M02; TBSA) was administered to groups of 10 male and 10 female healthy young adult Wistar rats (F_0 parental generation) and 5 male and 5 female satellite animals/group as a constant homogeneous addition to the food at different dietary concentrations (0; 200 and 400 ppm). The satellite animals were sacrificed after about ten weeks. At least 75 days after the beginning of treatment, F_0 rats were mated to produce a litter (F_1). Mating pairs were from the same test concentration group. Treatment of the F_0 parental animals continued throughout mating, gestation and the three-week lactation period until about 16 hours before terminal sacrifice. The F_1 pups were raised up until day 4 (culling) or 21 post partum (p.p.). Thereafter, the F_1 weanlings and the F_0 adult animals were sacrificed. The examinations and parameters recorded in this study were identical to those of the 4-week premating range-finding study. For details, see the Material and Methods section of BASF DocID 2004/1017198.

Findings:
Sufficient stability over the study period and homogenous distribution of the test substance in the diet was demonstrated. Concentration control analyses showed that actual concentrations in the diet were within a range of 94.5–104.7 % of the nominal concentrations.

Test substance intake
The calculated test substance intakes are presented in the table below:

Table B.6.8.1-14: Test substance intake (mg/kg bw/d)

	200 ppm	400 ppm
F_0 males	17.7	35.0
F_0 females		
Premating	20.7	40.9
Gestation period	17.3	33.1
Lactation period	30.0	62.3
Satellite males	17.6	35.3
Satellite females	20.3	41.2

Clinical findings
No unscheduled mortalities or substance-induced clinical findings were observed.

Body weights, food consumption, food efficiency, water consumption
Mean body weights gains of the treatment-group F_0 females were slightly below the corresponding control values during gestation and lactation periods (up to 17 % - 18 %, statistically not significant). Food consumption of both genders and body weight data of the males (parental and satellite animals) were unaffected by treatment.

Male reproduction data
Mating was confirmed for all F0 parental males, which were placed with females to generate F1 pups. Thus the mating index was 100 % in all groups. The male fertility index was unaffected by the treatment.

Table B.6.8.1-15: Fertility indices of F0 males/females

	0 ppm	200 ppm	400 ppm
Concerning F1 litters	8/10 (80 %)	10/10 (100 %)	10/10 (100 %)

Sperm parameters
Sperm parameters were not influenced by the treatment and values obtained were comparable among control and treatment groups.

Female reproduction data
The female fertility index varied between 80 % and 100 % (see table above), with 2/10 non-pregnant females in the control group. The postimplantation loss appeared slightly increased in the 400 ppm group. This increase was considered to be due to high dose female no. 122, which did not deliver pups but had 6 implantation sites in utero at terminal sacrifice.
There was no further substance-related effect on female reproduction, delivery and litter data. The viability index (pups surviving days 1 to 4) was 100 %, 100 % and 97 % in the control, 200 and 400 ppm group, the lactation index was 100 % in all groups.

Table B.6.8.1-16: Summary of female reproduction, delivery and litter data

	0 ppm	200 ppm	400 ppm
Females with liveborn (N) Gestation index (%)	8 100	10 100	9 90
With stillborn pups (N)	1	0	0
Pups delivered (mean)	11.6	10.4	11.7
Liveborn (N) Live birth index (%)	90 97	104 100	105 100
Pups died (N)	0	0	0
pups dead day 1-4	0	0	0
pups dead days 15-21	0	0	0
Implantation sites / Postimplantation loss (N)	96/3	112/8	116/ 11

Laboratory investigations (satellite animals)

Slight increases in white blood cell counts were observed in the 200 ppm and 400 ppm groups satellite males, which were coupled with an increase of lymphocytes, polymorphonuclear neutrophils and eosinophils.

Red blood cell count, hemoglobin and hematocrit was decreased in animals of the 400 ppm group, reaching statistical significance in females only. Reticulocyte count was slightly increased.

Table B.6.8.1-17: Hematological investigations

	0 ppm	200 ppm	400 ppm
Males			
WBC (giga/L)	4.32	5.64**	5.82**
RBC (tera/L)	8.45	8.44	8.20
HGB (mmol/L)	9.2	9.2	8.9
HCT (L/L)	0.422	0.426	0.414
Reti (%)	2.1	2.1	2.6
Females			
WBC (giga/L)	3.81	3.97	3.83
RBC (tera/L)	7.84	7.53	7.18
HGB (mmol/L)	9.2	9.0	8.7**
HCT (L/L)	0.414	0.406	0.395
Reti (%)	2.0	2.3	3.2

* $p \leq 0.05$, ** $p \leq 0.01$ Kruskal-Wallis + Wilcoxon-test

Serum cholesterol was increased in males and females at 200 and 400 ppm, reaching statistical significance in females at 400 ppm only.

Finally, magnesium serum concentrations were slightly decreased in females administered 200 or 400 ppm of the test substance.

Hormone examinations (T3, T4, TSH) revealed slightly lower T4 and TSH values in males at 400 ppm.

Table B.6.8.1-18: Laboratory investigations at day 71

	0 ppm	200 ppm	400 ppm
Males			
T3 (nmol/L)	1.11	1.16	1.18
T4 (nmol/L)	59.57	55.33	50.13*
TSH (µg/L)	10.86	9.65	8.96
Cholesterol (mmol/L)	1.71	1.91	2.17
Magnesium (mmol/L)	0.88	0.87	0.86
Females			
T3 (nmol/L)	1.15	1.06	1.36
T4 (nmol/L)	38.32	36.75	38.17
TSH (µg/L)	6.23	8.10	6.80
Cholesterol (mmol/L)	1.27	1.65	2.04*
Magnesium (mmol/L)	0.98	0.88*	0.89*

* $p \leq 0.05$ Kruskal-Wallis + Wilcoxon-test

Organ weights

The mean relative liver and kidney weights were significantly increased in high dose F_0 parental males, when compared with controls. The relative spleen weight was significantly increased in high dose F_0 parental females and minimally higher in males. The increased liver, kidney and spleen weights in the high dose F_0 parents are related to the administration of the test substance.

In the satellite groups, the mean relative kidney and liver weight was increased in high dose males, when compared with controls. In high dose females, the relative liver weight as well as the absolute and relative spleen weights were significantly increased. The increased liver, kidney and spleen weights in the high dose satellites are related to the administration of the test substance.

The absolute and relative weights of the thyroid glands were significantly increased in treated F_0 parental females. However an increased thyroid weight could not be reproduced in male or female animals from the satellite groups of this study, which had been administered the test substance for 10 weeks. In F0 males the absolute thyroid weights also appear increased in both treatment groups, although without dose-relation. Altogether these weight changes are considered incidental.

There were no substance-related gross lesions or microscopic findings in any of the organs and tissues examined, neither in the F_0 rats nor in the satellite animals.

Differential ovarian follicle count (DOFC)

The DOFC revealed no changes in the number of primordial and/or growing follicles between the control and treated groups.

F1-generation pups/litters

The F1 pups body weights and body weight gains were minimally lower in the 400 ppm group at all investigation points, although without statistical significance and with values lying mainly within the historical control values. In addition, at the 400 ppm level maternal toxicity was noted. Therefore, these changes were not considered toxicologically relevant.

Organ weights revealed no differences between control and treatment groups.

Table B.6.8.1-19: Pups body weight data

	0 ppm	200 ppm	400 ppm
Body weight in grams (mean male and female pups)			
day 1	6.0	6.1	5.4
day 4 (postculling)	8.8	9.2	8.1
day 7	14.0	14.7	13.0
day 14	28.5	29.4	26.6
day 21	45.5	47.7	42.8

Pups necropsy observations
The few changes noted at necropsy were considered not related to the treatment with the test article.

Discussion:
Under the conditions of this study, there were no indications from the clinical, clinical pathology and from gross and histopathological examinations, that the administration of the test substance at concentrations of 200 or 400 ppm (about 19 or 38 mg/kg bw/d) adversely affected fertility of the F_0 parental animals. The reproductive performance was impaired in one high dose F0 female. At necropsy 6 implantation sites were counted indicating that this female was pregnant and, therefore, fertility was not affected. Anyhow, it cannot be concluded with certainty that the total litter loss in this female was not substance-related. Historical control data for this endpoint were not available.
Estrous cycle data, mating behavior, conception, gestation, parturition, lactation and weaning (F_0 parental rats) as well as sperm parameters (satellite males), sexual organ weights, gross and histopathological findings of the reproductive organs (including differential ovarian follicle counts) were unaffected by treatment with 200 ppm or 400 ppm of the test substance.
F_1-pups of the 400 ppm group had minimally lower body weights when compared to the control and low dose group, but they were well within the range of historical control data. If at all, these weight changes occurred in the presence of maternal toxicity. Therefore, no relevance is attributed. No substance-induced signs of developmental toxicity occurred in the progeny of the F_0 parents. Pup mortality and survival rate, sex ratio, clinical and necropsy findings and organ weights were unaffected by treatment.

Conclusion:
Under the conditions of this study the NOAEL for reproductive toxicity is 200 ppm (corresponding to approximately 17.6 mg/kg bw/d and 17.3 mg/kg bw/d in males and females respectively) based on postimplantation loss noted in one female at the next higher dose of 400 ppm (corresponding to approximately 35.0 mg/kg bw/d and 40.9 mg/kg bw/d in males and females respectively).
The NOAEL for general toxicity is below 200 ppm based on lower body weight and clinico-chemical findings (higher white blood cell count in males, higher cholesterol concentrations in males and females, lower magnesium concentrations in females) at this dose level.

B.6.8.1.1.10 Re-evaluation of the third mutagenicity study

Report: Engelhardt G., Hoffmann H. D., 1999
Report: *In vitro* chromosome aberration assay with Reg. No. 292 564; BH 635-2 in V79 cells
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 1999/11684

GLP: Yes (Laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 473, EEC 92/69 B 10

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: 635M02 (Reg. No. 292 564; BH 635-2; batch no. 00831-201, purity: 98.2 %.
Test system: V79 cells
635M02 (BH 635-2; Reg. No. 292 564) was assessed for its potential to induce structural chromosomal aberrations in V79 cells *in vitro* both in the presence and in the absence of a metabolising system (S-9 mix of Aroclor 1254-induced Sprague-Dawley rat liver). According to an initial range-finding cytotoxicity test the following doses were evaluated.
1st experiment:
4 hours exposure, 18 hours harvest time, with and without S-9 mix:
0; 575; 1,150; and 2,300 µg/mL,
2nd experiment:
18 hours exposure, 18 hours harvest time, without S-9 mix:
0; 287.5; 575; and 1150 µg/mL
18 hours exposure, 28 hours harvest time, without S-9 mix:
0; 2300 µg/mL
4 hours exposure, 28 hours harvest time, with S-9 mix:
0; 575; 1150; and 2300 µg/mL.
The cell cycle of the untreated V79 cells is about 13 - 14 hours under the selected culture conditions. Thus, the selected 1st sampling time of 18 hours was within the 1 - 1.5 x the normal cell cycle time, as recommended by the OECD Guideline No. 473. The later sampling time of 28 hours was chosen to cover a possible cell cycle delay. About 2 - 3 hours prior to harvesting the cells, colcemid was added to arrest cells in a metaphase-like stage of mitosis (c-metaphases). After preparation of the chromosomes and staining with Giemsa, 100 metaphases for each culture in the case of the test substance and vehicle controls, or 50 or 100 cells for each culture in the case of the concurrent positive controls, were analysed for chromosomal aberrations. The criteria for a positive response are:

A dose-related and reproducible significant increase in the number of structural chromosomal aberrations.

The proportion of aberrations exceeded both the concurrent negative control range and the negative historical control range.

A test substance is generally considered non clastogenic in this test system if:

There was no significant increase in the number of chromosomally damaged cells at any dose above concurrent control frequencies.

The aberration frequencies were within the historical control range.

Findings:

The stability of the test substance throughout the study period was verified by reanalysis. Homogeneity of the test substance was achieved by mixing. The stability of 635M02 in the vehicle DMSO and in water each over a period of 4 hours has been determined analytically. Test substance precipitation occurred at concentrations of 575 µg/mL and higher. According to the results of the determination of the mitotic index, no suppression of the mitotic activity was observed under any of the experimental conditions. Cell count indicated a slight growth inhibition at 1150 µg/mL (18 hours exposure, 18 hours harvest) and 2300 µg/mL (4 hours exposure, 18 hours harvest and 18 hours exposure, 28 hours harvest) both without metabolic activation. Cell attachment was occasionally slightly reduced. Osmolarity and pH values were not influenced by test substance treatment. The negative controls (vehicle controls) gave frequencies of aberrations within the range expected for the V79 cell line. Both of the positive control chemicals, EMS (ethyl methane sulfonate) and cyclophosphamide, led to the expected increase in the number of cells containing structural chromosomal aberrations.

At 1150 and 2300 µg/mL (with S-9 mix), in the presence of precipitation, there were some significant dose-related increases in the number of aberrations. The results including gaps (gaps are not considered an indication of clastogenicity) and excluding gaps were still within the upper range of the historical control data.

Table B.6.8.1-20: Summary of metaphases with chromosome aberrations – 1st experiment

Dose (µg/mL)	No of Metaphases	Including gaps		Excluding gaps		Exchanges	
		No	%	No	%	No	%
1st experiment (4 hours exposure, 18 hours harvest time, without S-9 mix)							
DMSO	200	6	3.0	1	0.5	0	0.0
575	200	14	7.0	7	3.5	2	1.0
1150	200	14	7.0	5	2.5	4	2.0
2300	200	8	4.0	3	1.5	1	0.5
EMS 350	100	28	28.0**	24	24.0**	12	12.0**

Dose (µg/mL)	No of Metaphases	Including gaps		Excluding gaps		Exchanges	
		No	%	No	%	No	%
1st experiment (4 hours exposure, 18 hours harvest time, with S-9 mix)							
DMSO	200	6	3.0	2	1.0	1.0	0.5
575	200	4	2.0	1	0.5	1.0	0.5
1150	200	18	9.0*	7	3.5	2	1.0
2300	200	21	10.5**	10	5.0	5	2.5
CPP 0.5	100	21**	21.0**	21**	21.0**	18	18.0**

Table B.6.8.1-21: Individual data

Dose (µg/mL)	No of Metaphases	Including gaps		Excluding gaps		Exchanges	
		No	%	No	%	No	%
1st experiment (4 hours exposure, 18 hours harvest time, without S-9 mix)							
2300	100	3	3.0	1	1.0	0	0.0
2300	100	5	5.0	2	2.0	1	1.0
EMS 350	50	16	32.0	12	24.0	8	16.0
EMS 350	50	12	24.0	12	24.0	4	8.0

Dose (µg/mL)	No of Metaphases	Including gaps		Excluding gaps		Exchanges	
		No	%	No	%	No	%
1st experiment (4 hours exposure, 18 hours harvest time, with S-9 mix)							
2300	100	11	11.0	5	5.0	2	2.0
2300	100	10	10.0	5	5.0	3	3.0
CPP 0.5	50	11	22.0	11	22.0	9	18.0
CPP 0.5	50	10	20.0	10	20.0	9	18.0

Table B.6.8.1-22: Summary of metaphases with chromosome aberrations – 2nd experiment

2nd experiment (18 hours exposure, 18 hours harvest time, without S-9 mix)							
DMSO	200	4	2.0	1	0.5	1.0	0.5
287.5	200	14	7.0*	5	2.5	4.0	2.0
575	200	7	3.5	1	0.5	1.0	0.5
1150	200	7	3.5	1	0.5	0	0.0
EMS 350	100	19	19.0**	17	17.0**	16	16.0**

* P<0.05, ** P<0.01

Dose (µg/mL)	No of Metaphases	Including gaps		Excluding gaps		Exchanges	
		No	%	No	%	No	%
2nd experiment (4 hours exposure, 28 hours harvest time, with S-9 mix)							
DMSO	200	9	4.5	1	0.5	0	0.0
575	200	8	4.0	5	2.5	3	1.5
1150	200	9	4.5	3	1.5	2	1.0
2300	200	10	5.0	5	2.5	3	1.5
CPP 0.5	100	18	18.0**	14	14.0**	11	11.0**

Table B.6.8.1-23: Individual data

Dose (µg/mL)	No of Metaphases	Including gaps		Excluding gaps		Exchanges	
		No	%	No	%	No	%
2nd experiment (4 hours exposure, 28 hours harvest time, with S-9 mix)							
2300	100	5	5.0	3	3.0	2	2.0
2300	100	5	5.0	2	2.0	1	1.0
CPP 0.5	50	9	18.0	8	16.0	7	14.0
CPP 0.5	50	9	18.0	6	12.0	4	8.0

The results of this study indicate that the increase in aberrations (excluding gaps) at 1150 and 2300 µg/mL (with S-9 mix) occurred in the first experiment only, was not reproducible in the 2nd experiment and the values were still at the upper range of the historical control data (see below).

Table B.6.8.1-24: Historical negative control data

Untreated controls (without S-9 mix)						
Treatment/harvest	4/18 hours			18/18 hours		
No of experiments	46			3		
Aberrations	Incl gaps	Excl gaps	Exch	Incl gaps	Excl gaps	Exch
Mean (%)	4.6	1.8	0.8	2.3	1.0	0.3
Minimum (%)	1.5	0.0	0.0	1.5	0.5	0.0
Maximum (%)	9.0	4.0	3.0	3.5	1.5	0.5

Untreated controls (without S-9 mix)						
Treatment/harvest	4/28 hours			18/28 hours		
No of experiments	15			3		
Aberrations	Incl gaps	Excl gaps	Exch	Incl gaps	Excl gaps	Exch
Mean (%)	4.6	1.8	0.8	5.0	1.3	1.2
Minimum (%)	1.5	0.5	0.0	3.5	0.5	0.0
Maximum (%)	7.5	3.0	1.5	7.0	2.5	2.5

Untreated controls (with S-9 mix)						
Treatment/harvest	4/18 hours			4/28 hours		
No of experiments	46			18		
Aberrations	Incl gaps	Excl gaps	Exch	Incl gaps	Excl gaps	Exch
Mean (%)	4.4	1.7	0.7	4.5	1.6	0.5
Minimum (%)	1.5	0.0	0.0	2.0	0.5	0.0
Maximum (%)	10.5	5.0	2.5	9.0	3.0	1.0

Discussion: Based on the comments of the UK at ECCO 136 it was concluded that a clastogenic potential of the metabolite 635M02 cannot be excluded and this test was considered positive in the endpoint list. The re-evaluation of this study cannot support this conclusion anymore. The slight increases were within the range of the historical control data, were not reproducible in the second experiment and occurred in the presence of precipitation. Thus, the criteria for a positive response are not fulfilled.

Conclusion:
Under the conditions of this study the metabolite 635M02 has no clastogenic potential under *in vitro* conditions in V79 cells.

B.6.8.1.3 635M01

Reg. No. 335 184 (BH 635-4) is a soil metabolite. It was detected in the rat metabolism study; nevertheless, it was tested in three mutagenicity assays and in an acute oral test. The present 28-day dietary toxicity study in rats was submitted after the completion of the DAR.

Table B.6.8-4: Summary of toxicity studies of metabolite Reg. 635M01

Study/strains/species	Test material/conditions	Results
Acute oral toxicity in rats Wistar rats (Chbb:THOM (SPF))	Batch No. 01185-088, purity: 97.0 %	LD_{50}: > 5000 mg/kg bw
Salmonella typhimurium/Escherichia coli reverse mutation assay S. typhimurium strains TA 100, TA 1535, TA 1537, TA 98 and E. coli strain WP2 uvrA	Batch No. 00831-277, purity: 97.9 %	Not mutagenic
Gene mutation assay in Chinese hamster V79 cells *in vitro* (V79 / HPRT) Chinese hamster ovary (CHO) cells	Batch No. 01185-088, purity: 97.0 %	Not mutagenic
In vitro chromosome aberration assay in V79 cells (cells derived Chinese hamster)	Batch No. 01185-088, purity: 97.0 %	Not mutagenic
28-d oral (diet) toxicity study in Wistar rats (CrlGlxBrlHan:WI) 10 male / 10 female 0; 430; 1300 and 3900 ppm (corresponding to approximately 38, 115 and 344 mg/kg bw/d)	Batch no. 2059-011, purity: 96.4 %.	NOAEL: 3900 ppm No signs of toxicity

B.6.8.1.3.5 28-day toxicity study in Wistar rats

Report: Kaspers U. et al., 2003
Reg. No. 335 184 (metabolite of BAS 635 H) - Subacute toxicity study in Wistar rats administration in the diet for 4 weeks
BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep., unpublished

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 96/54, OECD 407

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Reg. No. 335 184; batch no. 2059-011, purity: 96.4 %.
Test animals: Male and female Wistar rats (CrlGlxBrlHan:WI), supplied by Charles River, Germany. Age: 33-35 days.
Reg. No. 335 184 (635M01) was administered for 4 weeks to groups of 10 male and 10 female Wistar rats at dietary concentrations of 0; 430; 1300 and 3900 ppm (corresponding to approximately 38, 115 and 344 mg/kg bw/d). Food consumption and body weight were determined weekly. The animals were examined for signs of toxicity or mortality at least once a day. Detailed clinical examinations in an open field were conducted prior to the start of the administration period and weekly thereafter. A functional observational battery (FOB) and measurement of motor activity was carried out at the end of the study The FOB included evaluation of home cage observations, Open Field examinations, sensimotor tests/reflexes, quantitative parameters (feces, rearing, grip strength, landing foot splay). Ophthalmologic examinations were performed in all animals before and in control and high dose animals at the

end of the administration period. Clinicochemical, hematological examinations and urinalyses were performed towards the end of the administration period. Finally, animals were assessed by gross pathology, followed by histopathological examinations.

Findings:
Sufficient stability over the study period and homogenous distribution of the test substance in the diet was demonstrated. Concentration control analyses showed that actual concentrations in the diet were within a range of 95.0–109.9 % of the nominal concentrations.

Mortality
No animal died during the study.

Clinical examinations
No substance-related effects were observed.

Food consumption, water consumption and body weight data
No substance-related effects were obtained.

Food efficiency
Food efficiency was statistically significantly reduced in male rats at 430 ppm on day 14. This isolated finding in low dose males was considered incidental and not related to the treatment.

Functional observation battery
No treatment-related effects were evident in the FOB evaluations. Regarding the overall motor activity (summation of all intervals) no substance-related findings were observed in both sexes.
Single intervals were statistically significantly increased in animals administered 1300 ppm (in males at interval 1 and in females at interval 4). These two isolated findings were assessed as fortuitous and not related to the treatment with the test compound, due to the lack of a dose-response relationship connected with no influence on the overall motor activity.

Opthalmologic examinations
No effects that could be related to treatment were observed.

Hematology, clinicochemistry and urinalyses
Substance-related effects were not evident at any dose level in either males or females.

Organ weight changes
Absolute and relative spleen weights were statistically significantly increased in low-dose group females (+16.4 % and 15.7 %, respectively). In the absence of a dose-response relationship, this finding is judged to be incidental. All other weight parameters did not show significant differences when compared to the control group.

Gross lesions and histopathology
No treatment-related findings were observed.

Conclusion:
In conclusion, no signs of toxicity were observed in treated animals at any of the dietary concentrations tested. Thus, the NOAEL was 3900 ppm for both sexes (corresponding to daily intakes of 334.4 and 352.6 mg/kg bw/d in males and females, respectively).

Conclusion for Reg. No. 335 184 (635M01):
Reg. No. 335 184 was found to be not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay, not mutagenic *in vitro* in the CHO/HPRT mutation assay and not chromosome-damaging (clastogenic) under *in vitro* conditions in V79 cells. An acute oral toxicity study revealed an LD_{50} of > 5000 mg/kg bw. In a 28-day dietary study, no signs of toxicity were observed in treated Wistar rats up to 4300 ppm (corresponding to daily intakes of 334.4 and 352.6 mg/kg bw, respectively), which was the highest dose level tested. Thus, the metabolite 635M01 is not of toxicological relevance.

B.6.8.2 Supplementary studies - AMTT (635M04)

AMTT was an impurity in the batch N24 which caused severe effects in the long-term rat studies and in the two-generation reproduction toxicity study in rats.
A separate metabolism study was conducted with AMTT, it was tested in an acute oral test, as well as in four genotoxicity tests.
After the completion of the DAR, an additional study was submitted in which AMTT was assessed for its potential to induce structural chromosomal aberrations (clastogenic activity) and/or changes in the number of chromosomes (aneugenic activity) in V79 cells *in vitro* both in the presence and in the absence of a metabolising system.

B.6.8.2.9 *In vitro* chromosome aberration assay with AMTT

Report: Engelhardt G., Leibold E., 2004
Report: *In vitro* chromosome aberration assay with Reg. No. 231700 (metabolite of BAS 635 H) in V79 cells
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep., unpublished,
BASF RegDoc# 2004/1014204

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 473, EEC 92/69 B 10

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Reg. No. 231 700 (BH 635-5 = AMTT); batch No. 00831-237, purity: 99.9 %.
Test system: V79 cells, with and without metabolising system.
The substance Reg. No. 231 700 (= AMTT) was assessed for its potential to induce structural chromosomal aberrations (clastogenic activity) and/or changes in the number of chromosomes (aneugenic activity) in V79 cells *in vitro* both in the presence and in the absence of a metabolising system.
According to an initial range-finding cytotoxicity test for the determination of the experimental doses and taking into account the cytotoxicity actually found in the main experiments, the following doses were evaluated in two independent experiments:

Table B.6.8.2-1: Experimental design

Experiment	Exposure period	Sampling time	S-9 mix	Dose levels [µg/mL]]
1st assay	4 hours	18 hours	With & without	0 - 500 - 1000 – 2000
2nd assay	18 hours	18 hours	Without	0 - 125 - 250 – 500
	18 hours	28 hours	Without	0 – 1000
	4 hours	28 hours	With	0 - 500 - 1000 – 2000

About 2 - 3 hours prior to harvesting the cells, Colcemid was added to arrest cells at a metaphase-like stage of mitosis (c-metaphases). After preparation of the chromosomes and staining with Giemsa, 100 metaphases for each culture in the case of the test substance and vehicle controls, or 50 cells for each culture in the case of the concurrent positive controls, were analysed for chromosomal aberrations.

Findings:
The negative controls (vehicle controls) gave frequencies of aberrations within the range expected for the V79 cell line. Both of the positive control chemicals, i.e. EMS and cyclophosphamide, led to the expected increase in the number of cells containing structural chromosomal aberrations.

Chromosome analysis – 1st experiment

Clastogenic mode of action
After a treatment time of 4 hours no relevant increase in the number of chromosomally damaged cells was observed either without S-9 mix or after the addition of a metabolising system. The types and frequencies of aberrations are given in the following tables.

Aneugenic mode of action
No increase in the number of cells with changes in the number of chromosomes was demonstrated either without S-9 mix or after the addition of a metabolising system.

Table B.6.8.2-2: Chromosome analysis-1st experiment

	Vehicle control	500 µg/mL	1000 µg/mL	2000 µg/mL	pos. control
Assay without S-9 mix; 4-hour exposure, 18-hour sampling time, positive control: 350 µg EMS/mL					
Metaphases incl. gaps	5 (2.5 %)	13 (6.5 %)	6 (3.0 %)	12 (6.0 %)	21 %**
Metaphases excl. gaps	1 (0.5 %)	9 (4.5 %*)	2 (1.0 %)	3 (1.5 %)	16 %**
Assay with S-9 mix; 4-hour exposure, 18-hour sampling time, positive control: 0.5 µg CPP/mL					
Metaphases incl. gaps	10 (5.0 %)	8 (4.0 %)	14 (7.0 %)	12 (6.0 %)	18 %**
Metaphases excl. gaps	4 (2.0 %)	1 (0.5 %)	6 (3.0 %)	7 (3.5 %)	17 %**

* $p \leq 0.05$, ** $p \leq 0.01$; Fisher's exact test with Bonferroni-Holm correction

Chromosome analysis – 2nd experiment

Clastogenic mode of action
Both with and without S-9 mix, no increase in the number of structurally damaged metaphases was observed either after a treatment time of 4 hours or after a continuous treatment of 18 hours at both sampling times, i.e. 18 and 28 hours. The types and frequencies of aberrations are given in the following table.

Aneugenic mode of action
No increase in the number of cells with changes in the number of chromosomes was demonstrated either with and without S-9 mix.

Table B.6.8.2-3: Chromosome analysis-2nd experiment

	Vehicle control	125 µg/mL	250 µg/mL	500 µg/mL	pos. control
Assay without S-9 mix; 18-hour exposure, 18-hour sampling time, positive control: 350 µg EMS/mL					
Metaphases incl. gaps	13 (6.5 %)	8 (4.0 %)	13 (6.5 %)	6 (3.0 %)	19 %**
Metaphases excl. gaps	3 (1.5 %)	3 (1.5 %)	4 (2.0 %)	4 (2.0 %)	17 %**

* $p \leq 0.05$, ** $p \leq 0.01$; Fisher's exact test with Bonferroni-Holm correction

	Vehicle control	1000 µg/mL	pos. control	-	-
Assay without S-9 mix; 18-hour exposure, 28-hour sampling time, positive control: 0.5 µg CPP/mL					
Metaphases incl. gaps	11 (5.5 %)	8 (4.0 %)	25 %**	-	-
Metaphases excl. gaps	7 (3.5 %)	5 (2.5 %)	25 %**	-	-

* $p \leq 0.05$, ** $p \leq 0.01$; Fisher's exact test with Bonferroni-Holm correction

	Vehicle control	500 µg/mL	1000 µg/mL	2000 µg/mL	pos. control
Assay with S-9 mix; 4-hour exposure, 28-hour sampling time, positive control: 0.5 µg CPP/mL					
Metaphases incl. gaps	7 (3.5 %)	12 (6.0 %)	13 (6.5 %)	7 (3.5 %)	21 %**
Metaphases excl. gaps	6 (3.0 %)	4 (2.0 %)	3 (1.5 %)	3 (1.5 %)	18 %**

* $p \leq 0.05$, ** $p \leq 0.01$; Fisher's exact test with Bonferroni-Holm correction

Mitotic Index
A dose-dependent suppression of the mitotic activity was observed after continuous treatment of 18 hours at both sampling times, i.e. 18 and 28 hours without metabolic system. Likewise, cell count was reduced and growth inhibition was observed under all experimental conditions.

Table B.6.8.2-4: Mitotic Index

Time (hours)		Dose level	% Rel.
Exposure	Sampling	Vehicle control DMSO	100
18	18	125 µg/mL	73.8
18	18	250 µg/mL	58.4
18	18	500 µg/mL	43.9
18	18	1000 µg/mL	*
18	18	1500 µg/mL	**
18	18	350 µg EMS /mL	42.1
Exposure	Sampling	Vehicle control DMSO	100
18	28	250 µg/mL	87.5
18	28	500 µg/mL	81.7
18	28	1000 µg/mL	54.9
18	28	1500 µg/mL	*
18	28	350 µg EMS /mL	68.9

* no or only few metaphases for evaluation
** no or only few metaphases for evaluation and of poor quality

Discussion:
In the present study a suppression of the mitotic activity was observed. The cause for that suppression remained unclear. It might be an indication for cytotoxicity already occurring in

the lowest dose of 125 µg/mL. In this case the cell line V79 is proved to be too sensitive for this test substance. On the other hand a lower mitotic activity might be caused by spindle cell poisons, for example carbendazim. Since the micronucleus test in NMRI mice revealed negative results it might be concluded that AMTT does not exert clastogenic properties.

Conclusion:
On the basis of the results of the present study, AMTT did not cause any relevant increase in the number of structurally aberrant metaphases incl. and excl. gaps at both sampling times either without S-9 mix or after adding a metabolising system in two experiments performed independently of each other. No increase in the frequency of cells containing numerical aberrations was demonstrated either.
AMTT is not considered to be a clastogenic or an aneugenic agent under in-vitro conditions in V79 cells.

B.6.12 Dermal absorption (Annex IIIA 7.3)

The absorption, distribution and excretion of radioactivity was studied in male rats following a single dermal administration of [^{14}C]-tritosulfuron mixed with the blank of a commercial formulation (BAS 635 01 H) and taken up in water. Nominal dose levels were 0.02, 0.2 and 2.0 mg/animal. Animals were exposed for 4 or 8 hours. About 3 % of the radioactivity applied were maximally absorbed at the low dose level of 0.002 mg/cm^3, about 0.6 – 0.7 % was absorbed at the higher doses. *In vitro* investigations on dermal absorption have been performed. The results of this study demonstrated that the rate of dermal penetration (µg/cm^2/h) through human skin was at least 2.2 fold less than through rat skin. Based on the *in vivo* results outlined above the dermal absorption of tritosulfuron in rats is determined to be 3.0 % at the most. Taking into account the difference between rat and human skin permeability, human skin penetration is 2-fold lower than rat skin penetration as demonstrated by *in vitro* investigations. Taking the *in vivo* and *in vitro* dermal penetration data together, they support the use of a dermal penetration figure of 1 % for the concentrate and 2 % for the in-use dilution in the operator exposure calculations.

B.6.14 Exposure data (Annex IIIA 7.2)

In the context of the European assessment of tritosulfuron (ECCO 136) to decide on its inclusion in Annex I of Directive 91/414/EEC, in contrast to the monograph the systemic AOEL for tritosulfuron was derived to be 0.15 mg/kg bw/d and the exposure risk assessment should be calculated with dermal absorption rates of 1 % for the concentrate and 2 % for the spray dilution of the product BAS 635 00 H. Additionally concern was raised by Member States that hydrolytical cleavage of tritosulfuron in the spray tank may result in increased concentrations of AMTT and lead to high-level exposure of operators to AMTT.
Therefore a re-assessment is given for the active ingredient tritosulfuron and also a specific risk assessment regarding the possible exposure to the metabolite AMTT resulting from field application of tritosulfuron-containing products.

B.6.14.4 Exposure data: active substance tritosulfuron

Information on product and use:
BAS 635 00 H is intended to be used as a post emergence herbicide in cereals. The maximum application rate is 0.07 kg product/ha and therefore 0.050 kg as/ha. The intended use is in a tank-mix with surfactants like BAS 152 00 S (preferably), Dash HC (BAS 904 70 S) or other additives. Its recommended application is in growth stage (BBCH-Code) 13 - 39 of the cereal crops. Applications of BAS 635 00 H will be carried out by using vehicle-mounted or drawn boom sprayers with hydraulic nozzles. Water will be the diluent/carrier in all situations. The spray volume will be in the range of 150 - 400 litres per hectar.

B.6.14.4.1 Operator exposure

B.6.14.1.1 Estimation of operator exposure

Using the German model a summary of the expected/potential operator exposure is provided in Table 6.14-1 (see also in the monograph: Table B.6.14-1). The assessment was made with the assumption of three individual levels of personal protective equipment (PPE) used by the operators:
- No PPE is used when handling both the undiluted product during mixing/loading (m/l) and the diluted product during application (appl.).
- Protective gloves are used when handling the undiluted product during m/l.
- PPE including gloves, standard protective garment and sturdy footwear is used when handling the undiluted and the diluted product (handling of product during m/l and appl.).

Table B.6.14.4-1: German model: Summary of the estimated operator exposure for tritosulfuron

Route of exposure and type of work	No PPE	Gloves: m/l	Gloves: m/l and appl.; garment: appl.
Dermal exposure (mg/person/d)			
- Mixing/loading	2.00	0.02	0.02
- Application	2.04	2.04	0.14
Total dermal	4.04	2.06	0.16
Inhalation exposure (mg/person/d)			
- Mixing/loading	0.008	0.008	0.008
- Application	0.001	0.001	0.001
Total inhalation	0.009	0.009	0.009
Total exposure (mg/person/d): (dermal + inhalation)	**4.049** (0.0578 mg/kg bw/d)	**2.069** (0.0296 mg/kg bw/d)	**0.169** (0.0024 mg/kg bw/d)
Total systemic exposure (mg/person/d) (absorbed dose)**	**0.070** (0.0010 mg/kg bw/d)	**0.050** (0.0007 mg/kg bw/d)	**0.012** (0.0002 mg/kg bw/d)

* dermal absorption: conc. = 1 %, dil. = 2 %; inhal. absorption: 100 %

Using the UK-POEM a summary of the operator exposure for the different levels of PPE is provided in Table 6.14-2 (see also in the monograph: Table B.6.14-2). However, BAS 635 00 H is a WG formulation and UK POEM does not have the appropriate data to estimate the level of exposure arising during mixing and loading for a WG formulation. Thus as in the monograph for the calculated operator exposure a strong overestimation is given.

Table B.6.14.4-2: UK POEM: Summary of the estimated operator exposure for tritosulfuron

Dermal exposure (mg/person/d)			Inhalation exposure (mg/person/d)	Systemic* exposure/absorbed dose (mg/person/d)		
Mix/load	**Spray**	**Total**	**Spray**	**Mix/load**	**Spray**	**Total**
No PPE						
42.84	13.90	56.74	0.02	0.4284	0.298	**0.726**
Gloves during m/l						
0.43	13.90	14.33	0.02	0.0043	0.298	**0.302**
Gloves during m/l and appl.						
0.43	2.16	2.59	0.02	0.0043	0.063	**0.068**

* dermal absorption: conc. = 1 %, dil. = 2 %; inhal. absorption: 100 %

Risk assessment, comparison of estimated and acceptable operator exposure

For direct extrapolation of the inhalation or dermal exposure, there are no appropriate toxicological studies available investigating the critical endpoints via specific routes. Therefore, the calculated systemic exposure from both models were compared directly with the proposed systemic AOEL of 0.15 mg/kg bw/d. The values are given in Table 6.14.4-3.

Table B.6.14.4-3: Results of the model calculations and a comparison with the proposed systemic AOEL

German model	**Treated area per day** (ha/d)	**PPE**	**Systemic exposure*** (mg/kg bw/d)	**% of AOEL** (0.15 mg/kg bw/d)
	20	No PPE	0.0010	0.66
		Gloves: m/l	0.0007	0.48
		Gloves: m/l and appl.; garment: appl.	0.0002	0.12
UK POEM	**Treated area per day** (ha/d)	**PPE**	**Systemic exposure*** (mg/kg bw/d)	**% of AOEL** (0.15 mg/kg bw/d)
	50	No PPE	0.726	8.07
		Gloves: m/l	0.302	3.36
		Gloves: m/l and appl.;	0.068	0.75
* body weight: 70 kg (German model) or 60 kg (UK POEM) dermal absorption: concentrate (m/l).: 1 %, dilution (appl.): 2 % inhalation absorption: 100 %				

The results show that the operator exposure for the proposed use is acceptable even if no personal protection is used (German model: exposure = 0.66 % of the systemic AOEL; UK-POEM: exposure = 8.07 % of the systemic AOEL).

With regard to the active ingredient tritosulfuron it is concluded that BAS 635 00 H can be handled safely under the recommended conditions of use.

B.6.14.4.2 Worker exposure

B.6.14.4.2.1 Estimation of worker exposure

The potential dermal exposure of workers is derived in the monograph (B.6.14.2.1). The calculation with a dermal absorption rate of 2 % results in values for a respective systemic exposure/absorbed dose.

Estimated absorbed dose:

Potential dermal exposure		x	0.02	=	Systemic exposure
Without PPE:	0.00143	x	0.02	=	0.0000286 mg/kg bw/d
Protected worker:	0.000072	x	0.02	=	0.0000014 mg/kg bw/d

Risk assessment
The estimated dermal exposure as percentage of the systemic AOEL (0.15 mg/kg bw/d) is calculated to be:

Without PPE:	0.019 %
Protected worker:	0.001 %

With regard to the active ingredient tritosulfuron the results of the risk assessment indicate that re-entry of treated fields is possible with a sufficient margin of safety after the spray solution has dried up. Specific protective measures for worker re-entry are not necessary.

B.6.14.4.3 Bystander exposure

With regard to the active ingredient tritosulfuron the risk assessment for the operator indicates that the exposure is always below the proposed systemic AOEL (German model and UK POEM, without PPE). Therefore, during application the field bystander exposure seems of no special concern and a more quantitative estimation of bystander exposure was not performed.

B.6.14.5 Exposure data: AMTT

AMTT has been identified as an impurity in batches of the technical active substance tritosulfuron. For technical tritosulfuron, a maximum of 0.2 g AMTT/kg tritosulfuron has been guaranteed equivalent to an AMTT content of 0.02 %. Formulated products like BAS 635 00 H are described to have a maximum AMTT content of 0.2 % relative to the amount (content) of tritosulfuron. Due to the toxicological properties of AMTT, it was considered necessary to perform an operator risk assessment for AMTT, taking into account the possible formation of additional AMTT, e.g. during prolonged storage of the product or via hydrolytic cleavage of the tritosulfuron molecule in the aqueous spray-mix dilution.
By the notifier, in addition to data from laboratory experiments that were already available, an outdoor trial was conducted under realistic worst-case conditions to investigate the extent of AMTT formation in spray-strength dilutions of the representative tritosulfuron formulation BAS 635 00 H.

Formation of AMTT during storage of BAS 635 00 H
Two studies have investigated the stability of the product BAS 635 00 H. In an accelerated study the storage stability of BAS 635 00 H was determined following a 14-day incubation period at 54 °C. The AMTT content increased from 0.5 to 1.7 g AMTT/kg product, corresponding to an AMTT increase relative to active substance from 0.07 to 0.24 % = Δ + 0.17 % [AIIIA-2.7: Kaestel R., (2000), BASF DocID 2000/1000089].
However, under practically relevant storage conditions, the build-up of AMTT in BAS 635 00 H was found to be limited: When BAS 635 00 H was stored in the original container for two years at 20 °C, an increase from 0.5 to 0.8 g AMTT/kg BAS 635 00 H was

observed; corresponding to an AMTT increase relative to active substance from 0.07 to 0.11 % = Δ +0.04 % [BASF DocID 2002/1004730 and BASF DocID 2002/1004731].
For tritosulfuron containing products BASF guarantees a maximum AMTT concentration level of 0.2 % (relative to tritosulfuron). For BAS 635 00 H, this means that the AMTT concentration will not exceed 1.428 g AMTT/kg product.

Formation of AMTT in tank mixes containing BAS 635 00 H

Laboratory study

In laboratory studies it was found that acidic conditions may facilitate formation of AMTT in spray-strength dilutions of BAS 635 00 H [Hassink J. (1999), BASF DocID 1999/10894]. A maximum AMTT increase was obtained by using the most acidic standard water "CIPAC A" for dilution of the product, which resulted in a pH of 4.7 in the spray broth. Under these study conditions, the AMTT content increased in the spray dilution at room temperature from 0.037 % to 0.093 % (24-h) to 0.155 % (48-h), corresponding to an AMTT increase of Δ + 0.056 % within 24 hours and Δ + 0.118 % within 48 hours (relative to active substance content), respectively.

Outdoor study [Stadler R. (2004) BASF DocID 2004/1016478]

Tritosulfuron may be hydrolytically cleaved in aqueous media, resulting in the formation of AMTT. Cleavage is favoured at increased temperatures and at acidic conditions. In order to cover a realistic worst-case situation under outdoor conditions (involving heating up of the spray tank during a warm sunny day; acidic conditions by use of the adjuvant DASH HC), the following experiment was conducted:

On June 30th 2004 (11:30 a.m., air temperature: 24.6 °C), 280 g BAS 635 00 H (containing 714 g/kg tritosulfuron) and 4 L of DASH HC were mixed with 800 L of water in a spray tank (corresponding to a concentration of 0.025 % tritosulfuron in the spray mix, according to use recommendation of 50 g as in 200 L water/ha). A second spray tank was filled in the same way, however without addition of the adjuvant DASH HC. The airborne and spray mix temperature as well as the actual AMTT content in the spray mix was determined immediately after mixing (t = 0) and after approx. 2, 4, 21 or 28 hours of outdoor incubation.

Table B.6.14.5-1: Outdoor study on AMTT formation in spray tank

Determination time point	Time elapsed after mixing	Temperature		pH	Concentration in spray mix		
		air	spray		AMTT (mg/L)	BAS 635 H (mg/L) (calculated)	% AMTT relative to parent
BAS 635 00 H							
30/06/2004 11:30	0 h	24.6 °C	14.6 °C	7.12	0.28	250	0.112
30/06/2004 13:30	2 h	28.6 °C	16.6 °C	7.16	0.30	250	0.120
30/06/2004 15:15	3 h 45 min	29.9 °C	17.8 °C	7.36	0.32	250	0.128
01/07/2004 08:10	20 h 40 min	19.4 °C	20.5 °C	7.75	0.35	250	0.14
01/07/2004 15:10	27 h 40 min	22.5 °C	21.6 °C	7.76	0.35	250	0.14
BAS 635 00 H with DASH HC							
30/06/2004 11:30	0 h	24.6 °C	14.3 °C	5.12	0.29	250	0.11
30/06/2004 13:30	2 h	28.6 °C	16.2 °C	5.12	0.32	250	0.12
30/06/2004 15:15	3 h 45 min	29.9 °C	17.7 °C	5.09	0.32	250	0.12
01/07/2004 08:10	20 h 40 min	19.4 °C	20.6 °C	5.19	0.38	250	0.15
01/07/2004 15:10	27 h 40 min	22.5 °C	21.6 °C	5.32	0.38	250	0.15

Results of the AMTT measurements are summarised in Table 6.14-4. By addition of DASH HC, the pH was lowered from approx. pH 7 to approx. pH 5. Within 28 hours, a slight

increase of AMTT concentration in the spray tank was observed, i.e. Δ + 0.028 % (without DASH HC) or Δ +0.036 % (with DASH HC) relative to parent.

The storage stability data confirm that even under worst-case conditions, the AMTT concentration in BAS 635 00 H will not exceed 0.2 % relative to tritosulfuron content in the product. In case the application after mixing and loading is postponed by 24 hours, results of the outdoor trial (performed under realistic worst-case conditions) confirm previous laboratory investigations that a further AMTT increase in the spray mix is not expected to exceed Δ + 0.1 % relative to tritosulfuron content in the spray mix within 24 hours.

For estimation of operator exposure to AMTT resulting from **mixing and loading** of BAS 635 00 H, the AMTT concentration of (0.2 % x 714 g/kg =) **1.428 g AMTT/kg product** was used as input parameter in the German and UK exposure models.
For estimation of operator exposure to AMTT resulting from spray application of BAS 635 00 H, it was assumed by the notifier that of five from six operations, spray application will immediately occur after the mixing/loading, while for the sixth operation, the spray dilution would be applied 24 h after the mixing/loading process (resulting in an increase of the AMTT concentration in the spray dilution to yield 0.3 % AMTT relative to tritosulfuron content in the spray broth). According to this assumption, a weighted AMTT content in BAS 635 00 H as shown in the formula below can be calculated:

$$AMTT\ (g/kg) = \frac{714 \times 5 \times 0.2}{6 \times 100} + \frac{714 \times 0.3}{6 \times 100} = 1.547$$

Thus, the AMTT concentration of **1.547 g AMTT/kg product** was used in the German and UK exposure models as input parameter for assessment of operator exposure to AMTT during **spray application**.

B.6.14.5.1 Operator exposure

B.6.14.5.1.1 Estimation of operator exposure

Operator exposure estimations using the German model and the UK POEM are presented by the notifier. However, BAS 635 00 H is a WG formulation and UK POEM does not have the appropriate data to estimate the level of exposure arising during mixing and loading for a WG formulation. Therefore, the notifier calculated with a combination of the German model and the UK POEM.
In the absence of experimental data, 100 % dermal absorption was assumed as a worst case, and employed for the calculation of the systemic exposure resulting from dermal contact to AMTT. Personal protective equipment (PPE) were taken into account as done for the active ingredient.

Using the German model the assessment was made with consideration of three individual levels of PPE used by operators:
- No PPE is used when handling both the undiluted product during mixing/loading (m/l) and the diluted product during application (appl.).
- Protective gloves are used when handling the undiluted product during m/l.
- PPE with gloves, standard protective garment and sturdy footwear is used when handling the undiluted and the diluted product (handling of product during m/l and appl.).

With respect to AMTT as outlined above the "maximum application rate" is splitted:
Input parameter used for m/l = 0.000100 kg AMTT/ha (i.e. 0.2 % of the maximum application rate of 0.05 kg tritosulfuron/ha corresponding to 1.428 g AMTT/kg product).
Input parameter used for spray application calculations = 0.000108 kg AMTT/ha (as **a** result of an increase of AMTT; the mean over six applications; corresponding to 1.547 g AMTT/kg product).

Table B.6.14.5-2: German model: Estimation of operator exposure for AMTT

Formulation type:	WG	$D_{M(H)}$	=	2.0 mg/person x kg as
Application technique:	tractor mounted	$D_{A(H)}$	=	0.38 mg/person x kg as
Application rate:	(0.05 kg tritosulfuron/ha)	$D_{A(B)}$	=	1.6 mg/person x kg as
- concentrate for mixing/loading	0.000100 kg AMTT/ha	$D_{A(C)}$	=	0.06 mg/person x kg as
- spray dilution for application	0.000108 kg AMTT/ha	I_M	=	0.008 mg/person x kg as
Area treated per day:	20 ha	I_A	=	0.001 mg/person x kg as

Route of exposure			**No PPE**	**Gloves: m/l**	**Gloves: m/l and appl.; garment: appl.**
Dermal: m/l					
exposure (hands):	$D_{M(H)}$	=	2.0 x 0.0001 x 20		
		=	0.004 mg/pers./d	0.00004 mg/pers./d [*1]	0.00004 mg/pers./d [*1]
Dermal: appl.					
exposure (hands, body, head)	$D_{A(H)}$	=	0.38 x 0.000108 x 20		
		=	0.0008208 mg/pers./d	0.0008208 mg/pers./d	0.0000082 mg/pers./d [*1]
	$D_{A(B)}$	=	1.6 x 0.000108 x 20		
		=	0.003456 mg/pers./d	0.003456 mg/pers./d	0.0001728 mg/pers./d [*2]
	$D_{A(C)}$	=	0.06 x 0.000108 x 20		
		=	0.0001296 mg/pers./d	0.0001296 mg/pers./d	0.0001296 mg/pers./d
Total dermal exposure		=	**0.00841 mg/pers./d**	**0.00445 mg/pers./d**	**0.00035 mg/pers./d**
Inhalation: m/l					
	I_M	=	0.008 x 0.0001 x 20		
		=	0.000016 mg/pers./d	0.000016 mg/pers./d	0.000016 mg/pers./d
Inhalation: appl.					
	I_A	=	0.001 x 0.000108 x 20		
		=	0.00000216 mg/pers./d	0.000016 mg/pers./d	0.000016 mg/pers./d
Total inhalation exposure		=	**0.000018 mg/pers./d**	**0.000018 mg/pers./d**	**0.000018 mg/pers./d**
Total exposure = total systemic exposure (absorbed dose) [*3]			**0.008428 mg/pers./d** (0.0001204 mg/kg bw/d)	**0.004468 mg/pers./d** (0.0000638 mg/kg bw/d)	**0.000368 mg/pers./d** (0.0000052 mg/kg bw/d)

[*1] reduction factor of gloves = 0.01
[*2] reduction factor of protective clothing = 0.05
[*3] dermal and inhalation absorption rate = 100 % (worst case)

Using the UK-POEM an estimation of the operator exposure is given. However, BAS 635 00 H is a WG formulation and UK POEM does not have the appropriate data to estimate the level of exposure arising during mixing and loading for a WG formulation. Therefore, to avoid a strong overestimation, the notifier as it is appropriate used a combination of the German model and the UK POEM; the German model (75th percentile values) to obtain a figure for exposure during mixing and loading and UK POEM to derive an estimate for application exposure ("UK POEM, modified"). Using the combination of these exposure models the exposure for different scenarios have been summarised in Table

B.6.14.5-2. Full exposure calculations are given in Appendix 1 - 3. To run the model the input parameters were chosen as shown below.
Packaging is not an input parameter in this assessment, which uses the German model for the mixing/loading scenario. However the product will be packed in 0.25 litre, 0.5 litre or 1.0 litre containers. Considering the low application rate of 70 g product/ha the container size of 1 litre could be used in the UK POEM assessment as a reasonable worst-case assumption. Taking into consideration that the bulk density of the product (loose) is 645 g/L the actual content of the 1 litre container corresponds to 0.645 kg product.
The application volume used in the following modelling was 150 L/ha.
Three levels of PPE were regarded as done for the active ingredient: No PPE, gloves worn during mixing/loading only, and gloves worn during mixing/loading and application

Table B.6.14.5-3: UK POEM, modified: Summary of the estimated operator exposure for AMTT

Dermal exposure[1)] (mg/person/d)			Inhalation exposure[1)] (mg/person/d)			Systemic* exposure / absorbed dose (mg/person/d)		
Mix/load	**Spray**	**Total**	**Mix/load**	**Spray**	**Total**	**Mix/load**	**Spray****	**Total****
No PPE								
0.028589	0.0299963	0.0585853	0.0012095	0.0000433	0.0012528	0.0297981	0.0300396	**0.0598377**
Gloves during m/l								
0.0002859	0.0299963	0.0302822	0.0012095	0.0000433	0.0012528	0.0014954	0.0300396	**0.0315350**
Gloves during m/l and appl.								
0.0002859	0.0046565	0.0049424	0.0012095	0.0000433	0.0012528	0.0014954	0.0046998	**0.0061952**

1) Source: Appendix 1 - 3
* dermal absorption: 100 %; inhal. absorption: 100 %
** dermal and inhalation exposure

Risk assessment, comparison of estimated and acceptable exposure

Determination of acceptable operator exposure level systemic AOEL for AMTT

The 2-generation reproduction toxicity study with tritosulfuron batch "N24" was considered to be the most appropriate study as the basis for deriving a systemic AOEL for AMTT. The NOAEL for reproductive performance of the F_0 parental females and the NOAEL for developmental toxicity for the F_{1a}/F_{1b} progeny for tritosulfuron containing 2.45 % AMTT was seen at 50 ppm (4.8 – 5.4 mg/kg bw/d) during the premating periods and for the F_2 progeny at 25 ppm (2.4 – 2.7 mg/kg bw/d). Using the lowest value and calculating with a Safety factor of 500 (see 2.3.1 and list of endpoints) the systemic $AOEL_{AMTT}$ is derived to be:

$NOAEL_{AMTT}$ = 2.4 mg/kg bw/d x 2.45 % = 0.0588 (0.06) mg/kg bw/d
systemic $AOEL_{AMTT}$ = 0.06 mg/kg bw/d / 500 = 0.0001 mg/kg bw/d

By the notifier the NOAEL at 25 ppm established for the F_2 progeny is not considered to be appropriate because the effects seen in this study on the F_1 parental and F_1 progeny mimic the short term exposure of operators in a more appropriate way for the risk assessment. Based on a NOAEL of 5.1 mg/kg bw/d (50 ppm) for developmental toxicity of tritosulfuron containing 2.45 % AMTT, the NOAEL for AMTT was: 5.1 mg/kg bw/d x 2.45 %= 0.125 mg/kg bw/d. Applying a Safety factor of 200 a systemic $AOEL_{AMTT}$ of 0.000625 mg/kg bw/d was proposed.

Comparison of estimated and acceptable operator exposure

For direct extrapolation of the inhalation or dermal exposure, there are no appropriate toxicological studies available investigating the critical endpoints via specific routes. Therefore, the calculated systemic exposure from both models (Table B.6.14.5-2 and Table B.6.14.5-3) were directly compared with the proposed systemic $AOEL_{AMTT}$ of 0.0001 mg/kg bw/d. The values are given in Table B.6.14.5-4.

Table B.6.14.5-4: Results of the model calculations (German model; UK POEM, modified) and a comparison with the proposed systemic AOEL

Treated area per day (ha/d)	PPE	Systemic exposure* (mg/kg bw/d)	% of $AOEL_{AMTT}$ (0.0001 mg/kg bw/d)
20	**German model**		
	No PPE	0.0001204	120.4
	Gloves: m/l	0.0000638	63.8
	Gloves: m/l and appl.; garment: appl.	0.0000052	5.2
50	**UK POEM (modified)**		
	No PPE	0.0009973	997.3
	Gloves: m/l	0.0005256	525.6
	Gloves: m/l and appl.	0.0001033	103.3
* dermal and inhalation absorption: 100 % (worst case); body weight: 70 kg (German model), 60 kg (UK POEM)			

The results show that under the given conditions the operator exposure to AMTT for the proposed use is acceptable if personal protection is used. For the German model the exposure is estimated to be 63.8 % of the derived systemic $AOEL_{AMTT}$ if gloves are worn during mixing/loading and for the modified UK POEM 103.3 % of the $AOEL_{AMTT}$ if gloves are worn during mixing/loading and application (however in this model it is not possible to consider a protective garment in the calculation).

B.6.14.5.2 Worker exposure

B.6.14.5.2.1 Estimation of worker exposure

The potential dermal exposure of workers is derived in the monograph for tritosulfuron (B.6.14.2.1) and re-assessed with the new data proposed by the ECCO 136 meeting. The calculation for the metabolite AMTT is carried out with the same assumptions. A worst case dermal absorption rate of 100 % is used to estimate a respective systemic exposure/absorbed dose. Thus the potential dermal exposure is identical with the estimated systemic exposure.

The following parameters were considered:

Dislodgeable foliar residue	DFR	=	1 µg/cm² x kg as applied
Transfer factor, according to EPA	TF*	=	1000 cm²/h x person
Working period	A	=	2 h/day
Penetration of protective material	P	=	5 % (= factor 0.05)
Rate of application	R	=	0.00015 kg AMTT/ha**

* A transfer factor of 1000 cm^2/h x person was chosen since the operations following the application of BAS 635 00 H at growth stages up to GS 39 do imply the exposure of feet and lower legs only.

** AMTT content max. 0.3 % relative to parent
(AMTT application rate = 0.05 kg tritosulfuron/ha x 0.3 %)

Calculation of potential dermal exposure:

Dermal exposure (D) of the unprotected worker

D	=	DFR	x	TF	x	A	x	R
	=	1	x	1000	x	2	x	0.00015

= 0.3 µg/person x day
= 0.0003 mg/person x day

considering a body weight of 70 kg

D = 0.0000042 mg/kg bw/d

Dermal exposure (D(PPE)) for the protected worker

D (PPE)	=	D	x	P
	=	0.0000042	x	0.05

D (PPE) = 0.0000002 mg/kg bw/d

Estimated absorbed dose = potential dermal exposure (dermal absorption rate: 100 %)

without PPE:	0.0000042	mg/kg bw/d
protected worker:	0.0000002	mg/kg bw/d

Risk assessment

The estimated dermal exposure as percentage of the systemic $AOEL_{AMTT}$ (0.0001 mg/kg bw/d) are calculated to be:

without PPE:	4.2 %
protected worker:	0.2 %

With regard to the metabolite AMTT as in the case of the active ingredient tritosulfuron the results of the risk assessment indicate that re-entry of treated fields is possible with a sufficient margin of safety after the spray solution has dried up. Specific protective measures for worker re-entry are not necessary.

B.6.14.5.3 Bystander exposure

BAS 635 00 H is a herbicide applied in field crops. The usual form of application is by tractor-mounted sprayers without bystanders. As outlined by the notifier the spray solution as applied will contain a maximum of 0.047 % (w/v) of the formulated product, 0.033 % (w/v) of tritosulfuron, and as a realistic worst-case assumption 0.3 % AMTT (relative to tritosulfuron content = 0.0001 % AMTT). In view of the recommended application technique in combination with Good Agricultural Practice bystanders may be exposed briefly and to relatively low quantities of spray compared to an operator.
A possible situation in which bystander exposure may occur would be a person walking on a footpath alongside an area, which is being treated at the same time. Even under these conditions the bystander will never walk directly next to the outer spraying nozzle. A distance of some meters from the downwind edge of the treated swath can always be expected. An estimation of bystander exposure has been presented by the notifier.

B.6.14.5.3.1 Estimation of bystander exposure

An approach for the assessment of the potential dermal exposure of bystanders can be made using drift estimates based on data published by EPPO [Kohsiek H. (2000); Bekanntmachung

über die Abdrifteckwerte, die bei der Prüfung von Pflanzenschutzmitteln herangezogen werden; Bundesanzeiger 100/26.05.2000, p. 9878 - 9880]:
Assuming a bystander may be at a 10 meter distance from the spray application, the data show that for field crops, the estimated drift may be about 0.29 % of the nominal application rate per surface unit. If a bystander with a body surface of approximately 2 m² (which is considered to be an average value for adults) is unilaterally exposed whilst passing a field being treated it can be estimated that the amount of active substance that may drift off on a square metre of ground outside the application area is deposited on the bystander instead. Assuming that the bystander is wearing only few clothing this amount can be considered to result in dermal exposure as a worst-case assumption.
By application of BAS 635 00 H, bystanders may be exposed to AMTT beside of the active substance tritosulfuron. For a maximum recommended application rate of 50 g tritosulfuron/ha and assuming (worst-case) the operator is applying a spray-broth that contains a maximum of 0.3 % AMTT relative to total tritosulfuron content, the corresponding application rate for AMTT would be 0.15 g/ha. This nominal application rate corresponds to 0.015 mg AMTT/m², and 0.29 % (drift) thereof is 0.0000435 mg AMTT/m².
Considering a worst-case dermal absorption of 100 % for the spray dilution the potential systemic exposure can be assessed to be 0.0000435 mg/bystander corresponding to 0.0000006 mg/kg bw if a body weight of 70 kg is assumed.

Risk assessment
The estimated bystander exposure is 0.0000006 mg/kg bw. Compared to the proposed systemic $AOEL_{AMTT}$ of 0.0001 mg/kg bw/d *(0.000625 mg/kg bw/d by the notifier)*, the bystander exposure is < 1 % of the systemic $AOEL_{AMTT}$.
With regard to the metabolite AMTT as in the case of the active ingredient tritosulfuron the results of the risk assessment submitted by the notifier indicate that for the bystander no risk is anticipated also if some assumptions should be changed.

B.6.14.6 Overall conclusion

BAS 635 00 H is intended as a post emergence herbicide in cereals. The maximum application rate is 0.07 kg product/ha and therefore 0.050 kg as/ha (WG: 714 g tritosulfuron/kg product). The intended use is in a tank-mix with surfactants. Hydrolytical cleavage of tritosulfuron in the spray tank results in increased concentrations of AMTT and hence leads to higher exposures to AMTT.
At the ECCO 136 meeting in contrast to the monograph the systemic AOEL for tritosulfuron was derived to be 0.15 mg/kg bw/d and the dermal absorption rates to be 1 % for the concentrate and 2 % for the spray dilution of the product BAS 635 00 H. Therefore a re-assessment is given for the active ingredient tritosulfuron.
On the basis of the data from laboratory experiments and an outdoor study a specific risk assessment regarding the possible exposure to the metabolite AMTT resulting from field application of tritosulfuron-containing products was prepared.
The different conditions for the risk assessment and the respective results are summarised in Table B.6.14.6-1.

Table B.6.14.6-1: Overwiev regarding the risk assessment of tritosulfuron and the metabolite AMTT

	PPE	Tritosulfuron		Metabolite AMTT	
		Syst. expos.* (mg/kg bw/d)	**% of AOEL (0.15 mg/kg bw/d)**	**Syst. expos.* (mg/kg bw/d)**	**% of AOEL (0.0001 mg/kg bw/d)**
Operator exposure (treated area: 20 ha/d)		German model		German model	
	No PPE	0.0010	0.66	0.0001204	120.4
	Gloves: m/l	0.0007	0.48	0.0000638	63.8
	Gloves: m/l and appl.; garment: appl.	0.0002	0.12	0.0000052	5.2
Operator exposure, (treated area: 50 ha/d)		UK POEM, not modified		UK POEM, modified	
	No PPE	0.726	8.07	0.0009973	997.3
	Gloves: m/l	0.302	3.36	0.0005256	525.6
	Gloves: m/l and appl.;	0.068	0.75	0.0001033	103.3
Worker exposure					
	No PPE	0.0000286	0.02	0.0000042	4.2
	With PPE	0.0000014	0.001	0.0000002	0.2
Bystander exposure					
-	-	Operator-expos. << syst. AOEL (no PPE)	not calculated	0.0000006	0.6

- Body weight: 70 kg (German model) or 60 kg (UK POEM)
- Dermal absorption tritosulfuron: concentrate (m/l): 1 %, dilution (appl.): 2 %; inhalation absorption: 100 %
- Dermal and inhalation absorption AMTT: 100 %

The results show that under the given conditions the operator and worker exposure for the active ingredient tritosulfuron is below the respective systemic AOEL even if no personal protection is used. In result of the risk assessment for the metabolite AMTT, PPE is needed for the operator to stay below the systemic $AOEL_{AMTT}$ (German model). Calculating with the modified UK POEM the $AOEL_{AMTT}$ is exceeded (103.3 %) even if gloves are worn during mixing/loading and application (however in this model it is not possible to consider a protective garment in the calculation).

With regard to the active ingredient tritosulfuron as well as to the metabolite AMTT it is concluded that for BAS 635 00 H the intended use is acceptable if PPE is worn by the operator which is in line with the notifier´s view. Although the exposure calculation for both the worker and the bystander results in an acceptable level considering the possible classification/labelling of AMTT [T; R 40-48/22-61(64)] the product should be handled carefully.

Appendix 1

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM) PPE: None

Application method:	Tractor-mounted/drawn field crop sprayer with hydraulic nozzles		
Product:	BAS 635 00 H	"Active Substance" (as):	**AMTT in BAS 635 H**
Formulation type:	WG	as concentration in product:	1.428 mg/g
PPE during mix/loading:	None	PPE during application:	None
Dose of product:	0.07 kg/ha	Work rate/day:	50 ha
Max. in-use as concentration*:	0.0007219 mg/mL	Duration of spraying:	6 h

DERMAL EXPOSURE DURING MIXING AND LOADING

Hand contamination/kg as	5.72	mg/kg as
Hand contamination/d:	0.028589	mg/d
Protective clothing:	None	
Transmission to skin:	100	%
Dermal exposure to formulation.	0.028589	mg/d

INHALATION EXPOSURE DURING MIXING AND LOADING

Inhalation exposure/kg as	0.242	mg/kg as
Inhalation exposure/day	0.0012095	mg/d
Respiration protective equipment (RPE)	None	
Transmission through RPE	100	%
Inhalation exposure to as	0.0012095	mg/d

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application volume:	150	L spray/ha		
Volume of surface contamination:	10	mL/h		
Distribution:	Hands	Trunk	Legs	
	65 %	10 %	25 %	
Clothing:	None	Permeable	Permeable	
Penetration:	100 %	5 %	15 %	
Dermal exposure:	6.5	0.05	0.375	mL/h
Duration of exposure:	6	h		
Total dermal exposure to spray:	41.55	mL/d		
Concentration of as in spray solution:	0.0007219	mg/mL		
Dermal exposure to as	0.0299963	mg/d		

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure:	0.01	mL/h
Duration of exposure:	6	h
Concentration of as in spray solution:	0.0007219	mg/mL
Inhalation exposure to as	0.0000433	mg/d
Percent absorbed:	100	%
Absorbed dose:	0.0000433	mg/d

ABSORBED DOSE

	Mix/load		Application	
Dermal exposure to as	0.028589	mg/d	0.0299963	mg/d
Percent absorbed:	100	%	100	%
Absorbed dose:	0.028589	mg/d	0.0299963	mg/d
Inhalation exposure to as	0.0012095	mg/d	0.0000433	mg/d
Subtotal exposure	0.0297981	mg/d	0.0300396	mg/d

PREDICTED EXPOSURE

Total absorbed dose:	0.0598377	mg/d
Operator body weight:	60	kg
Operator exposure:	0.0009973	mg/kg bw/d

Appendix 2

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM) PPE: Mixing/Loading

Application method:	Tractor-mounted/drawn field crop sprayer with hydraulic nozzles				
Product:	BAS 635 00 H		"Active Substance" (as):	**AMTT in BAS 635 H**	
Formulation type:	WG		as concentration in product:	1.428	mg/g
PPE during mix/loading:	Gloves		PPE during application:	None	
Dose of product:	0.07	kg/ha	Work rate/d:	50	ha
Max. in-use as concentration*:	0.0007219	mg/mL	Duration of spraying:	6	h

DERMAL EXPOSURE DURING MIXING AND LOADING

Hand contamination/kg as	5.72	mg/kg as
Hand contamination/d:	0.028589	mg/d
Protective clothing:	None	
Transmission to skin:	1	%
Dermal exposure to formulation.	0.00028589	mg/d

INHALATION EXPOSURE DURING MIXING AND LOADING

Inhalation exposure/kg as	0.242	mg/kg as
Inhalation exposure/day	0.0012095	mg/d
Respiration protective equipment (RPE)	None	
Transmission through RPE	100	%
Inhalation exposure to as	0.0012095	mg/d

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application volume:	150	L spray/ha		
Volume of surface contamination:	10	mL/h		
Distribution:	Hands	Trunk	Legs	
	65 %	10 %	25 %	
Clothing:	None	Permeable	Permeable	
Penetration:	100 %	5 %	15 %	
Dermal exposure:	6.5	0.05	0.375	mL/h
Duration of exposure:	6	h		
Total dermal exposure to spray:	41.55	mL/d		
Concentration of as in spray solution:	0.0007219	mg/mL		
Dermal exposure to as	0.0299963	mg/d		

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure:	0.01	mL/h
Duration of exposure:	6	h
Concentration of as in spray solution:	0.0007219	mg/mL
Inhalation exposure to as	0.0000433	mg/d
Percent absorbed:	100	%
Absorbed dose:	0.0000433	mg/d

ABSORBED DOSE

	Mix/load		Application	
Dermal exposure to as	0.0002859	mg/d	0.0299963	mg/d
Percent absorbed:	100	%	100	%
Absorbed dose:	0.0002859	mg/d	0.0299963	mg/d
Inhalation exposure to as	0.0012095	mg/d	0.0000433	mg/d
Subtotal exposure	0.0014954	mg/d	0.0300396	mg/d

PREDICTED EXPOSURE

Total absorbed dose:	0.0315350	mg/d
Operator body weight:	60	kg
Operator exposure:	0.0005256	mg/kg bw/d

Appendix 3

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM) PPE: Mixing/Loading & Application

Application method:	Tractor-mounted/drawn field crop sprayer with hydraulic nozzles				
Product:	BAS 635 00 H		"Active Substance" (as):	**AMTT in BAS 635 H**	
Formulation type:	WG		as concentration in product:	1.428	mg/g
PPE during mix/loading:	Gloves		PPE during application:	Gloves	
Dose of product:	0.07	kg/ha	Work rate/day:	50	ha
Max. in-use as concentration*:	0.0007219	mg/mL	Duration of spraying:	6	h

DERMAL EXPOSURE DURING MIXING AND LOADING

Hand contamination/kg as	5.72	mg/kg as
Hand contamination/ day:	0.028589	mg/day
Protective clothing:	None	
Transmission to skin:	100	%
Dermal exposure to formulation.	0.00028589	mg/day

INHALATION EXPOSURE DURING MIXING AND LOADING

Inhalation exposure/kg as	0.242	mg/kg as
Inhalation exposure/day	0.0012095	mg/day
Respiration protective equipment (RPE)	None	
Transmission through RPE	100	%
Inhalation exposure to as	0.0012095	mg/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application volume:	150	l spray/ha		
Volume of surface contamination:	10	mL/h		
Distribution:	Hands	Trunk	Legs	
	65 %	10 %	25 %	
Clothing:	Gloves	Permeable	Permeable	
Penetration:	10 %	5 %	15 %	
Dermal exposure:	0.65	0.05	0.375	mL/h
Duration of exposure:	6	h		
Total dermal exposure to spray:	6.45	mL/day		
Concentration of as in spray solution:	0.0007219	mg/mL		
Dermal exposure to as	0.0046565	mg/day		

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure:	0.01	mL/h
Duration of exposure:	6	h
Concentration of as in spray solution:	0.0007219	mg/mL
Inhalation exposure to as	0.0000433	mg/day
Percent absorbed:	100	%
Absorbed dose:	0.0000433	mg/day

ABSORBED DOSE

	Mix/load		Application	
Dermal exposure to as	0.0002859	mg/day	0.0046565	mg/day
Percent absorbed:	100	%	100	%
Absorbed dose:	0.0002859	mg/day	0.0046565	mg/day
Inhalation exposure to as	0.0012095	mg/day	0.0000433	mg/day
Subtotal exposure	0.0014954	mg/day	0.0046998	mg/day

PREDICTED EXPOSURE

Total absorbed dose:	0.0061952	mg/day
Operator body weight:	60	kg
Operator exposure:	0.0001033	mg/kg bw/day

B.6.15 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
AIIA-5.8.1/24	Gamer, A.O. and Leibold, E.	2003	TBSA - Acute oral toxicity study in rats BASF DocID 2003/1021650 GLP, unpublished	Y	BAS
AIIA-5.8.1/25	Kaspers, U. et al.	2003	TBSA - Subacute toxicity study in Wistar rats administration in the diet for 4 weeks BASF DocID 2003/1004048 GLP, unpublished	Y	BAS
AIIA-5.8.1/26	Kaspers, U. et al.	2003	TBSA - Repeated dose oral toxicity study in Wistar rats administration in the diet for 4 weeks BASF DocID 2003/1004049 GLP, unpublished	Y	BAS
AIIA-5.8.1/28	Schneider, S. et al.	2004	TBSA - One-Generation Reproduction Toxicity Study in Wistar Rats Range finding study (with 4 weeks premating) continuous dietary administration BASF DocID 2004/1017198 GLP, unpublished	Y	BAS
AIIA-5.8.1/29	Schneider, S. et al.	2004	TBSA - One-generation reproduction toxicity study in Wistar rats range finding study (with 10 weeks premating) continuous dietary administration BASF DocID 2004/1017197 GLP, unpublished	Y	BAS
AIIA-5.8.1/30	Kaspers, U. et al.	2003	Reg. No. 335 184 (metabolite of BAS 635 H) - Subacute toxicity study in Wistar rats administration in the diet for 4 weeks BASF DocID 2003/1022011 GLP, unpublished	Y	BAS
5.8.2/11	Engelhardt, G. and Leibold, E.	2004	*In vitro* chromosome aberration assay with Reg. No. 231700 (metabolite of BAS 635 H) in V79 cells BASF DocID 2004/1014204 GLP, unpublished	Y	BAS
AIIA-5.10/1	Stinchcombe, S.	2003	Tritosulfuron ECCO Evaluation. Position paper on open points and data requirements related to mammalian toxicology BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep 2003/1013468 No GLP, unpublished	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
AIIA-5.10/2	Stinchcombe, S.	2004	Tritosulfuron (BAS 635 H) - Summary of additional toxicity studies with tritosulfuron metabolites conducted during the EU Peer Review Process - EU dossier format BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 2004/1017898 No GLP, unpublished	Y	BAS
AIIIA-2.9.2/2	Hassink, J.	1999	Determination of Reg. No. 231 700 (AMTT) in the spray volume of BAS 635 H formulations BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed. Rep. 1999/10894 No GLP, unpublished	Y	BAS
AIIIA-2.9.2/3	Stadler, R.	2004	Hydrolysis Stability of BAS 635 00 H in Spray Tank under Field Conditions -- Determination of AMTT (635M04, BH 635-5, Reg. No. 231 700) BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed. Rep. 2004/1016478 No GLP, unpublished	Y	BAS
AIIIA-7.2.1.1.1/1	Lungershausen, R.	2002	Risk assessment for AMTT (Reg. No. 231 700) as an impurity in the active substance BAS 635 H BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 2002/1004227 No GLP, unpublished	Y	BAS
AIIIA-7.2.1.1.1/2	Stinchcombe, S.	2004	Tritosulfuron (BAS 635 H) - Operator, worker and bystander risk assessment of possible exposure to AMTT (Reg. No. 231 700) resulting from field application of tritosulfuron-containing products BASF AG; Ludwigshafen/Rhein; Germany Fed. Rep. 2004/1017899 No GLP, unpublished	Y	BAS

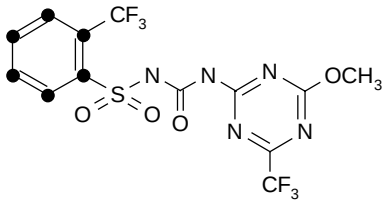
B.7 Residue data

B.7.1 Metabolism, distribution and expression of residues in plants (Annex IIA 6.1; Annex IIIA 8.1)

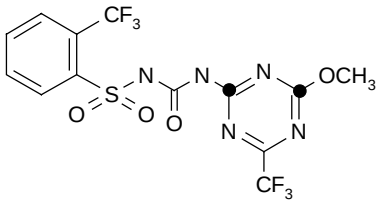
Metabolism in wheat

The purpose of the new wheat metabolism study is to compare the results with the maize metabolism and the confined rotational crop study and to propose a suitable definition of the relevant residue in plant matrices based on all studies.

The metabolism and distribution of tritosulfuron in wheat plants was investigated using [phenyl-U-^{14}C]- tritosulfuron and [triazine-2,4-^{14}C]- tritosulfuron. The same label positions have been used in the maize metabolism and the confined rotational crop study.



phenyl labelled tritosulfuron



triazine labelled tritosulfuron

Report:	Bross M., Mackenroth C., 2004 Metabolism of ^{14}C-tritosulfuron (^{14}C- BAS 635 H) in wheat BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. unpublished BASF DocID 2004/1010546
Guidelines:	US EPA Residue Chemistry Test Guidelines, OPPTS 860.1300: Nature of the Residue - Plants, Livestock and EPA 860.1000: Background, EEC working document 7028/VI/95 rev. 2 (22.07.1997): Appendix A - Metabolism and distribution in plants
GLP:	Yes (laboratory certified by Landesanstalt für Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

B.7.1.4 Test system

During the plant uptake part of the study, wheat plants (variety: Star) were grown in a loamy sand soil in a glass house located in Limburgerhof. Two parallel investigations were performed using radioactive material labelled either in the triazine or in the phenyl ring system. The post emergence applications of both labels took place at BBCH growth stage 37 - 39 (flag leaf visible - flag leaf fully unrolled). The test compound was applied in form of the WG formulation BAS 635 00 H to the plants at a rate of 50 g as/ha. As adjuvant Dash HC was added. The foliar application was performed by an automatic spray track system. Samples

were taken immediately after application, 64/69 DAT and 96 DAT. Harvest of the ripe plants took place 118 days after application. The ripe samples were separated into straw, chaff and grain. An overview of the study design is given in Table B.7.1.4-1.

Table B.7.1.4-1: Design of the plant uptake part - wheat

	triazine label	**phenyl label**
Intended application rate [g as/ha]	50	50
Treatment	Post-emergence	Post-emergence
Number of applications	1	1
Interval between applications [days]	Not applicable	Not applicable
Comparison to the maximum recommended use rate	1 x	1 x
Sampling of unripe material [days after treatment]	0, 64, 96	0, 69, 96
DAT [days after last application]	118	118

After homogenisation, all samples taken were extracted with methanol followed by water. The total radioactive residue was calculated by adding the extractable radioactivity and the residual radioactivity. For further characterisation, the methanol extracts were partitioned between organic solvents and water. All extracts were analysed by chromatographic means (radio-HPLC and radio-TLC). Identification of metabolites is based on LC-MS investigations and HPLC retention time comparison with certified reference standards or well characterised metabolites. The non-released radioactive residues were further treated with ammonia.

B.7.1.5 Findings

The total radioactive residues calculated are summarised in Table B.7.1.5-1. Immediately after spray application, TRR values of 2.002 and 1.942 mg/kg were determined in forage. At the second sampling (64/69 DAT) 0.63 mg/kg were found with both labels. In hay (96 DAT), the TRRs amounted to 2.381 and 1.115 mg/kg. At harvest (GS 89), higher amounts of radioactivity were detected in straw (0.705 and 0.638 mg/kg) whereas in grain the TRR was very low (0.005 and 0.004 mg/kg). In chaff, 0.133 and 0.181 mg/kg were found. In general, the total radioactive residues were comparable for both labels.

Table B.7.1.5-1: Total radioactive residue in wheat samples after post-emergence treatment with ^{14}C-tritosulfuron

Sample description	Days after treatment	TRR[1)] calculated [mg/kg]
Triazine label		
Forage	0 DAT	2.002
Forage	64 DAT	0.633
Hay	96 DAT	2.381
Grain	118 DAT	0.004
Chaff	118 DAT	0.133
Straw	118 DAT	0.705
Phenyl label		
Forage	0 DAT	1.942
Forage	69 DAT	0.630
Hay	96 DAT	1.115
Grain	118 DAT	0.002
Chaff	118 DAT	0.181
Straw	118 DAT	0.638

1) TRR calculated = extractable radioactive residue (ERR) + residual radioactive residue (RRR)
2) DAT = days after treatment

The samples were extracted with methanol followed by water. The extractability in the forage samples was very good; more than 97 % of the TRR were extractable. From hay, 95 - 98 % TRR were obtained. At later sampling intervals, the extractability slightly decreased. The amounts extracted from grain ranged between 67 and 79 % of the TRR. From chaff, 88 and 93 % TRR could be extracted, from straw 96 % TRR with both labels. After methanol and water extraction, ammonia extraction was performed. The amounts of additionally released radioactivity ranged between 0.2 and 7.0 % of the TRR.

In addition to methanol and water extraction, selected sample materials of both labels were extracted according to the residue analytical methods 555/0 and 405/1. The extraction with an acetonitrile / buffer system consisting of tris(hydroxymethyl)aminomethan and hydrochloric acid (555/0) yielded about 90 % TRR from forage, 64 % from grain and 70 and 75 % from straw. Acetone / water extraction (405/1) released about 77 % TRR from forage, 39 % from grain and 71 % from straw. The method 555/0 was developed for the quantitation of tritosulfuron and metabolites whereas method 405/1 is used for parent only.

Table B.7.1.5-2: Extractability of wheat samples after post emergence treatment with ^{14}C- tritosulfuron

Sample description (DAT)[4]	TRR[1] [mg/kg]	MeOH extract [mg/kg] (%TRR)	Aqueous extract [mg/kg] (% TRR)	ERR[2] [mg/kg] (% TRR)	RRR[3] [mg/kg] (% TRR)
Triazine label					
Forage (0 DAT)	**2.002 (100.0%)**	1.977 (98.8%)	0.012 (0.6%)	**1.989 (99.4%)**	**0.013 (0.6%)**
Forage (64 DAT)	**0.633 (100.0%)**	0.592 (93.5%)	0.033 (5.1%)	**0.625 (98.7%)**	**0.009 (1.3%)**
Forage (64 DAT): Extractability[4]	**0.605 (100.0%)**	0.543 (89.7%)		**0.543 (89.7%)**	0.063 **(10.3%)**
Forage (64 DAT): Extractability[5]	**0.616 (100.0%)**	0.472 (76.5%)		**0.472 (76.5%)**	0.145 **(23.5%)**
Hay (96 DAT)	**2.381 (100.0%)**	2.182 (91.6%)	0.153 (6.4%)	**2.335 (98.1%)**	**0.047 (2.0%)**
Grain (118 DAT)	**0.004 (100.0%)**	0.002 (41.6%)	0.001 (25.0%)	**0.003 (66.6%)**	**0.001 (33.4%)**
Grain (118 DAT): Extractability[4]	**0.005 (100.0%)**	0.003 (64.1%)		**0.003 (64.1%)**	**0.002 (35.9%)**
Grain (118 DAT): Extractability[5]	**0.004 (100.0%)**	0.002 (38.6%)		**0.002 (38.6%)**	**0.003 (61.4%)**
Chaff (118 DAT)	**0.133 (100.0%)**	0.102 (76.5%)	0.016 (11.9%)	**0.118 (88.4%)**	**0.015 (11.6%)**
Straw (118 DAT)	**0.705 (100.0%)**	0.637 (90.3%)	0.043 (6.1%)	**0.680 (96.4%)**	**0.025 (3.6%)**
Straw (118 DAT): Extractability[4]	**0.705 (100.0%)**	0.495 (70.2%)		**0.495 (70.2%)**	**0.210 (29.8%)**
Phenyl label					
Forage (0 DAT)	**1.942 (100.0%)**	1.918 (98.8%)	0.017 (0.9%)	**1.935 (99.7%)**	**0.007 (0.3%)**
Forage (69 DAT)	**0.630 (100.0%)**	0.574 (91.1%)	0.047 (7.4%)	**0.621 (98.6%)**	**0.009 (1.5%)**
Hay (96 DAT)	**1.115 (100.0%)**	0.991 (88.9%)	0.101 (9.0%)	**1.092 (97.9%)**	**0.023 (2.0%)**
Grain (118 DAT)	**0.002 (100.0%)**	0.001 (54.3%)	0.000 (17.1%)	**0.002 (71.4%)**	**0.001 (28.6%)**
Chaff (118 DAT)	**0.181 (100.0%)**	0.149 (82.2%)	0.019 (10.3%)	**0.168 (92.5%)**	**0.013 (7.4%)**
Straw (118 DAT)	**0.638 (100.0%)**	0.567 (88.8%)	0.047 (7.4%)	**0.614 (96.2%)**	**0.025 (3.8%)**
Straw (118 DAT): Extractability[4]	**0.480 (100.0%)**	0.363 (75.7%)		**0.363 (75.7%)**	**0.117 (24.3%)**
Straw (118 DAT): Extractability[5]	**0.558 (100.0%)**	0.395 (70.8%)		**0.395 (70.8%)**	**0.163 (29.2%)**

1) TRR determined by calculation
2) ERR: extractable radioactive residue
3) RRR: residual radioactive residue
4) Extractability investigations using an acetonitrile/ buffer mixture (method 555/0)
5) Extractability investigations according to method 405/1

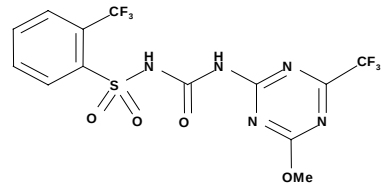
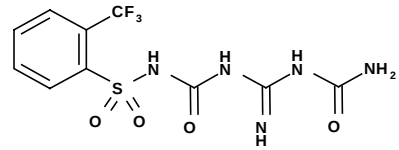
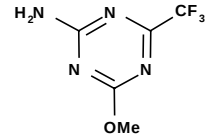
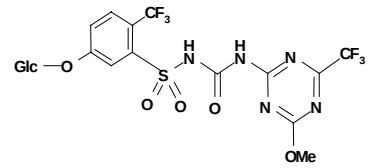
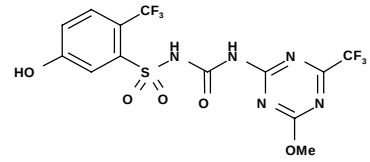
In order to classify the metabolites into organosoluble and water-soluble ones, the methanol extracts were partitioned between iso-hexane, dichloromethane, ethyl acetate and water. The predominant proportion of the extractable radioactivity was found to be organosoluble: In case of the wheat forage 0 DAT 97 - 98 % TRR could be partitioned into organic solvents. From the forage taken later as well as from hay and straw, the organosoluble portion ranged between 66 and 77 % TRR. The lowest organosoluble portion was found in chaff (51 and 60 % TRR).

The metabolic profiles of both labels were almost comparable. Tritosulfuron formed the major part of the extractable radioactivity in almost all matrices. Second most abundant peak in almost all samples under investigation was the glucoside 635M13, which is formed after hydroxylation of the phenyl ring system. The long chain guanidine metabolite 635M01 is also common for most of the wheat matrices. It is formed by cleavage of the triazine ring system. In wheat grain, as edible portion the most important one, no individual compound above 0.001 mg/kg was detected. The direct cleavage of the sulfonyl urea bridge is not an important degradation pathway of tritosulfuron in wheat. The resulting metabolites 635M04 and 635M02 were only found in trace amounts.
The amounts of the individual metabolites are summarised in Table B.7.1.6-1 to Table B.7.1.6-4.

B.7.1.6 Conclusion

The metabolic pathway of BAS 635 H in cereals was investigated after post emergence application of material labelled in either the phenyl or the triazine ring system. For this purpose, BAS 635 H was applied in form of a WG formulation to plants. As adjuvant, Dash HC was added.
In general, the total radioactive residues (TRR) were comparable for both labels. The TRRs in grain, which is the only raw agricultural commodity destined for human food, were very low. Higher values were found in the plant matrices used as animal feed.
The parent molecule was the predominant residue in most of the samples under investigation. Second most abundant peak is the conjugate 635M13, which is formed after hydroxylation of the phenyl ring system. As further degradation pathway, cleavage of the triazine ring system occurred resulting in the guanidine metabolites 635M01 and 635M03.
The direct cleavage of the sulfonyl urea bridge is not an important degradation pathway of BAS 635 H in cereals. The resulting metabolites 635M04 and 635M02 were only found in trace amounts. No significant incorporation of BAS 635 H and its metabolites into natural products were observed. The results of this study are in good accordance with the metabolism study in maize and in rotational crops. The metabolic pathway of BAS 635 H in wheat is depicted in Figure B.7.1.6-1.

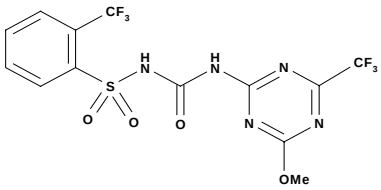
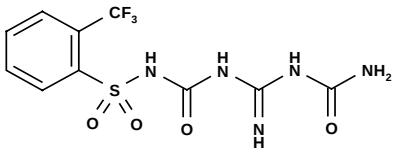
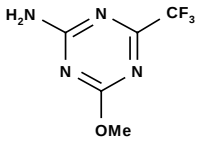
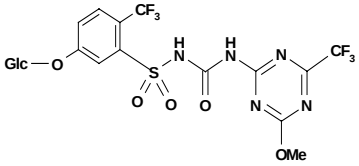
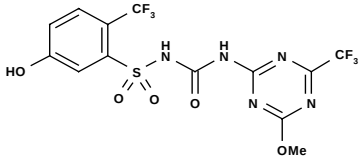
Table B.7.1.6-1: Summary of identified components in unripe wheat samples after post-emergence treatment with ^{14}C-tritosulfuron (triazine label)

Designation Metabolite Code (Reg. No.)	Structure	Forage (0 DAT)		Forage (64 DAT)		Hay (96 DAT)	
		mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
BAS 635 H (271272)		1.841	92.0	0.476	75.2	1.582	66.4
635M01 (335184)		0.012	0.6	0.018	2.9	0.098	4.1
635M04 (231700)		0.062	3.1	0.019	3.0	0.111	4.7
635M13 (n.a.)		0.133	6.6	0.085	13.5	0.356	14.9
635M17 (373906)		n.d.	n.d.	0.022	3.5	0.098	4.1

n.d.: not detected

In case of the metabolite 635M04, the peak is overlapped by unknowns. The total amount of the relevant peak is given.

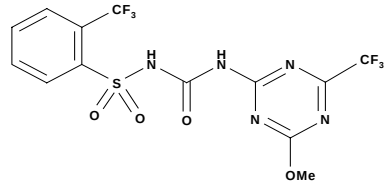
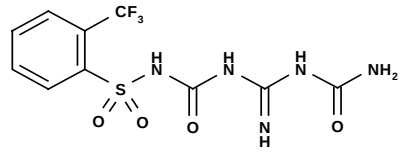
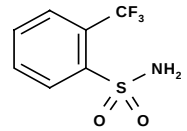
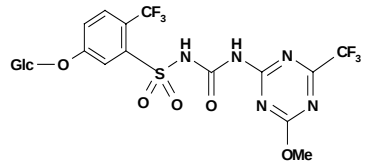
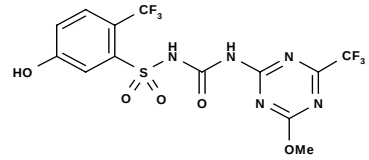
Table B.7.1.6-2: Summary of identified components in wheat samples at harvest after post emergence treatment with ^{14}C-tritosulfuron (triazine label)

Designation Metabolite Code (Reg. No.)	Structure	Grain (118 DAT)		Chaff (118 DAT)		Straw 118 DAT	
		mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
BAS 635 H (271272)		< 0.001	6.3	0.037	27.8	0.372	52.8
635M01 (335184)		n.d.	n.d.	0.007	5.4	0.042	6.0
635M04 (231700)		< 0.001	1.8	0.006	4.5	0.044	6.2
635M13 (n.a.)		n.d.	n.d.	0.021	16.1	0.113	16.0
635M17 (373906)		n.d.	n.d.	0.005	3.4	0.018	2.5

n.d.: not detected

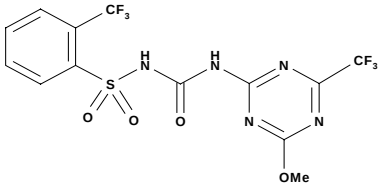
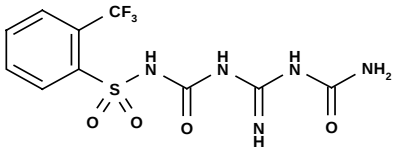
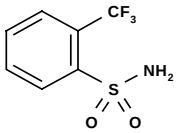
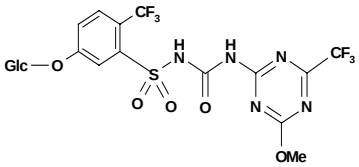
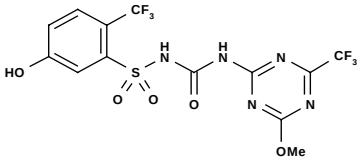
In case of the metabolite 635M04, the peak is overlapped by unknowns. The total amount of the relevant peak is given.

Table B.7.1.6-3: Summary of identified and characterised components in unripe wheat samples after post emergence treatment with ^{14}C-tritosulfuron (phenyl label)

Designation Metabolite Code (Reg. No.)	Structure	Forage (0 DAT)		Forage (69 DAT)		Hay (96 DAT)	
		mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
BAS 635 H (271272)		1.744	89.8	0.377	59.9	0.594	53.3
635M01 (335184)		0.014	0.7	0.024	3.8	0.051	4.6
635M02 (292564)		0.049	2.5	0.014	2.2	0.038	3.4
635M13 (n.a.)		0.098	5.0	0.146	23.1	0.255	22.8
635M17 (373906)		n.d.	n.d.	0.029	4.7	0.051	4.6

n.d.: not detected

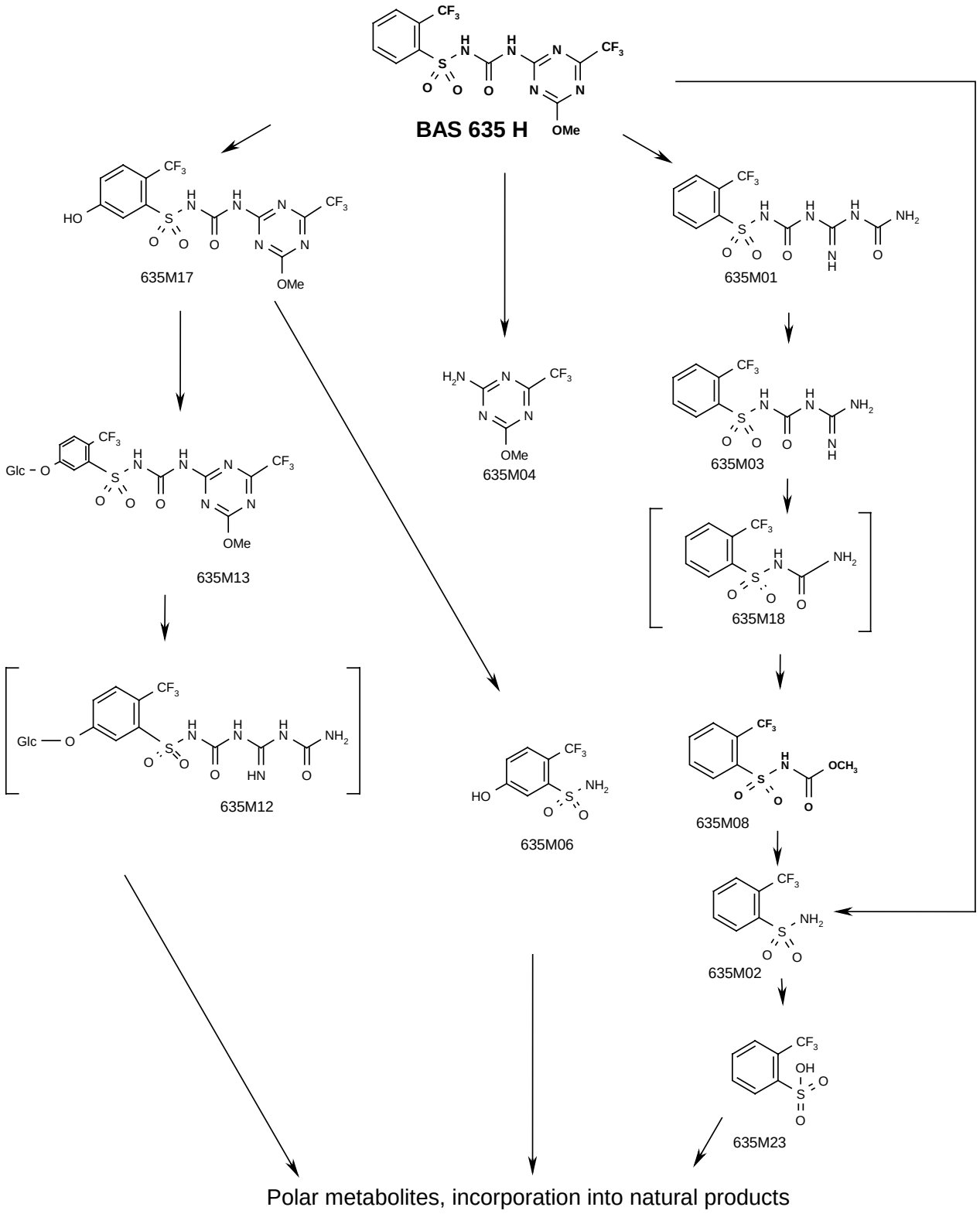
Table B.7.1.6-4: Summary of identified and characterised components in wheat samples at harvest after post-emergence treatment with ^{14}C-tritosulfuron (phenyl label)

Designation Metabolite Code (Reg. No.)	Structure	Grain (118 DAT)		Chaff (118 DAT)		Straw 118 DAT	
		mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
BAS 635 H (271272)		< 0.001	7.3	0.043	24.0	0.268	42.0
635M01 (335184)		< 0.001	1.8	0.015	8.4	0.034	5.4
635M02 (292564)		< 0.001	6.7	0.012	6.5	0.025	4.0
635M13 (n.a.)		< 0.001	3.5	0.048	26.6	0.142	22.3
635M17 (373906)		n.d.	n.d.	0.007	4.1	0.025	4.0

n.d.: not detected

In case of the metabolite 635M02 in grain, the peak is overlapped by unknowns. The total amount of the relevant peak is given.

Figure B.7.1.6-1: Proposed metabolic pathway of tritosulfuron in wheat



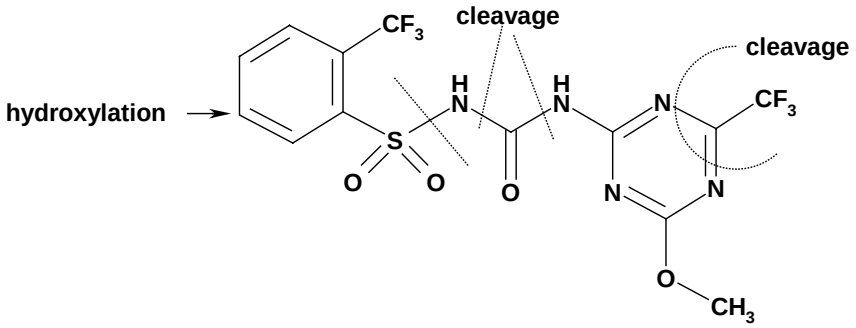
B.7.1.7 Comparison of the plant metabolism studies

For tritosulfuron, two plant metabolism studies in wheat and maize are available; both studies cover the crop category cereals. The study design of both studies was slightly different. In the wheat metabolism study, the exact label rate was applied whereas the maize study was conducted at an exaggerated rate. In case of the wheat study, an adjuvant (Dash HC) was added to the test formulation. For investigating the nature of the residue in succeeding crops, a confined rotational crop study (crops: wheat, carrot, lettuce, beans) was performed at a rate of 60 g as/ha. A detailed summary of maize metabolism and the confined rotational crop study can be found in the draft assessment report Vol. 3, chapter B.7 "Residue data".
The degradation pathway in all crops investigated was qualitatively identical. The observed metabolic transformations in plants are summarised in Figure B.7.1.7-1.
Tritosulfuron is intensively metabolised by mainly three key steps:

- Hydroxylation of the phenyl ring system
- Cleavage of the triazine ring system
- Cleavage of the sulfonyl urea bond

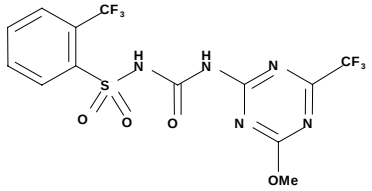
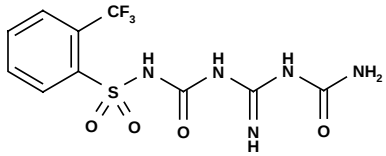
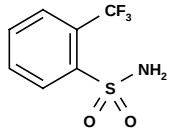
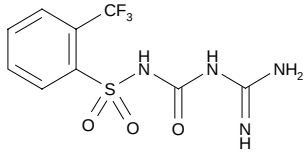
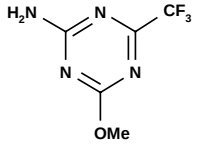
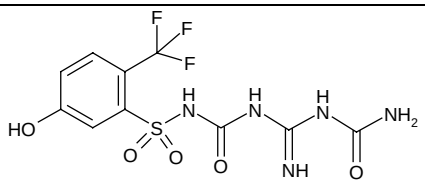
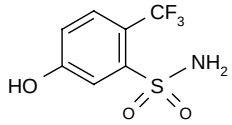
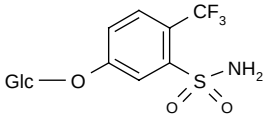
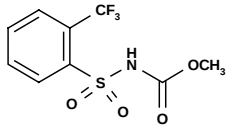
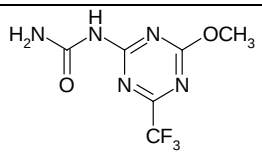
The contribution of these reactions followed by subsequent conjugation leads to a large number of metabolites.

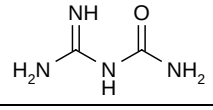
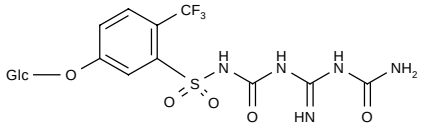
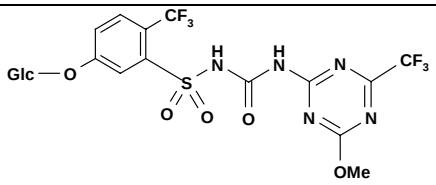
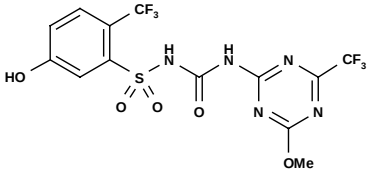
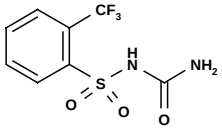
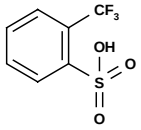
Figure B.7.1.7-1: Targets of metabolic transformation of BAS 635 H in plants



A summary of the metabolites identified is given in Table B.7.1.7-1:

Table B.7.1.7-1: Overview: Summary of identified tritosulfuron metabolites in cell culture experiments, plant metabolism and confined rotational crop studies

Metabolite designation		Structure	Cell culture	Maize	Wheat	Confined rot. crop
Code	Reg. No. (BH code)					
635M00	271 272 (BAS 635H)			X	X	X
635M01	335 184 (BH 635-4)		X	X	X	X
635M02	292 564 (BH 635-2)		X		X	X
635M03	335 182 (BH 635-3)				X	X
635M04	231 700 (BH 635-5)		X		X	X
n.a.	347 664 (BH 635-6)		X			
635M06	347 665 (BH 635-7)		X	X	X	X
635M07	n.a.		(X)	X		
635M08	311313 (BH 635-9)		X		X	
635M09	347 667 (BH 635-10)		X			

Metabolite designation		Structure	Cell culture	Maize	Wheat	Confined rot. crop
Code	Reg. No. (BH code)					
635M10	224 143 (BH 635-11)		X			X
635M12	n.a.		X		(X)	X
635M13	n.a.		X	X	X	X
635M17	373 906 (BH 635-16)			X	X	X
635M18	n.a.				(X)	X
635M23	324 543 (BH 635-1 as Na-Salt)				X	X

All key transformation steps specified above are also known from other herbicides belonging to the same class of compounds. A common pathway for metabolic deactivation for most triazinyl sulfonylureas in cereal crops is hydroxylation of the phenyl ring in position 5.

B.7.3 Definition of the residue

B.7.3.1 Plants

The following conclusions can be drawn from all previous ^{14}C-studies in plants including the new wheat metabolism study:

- Unchanged tritosulfuron was detected in almost all matrices of the plant metabolism studies as major and predominant component. It was also found in a majority of the matrices investigated during the rotational crop study (exception: wheat). In most of the samples, tritosulfuron is present in amounts significantly greater than 10 % TRR and 0.05 mg/kg. Compared with parent all other metabolites are present in considerably lower amounts.
- As major degradation pathway in maize, wheat, carrot and lettuce (both succeeding crops), hydroxylation of the phenyl ring system followed by conjugation (635M13) or cleavage (635M06) was found.
- The metabolite 635M01, which is formed by cleavage of the triazine ring system was detected in all studies.
- The cleavage reaction of the sulfonyl urea bridge does not play an important role in plants. Metabolites 635M02 (TBSA) and 635M04 (AMTT) were only detected in minor amounts in wheat. Both metabolites were not found in maize. In the edible portions of the confined rotational crop study, they also occurred in trace amounts (about 0.001 mg/kg). Significant hydrolytical cleavage of tritosulfuron was only observed in the hydrolysis study performed at exaggerated temperatures. Tritosulfuron was almost quantitatively cleaved to TBSA and AMTT.

Due to all findings of the hydroysis investigations, plant metabolism studies, supervised residue trials, rotational crop studies, and toxicological significance the following definitions of the relevant residues in plant matrices are proposed:

Risk assessment: Tritosulfuron and AMTT, expressed as tritosulfuron,
Monitoring purpose: Tritosulfuron.

B.7.17 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
AIIA-6.1	Bross, M. and Mackenroth, C.	2004	Metabolism of ^{14}C-tritosulfuron (^{14}C-BAS 635 H) in Wheat. REPORT NO. BASF DocID 2004/1010546 GLP, unpublished RIP2004-1274	Y	BAS
AIIA-6.1	Bross, M.	2004	Tritosulfuron (BAS 635 H): Summary of the plant metabolism studies including a proposal for the definition of the relevant residue REPORT NO. BASF DocID 2004/1015920 unpublished RIP2004-1275	Y	BAS
AIIIA-7.2	Stadler, R.	2004	Hydrolysis Stability of BAS 635 00 H in Spray Tank under Field Conditions - Determination of AMTT (635M04, BH 635-5, Reg. No. 231 700) REPORT NO. BASF DocID 2004/1016478 GLP, unpublished PHY2004-636 TOX2004-1952	N	BAS

B.8 Environmental fate and behaviour

B.8.1 Route and rate of degradation in soil (Annex IIA 7.1.1; Annex IIIA 9.1.1)

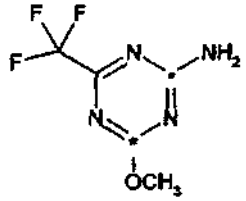
B.8.1.2 Rate of degradation

B.8.1.2.1 Laboratory studies

Annex Point:	IIA-7.1.1.1.1
Author:	Hein, Werner
Title:	Determination of the degradation rate of [triazine-2,4-^{14}C] AMTT in three different soils under aerobic conditions
Date:	2003-01-28
Doc ID:	BASF Doc ID 2003/1001038 BOD2004-16
Guidelines:	SETAC: "Procedures for Assessing the Fate and Ecotoxicity of Pesticides"
GLP:	Yes

Material and methods:

Test item
Test item was [triazin-2,4-^{14}C]AMTT (synonyms: Reg. No. 231700 = BH 635-5 = 635M04 = AMTT), batch 687-1008, specific radioactivity 4.68 MBq/mg. It was used for the application of the test vessels.



(* denotes position of radiolabel)

Test system
300 mL-Erlenmeyer flasks with ground joint and trap attachment for the adsorption of volatile compounds (i.e. $^{14}CO_2$) served as test system. The test system was chosen in order to meet the requirements of the mentioned test guideline.

Soils
Three different agriculturally used soils were tested in this study. Their characteristics are given in Table B.8.1.2-1.

Table B.8.1.2-1: Characteristics of the soils used in the degradation study with AMTT

Number	I	II	III
Name	Birkenheide	Mussbach	Lufa 6S
Soil type[1)]	weakly loamy sand	silty clayey sand	clayey loam
Texture (%)[1)]			
< 0.002 mm	8.0	14.9	41.0
0.002-0.006 mm	4.7	6.3	9.7
0.006-0.02 mm	6.9	13.1	14.4
0.02-0.063 mm	9.3	21.9	16.1
0.063-0.2 mm	30.2	17.3	9.1
0.2-0.63 mm	37.9	25.6	7.8
0.63-2.0 mm	3.0	0.9	2.2
Soil type[2)]	Sandy loam	Loam	Clay
Texture (%)[2)]			
< 0.002 mm	8.0	14.9	41.0
0.002-0.05 mm	18.0	39.6	36.3
0.05-2.0 mm	74.0	45.5	22.8
pH (0.01 M $CaCl_2$)	5.8	7.4	6.8
Organic carbon (OC) [%]	0.55	0.57	2.0
Organic matter [%] = OC × 1.72	0.95	0.98	3.44
Cation exchange capacity [mval/100 g]	6.1	10.9	18
WHC_{max} [g H_2O/100 g dry soil]	21.68	26.15	32.11
Bulk density [g/1000 mL]	1629	1739	1233
microbial biomass [mg C_{mikr}/100 g dry soil]	12.17	12.90	19.53
Optimum amount of glucose [ppm]	1000	6000	6000

1) According to DIN 19682
2) According to USDA

Test system preparation

The soils were freshly sampled at the field site, air-dried and screened trough a 2 mm-sieve. Determination of soil moisture was carried out by differential weighing after drying of 10 g aliquots of each soil in a microwave oven. Thereafter, 100 g of dry soil equivalents were weighed into each 300 mL-Erlenmeyer flask. The soil moisture in each test vessel was adjusted to about 40 % of the maximum water holding capacity of the soil. Afterwards, the vessels were closed with a cotton wool stopper and pre-incubated at 20 ± 2 °C in the dark for 14 days.

Application and incubation of test system

The initial concentration of AMTT was chosen as 5 µg/100 g dry soil, as to represent the actual use rate of BAS 635 H (50 g/ha, being roughly equivalent to 50 µg/kg dry soil) and 100 % degradation of the parent compound to AMTT. No molecular weight correction factor was used.

AMTT was added to each test vessel individually by pipetting the appropriate amount of an aqueous application solution in small drops onto the soil surface. The actual weight of each test vessel was recorded. Afterwards the test vessels were closed with a trap attachment (containing soda lime and oil wetted quartzwool) which allowed for some gas exchange but

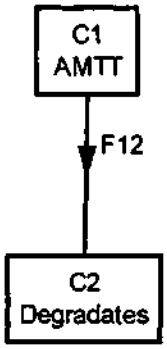
which adsorbed all volatile metabolites (including CO_2). Samples were incubated for 0 (1 hour), 1, 3, 7, 14, 30, 62, 90 and 120 days at a temperature of 20 °C and a soil moisture of about 40 % of the respective soils' maximum water holding capacity.

Sampling and analysis

At sampling, the soils were extracted sequentially with acetonitrile, methanol and methanol/0.01 M $CaCl_2$ solution (1:1; v/v). Radioactivity in the extracts was determined by liquid scintillation counting. Identification of individual compounds in the soil extracts was done by HPLC with radiochemical detection. Non-extractable radioactivity in the soils was quantified by combustion and measuring of released $^{14}CO_2$. Soda lime from the trap attachments was dissolved in hydrochloric acid; the evolving $^{14}CO_2$ trapped in liquid sorbent with scintillator and measured in a liquid scintillation counter. The recovery from the individual test vessels ranged from 90.6 % to 98.9 % of the applied radioactivity.

Calculations

The results of the degradation study with AMTT were evaluated using ModelMaker™ version 4.0 (Cherwell Scientific Publishing, The Magdalen Centre, Oxford OX4 4 GA). For the determination of the DT_{50} and DT_{90} values, a model was set up in order to describe the dissipation of AMTT from the whole system by 1st order kinetics. A graphic description of the model is given below.



Findings:

Distribution of radioactivity

After 120 days between 38.7 % (Birkenheide), 58.2 % (Mussbach) and 64.1 % (Lufa 6S) of the applied radioactivity was still extractable. Relevant portions of $^{14}CO_2$ were determined in all soils. In soil Birkenheide nearly half of the applied radioactivity was released as $^{14}CO_2$ with 46.4 % after 120 days, while amounts of 17.2 % were found in soil Mussbach and of 16.1 % in soil Lufa 6S, respectively. Amounts of $^{14}CO_2$ were still increasing at the end of the incubation period. Formation of non-extractable radioactivity (NER) was negatively correlated with extractability and the formation of $^{14}CO_2$. Therefore, the biggest portion of NER was found in soil Lufa 6S with 18.7 % of the applied radioactivity, followed by soil Mussbach with 15.2 % and soil Birkenheide with 9.7 %, respectively (Table B.8.1.2-2 to Table B.8.1.2-4.

Table B.8.1.2-2: Distribution of radioactivity in soil "Birkenheide"

DAT	Extractable % TAR	NER % TAR	$^{14}CO_2$ % TAR	Recovery % TAR
0[1)]	94.41	1.00	0.00	95.41
1[1)]	93.52	1.82	1.04	96.37
3	90.91	2.93	2.00	95.84
7	84.74	6.09	4.74	95.57
14	78.37	8.11	9.82	96.30
30	65.31	9.23	20.24	94.78
60	51.09	12.21	29.86	93.16
90	45.03	10.20	38.61	93.84
120	38.69	9.67	46.42	94.78

1) mean out of two determinations

Table B.8.1.2-3: Distribution of radioactivity in soil "Mussbach"

DAT	Extractable % TAR	NER % TAR	$^{14}CO_2$ % TAR	Recovery % TAR
0[1)]	93.87	1.42	0,00	95.29
1[1)]	93.24	2.33	0.62	96.18
3	90.84	4.45	0.98	96.27
7	88.34	6.93	1.11	96.38
14	84.77	9.25	1.97	95.99
30	79.32	12.87	3.47	95.66
60	73.41	13.35	8.78	95.54
90	66.46	14.64	11.94	93.04
120	58.20	15.21	17.22	90.63

1) mean out of two determinations

Table B.8.1.2-4: Distribution of radioactivity in soil "Lufa 6S"

DAT	Extractable % TAR	NER % TAR	$^{14}CO_2$ % TAR	Recovery % TAR
0[1)]	91.56	1.80	0.00	93.36
1[1)]	90.10	2.64	0.43	93.17
3	88.92	4.31	0.81	94.04
7	86.60	6.09	1.21	93.90
14	84.15	8.81	1.87	94.83
30	80.60	10.22	3.44	94.26
60	73.04	13.91	7.44	94.39
90	66.55	17.03	7.68	91.26
120	64.09	18.71	16.05	98.85

1) mean out of two determinations

Degradation of AMTT

The test item AMTT was degraded under the actual incubation conditions. At the end of the incubation period, 10.1 %, 20.5 % and 41.4 % of the applied radioactivity were assigned to unchanged AMTT for the soils Birkenheide, Mussbach and Lufa 6S, respectively. Different non-identified metabolite zones were determined, amounting to 28.6 % of the applied radioactivity after 120 days incubation in soil Birkenheide, 37.7 % in soil Mussbach and 22.7 % in soil Lufa 6S, respectively. For all three soils a decrease of the portion of non-identified radioactivity was observed between 90 and 120 days (Table B.8.1.2-5).

Table B.8.1.2-5: Amounts of AMTT and non-identified radioactivity in the soil extracts from the different test soils

DAT	Birkenheide % TAR		Mussbach % TAR		Lufa 6S % TAR	
	AMTT	n.i.	AMTT	n.i.	AMTT	n.i.
0[1)]	91.65	2.76	90.38	3.50	88.13	3.43
1[1)]	83.67	9.85	84.43	8.81	85.57	4.54
3	80.38	10.53	82.04	8.80	83.82	5.10
7	66.06	18.68	73.54	14.80	79.08	7.52
14	63.03	15.34	66.90	17.87	73.65	10.50
30	30.97	34.34	51.12	28.20	63.23	17.37
60	22.69	28.40	33.90	39.51	49.92	23.12
90	12.51	32.52	26.35	40.11	40.32	26.23
120	10.10	28.59	20.53	37.67	41.44	22.65

1) mean out of two determinations

Valid: Yes

Conclusions:

The kinetic calculations with ModelMaker 4.0 revealed the following DT_{50} and DT_{90}-values for AMTT (Table B.8.1.2-6).

Table B.8.1.2-6: DT_{50} and DT_{90}-values for aerobic degradation of AMTT in soil

Soil no.	Name	DT_{50} [d]	DT_{90} [d]
I	Birkenheide	35.3	117.1
II	Mussbach	53.9	178.9
III	Lufa 6S	94.5	313.8

B.8.2

Adsorption, desorption and mobility in soil (Annex IIA 7.1.2 and 7.1.3; Annex IIIA 9.1.2)

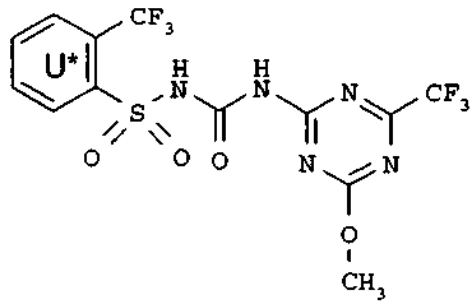
Sorption behaviour of tritosulfuron

Annex Point:	IIA-7.1.2
Author:	Richter, Thomas
Title:	Study for the adsorption/desorption determination of BAS 635 H on 5 European soils
Date:	2003-08-14
Doc ID:	BASF Doc ID 2003/1005441 BOD2004-14
Guidelines:	OECD Guideline for Testing of Chemicals No. 106 US EPA Guideline - No. 540/9-82-021, Subdivision N, § 163-1
GLP:	Yes

Material and methods:

Test item

The study was conducted with [Phenyl-U-^{14}C]tritosulfuron (BAS 635 H), batch 538-0404, specific radioactivity 5.61 MBq/mg.



(U* denotes position of radiolabel)

Soils

Five European soils differing in organic carbon contents, pH-value, particle size distribution, and cation exchange capacity were used in the experiments. The soils were classified into five different soil classes (according to German classification scheme): Sl4 (strongly loamy sand), Ls2 (slightly sandy Loam), Ut2 (slightly clayey silt), Ut3 (medium clayey silt) and Ut4 (strongly clayey silt). Further soil parameters (non-GLP data) are given in Table B.8.2-10.

Table B.8.2-10: Characteristics of five European soils used in the adsorption/desorption study with BAS 635 H

Soil		Organic carbon [%]	pH $CaCl_2$	Particle size distribution [%][2)]			Cation exchange capacity [meq/100g]	Water content[3)] [%]
Origin	Type[1)]			< 2 µm (clay)	2-63 µm (silt)	63-2000 µm (sand)		
Las Cabezas	Ut4	0.8	7.6	22.4	76.5	1.1	30.5	6.1
Manzanilla	SI4	0.7	7.6	14.3	33.5	52.2	10.6	1.2
Almonacid de la Sierra	Ls2	1.5	7.7	17.4	49.0	33.6	15.0	1.6
Nierstein	Ut3	2.0	7.1	13.2	64.4	22.4	11.3	2.0
Ploudalmezeau	Ut2	1.5	6.4	11.7	74.8	13.5	12.0	1.6

1) classification according to German scheme
2) determined according to German scheme
3) values were measured in this study

Experimental procedures

The soil samples used were sieved to a particle size < 2 mm. The soils were air-dried at ambient temperature. The actual water content of the soils was determined before starting the sorption experiments by drying at 160 °C by an infra-red drying device until constant weight. The residual water content of the soils ranged between 1.15 % and 6.05 % (see Table B.8.2-10) and was taken into account for all calculations. Application solutions of BAS 635 H were prepared by diluting a stock solution in methanol/0.01 M $CaCl_2$ solution (60:40, v/v) with 0.01 M $CaCl_2$ solution to the desired concentrations.

A soil/solution ratio of 1:1 was used for all tests except for the soil "La Cabezas", which was tested with a ratio of 1:2, due to the soil texture (highest silt and clay content). Adsorption time was 24 h for all experiments.

The adsorption isotherm determination was performed for the following five concentration levels (actual concentrations): 2.305, 0.436, 0.114, 0.023 and 0.004 µg/mL, with each of the five soils. Each experiment (one soil and one solution) was done in duplicate. 5 g soil and 5 mL of application solution were filled in centrifuge glass tubes, the tubes closed with screw caps and then gently shaken on a horizontal shaker for 24 hours. As with Las Cabezas soil, 5 g soil and 10 mL of application solution were used, because the samples of this soil with soil/solution ratio 1:1 could not be properly handled. The soil/water suspension was then centrifuged and the supernatant removed for analysis by liquid scintillation counting. The respective volumes of liquid phase and water retained in the soil after decantation were determined by weighing the supernatant.

Desorption isotherms were determined with the same soil samples used in the adsorption isotherm determination after removal of the supernatant. The removed supernatant was replaced by an equal volume of 0.01 M $CaCl_2$ solution and the experiment performed as described above. This was repeated once, to achieve a two-step desorption with steps I and II.

Calculations

The equilibrium concentrations (C_{AW}) and the total amounts (G_W) of BAS 635 H in the aqueous phase were calculated based on the results of the radio-assays of the supernatants, as described above. The amount of BAS 635 H adsorbed onto soil particles (G_{Soil}) and its concentration in soil (C_{ASoil}) were calculated using the difference between the initial and final amount of the compound in the aqueous phase and the soil dry weight. The distribution coefficient K_d was calculated as quotient C_{AW}/C_{ASoil}. For normalisation, K_d was divided by the organic carbon content of the respective soil to yield the K_{OC}.

The Freundlich adsorption isotherm equation relates the concentration of the test item adsorbed on soil to the concentration of the test item in solution at equilibrium via the Freundlich adsorption constant, K_F:

$$C_{ASoil} = K_F \times C_{AW}^{1/n} \quad \Leftrightarrow \quad \log C_{ASoil} = \log K_F + 1/n \log C_{AW}$$

K_F values were also divided by the organic carbon content of the respective soils to obtain normalised K_{FOC} values.
The desorption constants K_{Fdes} and K_{FOCdes} were calculated similar to the adsorption constants K_F and K_{FOC} using the measured concentrations from the desorption experiments.

Findings:

The results of the tests obtained in this study with ^{14}C-BAS 635 H are provided in Table B.8.2-11 to Table B.8.2-13.

Table B.8.2-11: Adsorption isotherm determination of BAS 635 H

Soil Origin Soil Type	Concentration of BAS 635 H			Adsorption				
	C_0 [µg/mL]	C_{Asoil} [µg/g]	C_{AW} [µg/mL]	K_d [mL/g]	K_{OC} [mL/g]	K_F [mL/g]	1/n	K_{FOC} [mL/g]
Las Cabezas Ut4	2.305	0.184 0.000	2.157 2.341	0.085 0.000	10.685 0.000	0.057	1.272	7.144
	0.436	0.000 0.000	0.448 0.453	0.000 0.000	0.000 0.000			
	0.114	0.002 0.000	0.110 0.112	0.018 0.000	2.249 0.000			
	0.023	0.000 0.001	0.028 0.022	0.000 0.029	0.000 3.648			
	0.004	0.000 0.000	0.004 0.004	0.000 0.000	0.000 0.000			
Manzanilla Sl4	2.305	0.034 0.046	2.241 2.243	0.015 0.021	2.197 2.932	0.019	0.851	2.660
	0.436	0.000 0.000	0.430 0.437	0.000 0.000	0.000 0.000			
	0.114	0.003 0.004	0.109 0.109	0.025 0.036	3.631 5.084			
	0.023	0.000 0.001	0.023 0.023	0.016 0.023	2.287 3.288			
	0.004	0.000 0.000	0.004 0.004	0.000 0.081	0.000 11.502			
Almonacid de la Sierra Ls2	2.305	0.152 0.139	2.110 2.131	0.072 0.065	4.817 4.342	0.055	1.154	3.684
	0.436	0.005 0.016	0.417 0.414	0.011 0.039	0.743 2.574			
	0.114	0.008 0.006	0.104 0.105	0.079 0.058	5.260 3.867			
	0.023	0.002 0.002	0.021 0.022	0.076 0.072	5.055 4.817			
	0.004	0.000 0.000	0.004 0.004	0.020 0.007	1.366 0.455			

Soil Origin Soil Type	Concentration of BAS 635 H			Adsorption				
	C_0 [µg/mL]	C_{Asoil} [µg/g]	C_{AW} [µg/mL]	K_d [mL/g]	K_{OC} [mL/g]	K_F [mL/g]	1/n	K_{FOC} [mL/g]
Nierstein Ut3	2.305	0.170 0.100	2.108 2.147	0.081 0.047	4.043 2.338	0.045	0.961	2.245
	0.436	0.017 0.002	0.411 0.419	0.043 0.005	2.126 0.240			
	0.114	0.012 0.009	0.101 0.104	0.120 0.084	5.988 4.176			
	0.023	0.002 0.002	0.021 0.021	0.101 0.091	5.036 4.561			
	0.004	0.000 0.000	0.004 0.004	0.021 0.000	1.058 0.000			
Ploudalmezeau Ut2	2.305	0.189 0.163	2.094 2.128	0.090 0.077	6.0235.117	0.089	1.100	5.952
	0.436	0.028 0.017	0.404 0.408	0.069 0.042	4.628 2.806			
	0.114	0.013 0.012	0.098 0.101	0.132 0.118	8.804 7.837			
	0.023	0.002 0.002	0.021 0.021	0.112 0.106	7.481 7.051			
	0.004	0.000 0.000	0.004 0.004	0.000 0.015	0.000 1.031			

"0.000" no values were obtained
C_0 Initial concentration of test item in aqueous solution
C_{ASoil} Concentration of test item on soil after adsorption
C_{AW} Concentration of test item in aqueous solution after adsorption
K_d Distribution coefficient
K_{OC} adsorption coefficient normalised by organic carbon content (calculated from $K_{d)}$
K_F Freundlich adsorption coefficient
K_{FOC} adsorption coefficient normalised by organic carbon content (calculated from $K_{F)}$
1/n Freundlich exponent

Table B.8.2-12: Desorption isotherm determination of BAS 635 H – step I

Soil Origin Soil Type	Concentration of BAS 635 H			Desorption			
	C_0 [µg/mL]	CRD_{Soil} [µg/g]	C_{desW} [µg/mL]	K_{ddes} [mL/g]	K_{Fdes} [mL/g]	$1/n_{des}$	K_{FOCdes} [mL/g]
Las Cabezas Ut4	2.305	0.119 0.148	0.744 0863	0.160 0.172	0.167	1.014	20.88
	0.436	0.023 0.033	0.166 0.160	0.141 0.204			
	0.114	0.008 0.009	0.042 0.043	0.198 0.199			
	0.023	0.004 0.000	0.010 0008	0.403 0019			
	0.004	0000 0.000	0002 0.001	-0.055 0.287			

Soil Origin Soil Type	Concentration of BAS 635 H			Desorption			
	C_0 [µg/mL]	CRD_{Soil} [µg/g]	C_{desW} [µg/mL]	K_{ddes} [mL/g]	K_{Fdes} [mL/g]	$1/n_{des}$	K_{FOCdes} [mL/g]
Manzanilla Sl4	2.305	0506 0.502	0531 0543	0952 0.925	0.911	1.011	130.20
	0.436	0.088 0.081	0110 0.117	0.800 0699			
	0114	0.026 0.026	0027 0.026	0.965 1.022			
	0023	0.005 0.005	O.OO6 0005	0908 1.049			
	0.004	0.001 0.001	0.001 0.001	0.764 0.757			
Almonacid de la Sierra Ls2	2.305	0.541 0531	0.454 0.457	1.191 1.162	0.554	0.913	36.93
	0.436	0.013 0.078	0.168 0.105	0.077 0.745			
	0.114	0.027 0.022	0023 0.027	1.159 0.806			
	0.023	0,005 0006	0.005 0004	0.942 1274			
	0.004	0.001 0.001	0.001 0001	1.294 0.646			
Nierstein Ut3	2.305	0.584 0557	0.493 0.507	1.185 1.099	1266	1.079	63.30
	0.436	0.084 0.089	0.095 0.100	0.882 0.889			
	0.114	0032 0.030	0.024 0023	1367 1.311			
	0.023	0.006 0.004	0005 0.007	1095 0.670			
	0.004	0001 0.001	0001 0.001	0.597 0683			
Ploudalmezeau Ut2	2.305	0.617 0.590	0.502 0523	1.227 1.129	1.308	1 056	8720
	0436	0.108 0.104	0104 0.100	1.037 1.034			
	0.114	0034 0.032	0.026 0.026	1.334 1.231			
	0.023	0006 0006	0005 0.005	1.172 1.226			
	0.004	0001 0001	0001 0.001	0.774 0674			

"0.000" no values were obtained
C_0 Initial concentration of test item in aqueous solution
CRD_{soil} Concentration of test item on soil after desorption
C_{desW} Concentration of test item in aqueous solution after desorption
K_{ddes} Distribution coefficient of desorption
K_{Fdes} Freundlich desorption coefficient
K_{FOCdes} adsorption coefficient normalised by organic carbon content (calculated from $K_{Fdes)}$
$1/n_{des}$ Freundlich exponent of desorption

Table B.8.2-13: Desorption isotherm determination of BAS 635 H – step II

Soil Origin Soil Type	Concentration of BAS 635 H			Desorption			
	C_0 [µg/mL]	CRD_{Soil} [µg/g]	C_{desW} [µg/mL]	K_{ddes} [mL/g]	K_{Fdes} [mL/g]	$1/n_{des}$	K_{FOCdes} [mL/g]
Las Cabezas Ut4	2.305	0.065 0.174	0277 0.327	0.235 0.532	0.579	1.162	7242
	0.436	0.028 0.032	0.065 0063	0.426 0502			
	0.114	0.008 0.009	0.017 0.017	0467 0525			
	0.023	0.004 0.000	0.004 0.003	0936 0.014			
	0.004	0.000 0.000	0.001 0.001	-0.102 0.306			
Manzanilla Sl4	2.305	0.551 0.543	0.179 0.188	3.071 2.893	2.978	1.035	425.42
	0.436	0093 0094	0.040 0038	2313 2.462			
	0114	0024 0028	0013 0.009	1.911 3.046			
	0023	0006 0006	0.002 0002	3287 2834			
	0.004	0001 0001	0.000 0000	1.720 2.284			
Almonacid de la Sierra Ls2	2.305	0.110 0.101	0577 0589	0.191 0.171	0.061	0.754	4.09
	0.436	0.002 0.002	0.076 0.111	0.026 0022			
	0.114	0.006 0.005	0.028 0.026	0.219 0.201			
	0.023	0.001 0.001	0.006 0.006	0.195 0217			
	0.004	0000 0000	0.001 0.001	0678 -0.152			
Nierstein Ut3	2.305	0620 0.589	0.164 0.183	3.787 3.217	4314	1072	215.72
	0.436	0089 0.100	0.030 0.031	2924 3181			
	0.114	0.033 0032	0.009 0007	3.543 4.298			
	0.023	0006 0.005	0002 0.002	3.186 3.066			
	0.004	0.001 0.001	0.000 0.000	2135 1.901			
Ploudalmezeau Ut2	2.305	0.261 0.560	0.565 0.260	0.462 2.155	1.324	0.936	8827
	0436	0.104 0.098	0.051 0.051	2047 1928			
	0.114	0.034 0031	0.013 0.012	2597 2481			
	0.023	0006 0.006	0003 0003	2508 2476			
	0.004	0001 0.001	0.001 0.001	1.426 1.184			

"0.000" means, no values were obtained
C_0 Initial concentration of test item in aqueous solution
CRD_{soil} Concentration of test item on soil after desorption
C_{desW} Concentration of test item in aqueous solution after desorption

K_{ddes}	Distribution coefficient of desorption
K_{Fdes}	Freundlich desorption coefficient
K_{FOCdes}	adsorption coefficient normalised by organic carbon content (calculated from $K_{Fdes)}$
$1/n_{des}$	Freundlich exponent of desorption

The Freundlich adsorption coefficients K_F of all five soils covered a range from 0.019 to 0.089 mL/g. The Freundlich adsorption exponents 1/n ranged from 1.272 to 0.851. The corresponding K_{FOC} values for all five soils were between 7.1 and 2.3 mL/g. The recovery of ^{14}C-BAS 635 H in control samples without soil was 95.6 to 109.0 %, showing that no adsorption of BAS 635 H had occurred at the glass wall of the tubes.
Desorption isotherms were established in the similar manner as the Freundlich adsorption isotherms. The values of the constants K_{Fdes} of the both desorption trials (desorption I and II) ranged from 0.057 to 4.201 mL/g for all five soils. Desorption exponents 1/n covered values from 0.677 to 1.062. The corresponding K_{FOCdes} values were between 3.8 and 413 mL/g.

Valid: Yes

Conclusions:

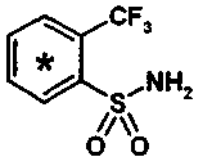
The adsorption and desorption behaviour of ^{14}C-BAS 635 H was determined in five European soils. The soils covered a range of pH from 6.4 to 7.7 and a range of organic carbon content from 0.7 % to 2.0 %. The soils were characterised into five different soil types according to German classification scheme: Sl4 (strongly loamy sand), Ls2 (slightly sandy loam), Ut2 (slightly clayey silt), Ut3 (medium clayey silt) and Ut4 (strongly clayey silt). Although the adsorption to soil is, depending on the soil and the concentration, partly very low, a dependence of the adsorption behaviour on soil pH could not be observed.

Sorption behaviour of metabolite 635M02

Annex Point:	IIA-7.1.2
Author:	Zirnstein, M.
Title:	Adsorption/desorption-study of BAS 635 H metabolite BH 635-2 (reg. no. 292564) on five European soils
Date:	2003-07-28
Doc ID:	BASF Doc ID 2003/1005442 BOD2004-57
Guidelines:	OECD Guideline for Testing of Chemicals No. 106 US EPA Guideline - No. 540/9-82-021, Subdivision N, § 163-1
GLP:	Yes

Material and methods:

Test item
The study was conducted with [Phenyl-U-^{14}C]-2-trifluormethyl-benzene-sulfonamide (synonyms: Reg. No. 292564 = BH 635-2 = 635M02 = TBSA), batch 603-1005, specific radioactivity 3.44 MBq/mg.



(* denotes position of radiolabel)

Soils

Five European soils differing in organic carbon contents, pH-value, particle size distribution, and cation exchange capacity were used in the experiments. These were the same soils as used in the study of Richter, 2003. Soil parameters (non-GLP data) are given above in Table B.8.2-10.

Experimental procedures

The experimental procedure was identical to that described in the study of Richter, 2003 (see above). The adsorption isotherm determination for BH 635-2 was performed for the following five concentration levels (actual concentrations): 5.09, 1.02, 0.509, 0.102 and 0.0203 µg/mL. Like in the study with BAS 635 H, a soil/solution ratio of 1:1 was used for all tests except for the soil "La Cabezas", which was tested with a ratio of 1:2, due to the soil texture (highest silt and clay content). Adsorption time was 24 h for all experiments.

Calculations

The equilibrium concentrations and the total amounts of BH 635-2 in aqueous and solid phase were calculated as described for BAS 635 H in the study of Richter, 2003 (see above). The equations for calculating the distribution coefficients K_d and K_{OC} as well as the Freundlich sorption parameters K_F, K_{FOC} and 1/n are also given above.

Findings:

The results of the tests obtained in this study with ^{14}C-BH 635-2 are provided in Table B.8.2-14 to Table B.8.2-16.

Table B.8.2-14: Adsorption isotherm determination of ^{14}C-BH 635-2

Soil Origin Soil Type	Concentration of BAS 635 H			Adsorption				
	C_0 [µg/mL]	C_{Asoil} [µg/g]	C_{AW} [µg/mL]	K_d [mL/g]	K_{OC} [mL/g]	K_F [mL/g]	1/n	K_{FOC} [mL/g]
Las Cabezas Ut4	5.0900	1.1199 1.0869	3.8047 3.8195	0.294 0.285	36.8 35.6	0.358	0.876	44.7
	1.0200	0.2756 0.2736	0.7145 0.7156	0.386 0.382	48.2 47.8			
	0.5090	0.1500 0.1456	0.3501 0.3514	0.429 0.414	53.6 51.8			
	0.1020	0.0339 0.0335	0.0662 0.0665	0.513 0.504	64.1 63.1			
	0.0203	0.0075 0.0074	0.0125 0.0126	0.599 0.588	74.9 73.5			
Manzanilla Sl4	5.0900	0.9873 1.0026	4.0421 4.0431	0.244 0.248	34.9 35.4	0.297	0.883	42.5
	1.0200	0.2451 -	0.7686 -	0.319 -	45.6 -			
	0.5090	0.1255 0.1285	0.3760 0.3791	0.334 0.339	47.7 48.4			
	0.1020	0.0301 -	0.0708 -	0.426 -	60.8 -			
	0.0203	0.0064 0.0067	0.0137 0.0136	0.468 0.492	66.9 70.2			

Soil Origin Soil Type	Concentration of BAS 635 H			Adsorption				
	C_0 [µg/mL]	C_{Asoil} [µg/g]	C_{AW} [µg/mL]	K_d [mL/g]	K_{OC} [mL/g]	K_F [mL/g]	1/n	K_{FOC} [mL/g]
Almonacid de la Sierra Ls2	5.0900	1.0560 -	3.9880 -	0.265 -	17.7 -	0.304	0.937	20.2
	1.0200	0.2442 -	0.7631 -	0.320 -	21.3 -			
	0.5090	0.1299 0.1241	0.3760 0.3832	0.345 0.324	23.0 21.6			
	0.1020	0.0255 -	0.0756 -	0.337 -	22.5 -			
	0.0203	0.0058 0.0057	0.0145 0.0144	0.400 0.396	26.6 26.4			
Nierstein Ut3	5.0900	1.2058 1.2931	3.7961 3.7423	0.318 0.346	15.9 17.3	0.380	0.924	19.0
	1.0200	0.2918 0.2930	0.7173 0.7176	0.407 0.408	20.3 20.4			
	0.5090	0.1503 0.1499	0.3541 0.3552	0.424 0.422	21.2 21.1			
	0.1020	0.0313 0.0314	0.0698 0.0698	0.448 0.450	22.4 22.5			
	0.0203	0.0070 0.0070	0.0132 0.0132	0.530 0.529	26.5 26.5			
Ploudalmezeau Ut2	5.0900	1.3641 1.3720	3.6706 3.6774	0.372 0.373	24.8 24.9	0.440	0.919	29.3
	1.0200	0.3323 0.3179	0.6827 0.6939	0.487 0.458	32.4 30.5			
	0.5090	0.1727 0.1712	0.3340 0.3361	0.517 0.509	34.5 33.9			
	0.1020	0.0366 0.0342	0.0648 0.0672	0.565 0.508	37.7 33.9			
	0.0203	0.0077 0.0076	0.0126 0.0126	0.612 0.607	40.8 40.5			

"-" no results available due to broken vial
C_0 Initial concentration of test item in aqueous solution
C_{ASoil} Concentration of test item on soil after adsorption
C_{AW} Concentration of test item in aqueous solution after adsorption
K_d Distribution coefficient
K_{OC} adsorption coefficient normalised by organic carbon content (calculated from $K_{d)}$
K_F Freundlich adsorption coefficient
K_{FOC} adsorption coefficient normalised by organic carbon content (calculated from $K_{F)}$
1/n Freundlich exponent

Table B.8.2-15: Desorption isotherm determination of ^{14}C-BH 635-2 – step I

Soil Origin Soil Type	Concentration of BAS 635 H			Desorption			
	C_0 [µg/mL]	CRD_{Soil} [µg/g]	C_{desW} [µg/mL]	K_{ddes} [mL/g]	K_{Fdes} [mL/g]	$1/n_{des}$	K_{FOCdes} [mL/g]
Las Cabezas Ut4	5.0900	1.0514 1.0052	2.5093 2.5355	0.419 0.396	0.451	0.890	56.4
	1.0200	0.2404 0.2383	0.4929 0.4951	0.488 0.481			
	0.5090	0.1283 0.1238	0.2372 0.2380	0.541 0.520			
	0.1020	0.0297 0.0287	0.0462 0.0458	0.643 0.627			
	0.0203	0.0065 0.0064	0.0085 0.0086	0.769 0.747			
Manzanilla Sl4	5.0900	0.6433 0.6332	1.9664 1.9788	0.327 0.320	0.361	0.888	51.6
	1.0200	0.1626 -	0.3898 -	0.417 -			
	0.5090	0.0803 0.0850	0.1862 0.1889	0.431 0.450			
	0.1020	0.0212 -	0.0370 -	0.574 -			
	0.0203	0.0043 0.0046	0.0074 0.0075	0.583 0.617			
Almonacid de la Sierra Ls2	5.0900	0.6363 -	1.9761 -	0.322 -	0.351	0.952	23.4
	1.0200	0.1504 -	0.3948 -	0.381 -			
	0.5090	0.0787 0.0758	0.1941 0.1968	0.405 0.385			
	0.1020	0.0156 -	0.0403 -	0.387 -			
	0.0203	0.0035 0.0035	0.0080 0.0079	0.440 0.443			
Nierstein Ut3	5.0900	0.7905 0.8934	2.0919 2.0852	0.378 0.428	0.439	0.928	22.0
	1.0200	0.1993 0.1942	0.4038 0.4031	0.494 0.482			
	0.5090	0.0993 0.1020	0.1948 0.2047	0.510 0.498			
	0.1020	0.0216 0.0219	0.0403 0.0402	0.536 0.545			
	0.0203	0.0050 0.0048	0.0079 0.0080	0.631 0.604			
Ploudalmezeau Ut2	5.0900	0.8988 0.8876	2.1505 2.1906	0.418 0.405	0.462	0.925	30.8
	1.0200	0.2175 0.2120	0.4229 0.4298	0.514 0.493			
	0.5090	0.1185 0.1164	0.2084 0.2087	0.569 0.558			
	0.1020	0.0257 0.0228	0.0414 0.0419	0.620 0.545			
	0.0203	0.0053 0.0052	0.0081 0.0082	0.657 0.628			

"-" no results available due to broken vial
C_0 Initial concentration of test item in aqueous solution
CRD_{soil} Concentration of test item on soil after desorption
C_{desW} Concentration of test item in aqueous solution after desorption

K_{ddes} Distribution coefficient of desorption
K_{Fdes} Freundlich desorption coefficient
K_{FOCdes} adsorption coefficient normalised by organic carbon content (calculated from $K_{Fdes)}$
$1/n_{des}$ Freundlich exponent of desorption

Table B.8.2-16: Desorption isotherm determination of ^{14}C-BH 635-2 – step II

Soil Origin Soil Type	Concentration of BAS 635 H			Desorption			
	C_0 [µg/mL]	CRD_{Soil} [µg/g]	C_{desW} [µg/mL]	K_{ddes} [mL/g]	K_{Fdes} [mL/g]	$1/n_{des}$	K_{FOCdes} [mL/g]
Las Cabezas Ut4	5.0900	0.7701 0.7441	1.7144 1.7563	0.449 0.424	0.499	0.863	62.4
	1.0200	0.2031 0.2009	0.3271 0.3348	0.621 0.600			
	0.5090	0.1098 0.1028	0.1604 0.1592	0.685 0.646			
	0.1020	0.0264 0.0249	0.0321 0.0310	0.824 0.803			
	0.0203	0.0058 0.0057	0.0059 0.0060	0.989 0.949			
Manzanilla Sl4	5.0900	0.4391 0.4131	0.9652 0.9701	0.455 0.426	0.446	0.886	63.8
	1.0200	0.1110 -	0.2039 -	0.544 -			
	0.5090	0.0538 0.0570	0.0954 0.0952	0.564 0.599			
	0.1020	0.0151 -	0.0197 -	0.767 -			
	0.0203	0.0031 0.0035	0.0040 0.0041	0.776 0.841			
Almonacid de la Sierra Ls2	5.0900	0.3727 -	0.9388 -	0.397 -	0.420	0.954	28.0
	1.0200	0.0926 -	0.2012 -	0.460 -			
	0.5090	0.0495 0.0486	0.0995 0.1003	0.498 0.485			
	0.1020	0.0101 -	0.0209 -	0.483 -			
	0.0203	0.0023 0.0023	0.0043 0.0042	0.527 0.543			
Nierstein Ut3	5.0900	0.5301 0.6417	1.1685 1.1701	0.454 0.548	0.519	0.929	26.0
	1.0200	0.1427 0.1334	0.2329 0.2286	0.613 0.584			
	0.5090	0.0720 0.0720	0.1113 0.1192	0.647 0.604			
	0.1020	0.0150 0.0150	0.0236 0.0234	0.636 0.642			
	0.0203	0.0037 0.0035	0.0046 0.0047	0.804 0.739			

Soil Origin Soil Type	Concentration of BAS 635 H			Desorption			
	C_0 [µg/mL]	CRD_{Soil} [µg/g]	C_{desW} [µg/mL]	K_{ddes} [mL/g]	K_{Fdes} [mL/g]	$1/n_{des}$	K_{FOCdes} [mL/g]
Ploudalmezeau Ut2	5.0900	0.5989 0.5902	1.2341 1.2511	0.485 0.472	0.513	0.926	34.2
	1.0200	0.1476 0.1414	0.2461 0.2541	0.600 0.557			
	0.5090	0.0830 0.0800	0.1270 0.1257	0.654 0.636			
	0.1020	0.0184 0.0153	0.0255 0.0246	0.720 0.622			
	0.0203	0.0038 0.0036	0.0051 0.0050	0.755 0.715			

"-" no results available due to broken vial
C_0 Initial concentration of test item in aqueous solution
CRD_{soil} Concentration of test item on soil after desorption
C_{desW} Concentration of test item in aqueous solution after desorption
K_{ddes} Distribution coefficient of desorption
K_{Fdes} Freundlich desorption coefficient
K_{FOCdes} adsorption coefficient normalised by organic carbon content (calculated from $K_{Fdes)}$
$1/n_{des}$ Freundlich exponent of desorption

The Freundlich adsorption coefficients K_F of all five soils covered a range from 0.297 to 0.440 mL/g. The Freundlich adsorption exponents 1/n ranged from 0.876 to 0.937, indicating a slight non-linearity of the adsorption with the concentration. The corresponding K_{FOC} values for all five soils were between 19.0 to 44.7 mL/g. The recovery of ^{14}C-BH 635-2 in control samples without soil was 100.0 to 100.4 %, showing that no adsorption of BH 635-2 had occurred at the glass wall of the tubes.
Desorption isotherms were established in the similar manner as the Freundlich adsorption isotherms. The values of the constants K_{Fdes} of both desorption trials (desorption I and II) ranged from 0.351 to 0.519 mL/g for all five soils. Desorption exponents 1/n covered values from 0.863 to 0.954. The corresponding K_{FOCdes} values were between 22.0 and 63.8 mL/g.

Valid: Yes

Conclusions:

The adsorption and desorption behaviour of ^{14}C-BH 635-2 was determined in five European soils. The soils covered a range of pH from 6.4 to 7.7 and a range of organic carbon content from 0.7 % to 2.0 %. The soils were characterised into five different soils types according to German classification scheme: Sl4 (strongly loamy sand), Ls2 (slightly sandy loam), Ut2 (slightly clayey silt), Ut3 (medium clayey silt) and Ut4 (strongly clayey silt). Within the five soils studied, a dependence of the adsorption behaviour on soil pH could not be observed.

B.8.2.2 Kinetic evaluation of the adsorption behaviour and estimation of effective relevant sorption values of BAS 635 H and its metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5 in soils

Annex Point:	IIA-7.1.2
Author:	Jene, B.
Title:	Estimation of effective relevant sorption values of BAS 635 H in soils
Date:	2002-02-13
Doc ID:	2001/1015001 BOD2004-57
GLP:	No

Annex Point:	IIA-7.1.2
Author:	Jene, B.
Title:	Kinetic evaluation of the adsorption behaviour of the metabolites of BAS 635 H (tritosulfuron): BH 635-2, BH 635-3, BH 635-4 and BH 635-5
Date:	2003-03-27
Doc ID:	2003/1000991 BOD2003-171
GLP:	Yes

Sorption parameters for the simulation of the transport of pesticides through the unsaturated zone of soils are usually determined with the help of standard adsorption-desorption batch experiments. For regulatory purposes these sorption batch studies are carried out following the OECD Guideline 106. However, for regulatory modelling as recommended by FOCUS or other groups, only the Freundlich isotherm for *adsorption* is used, although the *desorption* behaviour of the substance may often be markedly different and the so-called sorption hysteresis can have an decisive effect on the transport of the substance. Hence it is often reported that a greater effective sorption in the field or lysimeters in comparison with the adsorption batch-equilibrium study adsorption values was found which can be explained by kinetic/aged sorption processes. In such cases, the leaching of the respective substances to deeper soil layers was overpredicted if not considering kinetic/aged sorption, but could be well predicted by including kinetic/aged sorption.
Apart from theoretical considerations, more information is often available from outdoor field or lysimeter studies where the leaching behaviour of the respective substance is investigated under natural conditions. With that kind of studies, all processes like sorption hysteresis, kinetic or aged sorption which are relevant for the transport of the compound through the soil are reflected on the appropriate scale for which risk assessment is made.

In the following, all available information with regard to the adsorption behaviour combined with state-of-the-art theory were used in order to derive sound parameters for the process-relevant effective sorption behaviour of the herbicidal substance BAS 635 H and its metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5 in soils.
Hence, in a first step, transport-relevant sorption coefficients (for BAS 635 H only) will be determined using the outflow concentration of two lysimeter studies including 5 individual lysimeters. In a second step the relevance of the mean sorption value found in the lysimeter experiments will be compared by evaluation of the available sorption batch studies, using an approach which considers sorption kinetics (aged-sorption effects) as an expression of sorption hysteresis. This is done for BAS 635 H as well as for its metabolites. Finally, the terrestrial field dissipation studies will be analysed with regard to the transport behaviour of

BAS 635 H. Simulations with environmental fate models were carried out in order to test the relevance of the derived sorption parameters.

B.8.2.2.1 Estimation of sorption parameters from lysimeter studies

Introduction

The leaching behaviour of substances is often investigated in detail with the help of large undisturbed outdoor lysimeters. The translocation of a substance is determined by convection, depending on the water transport in the soil regime, whereas retention or retardation of the compound in the soil as well as transformation depends on the substance properties with respect to sorption and degradation.
Simulation models are commonly used to predict the leaching behaviour of substances under given scenarios by inserting environmental fate parameters of substances which were measured in field studies. However, if it is possible to describe the water-balance dynamics of a lysimeter study very well, important information can be obtained with respect to the process-relevant efficient sorption and degradation behaviour of the compound. This is possible by fitting the calculated breakthrough concentrations to the measured outflow concentrations of the substance by inverse parameter estimation, until the deviations between observed and predicted values are minimised. The resulting sorption parameters can be considered as effective environmental parameters which represent the compound's behaviour during the transport in the soil. These parameters are clearly identified by the measured outflow concentrations of the substance from the lysimeter, since lower sorption or degradation parameters would have caused lower retardation and higher concentration of the compound in the leachate.

The following section describes the lysimeter experiments as well as the inverse parameter estimation with the help of the simulation model FOCUS-PEARL 1.1.1.

The lysimeter experiments

Two lysimeter studies with two and three individual lysimeters with the herbicidal substance BAS 635 H were carried out at the lysimeter station of the BASF Agricultural Research Center Limburgerhof. The studies had already been documented and assessed in the monograph. Important characteristics and the main results of the lysimeter studies are shown in Table B.8.2.2-1 whereas Table B.8.2.2-2 shows the most important properties of the lysimeter soils.

Table B.8.2.2-1: Important characteristics of the lysimeter studies with BAS 635 H

Type of study	Lysimeter study following BBA Guideline PART IV, 4-3	
Experimental period	April 1994 - May 1996	May 1996 - May 1999
Study code	P94-E010	37537
Number of replicates	2 (Lys 5, Lys 6)	3 (Lys 16, Lys 17, Lys 18)
Location	BASF Agricultural Research Center Limburgerhof	
Soil name Soil type	Speyrer Wald luvic arenosol (loamy sand)	
Crop rotation	spring wheat winter barley winter oilseed rape	spring wheat winter barley winter oilseed rape spring rye
Application date and rate	28/04/1994: 0.05 kg/ha	Lys18 (1st appl.): 03/04/1996: 0.05 kg/ha Lys 16, 17, 18 (2nd appl.): 20/05/1996: 0.05 kg/ha
Total precipitation (fraction of irrigation) [mm]	Year 1: 808 (325) year 2: 835 (165)	year 1: 802 (317) year 2: 807 (285) year 3: 836 (315)
Amount of leachate [mm]	Lys 5 / Lys 6 year l: 271 / 199 year 2: 487 / 454	Lys 16 /Lys 17 / Lys 18 year l: 207 / 228 / 241 year 2: 272 / 290 / 283 year 3: - / 415 / 423
Annual BAS 635 H concentration in the leachate [µg/L]	Lys 5 / Lys 6 year l: 0.042 / 0.022 year 2: 0.019 / 0.014	Lys 16 /Lys 17 / Lys 18 year l: 0.023 / 0.020 / 0.014 year 2: 0.009 / 0.012 / 0.036 year 3: - / n.d. / n.d.
Maximum substance concentration of BAS 635 H in one sample [µg/L]	0.072 (sampling on 08/02/1995 of Lys 5 for a 15-days interval)	0.067 (sampling on 21/11/1997 of Lys 18 for a 77-days interval)

Table B.8.2.2-2: Characteristics of the lysimeter soils

Horizon	Ap	B	Cv	C
Lys 5, Lys 6				
Depth [cm]	0-35	35-60	55-80	80-100
pH-value (KCl)	5.7	6.3	6.5	6.8
C_{org}	0.9	0.4	0.1	0.2
Sand [%]	75.8	76.3	87.5	90.1
Silt [%]	16.6	14.1	5.3	6.3
Clay [%]	7.7	9.7	7.3	3.6
Lys 16, Lys 17, Lys 18				
Depth [cm]	0-35	35-57	55-75	75-100
pH-value (KCl)	5.7	5.9	6.2	6.2
C_{org}	0.6	0.2	0.1	0.1
Sand [%]	74.1	79.6	92.6	95.2
Silt [%]	21.8	18.6	6.6	3.9
Clay [%]	4.3	1.9	0.8	0.9

Modelling tools

In order to simulate the transport of BAS 635 H in the lysimeter studies the simulation model FOCUS-PEARL 1.1.1 was used, which solves the Richards equation for water transport and the convection dispersion equation (CDE) for solute transport. In order to estimate the substance transport parameters, the model was coupled with the parameter estimation tool WinPest 1.0.1 (Watermark numerical computing and Waterloo hydrogeologic, 1999). WinPest is a shell which runs the simulation model (here: FOCUS-PEARL) several times, thereby comparing the model output with the experimental results of the lysimeter study. It modifies the environmental fate parameters of the simulation input file until the model is able to describe the experimental data best. This means that the differences between the experimental results and the model were minimised.

Model parameterisation

In order to run the model, all required parameters apart from the environmental fate parameters of the substance (sorption and degradation parameters) have to be determined and inserted in the model input file. For the environmental fate parameters, initial values have to be given. The parameterisation of the model includes soil hydraulic, plant and application parameters as well as meteorological boundary conditions.

Soil hydraulic parameters

The soil hydraulic parameters were estimated using the parameter estimation tool ROSETTA (Pedotransfer functions based on artificial neural networks, US Salinity Laboratory, ARS-USDA, Riverside, http://www.ussl.ars.usda.gov/MODELS/rosetta/rosetta.htm). The results of the parameter estimation are shown in Table B.8.2.2-3. For both lysimeter studies, a specific set of hydraulic parameters was estimated on the basis of the specific soil texture (sand, silt, clay content) of the soils.

Table B.8.2.2-3: Hydraulic properties of the lysimeter soils

Horizon	Internal name	Θ_s [%}	Θ_r [%]	α [cm^{-1}]	n [-]	K_s [m/day]	L [-]
Lys 5, Lys 6							
Ap	SPWI-SU1 0-35cm	38.5	4.1	0.039	1.53	0.66	-1.03
B	SPWI-SU2 35-60cm	38.2	4.5	0.037	1.52	0.62	-1.05
Cv	SPWI-SU3 60-80cm	37.6	5.3	0.032	2.14	2.00	-0.86
C	SPWII-SU4 80-100cm	38.0	4.8	0.036	2.59	3.45	-0.86
Lys 16, Lys 17, Lys 18							
Ap	SPWII-SU1 0-35cm	39.2	3.3	0.043	1.52	0.74	-1.00
B	SPWII-SU2 35-57cm	39.4	3.3	0.048	1.72	1.12	-0.88
Cv	SPWII-SU3 57-75cm	38.4	4.5	0.039	3.11	5.42	-0.87
C	SPWII-SU4 75-100cm	38.1	4.8	0.036	3.52	7.82	-0.89

Crop data

The most important crop data (date of emergence, date of maximum leaf area index: LAI, and date of harvest) were taken from the lysimeter reports or estimated by expert judgement (max. LAI, crop growth factors, crop shape factors).

Application scenario

The application dates for the lysimeter simulations were as listed in Table B.8.2.2-1. Due to crop interception of the winter wheat canopy showing a BBCH crop stage of 20-39, the application rate of 50 g/ha BAS 635 H was corrected to a realistic effective amount of 25 g/ha reaching the soil in accordance with the recommendations of FOCUS.

Climatic data

Climatic data were available from the meteorological station Limburgerhof. Daily sum or average values were used for the simulation. The following parameters were available and used for calculating precipitation as well as evapotranspiration:

- precipitation
- irrigation
- max/min/mean air temperature
- global radiation
- wind speed
- relative humidity

Substance transport data

The substance data for the simulation of the lysimeter scenarios were estimated by the fitting algorithm of WinPest. Therefore, only starting values have to be given. Here, the mean degradation rate (14.1 days) as well as the mean sorption values (K_{om} = 4.3 mL/g) as used in the monograph were taken.

Modelling strategy

Due to the different climatic boundary conditions, the studies had to be modelled separately, while the individual lysimeters were considered as replicates.

In a first step it was tested whether the model is able to simulate the water transport of the lysimeters. If the simulated leachate outflow of the model is in sufficient agreement with the actual lysimeter outflow, it can be assumed that the time and the shape of the substance breakthrough curve (BTC) will predominantly depend on substance properties.
In a second step, the environmental fate parameters of the substance (sorption and degradation) were estimated by fitting the simulated substance BTC to the measured outflow concentrations. If a sufficient agreement between the measured and simulated BTC is achieved, it can be concluded that the estimated environmental fate parameters reflect the relevant sorption and degradation parameters for the transport process of the substance in the particular soil.

Since the model output is on a daily basis, the respective sampling intervals of the lysimeter studies (1-2 weeks during the drainage period) had to be considered by calculating average simulated concentrations (post-processing tool) for the corresponding sampling intervals, which could then be compared with the measured concentrations.
Simultaneous fitting of the environmental fate parameters of Lysimeters 16-18 required the sequential run of two application patterns, where the first run with only one application was fitted to Lysimeter 16-17 and the second run with an additional second application was simultaneously fitted to the values of Lysimeter 18. Nevertheless, for both runs, the same environmental fate parameters were fitted resulting, in a unique parameter set for that study.
For the estimation procedure, the Freundlich exponent (1/n) was fixed to the mean value of the sorption studies (1/n = 0.913), since this parameter could not be modelled with sufficient sensitivity using the actual outflow concentrations. Thus, the Freundlich factor (K_{om}) and the half-life were the only parameters which were fitted.

Results

Water transport

The simulated and measured cumulative water outflow of lysimeters 5 and 6 is shown in Figure B.8.2.2-1. The total amount as well as the dynamic of the outflow is well simulated by the model. The good agreement between simulated and observed leachate outflow is shown by the course of the simulation which is always between the observed outflow curves of the two replicates. Figure B.8.2.2-2 shows the simulation of the three replicates Lysimeter 16-18. The simulated outflow was in excellent agreement with the three replicates, which showed almost identical leachate outflow, with respect to the total amount as well as the dynamics of the outflow (shape of the outflow curve).
Due to the agreement between predicted and observed leachate outflow for both studies, it can be concluded that the model was able to sufficiently simulate the water transport during the lysimeter studies.

Figure B.8.2.2-1: Observed and predicted cumulative leachate outflow from lysimeter 5 and 6

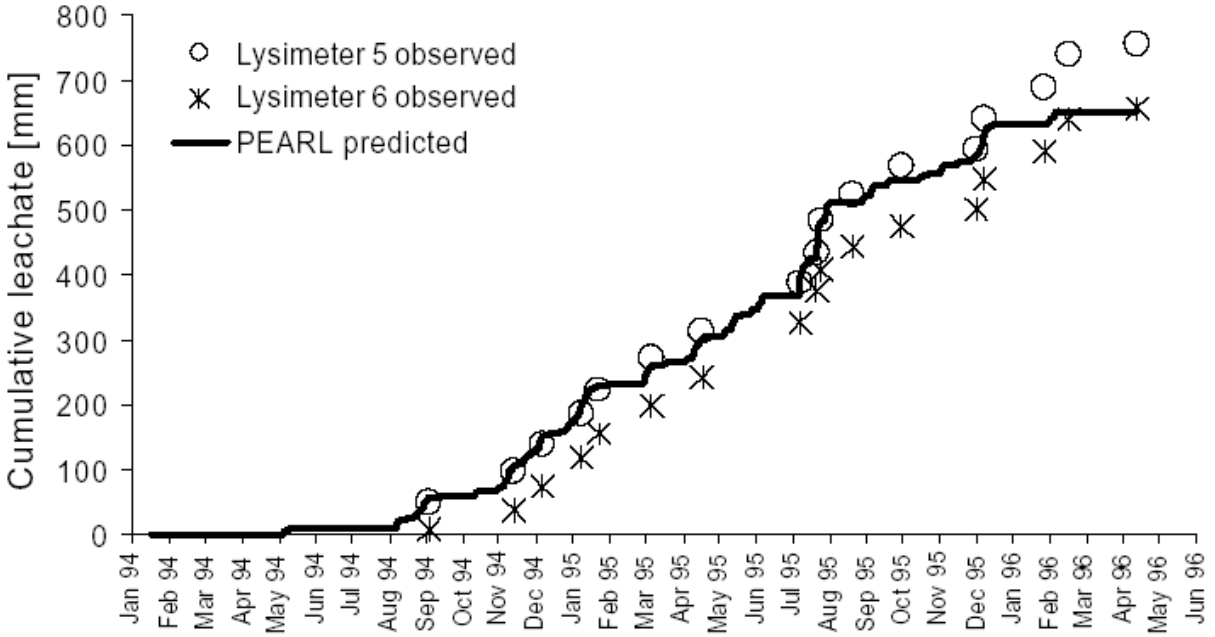
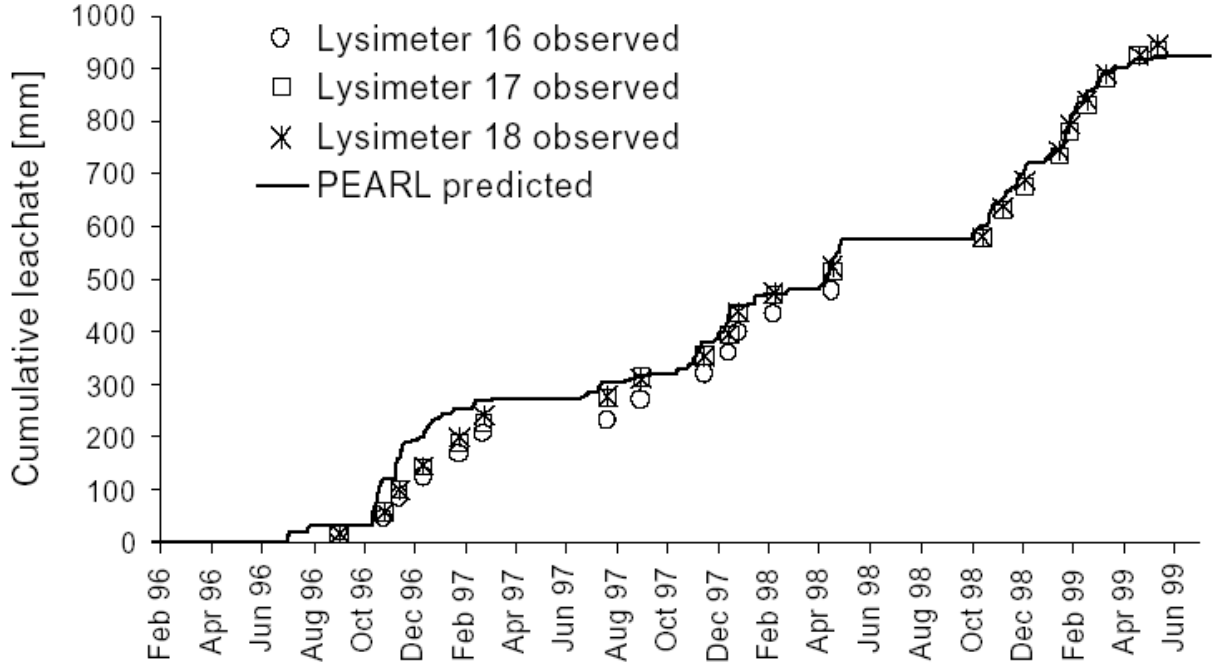


Figure B.8.2.2-2: Observed and predicted cumulative leachate outflow from lysimeter 16-18

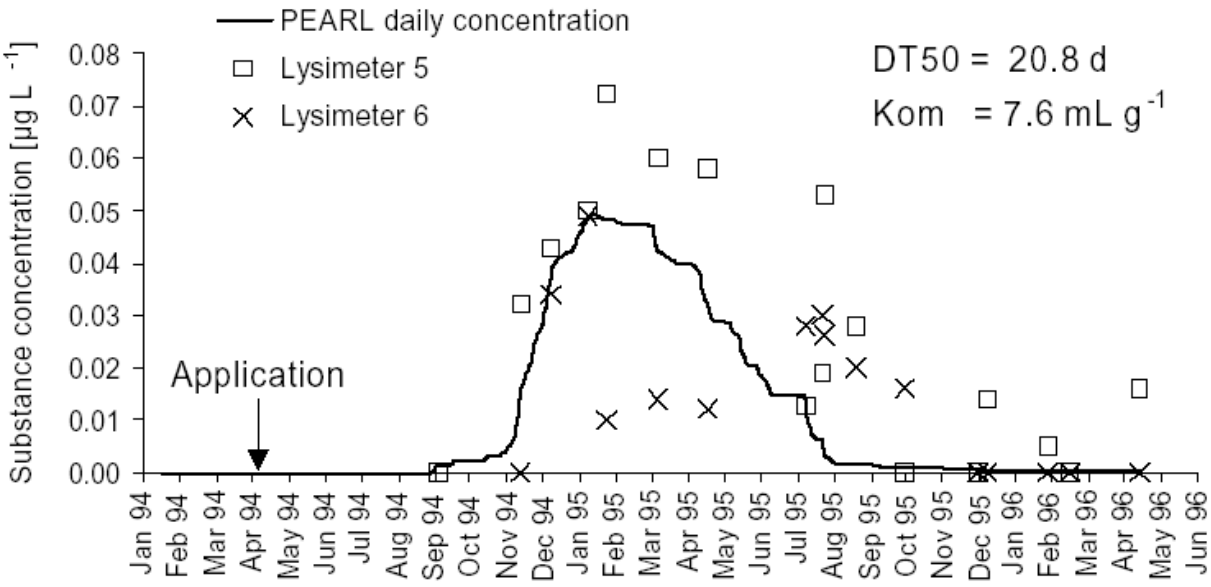


Inverse estimation of environmental fate parameters

The best fits of the inverse modelling procedure with regard to the breakthrough curves of BAS 635 H in lysimeters 5-6 are shown in Figure B.8.2.2-3. Due to the double peak of the breakthrough curve of lysimeter 6 and the resulting deviations between the measured concentrations from January 1995 until May 1995, the two lysimeters show high deviations

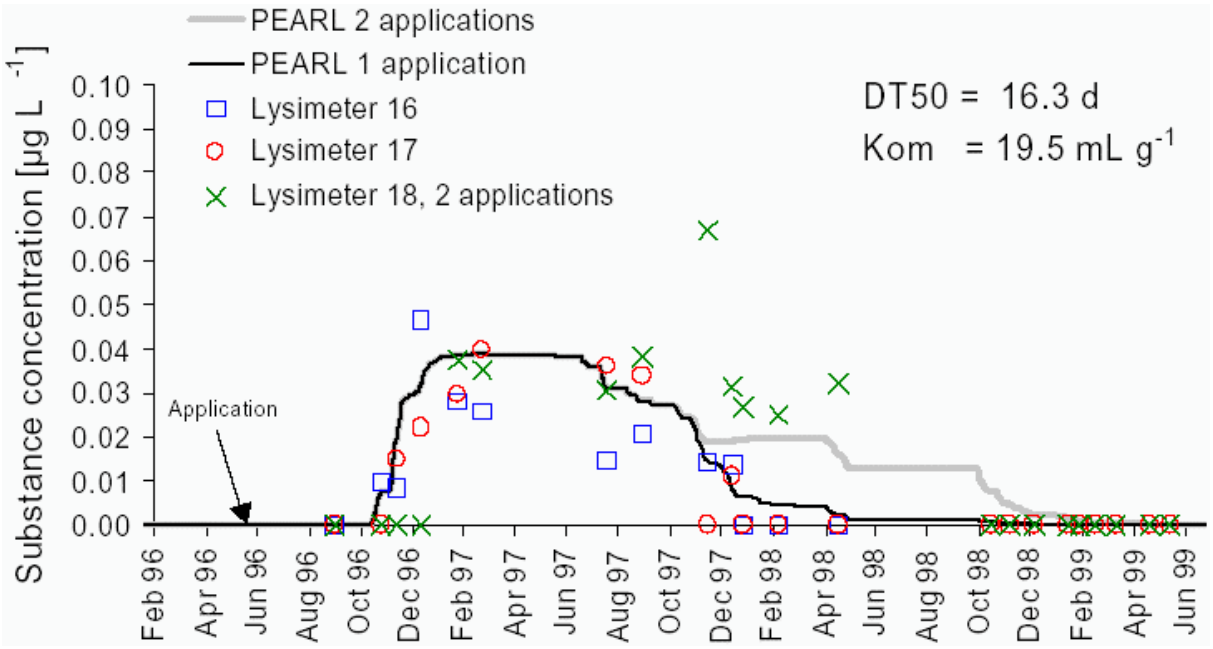
and therefore the overall correlation coefficient was only moderate. Nevertheless, the beginning of the BTC was well simulated and the measured breakthrough concentrations at later time points have the tendency to represent greater retardation than the simulated curve, which would imply an even higher effective sorption than derived from modelling.
The environmental fate parameters were estimated with a K_{om}-value of 7.6 mL/g and a DT_{50} of 20.8 days.

Figure B.8.2.2-3: Observed and inverse modelled BAS 635 H breakthrough curve from lysimeter 5 and 6



The inverse modelled breakthrough curves of lysimeter 16-18 are shown in Figure B.8.2.2-4. Taking into account the spatial heterogeneity of natural systems, the three lysimeters yield comparable BTCs with a similar shape. The fitted model breakthrough curve is in good agreement with the measured breakthrough concentrations. The maximum concentration as well as the shape of the simulated BTC sufficiently matches the measured breakthrough concentrations. This is additionally reflected by the overall squared regression coefficient of $r^2 = 0.85$. The estimated effective K_{om}-value is 19.5 mL/g and therefore significantly greater than for lysimeter 5-6. This may be explained by the fact that for lysimeter 5-6, the measured outflow concentrations seemed to be more retarded than the simulated BTC and therefore the effective sorption in lysimeter 5-6 appears slightly underestimated.
Due to the greater number of replicates, the greater similarity of the replicated BTCs as well as the better fitting result of the inverse modelling procedure, the results of the fitting of lysimeters 16-18 show the greater relevance compared to the estimated parameters of lysimeter 5-6.

Figure B.8.2.2-4: Observed and inverse modelled BAS 635 H breakthrough curve from lysimeter 16-18



The most important statistical results from inverse modelling of BAS 635 H breakthrough curves in lysimeters 5-6 and 16-18 are listed in Table B.8.2.2-4.

Table B.8.2.2-4: Statistics of the inverse modelling procedure

	Best fit	Standard deviation	95 % confidence limit	Initial guess	Overall r^2	Parameter correlation
Lysimeters 5-6						
K_{om} [mL/g]	7.6	1.2	5.1 - 10.0	4.3	0.63	0.84
DT_{50} [days]	20.8	1.1	18.5-23.0	14.1		
Lysimeters 16-18						
K_{om} [mL/g]	19.5	1.6	16.3-22.7	4.3	0.85	0.94
DT_{50} [days]	16.3	0.68	14.9-17.7	14.1		

Conclusion

Effective environmental fate parameters for BAS 635 H were derived from outdoor lysimeter studies by means of inverse parameter estimation technique, using the environmental fate model FOCUS-PEARL and the parameter estimation tool WinPest.
A good agreement of actual and simulated leachate outflow showed that the water balance was sufficiently simulated by the model for the lysimeters from both studies. Hence, the time as well as the shape of the substance BTC give important information about the effective sorption and degradation of the compound in an outdoor study in undisturbed soil.

Fitting the model BTC to the measured outflow concentrations resulted in a moderate fit for the study with lysimeter 5-6 and a good fit for lysimeter 16-18 where the breakthrough concentrations of the individual lysimeters were more in parallel. For lysimeter 5-6, the measured BTC showed a slightly greater retardation than the simulated curve, which would imply an underestimation of the sorption. In contrast, the modelled BTC for Lysimeter 16-18

matches the outflow concentrations of the three replicated lysimeters very well and showed that the effective process-relevant sorption coefficient (K_{om}) for BAS 635 H is significantly higher than the adsorption K_{om} values from the batch-equilibrium studies.
The effective K_{om} values for BAS 635 H derived from inverse modelling of lysimeter BTCs are 7.6 mL/g and 19.5 mL/g for lysimeter 5-6 and lysimeter 16-18, respectively, with an average value of 13.6 mL/g. The adsorption K_{om} values from batch equilibrium studies with different soils range from 1.2 mL/g to 6.3 mL/g with an average value of 4.3 mL/g (arithmetic means). Thus, the mean effective K_{om} from the lysimeter studies exceeds the mean batch-equilibrium K_{om} by a factor of 3.

With regard to degradation, the inverse-estimated half-lives of 20.6 days and 16.3 days for lysimeter 5-6 and lysimeter 16-18, respectively, are in good agreement with the observed field half-lives. When the lysimeter results are included in the calculation of average field half-lives, this results in a value of 15.1 days. The value without considering the lysimeter results is 14.1 days, which was used as initial value for the inverse modelling process (see Table B.8.2.2-4).

The greater effective sorption in the lysimeters, as compared to the adsorption batch-equilibrium study values, can thus be explained by kinetic/aged-sorption processes like already found and described for other herbicides. While leaching of such substances to deeper soil layers would be overestimated without considering kinetic/aged sorption, it could be well predicted by including aged sorption.

Comment of RMS:
The RMS agrees with notifier that adsorption coefficients derived from batch equilibrium studies will not always reflect actual sorption of a compound in soil when the sorption equilibrium has not been fully achieved in this laboratory studies. Modelling based on that values would in fact overestimate concentrations in the leachate. In contrast, the actual sorption behaviour of a compound will always be considered in lysimeter studies. Therefore, in the national authorisation process of the RMS, lysimeter studies are considered as higher-tier studies, which means that measured concentrations in lysimeter leachates will surpass contradictory results from modelling.
Inverse modelling of lysimeter studies can be used to link their results with those from modelling. However, no agreed position exists as yet on EU level with respect to such approaches. Therefore, assessment needs to be done on a case-by-case basis.
In the case of tritosulfuron, the two fitted parameters in the inverse modelling process were sorption coefficient and dissipation half-life. Since the latter was calculated to be close to the initial value obtained from field studies, the conclusion was drawn that the differences between lysimeter and modelling results could be predominantly attributed to the impact of the sorption coefficient. The RMS can agree with that assumption for this specific compound.

B.8.2.2.2 Kinetic sorption parameters of the laboratory sorption studies

Introduction

Laboratory batch equilibrium studies are commonly carried out following the OECD Guideline 106. Besides one adsorption step, the method includes at least two desorption steps. Most often, pronounced hysteresis of desorption as compared with adsorption isotherms is

observed, i.e. it is not possible to describe the system with one single sorption isotherm. Therefore the actual ratio between adsorbed and liquid concentration of a compound in the soil/aqueous phase system will depend on the fact whether the measurements were carried out after an adsorption or a desorption step. Streck and others (Streck et al. 1995; Streck & Richter, 1999; Altfelder et al., 2000) found that the sorption hysteresis is the result of kinetic or long-term sorption processes and indicates that the sorption equilibrium was not achieved within the equilibration time of the study (mostly < 24 h). They showed that the transport of simazine as well as chlortoluron could only be predicted when considering the effect of kinetic/aged sorption.
Since in the BAS 635 H adsorption studies, the experimental equilibration time for adsorption was only 16 h for the soil 'Speyrer Wald' and only 4 h for the other 6 tested soils, it can be expected that sorption equilibrium was not achieved during the adsorption equilibration time. Additionally, strong hysteretic behaviour resulting in great differences between the adsorption and the desorption isotherms could be observed for all soils.

Theory

The theory of the influence of long-term sorption on batch sorption studies is described in detail in Streck et al. (1995), Streck & Richter (1999) and Altfelder et al. (2000). The sorption model assumes two different types of sorption sites: The sorption sites of Fraction *f* are in instantaneous equilibrium with the concentration in the liquid phase according to a Freundlich isotherm, $C_{sol\text{-}1} = K_f \times C_{liqu}^{1/n}$. On the other hand, sorption on the sites of Fraction (1-*f*) is kinetically controlled according to:

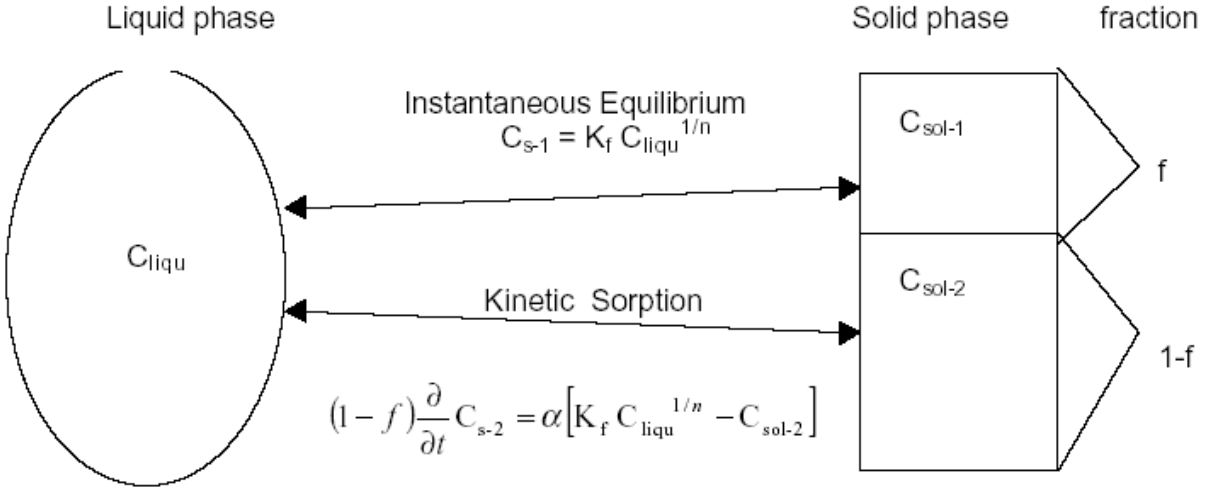
$$(1-f)\frac{\partial}{\partial t}C_{sol-2} = \alpha\left(K_f C_{liqu}^{1/n} - C_{sol-2}\right)$$

where:

f	=	Fraction of instantaneously sorbing sites
$C_{sol\text{-}1}$	=	Concentration of compound on instantaneously sorbing sites [mg/kg]
$C_{sol\text{-}2}$	=	Concentration of compound on kinetically sorbing sites [mg/kg]
C_{liqu}	=	Concentration of compound in liquid phase [mg/L]
α	=	sorption rate [day^{-1}]

The total concentration of the compound on the solid phase is thus composed of the concentrations on instantaneously and kinetically sorbing sites and therefore time-dependent. This model is able to describe the adsorption as well as the desorption processes and requires one single kinetic parameter a for both processes. Figure B.8.2.2-5 shows a schematic view of this so-called two-stage non-linear kinetic sorption model.

Figure B.8.2.2-5: Schematic view of the two-stage non-equilibrium model



The model implies that the true sorption equilibrium, which will be reached after sufficient sorption time, is decisively greater than the adsorption isotherm which is measured after the adsorption shaking time (mostly < 24 h) of the batch study, because only the sorption sites of Fraction *f* are in equilibrium and sorption of Fraction (1-*f*) has not reached equilibrium. Fitting the two-stage non-equilibrium model to the adsorption/desorption data of a given experiment yields the 'true' equilibrium isotherm as well as the corresponding kinetic parameter α.

The adsorption-desorption studies

The adsorption-desorption studies which were performed with BAS 635 H and its metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5 are described in detail in the monograph.
An overview of these adsorption-desorption studies is given in Table B.8.2.2-5. The studies were carried out with a soil/water-ratio of 1/2 [w/w] resulting in 20 mL solution and 10 g soil. Four different concentrations were used and two desorption steps were performed after adsorption. The duration of the adsorption procedure was 16 h for all compounds on the 'Speyrer Wald' soil and for the metabolites BH 635-2, BH 635-3 and BH 635-5 on the other soils that are listed in Table B.8.2.2-5. For BH 635-4, a shaking time for adsorption of 24 h on those other soils was used, while for the parent, BAS 635 H, the respective sorption time was only 4 h. For the desorption steps a constant shaking interval of 16 h was realised for all compounds and all soils.

Table B.8.2.2-5: Adsorption-desorption laboratory studies with BAS 635 H and its metabolites BH 635-2, BH 635-3,BH 635-4 and BH 635-5

Soil name	Texture (USDA)	BAS 635 H		BH 635-2, BH 635-3 BH 635-4		BH 635-5	
		OM [%]	pH	OC [%]	pH	OC [%]	pH
Speyrer Wald (Lysimeter soil)	Loam	1.21	6.0	0.7	6.0	0.7	6.0
LUFA Speyer 2.1	Sandy loam	1.21	5.7	0.5	5.8	0.7	5.8
LUFA Speyer 2.2	Sandy loam	4.48	6.0	1.8	5.8	2.5	5.8
LUFA Speyer 2.3	Sandy loam	2.59	6.5	1.0	6.5	1.0	6.8
Limburgerhof Bruch	Clay loam	5.64	7.8	1.8	7.5	1.5	7.5
USA 528-30-5	Loamy sand	0.60	8.7	0.35	6.7	0.4	5.8
USA 528-31 -2	Silty loam	0.60	5.4	0.35	5.4	0.5	5.2

Parameter estimation technique

The fitting tool FITHYST 3.00 (Streck et al., 1997) was used for parameter estimation. FITHYST 3.00 includes a set of numerical solutions including the non-linear (Freundlich-type) two-stage non-equilibrium model as described in Table B.8.2-14. The Levenberg-Marquardt algorithm is implemented as numerical fitting algorithm to reduce the sum of squared residuals.

For each sorption experiment an input file has to be edited, including the initial concentrations of the adsorption solution as well as the concentrations of the substance in the liquid and solid phase after the adsorption and the desorption steps, respectively. Furthermore, the decanted and replaced liquid volume and the shaking time have to be provided. It has to be noted that in the initial reports of the adsorption studies, only the adsorption and the 'apparent' desorption concentrations were reported. Therefore, the concentrations of the 'true' desorption isotherms had to be additionally calculated from the raw data.

For the adsorption study of BAS 635 H with the 'Speyrer Wald' soil, the replicated values were used as single data points for the fitting procedure. This was also done for all metabolite adsorption studies. For the study of BAS 635 H with the other soils, the mean concentrations of the replicates as reported in the monograph were used.

It is often useful to additionally include the data from the pre-equilibration test (i.e. the pre-test to determine the required shaking time for the main experiment: the change of %-adsorbed between two defined time-steps must be below 5 %), in order to obtain a greater data basis. However, regarding the BAS 635 H studies, the pre-equilibration data were obtained with a soil/water ratio of 1/5 [w/w]; therefore only very small changes in the concentration were observed, resulting in relatively strongly scattered data for the adsorbed phase. Thus, the pre-equilibration data were used only for the soil LUFA 2.2, since no convergence of the fit was obtained for this soil without these data. In contrast, all data from the pre-equilibration tests were included in the fitting procedure for each metabolite.

Four parameters of the two-stage non-equilibrium model are estimated by FITHYST. Besides the coefficients of the Freundlich-equation, K_f and 1/n, the instantaneously sorbing fraction *f* as well as the kinetic parameter α are estimated. The success of this fitting procedure strongly depends on the selection of the initial values, since the algorithm is often not robust enough to deliver a convergent result with a wide range of initial values. Therefore, the initial values had to be varied until a reasonable fit was obtained. With respect to the necessary experimental data, two desorption steps generally have to be considered as minimum data basis on which fitting of the two-stage non-equilibrium model can be applied (Streck, personal communication). However, even with two desorption steps it might occur that no unique solution is found and that one of the four parameters has to be fixed manually at a reasonable value where the sum of squared residuals is minimised and the overall quality of the fit is sufficient.

In the case of BAS 635 H, it was tested for each system whether the normal or logarithmic (logarithms of observed and predicted values) fitting technique yielded better results. With respect to the metabolite studies, it was always necessary to use a logarithmic fit, due to the great range of concentrations.

$$\chi^2 = \sum_{i=1}^{n} \left[\log(\text{obs}_i) - \log(\text{mod}_i)\right]^2$$

Results

In the following sections, results of adsorption-desorption isotherm fitting are documented for each compound and soil, but the graphical presentation is limited to the 'Speyrer Wald' soil. The graphs for the other soils are available in the notifier's original documents, which had been distributed to all Member States.

Parent compound BAS 635 H

A short summary of the fitting results is shown in Table B.8.2.2-6. Without exception, the soils show pronounced sorption hysteresis and decisive deviation between the adsorption isotherm and the desorption branches. In most cases, simultaneous fitting of all parameters was possible. Only for the soil 'Limburgerhof Bruch Ost', the Freundlich exponent had to be fixed. (The Freundlich exponent is the first parameter which is justified to be fixed, since it only slightly deviates from the fit of the apparent sorption isotherm; therefore a reasonable value for this parameter already exists.)
In most cases, the 'logarithmic' fit, where the low concentrations are equally weighted as compared to the high concentrations, yielded better results than the 'normal' fit, which is putting more emphasis on the high concentrations.

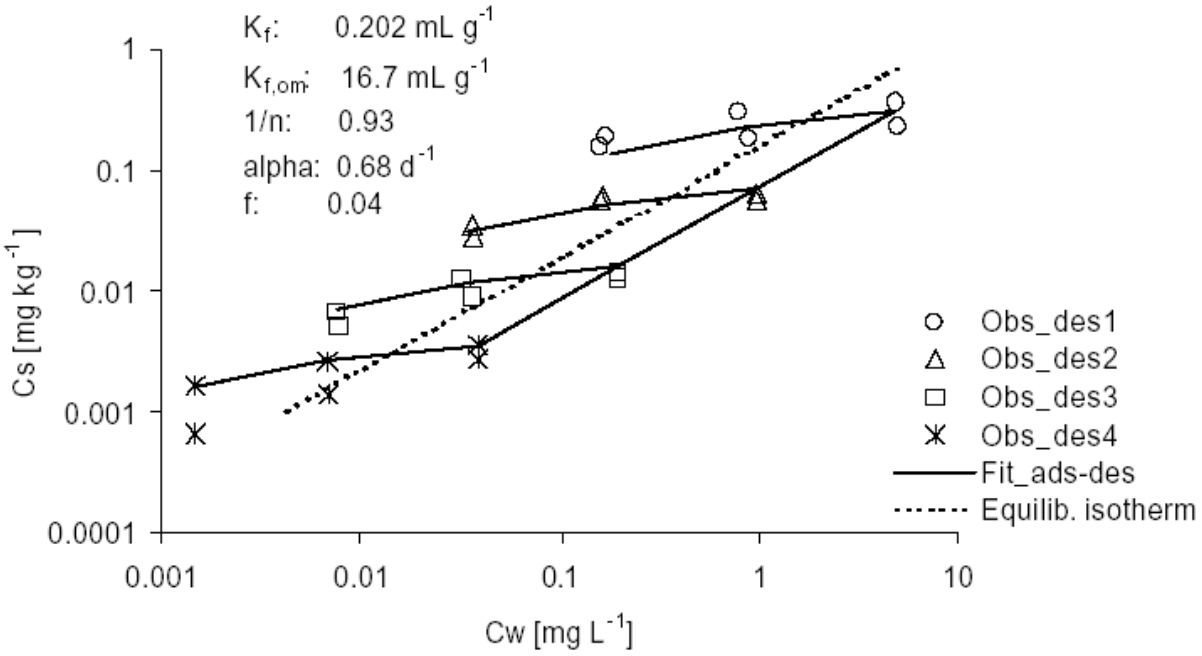
Table B.8.2.2-6: Overview of the kinetic sorption fitting results

	K_f [mL/g]	1/n [-]	*f* [-]	α [d^{-1}]	weighting	r^2
Speyrer Wald	0.202	0.93	0.04	0.68	log	0.92
LUFA Speyer 2.1	0.239	0.93	0.19	0.51	log	0.99
LUFA Speyer 2.2*	0.638	0.93	0.23	0.12	log	0.99
LUFA Speyer 2.3	0.287	1.0	0.055	0.91	normal	0.99
Li.-hof Bruch Ost	0.243	0.86§	0.21	0.54	log	0.88
USA 538-30-5	0.225	0.91	0.00	1.2	normal	0.99
USA 538-31-2	0.235	1.0	0.035	1.1	log	0.82

* kinetic data from pre-equilibration experiment was additionally used
§ value had to be fixed

The fit of BAS 635 H sorption in the Speyrer Wald soil is shown in Figure B.8.2.2-6. The plot shows the measured liquid and sorbed concentrations after adsorption and the two desorption steps as well as the fitted model concentrations.

Figure B.8.2.2-6: Observed and fitted adsorption-desorption isotherms of BAS 635 H in the Speyrer Wald soil (lysimeter soil)



Metabolite BH 635-2

An overview of the estimated equilibrium Freundlich coefficients and the kinetic parameters as well as the apparent sorption coefficients is given in Table B.8.2.2-7. Table B.8.2.2-8 shows an overview of the resulting $K_{f,oc}$-values and the difference of the equilibrium and the apparent sorption coefficients. The comparison between apparent and equilibrium K_f-values is shown in Table B.8.2-16. As an example of a kinetic fit, Figure B.8.2.2-8 shows the observed and fitted logarithmic adsorption-desorption isotherms as well as the predicted equilibrium isotherm of BH 635-2 in the Speyrer Wald soil.

Table B.8.2.2-7: Overview of the kinetic sorption fitting results for BH 635-2

	Equilibrium sorption coefficients		Kinetic parameters		Apparent sorption coefficients		r^2
	K_f [mL/g]	1/n [-]	A [d^{-1}]	*f* [-]	K_f [mL/g]	1/n [-]	
Speyrer Wald	0.555	0.957	0.766	0.077	0.255	0.972	0.973
LUFA Speyer 2.1	0.391	0.946	0.291	0.331	0.195	0.978	0.988
LUFA Speyer 2.2	0.912	0.932	0.137	0.509	0.517	0.943	0.995
LUFA Speyer 2.3	0.836	0.930	0.437	0.163	0.332	0.921	0.985
Li.-hof Bruch West	0.482	0.945	0.588	0.328	0.291	0.942	0.992
USA 538-30-5	0.434	0.881	0.604	0.074	0.179	0.964	0.979
USA 538-31-2	0.497	0.871	0.879	0.092	0.278	0.980	0.921

Table B.8.2.2-8: Overview of the equilibrium and apparent $K_{f,oc}$-values of BH 635-2

	Equilibrium sorption coefficients		Apparent sorption coefficients		Deviation factor
	$K_{f,oc}$ [mL/g]	1/n [-]	$K_{f,oc}$ [mL/g]	1/n [-]	[-]
Speyrer Wald	79.3	0.957	36.4	0.972	2.2
LUFA Speyer 2.1	78.2	0.946	39.0	0.978	2.0
LUFA Speyer 2.2	50.7	0.932	28.7	0.943	1.8
LUFA Speyer 2.3	83.6	0.93	33.2	0.921	2.5
Li.-hof Bruch West	26.8	0.945	16.2	0.942	1.7
USA 538-30-5	124.0	0.881	51.1	0.964	2.4
USA 538-31-2	142.0	0.871	79.4	0.980	1.8

Figure B.8.2.2-7: Apparent and equilibrium K_f-values of BH 635-2 in different soils

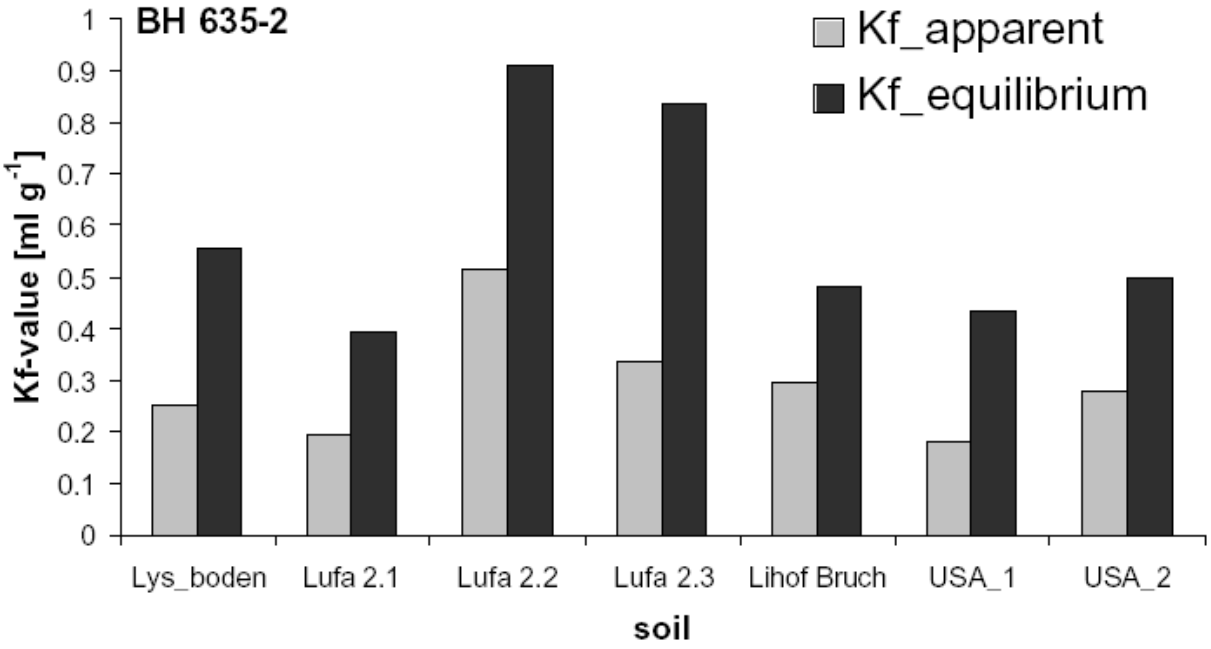
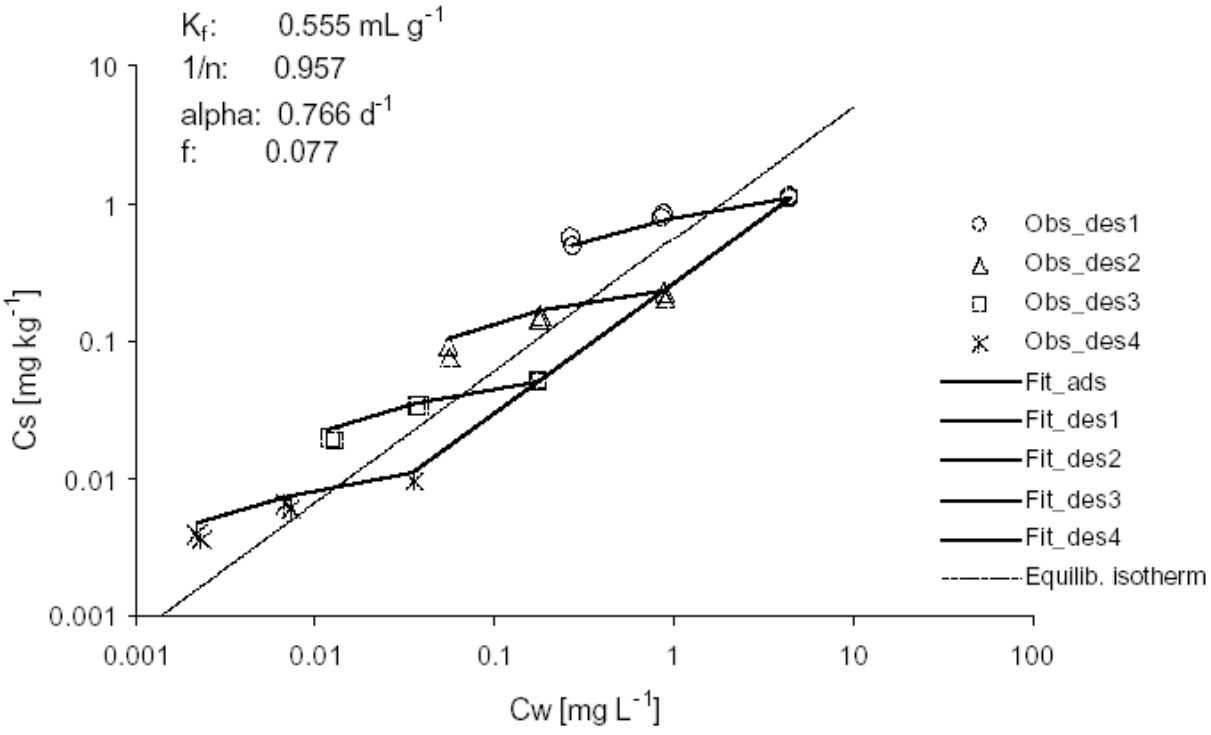


Figure B.8.2.2-8: Graphical representation of the kinetic fit of BH 635-2 in the Speyrer Wald soil



Metabolite BH 635-3

An overview of the estimated equilibrium Freundlich coefficients and the kinetic parameters as well as the apparent sorption coefficients is given in Table B.8.2.2-9. Table B.8.2.2-10 shows an overview of the resulting $K_{f,oc}$-values and the difference of the equilibrium and the apparent sorption coefficients. The comparison between apparent and equilibrium K_f-values is shown in Figure B.8.2.2-9. As an example of a kinetic fit, Figure B.8.2.2-10 shows the observed and fitted logarithmic adsorption-desorption isotherms as well as the predicted equilibrium isotherm of BH 635-3 in the Speyrer Wald soil.

Table B.8.2.2-9: Overview of the kinetic sorption fitting results for BH 635-3

	Equilibrium sorption coefficients		Kinetic parameters		Apparent sorption coefficients		r^2
	K_f [mL/g]	1/n [-]	A [d^{-1}]	*f* [-]	K_f [mL/g]	1/n [-]	
Speyrer Wald	0.560	0.950	0.737	0.036	0.232	0.969	0.965
LUFA Speyer 2.1	0.145	0.948	2.19	0.499	0.114	0.91	0.943
LUFA Speyer 2.2	0.617	0.942	0.051	0.640	0.421	0.935	0.991
LUFA Speyer 2.3	0.726	0.966	0.735	0.051	0.288	0.897	0.970
Li.-hof Bruch West	0.687	0.972	0.817	0.132	0.323	0.927	0.982
USA 538-30-5	0.264	0.922	0.911	0.145	0.119	0.854	0.939
USA 538-31-2	0.254	0.898	0.834	0.335	0.177	0.892	0.958

Table B.8.2.2-10: Overview of the equilibrium and apparent $K_{f,oc}$-values of BH 635-3

	Equilibrium sorption coefficients		Apparent sorption coefficients		Deviation factor
	$K_{f,oc}$ [mL/g]	1/n [-]	$K_{f,oc}$ [mL/g]	1/n [-]	[-]
Speyrer Wald	80.0	0.95	33.1	0.969	2.4
LUFA Speyer 2.1	29.0	0.948	22.8	0.910	1.3
LUFA Speyer 2.2	34.3	0.942	23.4	0.935	1.5
LUFA Speyer 2.3	72.6	0.966	28.8	0.897	2.5
Li.-hof Bruch West	38.2	0.972	17.9	0.927	2.1
USA 538-30-5	75.4	0.922	34.0	0.854	2.2
USA 538-31-2	72.6	0.898	50.6	0.892	1.4

Figure B.8.2.2-9: Apparent and equilibrium K_f-values of BH 635-3 in different soils

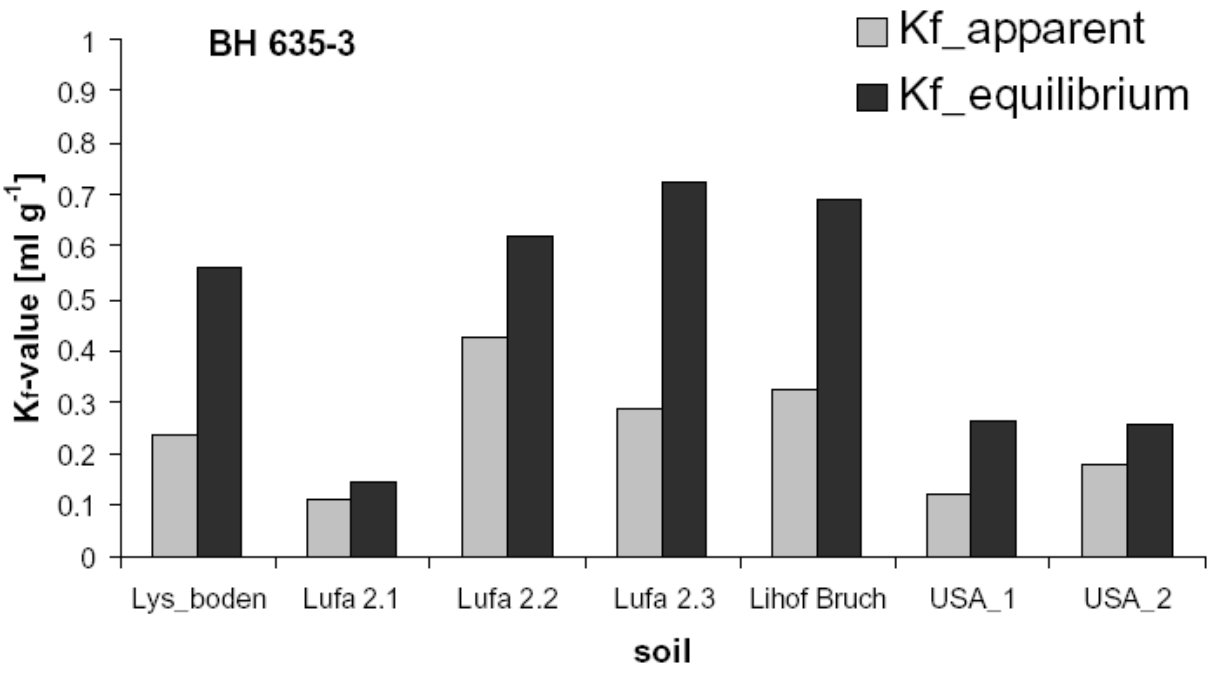
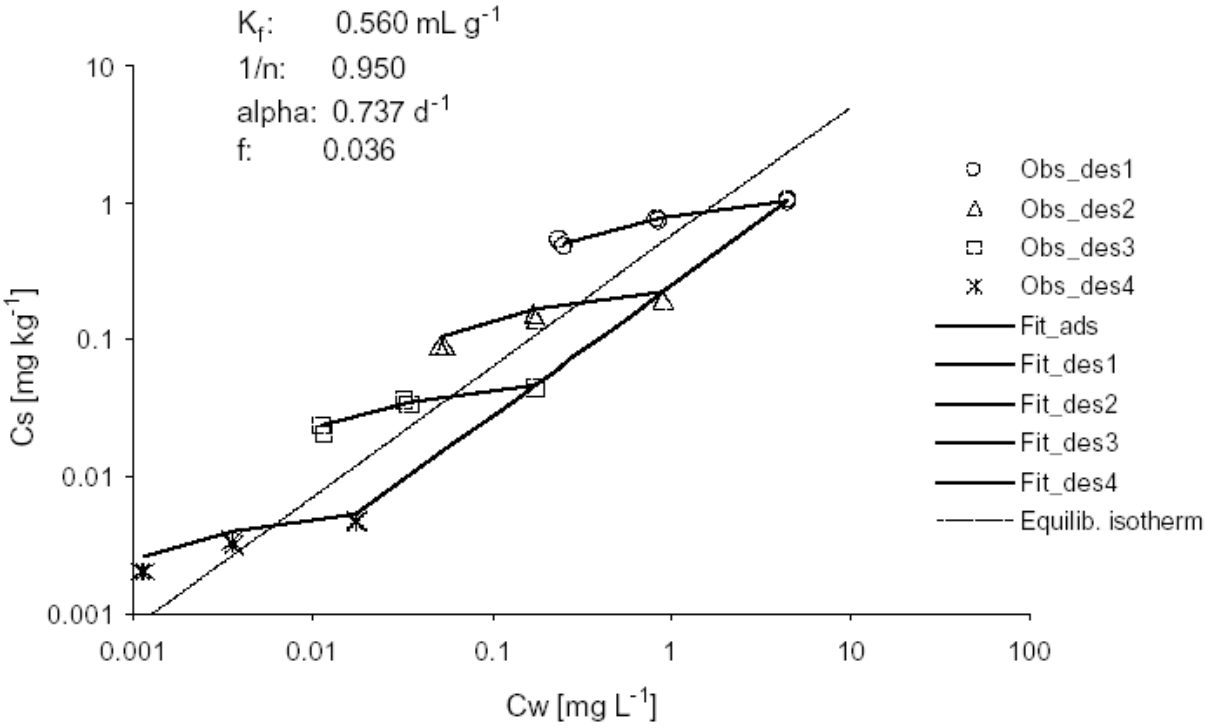


Figure B.8.2.2-10: Graphical representation of the kinetic fit of BH 635-3 in the Speyrer Wald soil



Metabolite BH 635-4

An overview of the estimated equilibrium Freundlich coefficients and the kinetic parameters as well as the apparent sorption coefficients is given in Table B.8.2.2-11. Table B.8.2.2-12 shows an overview of the resulting $K_{f,oc}$-values and the difference of the equilibrium and the apparent sorption coefficients. The comparison between apparent and equilibrium K_f-values is shown in Figure B.8.2.2-11. As an example of a kinetic fit, Figure B.8.2.2-12 shows the observed and fitted logarithmic adsorption-desorption isotherms as well as the predicted equilibrium isotherm of BH 635-4 in the Speyrer Wald soil.

Table B.8.2.2-11: Overview of the kinetic sorption fitting results for BH 635-4

	Equilibrium sorption coefficients		Kinetic parameters		Apparent sorption coefficients		r^2
	K_f [mL/g]	1/n [-]	α [d^{-1}]	*f* [-]	K_f [mL/g]	1/n [-]	
Speyrer Wald	1.07	0.908	0.784	0.067	0.521	0.936	0.975
LUFA Speyer 2.1	0.521	0.957	0.540	0.452	0.443	0.916	0.978
LUFA Speyer 2.2	2.14	0.976	0.274	0.518	1.47	0.94	0.979
LUFA Speyer 2.3	1.63	0.947	0.351	0.095	0.609	0.901	0.984
Li.-hof Bruch West	0.825	0.963	0.596	0.00*	0.319	0.958	0.914
USA 538-30-5	0.820	1.00	0.562	0.121	0.407	0.899	0.984
USA 538-31-2	1.46	0.951	0.466	0.062	0.642	0.914	0.982

* value had to be fixed

Table B.8.2.2-12: Overview of the equilibrium and apparent $K_{f,oc}$-values of BH 635-4

	Equilibrium sorption coefficients		Apparent sorption coefficients		Deviation factor
	$K_{f,oc}$ [mL/g]	1/n [-]	$K_{f,oc}$ [mL/g]	1/n [-]	[-]
Speyrer Wald	152.6	0.908	74.4	0.936	2.1
LUFA Speyer 2.1	104.2	0.957	88.6	0.916	1.2
LUFA Speyer 2.2	118.7	0.976	81.4	0.940	1.5
LUFA Speyer 2.3	162.5	0.947	60.9	0.901	2.7
Li.-hof Bruch West	45.8	0.963	17.7	0.958	2.6
USA 538-30-5	234.2	1.000	116.3	0.899	2.0
USA 538-31-2	418.6	0.951	183.4	0.914	2.3

Figure B.8.2.2-11: Apparent and equilibrium K_f-values of BH 635-4 in different soils

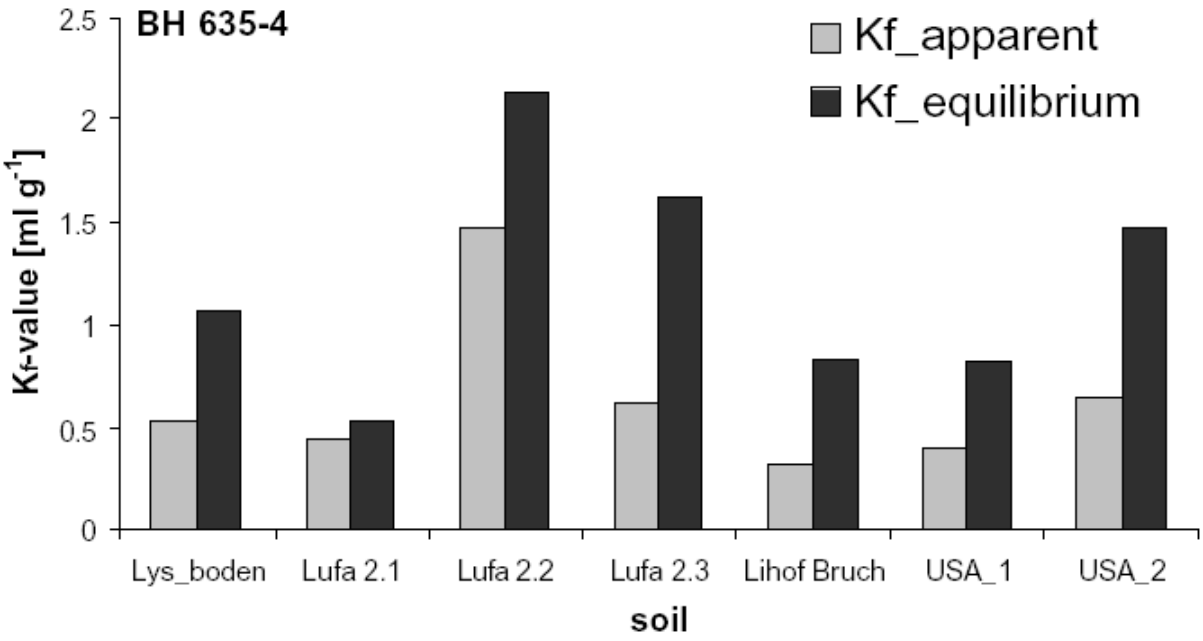
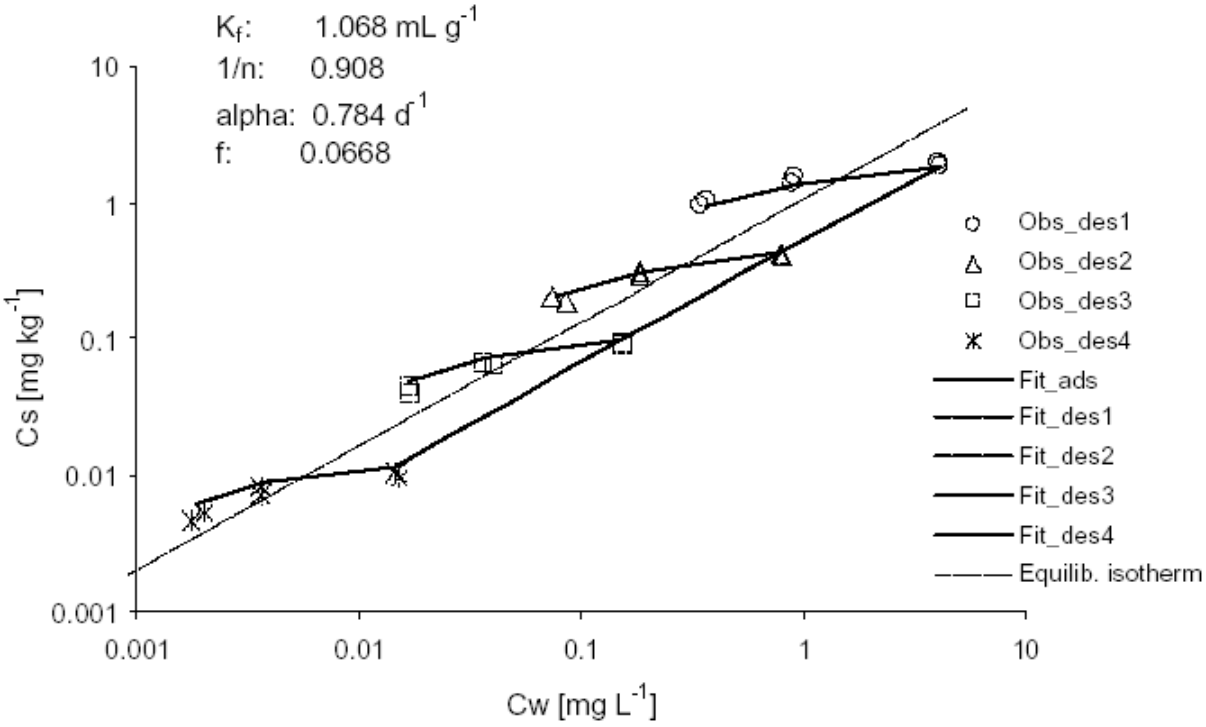


Figure B.8.2.2-12: Graphical representation of the kinetic fit of BH 635-4 in the Speyrer Wald soil



Metabolite BH 635-5

An overview of the estimated equilibrium Freundlich coefficients and the kinetic parameters as well as the apparent sorption coefficients is given in Table B.8.2.2-13. Table B.8.2.2-14 shows an overview of the resulting $K_{f,oc}$-values and the difference of the equilibrium and the apparent sorption coefficients. The comparison between apparent and equilibrium K_f-values is shown in Figure B.8.2.2-13. As an example of a kinetic fit, Figure B.8.2.2-14 shows the observed and fitted logarithmic adsorption-desorption isotherms as well as the predicted equilibrium isotherm of BH 635-5 in the Speyrer Wald soil.

Table B.8.2.2-13: Overview of the kinetic sorption fitting results for BH 635-5

	Equilibrium sorption coefficients		Kinetic parameters		Apparent sorption coefficients		r^2
	K_f [mL/g]	1/n [-]	α [d^{-1}]	*f* [-]	K_f [mL/g]	1/n [-]	
Speyrer Wald	0.217	0.962	0.768	0.129	0.108	0.9815	0.965
LUFASpeyer2.1	0.274	0.932	0.173	0.237	0.092	0.900	0.968
LUFA Speyer 2.2	0.513	0.961	0.082	0.444	0.249	0.951	0.993
LUFA Speyer 2.3	0.502	0.967	0.531	0.0953	0.180	0.898	0.931
Li.-hof Bruch West	0.288	0.946	0.445	0.191	0.123	0.933	0.983
USA 538-30-5	0.226	0.926	0.692	0.053	0.094	0.910	0.953
USA 538-31-2	0.466	0.983	0.206	0.444	0.287	0.971	0.971

Table B.8.2.2-14: Overview of the equilibrium and apparent $K_{f,oc}$-values of BH 635-5

	Equilibrium sorption coefficients		Apparent sorption coefficients		Deviation factor
	$K_{f,oc}$ [mL/g]	1/n [-]	$K_{f,oc}$ [mL/g]	1/n [-]	[-]
Speyrer Wald	31.0	0.962	15.4	0.982	2.0
LUFA Speyer 2.1	39.1	0.932	13.1	0.900	3.0
LUFA Speyer 2.2	20.5	0.961	9.9	0.951	2.1
LUFA Speyer 2.3	50.2	0.967	18.0	0.898	2.8
Li.-hof Bruch West	19.2	0.946	8.2	0.933	2.3
USA 538-30-5	56.5	0.926	23.5	0.910	2.4
USA 538-31-2	93.2	0.983	57.4	0.971	1.6

Figure B.8.2.2-13: Apparent and equilibrium K_f-values of BH 635-5 in different soils

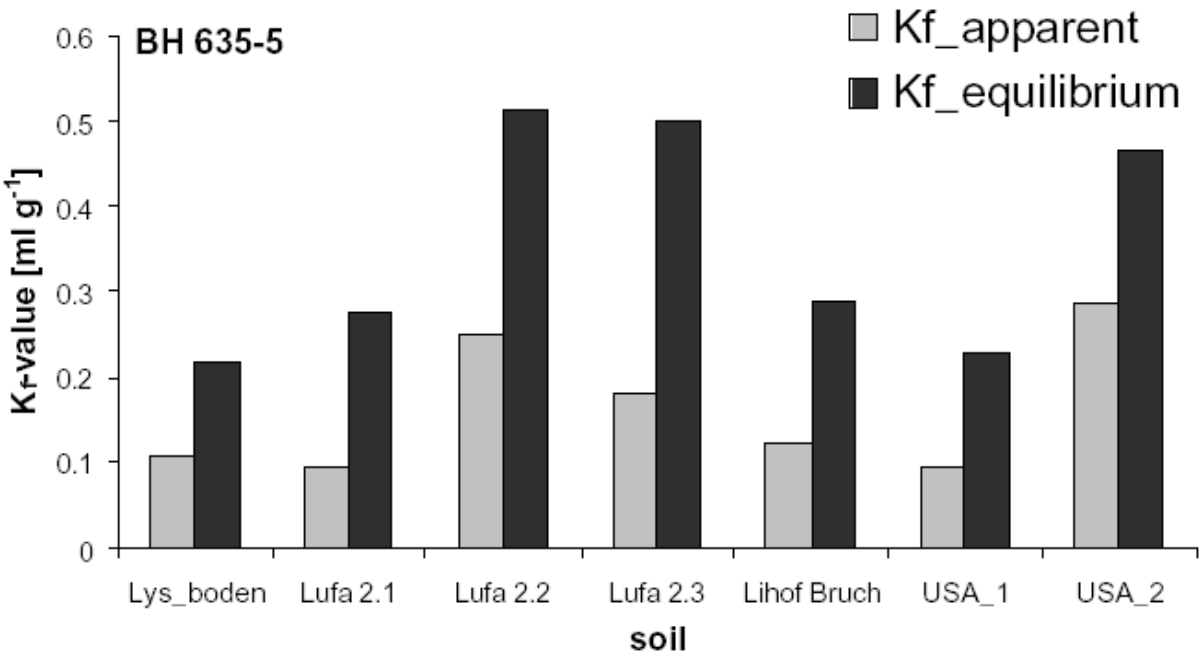
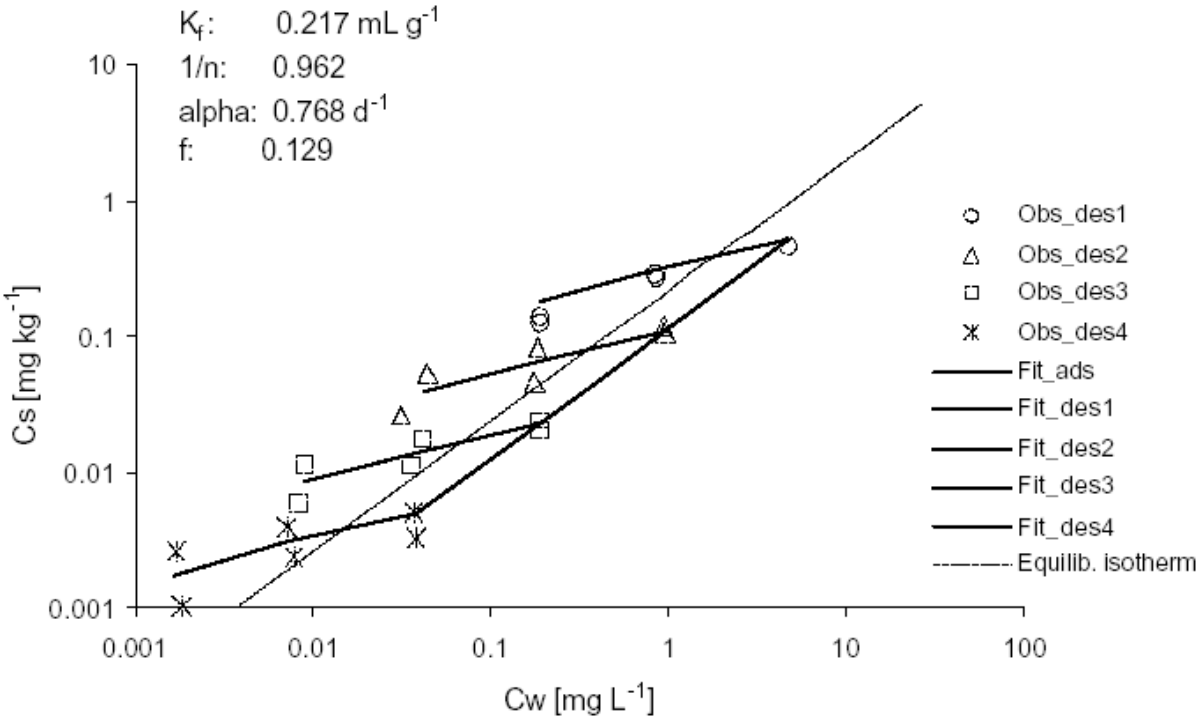


Figure B.8.2.2-14: Graphical representation of the kinetic fit of BH 635-5 in the Speyrer Wald soil

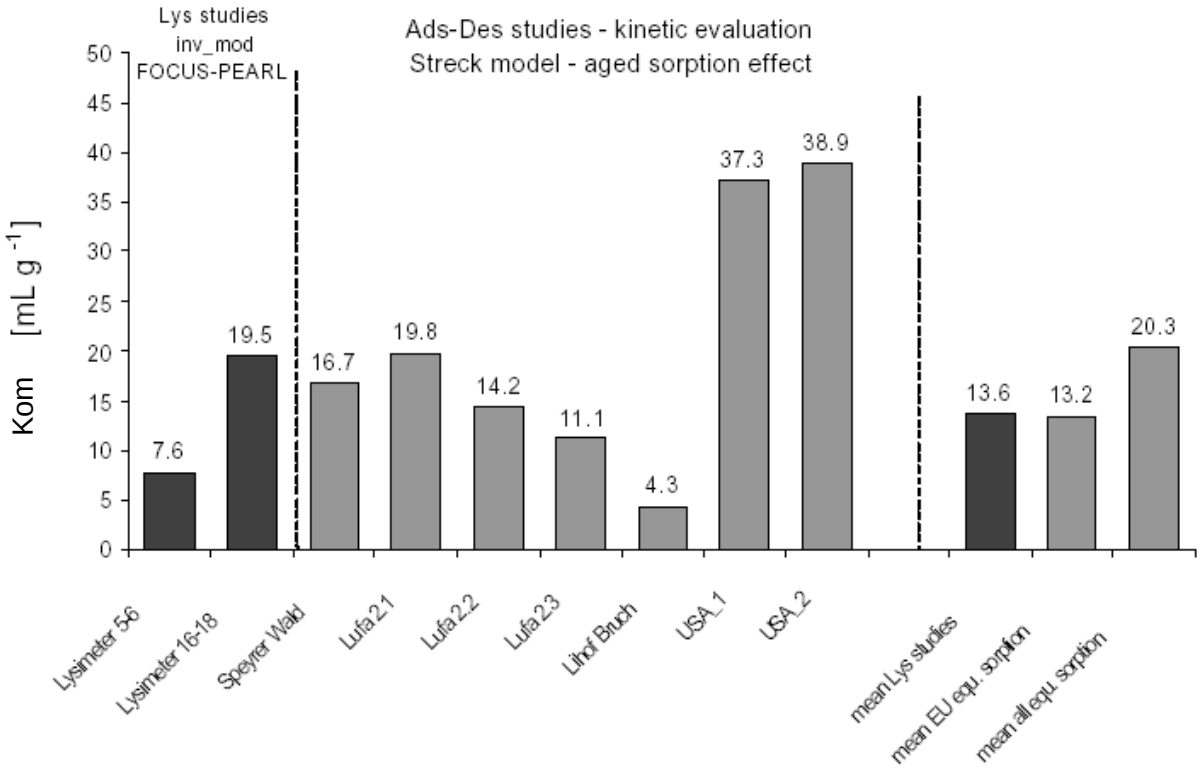


Conclusion

This section describes the kinetic evaluation of the adsorption-desorption studies with BAS 635 H and with its metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5 following the approach of Streck and others. The observed strong sorption hysteresis indicated that the sorption equilibrium was not achieved and that the sorption coefficients from long-term equilibrium sorption isotherms are considerably higher than those from apparent adsorption isotherms which are reported in the monograph. This could already be expected from the fact that the adsorption equilibrium times were comparatively short: for BH 635-5 in all soils 24 h, for the other metabolites in all soils and the parent compound BAS 635 H in the 'Speyrer Wald' soil 16 h, and for BAS 635 H in all other tested soils only 4 h.

The kinetic evaluation for BAS 635 H showed that under long-term conditions the expected K_{om} values were at least by a factor of 3 higher than under short-term (16 h and 4 h) conditions. A comparison of the long-term equilibrium sorption values with the inversely estimated effective K_{om}-values from the lysimeter studies (see B.8.2.2.1) is made in Figure B.8.2.2-15. The lysimeter sorption values are within the range of the long-term sorption values. The mean value of the European soils is in good agreement with the mean lysimeter K_{om}. Due to the strong sorption behaviour of the two US soils, the overall average K_{om} of the kinetic sorption evaluation considerably exceeds the lysimeter sorption.

Figure B.8.2.2-15: Comparison of effective lysimeter K_{om} and long-term equilibrium K_{om} from laboratory studies



It can be concluded that also the kinetic evaluation of the laboratory studies supports the conclusion drawn from the inversely estimated effective sorption values that the long-term equilibrium sorption values are the relevant sorption parameters for the transport of BAS 635 H in soils. In contrast, the apparent *adsorption* K_{om}-values from the batch equilibrium studies clearly underestimated the leaching-relevant sorption of BAS 635 H, since their equilibration times of only 16 h or 4 h are significantly shorter than the time-scale of leaching processes (several months).

Regarding the metabolites, the kinetic evaluation showed that under long-term conditions the expected K_f-values were by a factor of approximately 2 higher than under short-term (16 h and 24 h) conditions. Since the relevant time scale of outdoor transport conditions spans over several months up to years, the equilibrium sorption parameters are much more relevant to characterise the actual sorption under field conditions than the coefficients of the apparent isotherms after less than one day.

Comment of RMS:

As yet, there is no agreement on EU level on the use of complex sorption modelling approaches for regulatory purposes. Nevertheless, it is confirmed that the presented results for the parent compound tritosulfuron are well in line with the sorption coefficients derived from inverse modelling of the lysimeter studies as shown above.

As argued before, an assessment of complex sorption modelling approaches can only be done on a case-by-case basis. In the case of tritosulfuron, the conclusion is supported that the two-stage non-equilibrium model appropriately describes the actual sorption of the compound in soil. As regards the tritosulfuron metabolites, the appropriateness of this approach also appears plausible. Nevertheless, it must be stated that fewer experimental data than for the parent compound are available to support that

assumption; therefore a higher amount of uncertainty remains with the corresponding 'effective' sorption coefficients.

B.8.2.2.3 Effective sorption of BAS 635 H in field studies

Introduction

Field dissipation studies, where separated soil samples from different soil depths were analysed, will yield concentration profiles in depth for each sampling point after substance application. Provided the temporal and spatial resolution of sampling is sufficiently high, such studies can provide important information about the leaching behaviour of substances. A comparison of predicted soil concentration profiles versus the observed depth concentrations of a substance can show whether the translocation of the compound was under- or overpredicted. Since the retardation of chemicals in soil is a function of the sorption behaviour, the process-relevant effective sorption of a compound can be estimated by fitting the predicted depth profiles of the simulation model to the measured concentration profiles of a field experiment.
For the BAS 635 H field dissipation studies, no inverse parameter estimation is carried out, but a qualitative comparison between the observed concentration profiles and simulated depth distribution is made, therewith testing the relevance of the apparent as well as the long-term sorption values as shown in B.8.2.2.2.

The field dissipation experiments

Ten field dissipation studies were carried out with BAS 635 H at different sites in Europe (6 studies) and the US (4 Studies). The studies were already referred to in the monograph. For all studies, detailed information for the characterisation of the soils are available. Additionally, detailed climatic parameters were recorded at the US study sites. For the European studies, at least precipitation and air temperature data are available. Sampling of the US studies was carried out for four different soil depths down to 30 cm, while samples of three different soil depths down to 50 cm were analysed for the European sites. Sophisticated evaluation of the degradation behaviour of BAS 635 H in all field studies has been carried out before, resulting in standardised degradation rates, which could be used for the simulation.
A comparative overview of the field studies, including information about the experimental sites, the soil and the application time and rate, is given in Table B.8.2.2-15. An evaluation of the studies with regard to their suitability for the evaluation of the leaching behaviour of BAS 635 H in field soils is carried out in Table B.8.2.2-16. Due to this evaluation, two of the ten studies were excluded from the leaching simulation. In one US study (Waller County, Texas), the half-life was below three days and the dissipation of the substance was too fast for evaluating the transport behaviour. The field study in Utrera/Spain was not considered, because no precipitation occurred during the first 100 days after application of BAS 635 H and therefore no transport processes were possible.

Table B.8.2.2-15: Overview of the field dissipation experiments with BAS 635 H

Trial no. Study code	Trial number Trial site country	Texture of topsoil (USDA) Soil type (FAO) C_{org} [%] / pH	Application date and rate [g/ha]	Half-life [d]	Climatic data	Sampling [cm]
1 97002	RCN 97009 SD Marshall County South Dakota, US	loam Luvic Chernozem 2.3 / 7.9 (H_2O)	23/07/1997 72.8	32.5	precipitation, temperature, humidity, global radiation, wind speed	0-2.5 2.5-5 5-15 15-30
2 97002	RCN 97010 CA Tulare County California, US	sandy loam Chromic Luvisol 0.5 / 7.8 (H_2O)	28/05/1997 72.3	32.7	precipitation, temperature, humidity, global radiation, wind speed	0-2.5 2.5-5 5-15 15-30
3 97002	RCN 97011 IN Hamilton County Indiana, US	loam Orthic Luvisol 2.1 / 6.3 (H_2O)	07/07/1997 73.1	5.4	precipitation, temperature, humidity, global radiation, wind speed	0-2.5 2.5-5 5-15 15-30
4 97002	RCN 97012 TX Waller County Texas, US	sandy loam Chromic Luvisol 0.7 / 5.1 (H_2O)	14/05/1997 73.0	2.6	precipitation, temperature, humidity, global radiation, wind speed	0-2.5 2.5-5 5-15 15-30
5 ED/HA/044/97	ALO/11/97 Manzanilla Andalucia, Spain	sandy loam Arenosol 0.6 / 7.7 ($CaCl_2$)	29/04/1997 91	11.0	precipitation, temperature	0-10 10-25 25-50
6 EU/HA/044/97	ALO/12/97 Utrera Andalucia, Spain	sand Dystric Planosol 0.5 / 7.7 ($CaCl_2$)	18/06/1997 92	5.9	precipitation, temperature	0-10 10-25 25-50
7 EU/HA/044/97	HUS/11/97 Bjarred Sweden	loamy sand Chromic Cambisol 1.8 / 5.6 ($CaCl_2$)	23/05/1997 74	8.4	precipitation, temperature	0-10 10-25 25-50
8 DE/HA/042/96	D05/04/96 Großharrie Schleswig-Holstein, Germany	sandy loam Humic Podzol 1.1 / 6.2 ($CaCl_2$)	29/04/1996 99	14.1	precipitation, temperature	0-10 10-25 25-50
9 DE/HA/042/96	DU2/06/96 Niederhofen Baden-Württemberg, Germany	loam Orthic Luvisol 1.2 / 5.0 ($CaCl_2$)	18/04/1996 103	14.1	precipitation, temperature	0-10 10-25 25-50
10 DE/HA/042/96	DU3/02/96 Meckenheim Rheinland-Pfalz, Germany	sandy loam Orthic Luvisol 0.6 / 5.6 ($CaCl_2$)	23/04/1996 102	14.1	precipitation, temperature, humidity	0-10 10-25 25-50

Table B.8.2.2-16: Field dissipation experiments with BAS 635 H used for the leaching evaluation

Trial no. Study code	Trial number Trial site country	Used for leaching evaluation	Reason	Model used
1 97002	RCN 97009 SD Marshall County South Dakota, US	Yes	Detailed soil data available; climatic data for Haude as well as Penman available	FOCUS-PEARL 1.1.1 + FOCUS-PELMO 2.2.2
2 97002	RCN 97010 CA Tulare County California, US	Yes	Detailed soil data available; climatic data for Haude as well as Penman available	FOCUS-PEARL 1.1.1 + FOCUS-PELMO 2.2.2
3 97002	RCN 97011 IN Hamilton County Indiana, US	Yes	Detailed soil data available; climatic data for Haude as well as Penman available	FOCUS-PEARL 1.1.1 + FOCUS-PELMO 2.2.2
4 97002	RCN 97012 TX Waller County Texas, US	No	Half-life too short for sound evaluation (DT_{50} = 2.6 days)	
5 ED/HA/ 044/97	ALO/11/97 Manzanilla Andalucia, Spain	Yes	Appropriate soil data; Climatic data for Hamon model available	FOCUS-PELMO 2.2.2
6 EU/HA/ 044/97	ALO/12/97 Utrera Andalucia, Spain	No	No precipitation within the first 3 months after application (= 10 × DT_{50})	
7 EU/HA/ 044/97	HUS/11/97 Bjarred Sweden	Yes	Appropriate soil data; Climatic data for Hamon model available	FOCUS-PELMO 2.2.2
8 DE/HA/ 042/96	D05/04/96 Großharrie Schleswig-Holstein, Germany	Yes	Appropriate soil data; Climatic data for Hamon model available	FOCUS-PELMO 2.2.2
9 DE/HA/ 042/96	DU2/06/96 Niederhofen Baden-Württemberg, Germany	Yes	Appropriate soil data; Climatic data for Hamon model available	FOCUS-PELMO 2.2.2
10 DE/HA/ 042/96	DU3/02/96 Meckenheim Rheinland-Pfalz, Germany	Yes	Appropriate soil data; Climatic data for Haude model available	FOCUS-PELMO 2.2.2

Modelling strategy

In order to evaluate the leaching behaviour of the substances in the different field studies, parameterisation of the soil and climatic boundary conditions as well as the management technique (application, Table B.8.2.2-15) has to be performed. Generally, more detailed data, concerning climatic parameters as well as soil characteristics for different soil layers, were available for the US studies. Therefore, parameterisation of the PEARL model was possible including ET calculation following the Penman approach. For the European studies, at least precipitation and temperature data were available, which allows for simple estimation of the ET following the approach of Hamon, which is one option implemented in the PELMO model.

Soil hydraulic parameters

For the evaluation of the US studies with FOCUS-PEARL 1.1.1, hydraulic parameters were derived from depth depending soil texture values, using the database and the pedotransfer functions of ROSETTA. The estimated hydraulic parameters are shown in Table B.8.2.2-17. For the PELMO simulations, the field capacity as well as the wilting point was calculated with the help of the implemented pedotransfer functions. Table B.8.2.2-18 shows an overview of the most important soil characteristics as well as the resulting field capacity and wilting point.

Table B.8.2.2-17: Hydraulic properties of the US test fields

Soil	Depth [cm]	Θ_r [cm^3/cm^3]	Θ_s [cm^3/cm^3]	α [cm^{-1}]	N [-]	Ks [cm/day]	L [-]
Marshall County USA-SD	0-15	0.065	0.412	0.0082	1.52	2.5	-0.10
	15-31	0.076	0.435	0.0081	1.51	2.2	-0.14
	31-46	0.078	0.436	0.0097	1.47	2.6	-0.32
	46-61	0.080	0.435	0.0116	1.43	3.1	-0.52
	61-76	0.083	0.445	0.0110	1.43	2.9	-0.48
Tulare County USA-CA	0-31	0.035	0.390	0.0289	1.41	17.5	-0.95
	31-46	0.036	0.390	0.0316	1.41	19.5	-1.01
	46-61	0.032	0.394	0.0327	1.42	22.0	-0.97
	61-76	0.033	0.392	0.0379	1.44	26.7	-1.04
Hamilton County USA-IN	0-15	0.071	0.420	0.0102	1.47	3.0	-0.34
	15-31	0.078	0.432	0.0105	1.46	2.9	-0.40
	31-46	0.082	0.441	0.0118	1.42	3.1	-0.56
	46-61	0.086	0.450	0.0132	1.38	3.4	-0.74
	61-76	0.085	0.448	0.0121	1.40	3.1	-0.60

Table B.8.2.2-18: Important soil characteristics and PELMO estimated hydraulic parameters

Soil	Depth [cm]	Sand [%]	Silt [%]	Clay [%]	OM [%]	pH [-]	Field capacity [%]	Wilting point [%]
Marshall County USA-SD	0-15	36	34	21	4.0	7.9	0.370	0.104
	15-31	28	45	27	2.7	8.0	0.398	0.121
	31-46	30	41	29	1.4	8.1	0.398	0.125
	46-61	32	37	31	1.0	8.1	0.398	0.129
Tulare County USA-CA	0-15	63	30	7	0.8	7.8	0.288	0.060
	15-31	63	30	7	0.3	8.7	0.288	0.060
	31-46	65	28	7	0.1	9.1	0.284	0.060
	46-61	65	30	5	0.2	9.4	0.280	0.055
Hamilton County USA-IN	0-15	36	39	25	3.6	6.3	0.378	0.113
	15-31	32	39	29	2.7	6.7	0.394	0.124
	31-46	30	37	33	1.6	7.0	0.406	0.134
	46-61	28	35	37	1.1	7.1	0.418	0.144
Manzanilla Andalucia, Spain	0-30	62	27	11	1.0	7.7	0.298	0.071
Bjarred Sweden	0-30	84	12	4	3.1	5.6	0.240	0.046
Großharrie Schleswig-Holstein, Germany	0-30	67	20	13	1.9	6.2	0.292	0.073
Niederhof. Baden-Württemb., Germany	0-30	42	43	15	2.1	5.0	0.346	0.088
Meckenh. Rheinland-Pfalz, Germany	0-30	69	22	9	1.0	5.6	0.280	0.063

Application scenarios

The relevant application dates and rates can be found in Table B.8.2.2-15. All applications were carried out onto bare soil and no crops were grown during the duration of the studies. The application rates were around 72 g/ha and 100 g/ha for the US and European studies, respectively.

Substance parameters

In order to test the relevance of the laboratory and the effective outdoor $K_{f,om}$-values, runs were performed with the mean laboratory $K_{f,om}$-value of 4.3 mL/g. If overestimation of the leaching was observed, higher $K_{f,om}$-values were also tested. The mean Freundlich exponent of 0.913 from the laboratory studies was taken for all studies.
With regard to the degradation behaviour, the estimated standardised half-lives from the respective field studies were used. The different half-lives are listed in Table B.8.2.2-15 and range from 2.6 to 32.7 days. For the German studies, no half-lives were estimated, but it was demonstrated before that the average estimated half-life of 14.1 days was relevant for these studies. Therefore, the same value was also used for this evaluation. Since the half-lives were standardised to reference conditions, correction for temperature and moisture dependency was enabled in the models, using 20 °C and field capacity as reference values.

Climatic parameters

For the US studies, all parameters which are required for estimating the potential evapotranspiration (ET_{pot}) with the Penman-Monteith formula were available {$Temp_{min}$, $Temp_{max}$, vapour pressure, wind velocity, and global radiation) and therefore this option was used when calculating the substance fate with the PEARL model. For calculations of the US studies with the PELMO model, the ET_{pot} was calculated following the Haude formula which is implemented in the PELMO model, requiring $Temp_{14\,h}$, $Temp_{mean}$, $Temp_{delta\text{-}min\text{-}max}$ and relative humidity. For the European studies, only Temperature and precipitation values were available (apart from the Meckenheim site). Thus, the Hamon formula implemented in PELMO was used to calculated the ET_{pot}.

Results

Simulation runs with variation of the sorption values for BAS 635 H were performed with the model FOCUS-PELMO 2.2.2. For the US studies, for which detailed soil data as well as meteorological parameters were available, additional simulations with FOCUS-PEARL were carried out. Whereas PELMO is calculating water transport with a capacity approach, PEARL is solving the Richards equation. Potential evapotranspiration is calculated with the Haude formula in PELMO and with the Penman-Monteith approach in PEARL.

The full results including all graphs will only be shown for the two US sites Tulare County (CA) and Hamilton County (IN). Comparability of these sites to EU conditions has been explicitly shown (see B.8.6.6). More information can be derived from modelling of these US sites as compared to the EU site, owing to the finer depth resolution in the US studies as well as to the usage of two different models, PELMO and PEARL, for leaching simulation. The graphs for all other sites are available in the notifier's original documents, which had been distributed to all Member States.

Marshall County, USA-South Dacota

Simulations with FOCUS-PELMO as well as FOCUS-PEARL were carried out. Calculations were performed with the average laboratory K_{om}-value of 4.3 mL/g and stepwise incremented values, until rough correspondence between simulated and measured values was achieved.

With both models, the simulations with the laboratory K_{om} showed extreme overestimation of the leaching behaviour. The maximum concentration at all sampling dates until day 91 was consistently measured in the upper soil layer, whereas in the simulations with K_{om} = 4.3 mL/g, the maximum residue concentration was predicted in deeper soil layers already 14 days after application.
When increasing the K_{om}-value, better agreement between simulated and measured values could be achieved. A K_{om}-value of 150 mL/g finally showed the best agreement between the predictions and the observations with the PELMO as well as the PEARL model. Particularly for the last and most sensitive measured profile (day 91), good agreement was achieved with both models.

It is concluded that in the Marshall County study the mean laboratory K_{om}-value of 4.3 mL/g had no relevance and resulted in a considerable overestimation of the observed substance leaching. Very strong aged- or kinetic-sorption effects were the reason for the pronounced retention of the substance, resulting in an effective K_{om}-value around 150 mL/g, which was shown with the PELMO as well as the PEARL model.

Tulare County, USA-CA

Simulations with the models FOCUS-PELMO and FOCUS-PEARL were also performed for the Tulare County (CA) study. The results and comparison with the measured concentrations are shown in Figure B.8.2.2-16 and Figure B.8.2.2-17.

As it was already shown for the Marshall County (SD) field study, extreme overestimation of leaching was predicted when using the mean laboratory K_{om} of 4.3 mL/g. Due to the low OM-content of the profile, even a K_{om}-value for BAS 635 H of greater than 1000 mL/g still results in an over-prediction of the substance leaching with both models.
Again, consistent results were achieved with both models. No substance was predicted to remain in the upper 60 cm of the soil profile 91 days after treatment when using the mean laboratory K_{om} of 4.3 mL/g. For a K_{om}-value of 1000 mL/g, similar soil concentration profiles were simulated with both models.

Figure B.8.2.2-16: PELMO calculations of the Tulare County (CA) field study

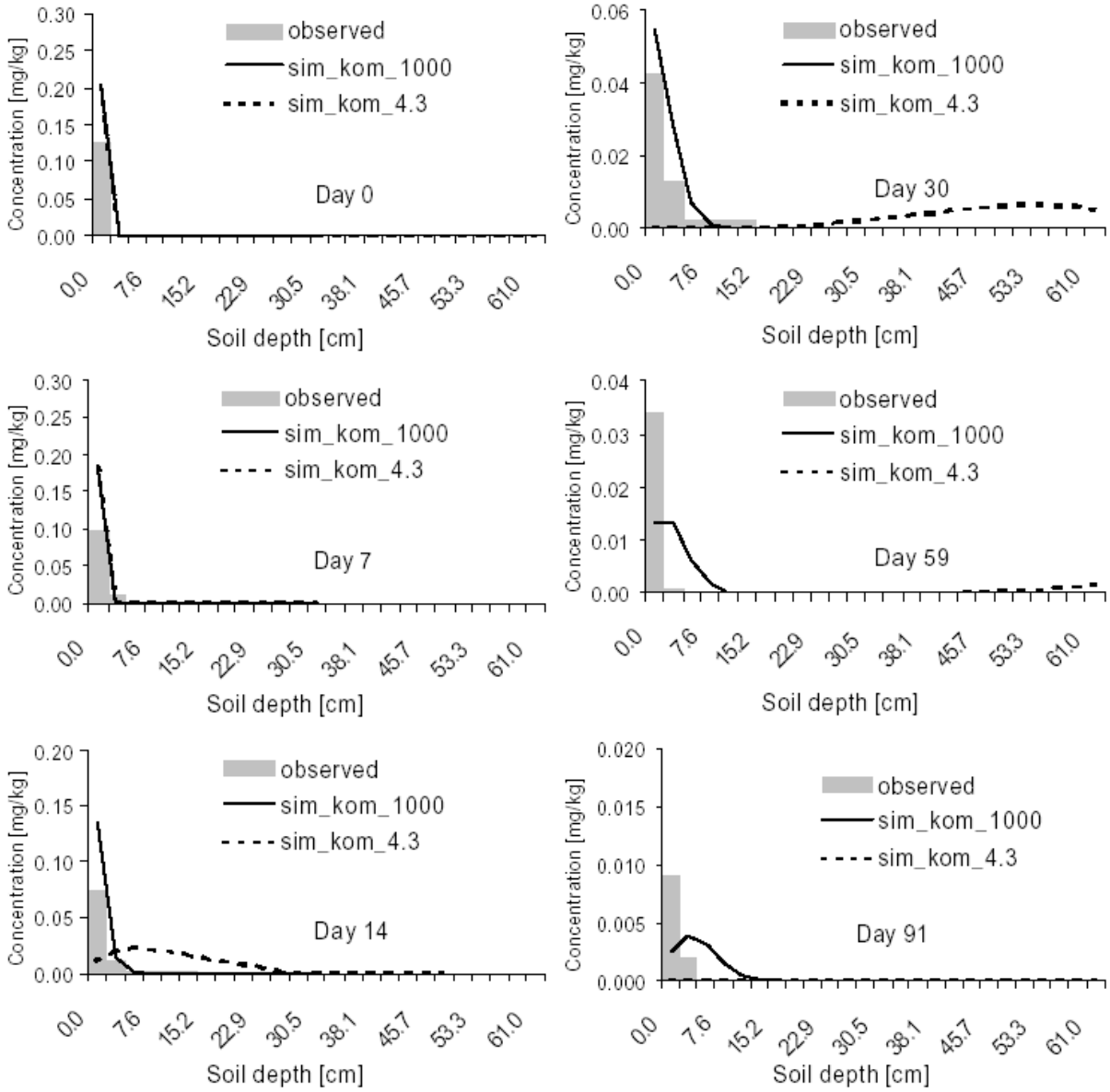
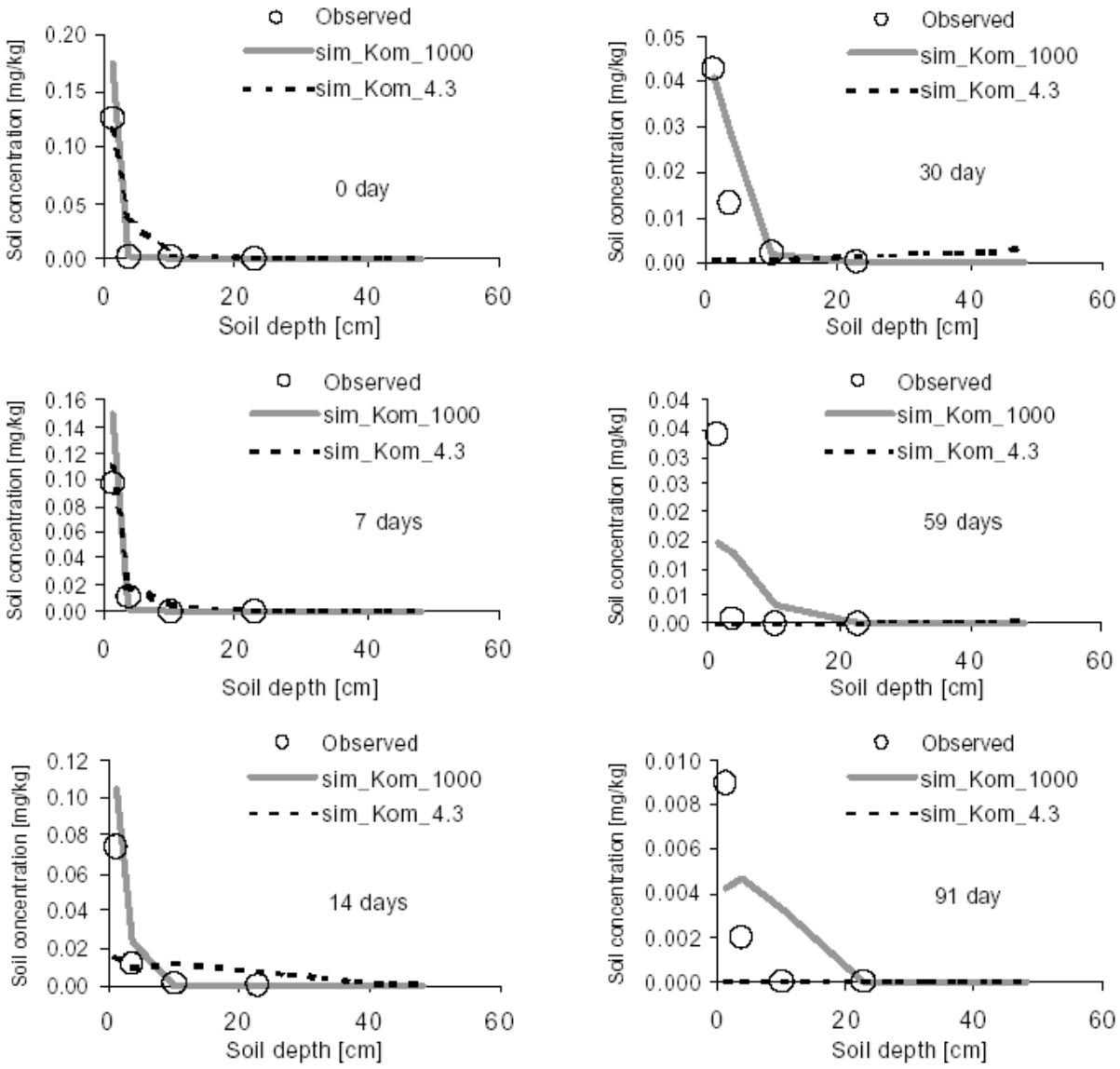


Figure B.8.2.2-17: PEARL calculations of the Tulare County (CA) field study



It is concluded that very strong aged-sorption effects must have been relevant during the field study of BAS 635 H in the Tulare County (CA) soil. Assuming an effective K_{om}-value of 1000 mL/g, the observed transport of the substance in the soil was still overpredicted with PEARL and PELMO. No relevance could be attributed to the mean laboratory K_{om}-value of 4.3 mL/g, which would result in enormous overestimation of substance leaching.

Hamilton County, USA-IN

The Hamilton County (IN) field study is the third US study that was modelled with both models FOCUS-PELMO and FOCUS-PEARL. The results of the PELMO simulations are shown in Figure 21 and the PEARL calculations are shown in Figure 22.

Like in the other studies, the use of the average laboratory K_{om}-value of 4.3 mL/g resulted in considerable overestimation of substance leaching. In reality, the maximum substance concentration 29 days after application was still located in the first 1-inch (2.54 cm) layer.
After increasing the K_{om}-value for the modelling to a value of 100 mL/g, sufficient agreement between predicted and simulated depth concentrations was found 3 and 10 days after

substance application, whereas for later sampling times the substance transport to depth was again overestimated, probably indicating further increase in time-dependent or aged sorption.

Figure B.8.2.2-18: PELMO calculations of the Hamilton County (IN) field study

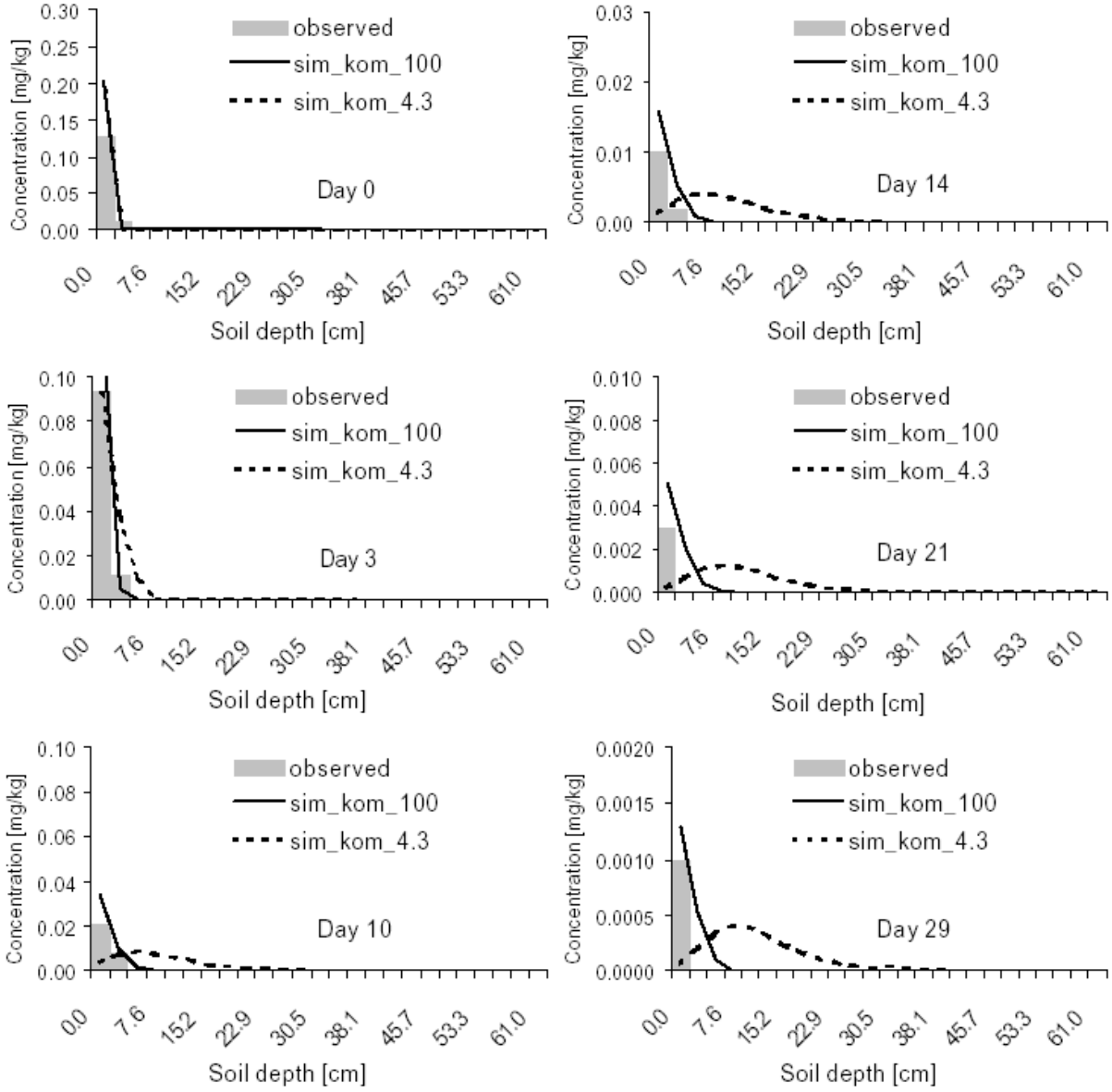
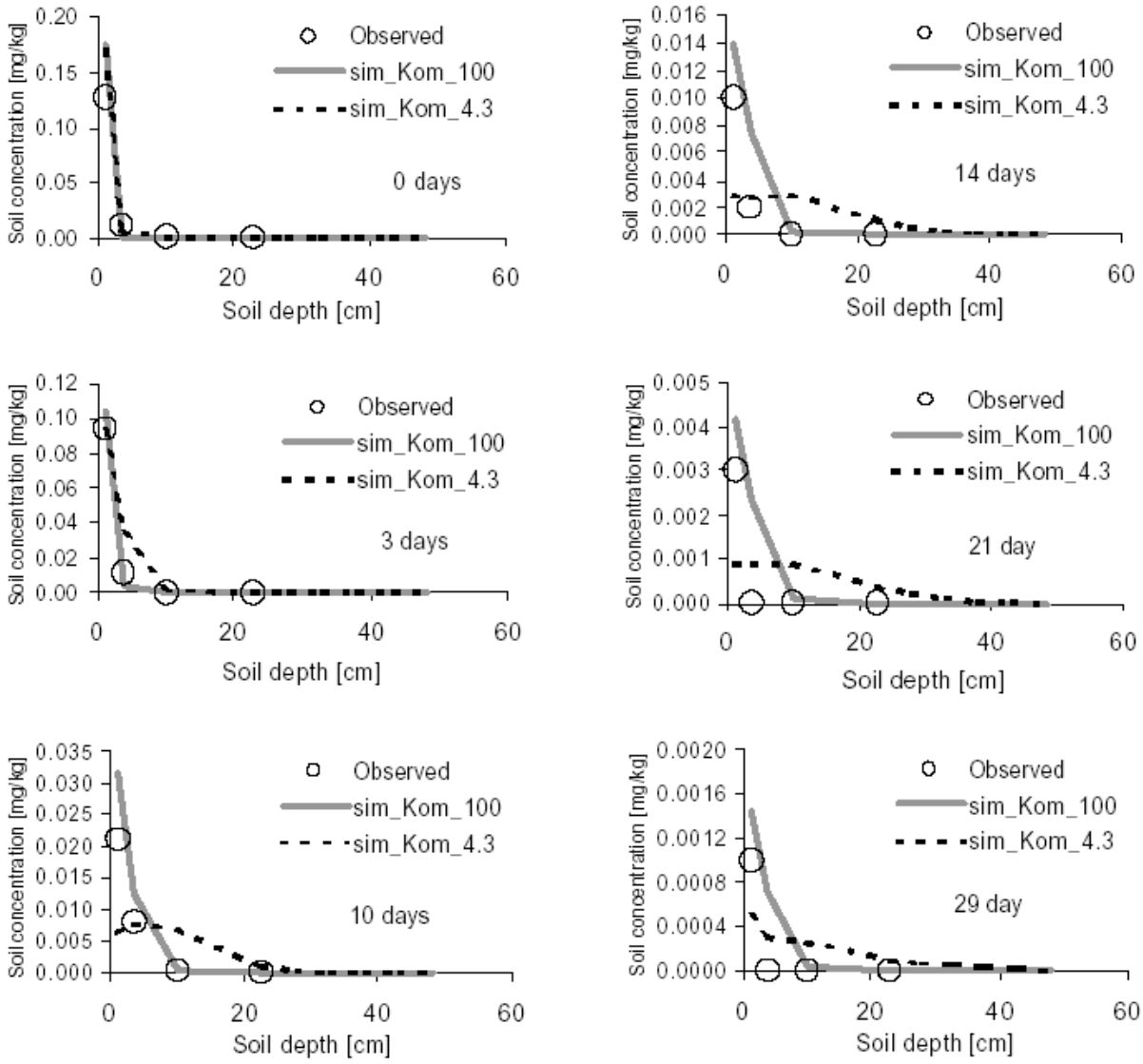


Figure B.8.2.2-19: PEARL calculations of the Hamilton County (IN) field study



Like for the other US-studies, it is concluded that evaluation of the Hamilton County (IN) field study showed that the mean laboratory K_{om}-value of 4.3 mL/g was of no relevance for the transport behaviour of BAS 635 H in the respective soil. An effective K_{om} of approximately 100 mL/g still gave a relatively conservative estimation for the retention of the substance. Long-term sorption processes (aged sorption) were considered to be the relevant processes.

Manzanilla, Andalucia, Spain

In contrast to the US-studies, much lower vertical resolution of the substance residue concentrations was realised in the European studies. Therefore, comparison between the measured and simulated concentrations can only be rough and differences are per se less pronounced. Due to the lack of more detailed soil and meteorological data, simulations were limited to calculations with the FOCUS-PELMO model.

Nevertheless, also the Manzanilla (Andalucia, Spain) field study shows that using the mean laboratory K_{om} of 4.3 mL/g leads to an overprediction of the substance transport to depth. In reality, the maximum concentration was still measured in the 0-10 cm layer 29 days after

BAS 635 H application, whereas the maximum simulated concentration was already predicted deeper than 10 cm. While approximately two third of the substance mass was actually still recovered in the first 10-cm layer, more than half the substance residues were simulated to have leached deeper than 10 cm.
By increasing the K_{om}-value in the simulation to 50 mL/g, much better agreement between observations and prediction could be achieved.

To conclude, overprediction of leaching was obtained with the mean laboratory K_{om}-value of 4.3 mL/g. Using an effective K_{om} of 50 mL/g results in better simulation of the predicted concentration profile, thereby demonstrating the relevance of aged-sorption processes.

Bjarred, Sweden

Simulation of the substance transport in the Bjarred (Sweden) field study was carried out with FOCUS-PELMO.

Like in all other field studies, the use of the laboratory K_{om}-value resulted in considerable overestimation of the leaching. BAS 635 H residues in the Bjarred (Sweden) field study were almost exclusively found in the top 10-cm layer. However, at 34 days after application, the simulated peak concentration suggested substance transport down to 25-30 cm depth.
Using a greater K_{om} of 50 mL/g, still a higher substance leaching is predicted as compared to the measurements, indicating that an even higher effective sorption value was relevant.

To sum up, overprediction of leaching was obtained when poerforming PELMO calculations with the mean laboratory K_{om}-value of 4.3 mL/g. An increased Kom of 50 mL/g would be a more realistic but still very conservative estimate for the relevant sorption parameter, thereby providing evidence for the relevance of aged sorption during the transport of BAS 635 H in the Bjarred soil.

Großharrie, Schleswig-Holstein. Germany

Simulations were conducted with FOCUS-PELMO.

Whereas the observed maximum concentration of BAS 635 H was always measured in the upper 10-cm soil layer, the simulated concentration peak using the average laboratory K_{om}-value of 4.3 mL/g moved downwards much faster. Thirty days after application of BAS 635 H, more than two third of the actual residual substance mass was still located in the upper layer, whereas more than half of the substance was simulated to be transported deeper than 10 cm.
Again, by using an effective K_{om} of 50 mL/g a much better agreement of predicted and simulated concentration profiles could be achieved, thereby indicating the relevance of aged sorption.

Increased (long-term) sorption was identified to be relevant for the Großharrie (Schleswig-Holstein. Germany) study, since the use of the laboratory K_{om}-value in PELMO-modelling results in overprediction of substance transport to depth. Better agreement between measurements and predictions could be achieved with an increased K_{om} of 50 mL/g.

Niederhofen, Baden-Württemberg, Germany

As for the Bjarred (Sweden) study, the majority of the substance mass remained in the upper 10-cm sampling layer. The simulated depth concentrations as calculated using FOCUS-PELMO and the average laboratory K_{om}-value of 4.3 mL/g resulted in considerable overestimation of the substance transport. Whereas at day 32 and 62 after substance application, the observed concentration peak was still within the first 10-cm soil layer, the predicted maximum concentration was already deeper than 13 cm.
Again, much better agreement was achieved with an enhanced K_{om}-value of 50 mL/g.

In conclusion, like for the other field studies, great overprediction of the downward substance transport was obtained by using the laboratory K_{om}-values for the simulation. An enhanced K_{om}-value of 50 mL/g resulted in much better simulation of the observed concentration profile.

Meckenheim, Rheinland-Pfalz, Germany

The field study in Meckenheim (Rheinland-Pfalz, Germany) differs from all other field studies in the point that the measured peak concentration of BAS 635 H in the soil profile at the end of the study was not found in the upper 10-cm sampling layer, but in the second layer (10-25 cm). Nevertheless, simulation with the mean laboratory Kom of 4.3 mL/g still results in significant overprediction of the downward movement. This is shown by a maximum of the predicted concentration depth profile between 30-35 cm at day 63 after substance treatment
Again, a K_{om}-value of 50 mL/g results in much better agreement of measured and simulated concentration profiles. Nevertheless, even with this K_{om} of 50 mL/g, a certain amount of the substance was calculated to be transported deeper than 25 cm at 63 days after treatment, while no substance was actually measured in the 25-50-cm soil sample.

Like for all other studies, it is concluded that simulation with the laboratory sorption value results in obvious overestimation of the downward transport of the substance. An increased K_{om}-value of 50 mL/g yields much better agreement of simulated and observed concentration profiles. This demonstrates the relevance of increased long-term sorption processes also for the Meckenheim study.

Summary and overall conclusion of the evaluations of the field studies

Scientific evidence for kinetic- or aged-sorption processes, resulting in increased long-term sorption of BAS 635 H, was already derived from the evaluation of the lysimeter studies (B.8.2.2.1) as well as from the kinetic evaluation of the desorption hysteresis in batch-equilibrium sorption experiments (B.8.2.2.2). As reality check for the actual applicability of these results, all available field dissipation studies, in which dissipation and translocation of BAS 635 H had been tested, were evaluated with the help of the simulation models FOCUS-PELMO and FOCUS-PEARL, By simulating the respective experimental scenarios, the relevance of the mean laboratory Kom-value versus the increased sorption values could be tested.
Ten field studies with BAS 635 H were available, of which four studies were carried out in the US and six studies in Europe. All but two studies could be used for evaluation. In one US-study, the half-life of BAS 635 H was too short and substance dissipation too fast for a sound evaluation and in one European study, no precipitation occurred for more than 3 months, consequently no downward transport of the substance could take place.

In all field studies the use of the average laboratory K_{om}-value of 4.3 mL/g results in more or less pronounced overestimation of the downward transport of BAS 635 H. It could be clearly shown, particularly for the US studies with their very high vertical spatial resolution of the soil samples, that measured and simulated concentration profiles where in better agreement when an effective K_{om}-value of 100 mL/g for the Marshall County (SD) field study, of 150 mL/g for the Hamilton County (IN) field study and of 1000 mL/g or even greater for the Tulare County (CA) field study was used. For the US studies, these results were found consistently in the simulations with FOCUS-PELMO as well as with FOCUS-PEARL.
Due to the relatively rough spatial resolution of the European studies, it could only be qualitatively shown that simulations with an increased sorption value of 50 mL/g yielded much better agreement of the simulated and observed concentration profiles in all studies. Also here, evidence is provided for the general relevance of enhanced sorption.

Summarising the evaluation of the field studies, it can be stated that the higher effective sorption, estimated from the lysimeter studies as well as from the evaluation of the sorption hysteresis in batch-equilibrium sorption studies, are fully supported by the evaluation of the field studies. It is therefore consistently demonstrated by all experimental approaches and evaluation methods that leaching of BAS 635 H will be significantly overestimated when using the average laboratory K_{om}-value of 4.3 mL/g in simulation model. This had to be considered an unrealistic worst-case.
Moreover, the field studies indicated that the average effective lysimeter K_{om}-value of 13.6 mL/g (B.8.2.2.1), which is almost identical to the equilibrium sorption value derived from the evaluation of the European laboratory sorption studies (B.8.2.2.2), still appears to be a very conservative approach. (Based on the field studies alone, values greater than 50 mL/g had to be considered relevant.)

Comment of RMS:
The RMS agrees to the notifier's statement that the results from reassessment of the field studies support the conclusions on the relevance of kinetic/aged sorption drawn from the inverse modelling of lysimeter experiments as well as from the kinetic evaluation of batch sorption experiments. The reassessment of the field studies is considered one important part of the case-by-case assessment for tritosulfuron.
Taking into account all presented information, the use of higher 'effective' sorption coefficients for tritosulfuron is considered to be justified for this individual compound.

B.8.2.3 Evaluation of the sorption of BAS 635 H - tritosulfuron and metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5 considering the dependency of sorption to soil parameters and sorption kinetics

Annex Point:	IIA-7.1.2
Author:	Gottesbüren, B.
Title:	Evaluation of the sorption of BAS 635 H - Tritosulfuron and metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5 considering the dependency of sorption to soil parameters and sorption kinetics
Date:	2003-03-00
Doc ID:	2003/1005456 BOD2003-300
GLP:	No

Introduction

This section aims at the evaluation of the adsorption parameters of the herbicidal active ingredient BAS 635 H (tritosulfuron) and its soil metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5 with respect to
i) the dependencies of the adsorption to soil properties and
ii) the kinetics of sorption processes.

The significance of correlations of soil adsorption and soil properties were analysed statistically. Moreover, effective sorption parameters representing true equilibrium sorption coefficients of BAS 635 H and metabolites are presented.

B.8.2.3.1 Relations between the adsorption of BAS 635 H and metabolites BH 635-2, BH 635 3, BH 635-4 and BH 635-5 and soil properties

The relationship between adsorption parameters of tritosulfuron and metabolites and soil properties was evaluated in detail and it was analysed if significant 'true' correlations do exist.

Statistical analysis of the relation between adsorption of BAS 635 H and soil properties

Sorption parameters for tritosulfuron in 7 soils have already been documented in the monograph as listed in Table B.8.2.3-1.

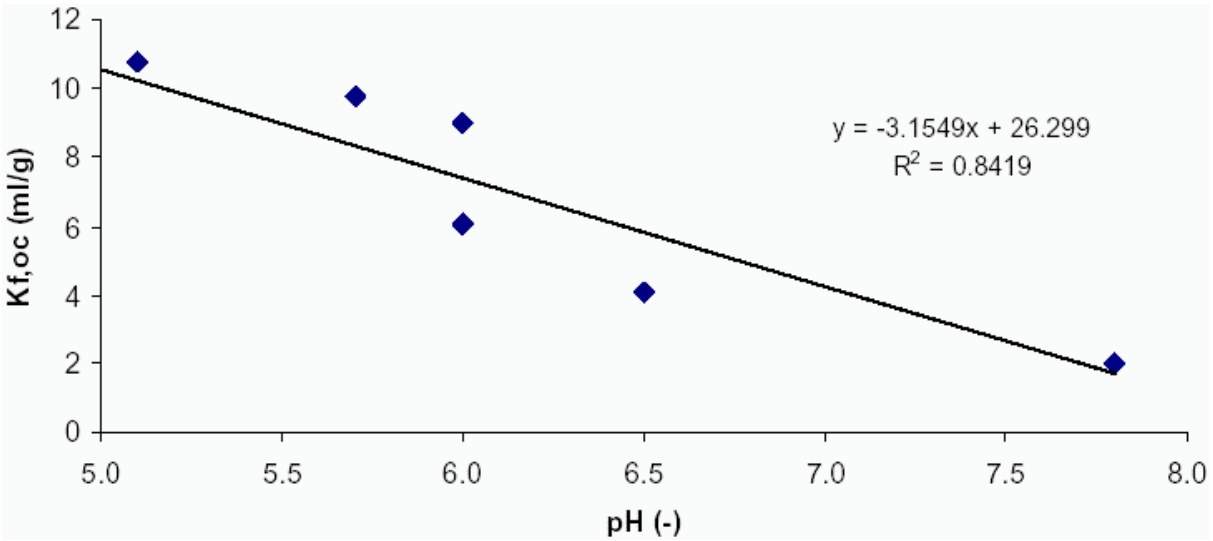
Table B.8.2.3-1: Adsorption of BAS 635 H in 7 different soils

Origin	Soil type (USDA)	$pH_{(CaCl2)}$ [-]	OC* [%]	Sand [%]	Silt [%]	Clay [%]	CEC** [meq/100 g]	K_f [mL/g]	1/n	$K_{f,oc}$ [mL/g]
LUFA2.1	sandy loam loamy sand	5.7	0.7	85	2	13	4.3	0.069	0.9551	0
LUFA2.2	sandy loam	6.0	2.6	79	7	14	11.5	0.158	0.945	6
LUFA2.3	sandy loam	6.5	1.5	68	17	15	11.4	0.062	0.980	4
Bruch Ost	cay loam	7.8	3.3	40	23	37	11.2	0.066	0.860	2
USA_538-30-5 Holly Springs	loamy sand	5.1	0.4	82	13	5	3.1	0.038	0.9611	1
USA_538-31-2 Greenville	silty loam	4.8	0.4	22	67	11	10.2	0.035	0.7611	0
Speyerer Wald	loamy sand	6.0	0.7	86	7	7	5	0.064	0.927	9

* OC = organic carbon
** CEC= cation exchange capacity

Graphical and statistical analysis of these sorption data lead to the first impression that the $K_{f,oc}$-values of BAS 635 H might be dependent on the pH-value (see Figure B.8.2.3-1), where $K_{f,oc}$-values are decreasing with increasing soil pH. This assumed dependency is statistically significant with a probability of 95 % (see also Table B.8.2.3-2).

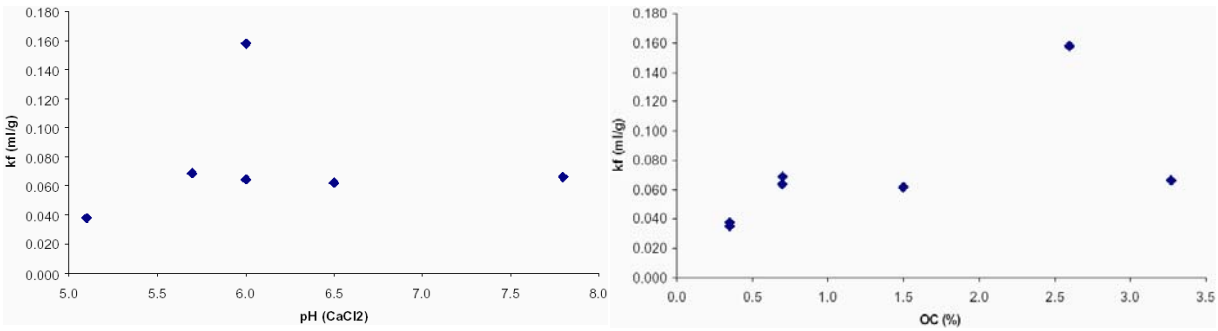
Figure B.8.2.3-1: $K_{f,oc}$-values of BAS 635 H and $pH_{(CaCl2)}$ values of the soils (n = 7, significant at p < 0.005)



In the following, the actual dependency of BAS 635 H adsorption on soil properties is investigated by means of a detailed statistical correlation and multiple stepwise regression analysis of the adsorption data and soil properties (as given in Table B.8.2.3-1), using the statistical package Statistica® (StatSoft Inc., 2001; http://www.statsoft.com).

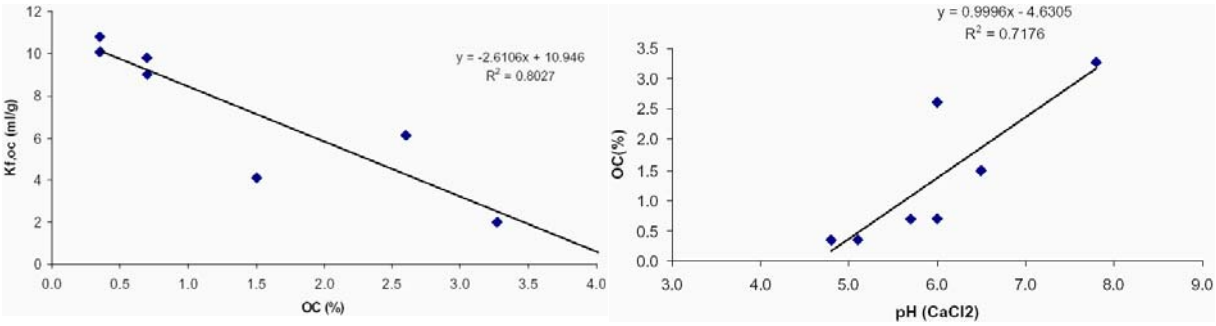
If the non-normalised K_f-values of tritosulfuron are plotted against the soil properties, no dependency of this sorption parameter from the pH-value is discernible (Figure B.8.2.3-2), which is in contrast to the apparent correlation observed for the $K_{f,oc}$-values. Further doubts on the applicability of the K_{oc}-concept (normalisation of sorption coefficients to the organic carbon content of the soil, implicitly assuming that the organic carbon is the most important sorbent therein) for BAS 635 H arise from the fact that the K_f-values are also not correlated to the organic carbon of the soils (Figure B.8.2.3-2 and Table B.8.2.3-2), whereas the $Kf._{oc}$-values are significantly negatively correlated to the organic carbon content (Figure B.8.2.3-3).

Figure B.8.2.3-2: left – Relation between K_f-values of BAS 635 H and the $pH_{(CaCl2)}$-values of the soils (n = 7, correlation not significant at p < 0.005)
right – Relation between the K_f-values of BAS 635 H and the organic carbon content (OC) of the soils (n = 7, correlation not significant at p < 0.005)



Since the organic carbon content of the soils used in the adsorption studies with BAS 635 H are positively correlated with the pH-values (see Figure B.8.2.3-3) it is therefore inevitable that the $Kf_{,oc}$-values of tritosulfuron become also correlated with the pH-values.

Figure B.8.2.3-3: left – Relation between the $K_{f,oc}$-values of BAS 635 H and the organic carbon content (OC) of the soils (n = 7, correlation significant at p < 0.005)
right – Relation between the organic carbon content (OC) of the soils and the $pH_{(CaCl2)}$-values of the soils from the sorption studies with tritosulfuron (n = 7; correlation significant at p < 0.005)



It can be concluded that the 'apparent' dependency of the $Kf_{,oc}$-values of tritosulfuron from the soil pH-values is not a 'true' correlation in a scientific sense (plausible cause-effect relation), but is caused by cross correlation and therefore virtual. The correlation matrix between the adsorption parameters of tritosulfuron and the soil characteristics as calculated with Statistica® is given in Table B.8.2.3-2.

Table B.8.2.3-2: Correlations (r) between the sorption parameters of BAS 635 H and soil characteristics (n = 7)

	OC [%]	$pH_{(CaCl2)}$	$pH_{(H2O)}$	Sand [%]	Silt [%]	Clay [%]	K_f [mL/g]	1/n	$K_{f,oc}$ [mL/g]
OC [%]	1.00								
$pH_{(CaCl2)}$	0.85*	1.00							
$pH_{(H2O)}$	0.83*	1.00*	1.00						
Sand [%]	-0.17	-0.09	-0.06	1.00					
Silt [%]	-0.20	-0.31	-0.33	-0.91	1.00				
Clay [%]	0.84*	0.86*	0.84*	-0.48	0.07	1.00			
K_f [mL/g]	0.60	0.25	0.24	0.34	-0.45	0.13	1.00		
1/n	0.03	0.14	0.15	0.90*	-0.90*	-0.26	0.32	1.00	
$K_{f,oc}$ [mL/g]	-0.90*	-0.92*	-0.90*	0.25	0.11	-0.84*	-0.34	-0.06	1.00

* significant correlation (r) with a probability of error of < 5 % (p < 0.005) – the coefficients of determination r^2 are given in the figures

It can be concluded that the K_f-values of tritosulfuron are not significantly correlated with a single soil characteristic alone. Therefore, a multiple stepwise regression was performed to evaluate if the sorption of tritosulfuron depends on multiple soil parameters. In the first step of this analysis, the soil parameter is included on which the dependent parameter K_f depends most strongly. In the following step, another parameter is included - the one which improves the correlation best. This process is iteratively continued, until the inclusion of additional parameters does not improve the correlation any more.
A significant correlation between K_f and the combined effect of the parameters %OC + %CLAY was achieved with the stepwise multiple regression. The significance level was 0.003 (F-test) and the coefficient of determination $r^2 = 0.83$, as given in Table B.8.2.3-3. The pH-value, for instance, did not significantly contribute to the improvement of the regression and was therefore not included.

Table B.8.2.3-3: Multiple stepwise regression between the K_f-values of tritosulfuron and soil properties

parameter	multiple r^2	r^2 change	F for incl/excl.	p-level	# of variables included
%OC	0.363	0.363	2.854	0.152	1
%CLAY	0.828	0.465	10.805	0.030	2

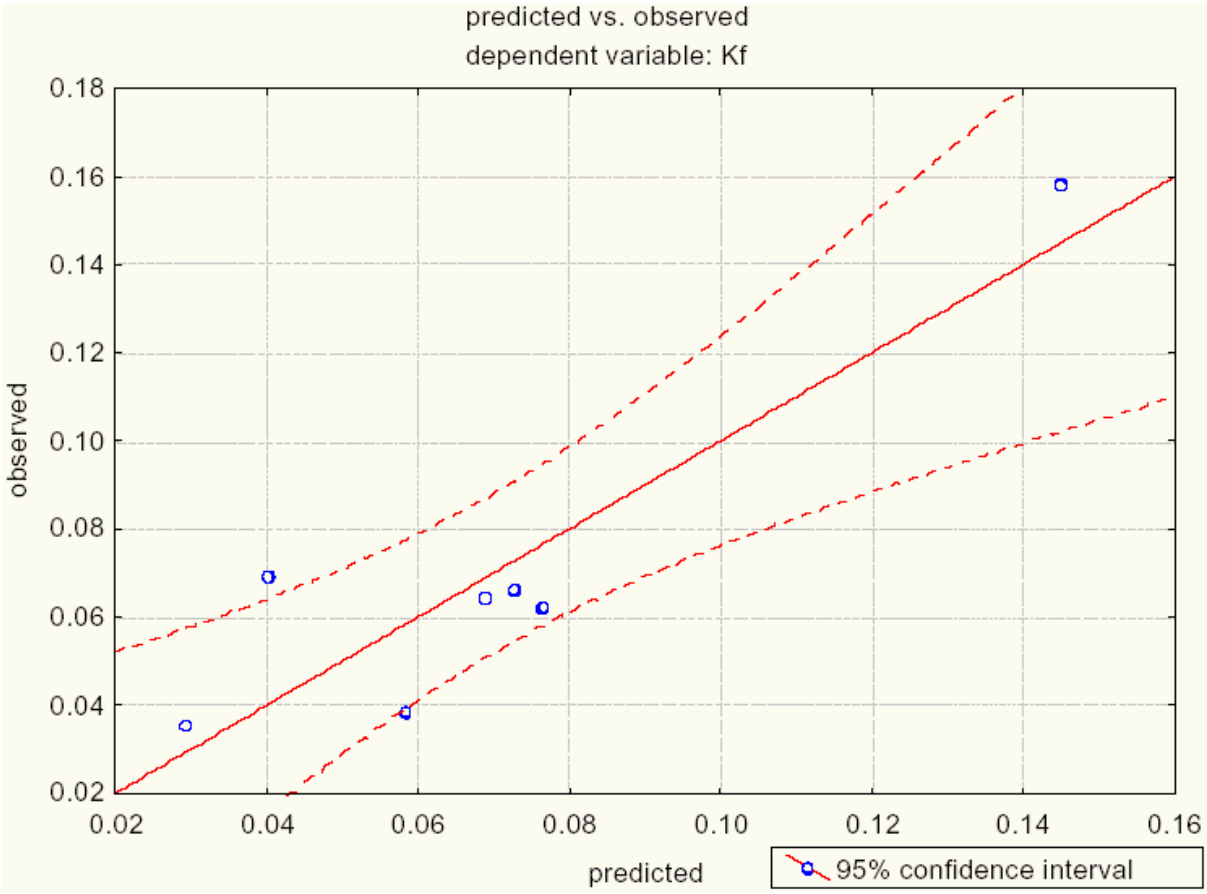
The parameters of the multiple regression equation are as follows:

$y = a + bx + cz$ with: $y = K_f$
x = %OC
z = %CLAY
a = constant
b and c = coefficients

$\Rightarrow Kf = a + b \times \%OC + c \times \%CLAY)$ with: $a = 0.062355 \pm 0.014155$ (p-level 0.012)
$b = 0.057837 \pm 0.013315$ (p-level 0.012)
$c = -0.004825 \pm 0.001468$ (p-level 0.030)

A comparison between the observed K_f-values of BAS 635 H and the predicted values using the multiple regression is shown in Figure B.8.2.3-4. It can be seen that the K_f-values of tritosulfuron are reasonably well predicted when the organic carbon plus the clay content of the soils are considered.

Figure B.8.2.3-4: Comparison between observed and predicted K_f-values of BAS 635 H



Additional experimental adsorption data set for BAS 635 H

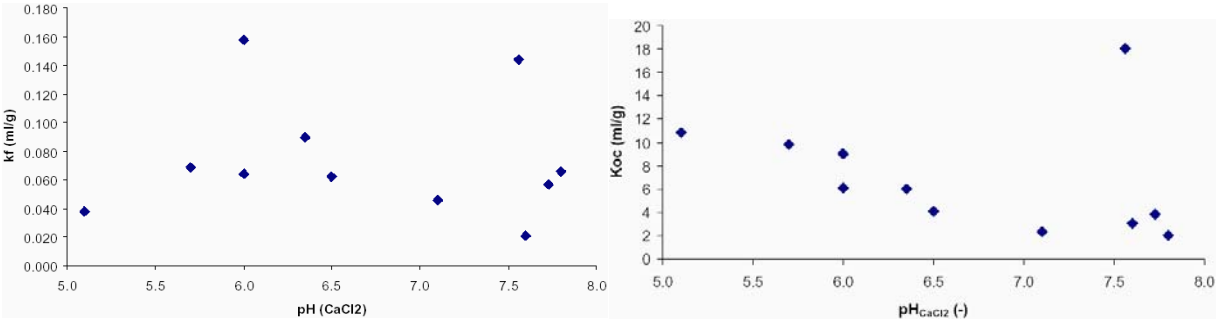
To improve the evaluation of the dependency of the sorption of BAS 635 H to soil properties, an additional sorption study was performed with 5 additional soils, putting the focus on higher pH-values as compared to the old studies (Richter, 2003; see Chapter B.8.2). The results of this study are compiled in Table B.8.2.3-4.

Table B.8.2.3-4: Adsorption of BAS 635 H in several soils with high pH-values (n = 5)

Origin	Soil type (German classification)	$pH_{(CaCl2)}$ [-]	OC [%]	Sand [%]	Silt [%]	Clay [%]	CEC [meq/100 g]	K_f [mL/g]	1/n [-]	$K_{f,oc}$ [mL/g]
Las Cabezas	clayey silt	7.6	0.8	11.1	76.5	22.4	30.5	0.144	1.215	18
Manzanilla	loamy sand	7.6	0.7	52.2	33.5	14.3	10.6	0.021	0.716	3
Almonacid de la Sierra	sandy loam	7.7	1.5	33.6	49	17.4	15	0.057	1.111	4
Nierstein	clayey silt	7.1	2.0	22.4	64.4	13.2	11.3	0.046	0.902	2
Ploudalmezeau	clayey silt	6.4	1.5	13.5	74.8	11.7	12	0.090	1.047	6

When the 5 ‘new’ soils are assessed together with the 7 ‘old’ soils, no dependency of K_f-values from soil pH can be seen. Moreover, also the previously postulated correlation between $K_{f,oc}$ and soil pH cannot be identified anymore (Figure B.8.2.3.-5).

**Figure B.8.2.3-5: left – Relation between the K_f-values of BAS 635 H and the $pH_{(CaCl2)}$-values of the soils (n = 12, data of the old and new studies)
right – Relation between the adsorption $K_{f,oc}$- values of BAS 635 H and the $pH_{(CaCl2)}$-values of the soils (n = 12, data of the old and new studies)**



Statistical analysis of the relation between adsorption of the soil metabolites of BAS 635 H and soil properties

Similar assessments as for the parent compound BAS 635 H have been performed also for the sorption of the soil metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5. In the following, the outcome of this assessment is presented in detail for BH 635-2, whereas for the other metabolites, documentation of result is limited to the tabular form.

Adsorption coefficients for the metabolite BH 635-2

Sorption parameters for metabolite BH 635-2 in 7 soils have already been documented in the monograph and are listed in Table B.8.2.3-5.

Table B.8.2.3-5: Adsorption of BH 635-2 in different soils (n = 7)

Origin	Soil type (USDA)	$pH_{(CaCl2)}$ [-]	OC* [%]	Sand [%]	Silt [%]	Clay [%]	CEC** [meq/100 g]	K_f [mL/g]	1/n	$K_{f,oc}$ [mL/g]
USA 538-31-2 Greenville	silty loam	5.4	0.35	22	67	11	10.2	0.278	0.98	79
Lihof Bruch West	sandy loam	7.5	1.8	54	29	17	16	0.291	0.942	16
LUFA 2.3	sandy loam	6.5	1	67	19	14	7.7	0.332	0.921	33
LUFA 2.2	sandy loam	5.8	1.8	78	11	11	12.1	0.517	0.943	29
USA 538-30-5 Holly Springs	loamy sand	6.7	0.35	82	13	5	3.1	0.179	0.964	51
LUFA 2.1	loamy sand	5.8	0.5	83	6	11	3.1	0.195	0.978	39
Speyrer Wald	loamy sand	6.0	0.7	86	7	7	5	0.255	0.972	36

* OC = organic carbon
** CEC= cation exchange capacity

Based on these data, a significant dependency of the $K_{f,oc}$-values for BH 635-2 from the pH-values of the soils, as tentatively might be seen in Figure B.8.2.3-6, cannot be confirmed by the correlation analysis given in Table B.8.2.3-6.

Figure B.8.2.3-6: Relation between the $K_{f,oc}$-values of BH 635-2 and the $pH_{(CaCl2)}$-values of the soils (n=7; not significant at p < 0.005)

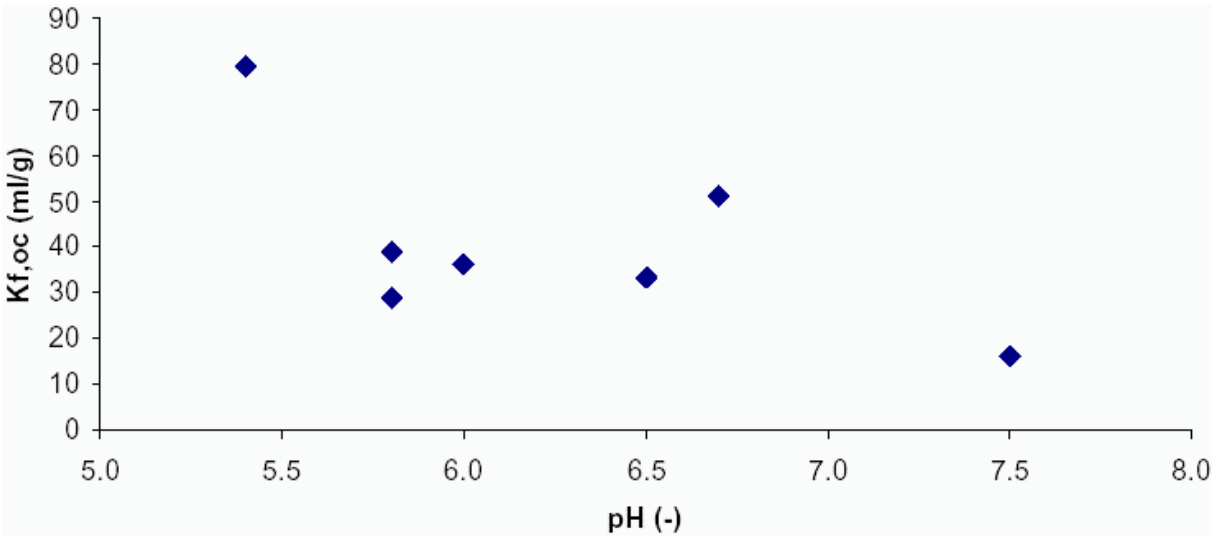


Table B.8.2.3-6: Correlations (r) between the adsorption parameters of BH 635-2 and soil properties (n = 7)

	OC [%]	$pH_{(CaCl2)}$	Sand [%]	Silt [%]	Clay [%]	CEC [meq/ 100 g]	K_f [mL/g]	1/n	$K_{f,oc}$ [mL/g]
OC [%]	1.00	0.44	0.05	-0.17	0.64	0.80*	0.75	-0.68	-0.77
$pH_{(CaCl2)}$		1.00	0.12	-0.20	0.38	0.34	-0.16	-0.54	-0.62
Sand [%]			1.00	-0.99*	-0.44	-0.55	-0.05	-0.09	-0.54
Silt [%]				1.00	0.28	0.44	-0.01	0.20	0.66
Clay [%]					1.00	0.75*	0.35	-0.56	-0.44
CEC [meq/ 100 g]						1.00	0.61	-0.46	-0.32

* correlation significant at p < 0.005

Additional experimental adsorption data set for BH 635-2

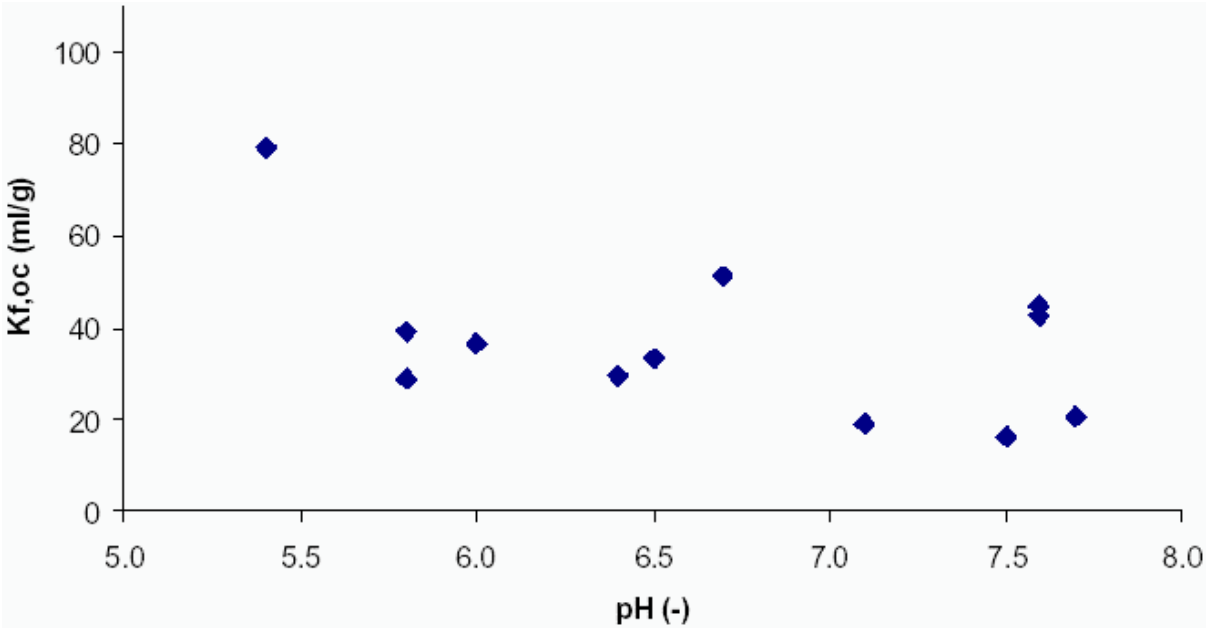
To improve the evaluation of the dependency of the sorption of the metabolite BH 635-2 to soil properties, an additional sorption study was performed with 5 additional soils, putting the focus on higher pH-values (pH_{CaCl2} 6.4-7.7) as compared to the old studies (Zirnstein, 2003; see Chapter B.8.2). The results of this study are compiled in Table B.8.2.3-7.

Table B.8.2.3-7: Adsorption of BH 635-2 in several soils with high pH-values (n = 5)

Origin	Soil type (German classification)	$pH_{(CaCl2)}$ [-]	OC [%]	Sand [%]	Silt [%]	Clay [%]	CEC [meq/100 g]	K_f [mL/g]	1/n [-]	$K_{f,oc}$ [mL/g]
Las Cabezas	Clayey silt	7.6	0.8	11.1	76.5	22.4	30.5	0.358	0.876	45
Manzanilla	loamy sand	7.6	0.7	52.2	33.5	14.3	10.6	0.297	0.883	42
Almonacid de la Sierra	sandy loam	7.7	1.5	33.6	49	17.4	15	0.304	0.937	20
Nierstein	Clayey silt	7.1	2.0	22.4	64.4	13.2	11.3	0.38	0.924	19
Ploudalmezeau	Clayey silt	6.4	1.5	13.5	74.8	11.7	12	0.44	0.919	29

When the 5 'new' soils are assessed together with the 7 'old' soils, no dependency of $K_{f,oc}$-values from soil pH can be seen (Figure B.8.2.3-7).

Figure B.8.2.3-7: Relation between the $K_{f,oc}$-values of BH 635-2 and the $pH_{(CaCl2)}$-values (n=12)



Statistical analysis of the relation between adsorption of the soil metabolites BH 635-3, BH 635-4 and BH 635-5 and soil properties

Sorption parameters for metabolites BH 635-3, BH 635-4 and BH 635-5 in 7 soils have already been documented in the monograph and are listed in Table B.8.2.3-8 to Table B.8.2.3-10.

Table B.8.2.3-8: Adsorption of BH 635-3 in different soils (n = 7)

Origin	Soil type (USDA)	$pH_{(CaCl2)}$ [-]	OC* [%]	Sand [%]	Silt [%]	Clay [%]	CEC** [meq/100 g]	K_f [mL/g]	1/n	$K_{f,oc}$ [mL/g]
USA 538-31-2 Greenville	silty loam	5.4	0.35	22	67	11	10.2	0.177	0.892	51
Lihof Bruch West	sandy loam	7.5	1.8	54	29	17	16	0.323	0.927	18
LUFA2.3	sandy loam	6.5	1	67	19	14	7.7	0.228	0.897	23
LUFA 2.2	sandy loam	5.8	1.8	78	11	11	12.1	0.421	0.935	23
USA 538-30-5 Holly Springs	loamy sand	6.7	0.35	82	13	5	3.1	0.119	0.854	34
LUFA 2.1	loamy sand	5.8	0.5	83	6	11	3.1	0.114	0.910	23
Speyrer Wald	loamy sand	6.0	0.7	86	7	7	5	0.232	0.970	33

* OC = organic carbon
** CEC= cation exchange capacity

Table B.8.2.3-9: Adsorption of BH 635-4 in different soils (n = 7)

Origin	Soil type (USDA)	$pH_{(CaCl2)}$ [-]	OC* [%]	Sand [%]	Silt [%]	Clay [%]	CEC** [meq/100 g]	K_f [mL/g]	1/n	$K_{f,oc}$ [mL/g]
USA 538-31-2 Greenville	silty loam	5.4	0.5	22	67	11	10.2	0.642	0.914	183
Lihof Bruch West	sandy loam	7.5	1.5	54	29	17	16	0.319	0.958	18
LUFA 2.3	sandy loam	6.5	1	67	19	14	7.7	0.609	0.901	61
LUFA2.2	sandy loam	5.8	2.5	78	11	11	12.1	1.466	0.94	81
USA 538-30-5 Holly Springs	loamy sand	6.7	0.4	82	13	5	3.1	0.407	0.899	116
LUFA 2.1	loamy sand	5.8	0.7	83	6	11	3.1	0.443	0.916	89
Speyrer Wald	loamy sand	6.0	0.7	86	7	7	5	0.521	0.936	74

* OC = organic carbon
** CEC= cation exchange capacity

Table B.8.2.3-10: Adsorption of BH 635-4 in different soils (n = 7)

Origin	Soil type (USDA)	$pH_{(CaCl2)}$ [-]	OC* [%]	Sand [%]	Silt [%]	Clay [%]	CEC** [meq/100 g]	K_f [mL/g]	1/n	$K_{f,oc}$ [mL/g]
USA 538-31-2 Greenville	Silty loam	5,4	0.5	22	67	11	10.2	0.287	0.971	57
Lihof Bruch West	sandy loam	7.5	1.5	54	29	17	16	0.123	0.933	8
LUFA 2.3	sandy loam	6.5	1	67	19	14	7.7	0.180	0.898	18
LUFA 2.2	sandy loam	5.8	2.5	78	11	11	12.1	0.249	0.951	10
USA 538-30-5 Holly Springs	loamy sand	6.7	0.4	82	13	5	3.1	0.094	0.910	24
LUFA 2.1	loamy sand	5.8	0.7	83	6	11	3.1	0.092	0.900	13
Speyrer Wald	loamy sand	6.0	0.7	86	7	7	5	0.108	0.982	15

* OC = organic carbon
** CEC= cation exchange capacity

Based on the data in Table B.8.2.3-8 to Table B.8.2.3-10, a significant dependency of the $K_{f,oc}$-values for BH 635-3, BH 635-4 or BH 635-5 from the pH-values of the soils cannot be confirmed by the correlation analyses given in Table B.8.2.3-11 to Table B.8.2.3-13.

Table B.8.2.3-11: Correlations (r) between the adsorption parameters of BH 635-3 and soil properties (n = 7)

	OC [%]	$pH_{(CaCl2)}$	Sand [%]	Silt [%]	Clay [%]	CEC [meq/ 100 g]	K_f [mL/g]	1/n	$K_{f,oc}$ [mL/g]
OC [%]	1.00	0.44	0.05	-0.17	0.64	0.80*	0.93*	0.46	-0.68
$pH_{(CaCl2)}$		1.00	0.12	-0.20	0.38	0.34	0.16	-0.11	-0.58
Sand [%]			1.00	-0.99*	-0.44	-0.55	-0.01	0.23	-0.55
Silt [%]				1.00	0.28	0.44	-0.07	-0.28	0.68
Clay [%]					1.00	0.75*	0.45	0.18	-0.49
CEC [meq/ 100 g]						1.00	0.78*	0.28	-0.21

* correlation significant at $p < 0.005$

Table B.8.2.3-12: Correlations (r) between the adsorption parameters of BH 635-4 and soil properties (n = 7)

	OC [%]	$pH_{(CaCl2)}$	Sand [%]	Silt [%]	Clay [%]	CEC [meq/ 100 g]	K_f [mL/g]	1/n	$K_{f,oc}$ [mL/g]
OC [%]	1.00	0.44	0.05	-0.17	0.64	0.80*	0.46	0.75	-0.70
$pH_{(CaCl2)}$		1.00	0.12	-0.20	0.38	0.34	-0.47	0.29	-0.72
Sand [%]			1.00	-0.99*	-0.44	-0.55	0.08	-0.03	-0.46
Silt [%]				1.00	0.28	0.44	-0.08	-0.05	0.59
Clay [%]					1.00	0.75*	-0.02	0.45	-0.50
CEC [meq/ 100 g]						1.00	0.29	0.70	-0.32

* correlation significant at $p < 0.005$

Table B.8.2.3-13: Correlations (r) between the adsorption parameters of BH 635-5 and soil properties (n = 7)

	OC [%]	$pH_{(CaCl2)}$	Sand [%]	Silt [%]	Clay [%]	CEC [meq/ 100 g]	K_f [mL/g]	1/n	$K_{f,oc}$ [mL/g]
OC [%]	1.00	0.29	0.26	-0.23	-0.31	0.66	0.36	0.11	-0.51
$pH_{(CaCl2)}$		1.00	0.16	-0.25	0.2CL	0.37	-0.37	-0.34	-0.61
Sand [%]			1.00	-0.99*	-0.86*	-0.50	-0.68	-0.32	-0.86*
Silt [%]				1.00	0.79*	0.49	0.72	0.36	0.88*
Clay [%]					1.00	0.46	0.43	0.10	0.62
CEC [meq/ 100 g]						1.00	0.67	0.33	0.09

* correlation significant at $p < 0.005$

B.8.2.3.2 Evaluation of the kinetic sorption behaviour of BAS 635 H and of metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5

A detailed analysis of the kinetic sorption behaviour of BAS 635 H and of metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5 was carried out by Jene (2002 and 2003) and is documented in this addendum under B.8.2.2. It could be shown that the original adsorption/desorption studies revealed a considerable sorption hysteresis, indicating that an adsorption equilibrium had not been achieved in the course of the study.

The original and the effective equilibrium sorption parameters of BAS 635 H in 7 different soils are listed synoptically in Table B.8.2.3-14. The results of the additional 'new' studies from Richter (2003) had not been available when the kinetic sorption model was set up and could not be included. The kinetic evaluation showed that the expected $K_{f,oc}$-values under long-term conditions for BAS 635 H were at least by a factor of 3 higher than under short-term (16 h and 4 h) conditions.

Table B.8.2.3-14: Original and effective equilibrium sorption parameters of BAS 635 H in 7 different soils

Origin	soil type (USDA)	$pH_{(CaCl2)}$	OC* [%]	'original' parameters			'effective' parameters		
				K_f [mL/g]	$K_{f,oc}$ [mL/g]	1/n	K_f [mL/g]	$K_{f,oc}$ [mL/g]	1/n
LUFA2.1	sandy loam loamy sand	5.7	0.7	0.069	10	0.955	0.239	34.1	0.93
LUFA 2.2	sandy loam	6.0	2.6	0.158	6	0.945	0.638	24.5	0.93
LUFA 2.3	sandy loam	6.5	1.5	0.062	4	0.980	0.287	19.1	1.00
Bruch Ost	clay loam	7.8	3.3	0.066	2	0.860	0.243	7.4	0.86
USA_538-30-5 Holly Springs	loamy sand	5.1	0.4	0.038	11	0.961	0.225	64.3	0.91
USA_538-31-2 Greenville	silty loam	4.8	0.4	0.035	10	0.761	0.235	49.8	1.00
Speyerer Wald	loamy sand	6.0	0.7	0.064	9	0.927	0.202	28.8	0.93

* OC = organic carbon

Due to the significant sorption hysteresis, the kinetic parameters could also be well estimated for all metabolites. The kinetic evaluation showed that the K_f-values under long-term (equilibrium) conditions were approximately by a factor of 2 higher than under short-term laboratory conditions (16 h and 24 h). The estimated equilibrium Freundlich sorption coefficients are given in Table B.8.2.3-15 for metabolites BH 635-2, BH 635-3 and BH 635-4 and in Table B.8.2.3-16 for metabolite BH 635-5.

Table B.8.2.3-15: Equilibrium sorption parameters of metabolites 635M02 (BH 635-2), 635M03 (BH 635-3) and 635M01 (BH 635-4)

Soil	OC	BH 635-2			BH 635-3			BH 635-4		
	[%]	K_f [mL/g]	$K_{f,oc}$ [mL/g]	1/n [-]	K_f [mL/g]	$K_{f,oc}$ [mL/g]	1/n [-]	K_f [mL/g]	$K_{f,oc}$ [mL/g]	1/n [-]
LUFA Speyer 2.1	0.5	0.391	78.2	0.946	0.145	29.0	0.948	0.521	104.2	0.957
LUFA Speyer 2.2	1.8	0.912	50.7	0.932	0.617	34.3	0.942	2.14	118.9	0.976
LUFA Speyer 2.3	1	0.836	83.6	0.93	0.726	72.6	0.966	1.63	163.0	0.947
Li. -hof Bruch West	1.8	0.482	26.8	0.945	0.687	38.2	0.972	0.825	45.8	0.963
USA 538-30-5	0.35	0.434	124.0	0.881	0.264	75.4	0.922	0.82	234.3	1
USA 538-31 -2	0.35	0.497	142.0	0.871	0.254	72.6	0.898	1.46	417.1	0.951
Speyrer Wald	0.7	0.555	79.3	0.957	0.56	80.0	0.95	1.07	152.9	0.908

Table B.8.2.3-16: Equilibrium sorption parameters of metabolite 635M04 (BH 635-5)

Soil	OC	BH 635-5		
	[%]	K_f [mL/g]	$K_{f,oc}$ [mL/g]	1/n [-]
LUFA Speyer 2.1	0.7	0.274	39.1	0.932
LUFA Speyer 2.2	2.5	0.513	20.5	0.961
LUFA Speyer 2.3	1.0	0.502	50.2	0.967
Li. -hof Bruch West	1.5	0.288	19.2	0.946
USA 538-30-5	0.4	0.226	56.5	0.926
USA 538-3 1-2	0.5	0.466	93.2	0.983
Speyrer Wald	0.7	0.217	31.0	0.962

B.8.2.3.3 Overall conclusions

The sorption parameters of BAS 635 H and its metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5 are not depending on soil pH. The lysimeter studies are therefore reliable higher tier experiments.
The effective equilibrium sorption parameters as determined under the assumption of kinetically controlled sorption are the most reliable estimates of relevant sorption of BAS 635 H and its metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5 in soil. They can therefore be used for the assessment of the predicted environmental concentrations in groundwater (PEC_{gw}) using the FOCUS models.

Comment of RMS:

The RMS agrees with the notifier's conclusion that adsorption of tritosulfuron is not pH-dependent. Therefore, leaching to groundwater can reliably be assessed based on the lysimeter studies.

With respect to the coefficients for kinetic/aged sorption derived from complex sorption modelling, it is stated that there is currently no agreed position on EU level on the appropriateness of such approaches for regulatory purposes. Therefore, assessment needs to be done on a case-by-case basis. In the case of tritosulfuron, the conclusion is supported that the two-stage non-equilibrium model appropriately describes the actual sorption of the compound in soil. With regard to the tritosulfuron metabolites, the appropriateness of this approach also appears plausible. Nevertheless, it must be stated that fewer experimental data than for the parent compound are available to support that assumption; therefore a higher amount of uncertainty remains with the corresponding 'effective' sorption coefficients.

B.8.2.4 Non-identified radioactivity in lysimeter leachates of lysimeters treated with BAS 635 H

Annex Point:	IIA-7.1.3.3
Author:	Staudenmaier, H.
Title:	Non-identified radioactivity in lysimeter leachates of lysimeters treated with BAS 635 H
Date:	2003-07-17
Doc ID:	2003/1009267; Li 740 BOD2003-301
GLP:	No

The total number and maximum concentration of individual chromatographic peaks of the non-identified radioactivity (NIR) in the leachate of lysimeters treated with BAS 635 H was re-investigated exemplary with lysimeter 18 of the study Staudenmaier (2001) "Outdoor lysimeter study with ^{14}C-BAS 635 H" (see monograph). This was the only double-treated BAS 635 H lysimeter and it showed the highest concentration of NIR of all lysimeters treated with BAS 635 H.

In addition to the identified compounds described in the original report, a total of 16 chromatographic peaks were summarised as NIR. However, at least five of these "peaks" were actually no peaks of individual compounds but represent more than one compound being integrated as a single 'region of interest' (ROI).

The average concentrations over the three year study duration of all of these ROIs, calculated as parent compound equivalents, were lower than 0.1 µg/L (max. 0.086 µg/L). 14 out of these 16 chromatographic ROIs had annual average concentrations of less than 0.1 µg/L (max. 0.084 µg/L). The two remaining ROIs were no individual compound peaks but regions consisting of more than one actual peak with the following total annual average concentrations (also given as equivalents of parent compound):

Region 1 (t_R = 4.6 min): 0.293 µg/L in the first year
Region 3 (t_R = 5.5 min): 0.110 µg/L in the second year

In the other experimental years these regions were clearly below 0.1 µg/L.

That means that the non-identified radioactivity can be attributed to very minor individual peaks and to a couple of inhomogeneous chromatographic regions, in which highly polar compounds of low molecular weight are to be expected.

The concentrations of the above regions are calculated as equivalents of parent compound. If one assumes a twofold to threefold lower molecular weight for these highly polar compounds as compared to the active substance, which is considered a reasonably conservative estimate in this case, the actual concentrations even of the above mentioned regions are below 0.1 µg/L in yearly average.

Further detailed characterisation of the polar radioactivity was not feasible with the material of individual samplings. Therefore selected samples of leachate water were combined for further investigation. The unidentified chromatographic region between about 4 and 20 min, which originally comprised 9 ROIs, was isolated from the combined samples. With some changes in the chromatographic system, these 9 ROIs were separated into at least 19 peaks. However, due to the mixing of several leachate samples, an exact quantification of individual peaks was no longer possible.

In a further attempt, subfractions of the radioactivity between 4 and 20 min (4-9 min, 9-15 min and 15-20 min) were chromatographed on a different system (Polar RP) with the result that also here a further separation into more peaks than before was achieved for each subfraction. It was concluded that the polar unidentified radioactivity – especially the regions that obviously consisted of more than one compound – could be further separated into different fractions. Taking into account the great number of polar chromatographic peaks and the expected molecular weight for those compounds, very low concentrations for individual polar compounds are to be expected.

Comment of RMS:

Since the lysimeter study was conducted with phenyl-labelled tritosulfuron, it can be assumed in a first approach that the molecules contributing to the non-identified radioactivity still contain this moiety. It can further be assumed that these molecules will have a lower molecular mass than the identified metabolite BH 635-2 (225.2 g/mol). This is about half of the molecular mass of tritosulfuron (445.3 g/mol). If a respective factor would be applied to the calculated concentration of 0.297 µg/L for Region 1 in the first year, the resulting value of 0.150 µg/L would still be above 0.1 µg/L. However, taking into account the relatively short retention time in RP-HPLC, which typically also corresponds to smaller molecule sizes, and the composition of Region 1 of more than one compound, the notifier's reasoning can be accepted that the concentrations of individual compounds in lysimeter leachates did not exceed 0.1 µg/L.

B.8.3 Predicted environmental concentrations in soil (PECs) (Annex IIIA 9.1.3)

B.8.3.1 Calculation of PECsoil for tritosulfuron and metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5 using worst case half-lives

Annex Point:	IIA-9.1.3
Author:	Hauck, T.
Title:	Calculation of predicted environmental concentrations for BAS 635 H (tritosulfuron) and metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5 in soil using worst case half-lives
Date:	2002-02-07
Doc ID:	BASF 2002/1000216 BOD2002-195
GLP:	No

Initial ($PEC_{s,ini}$), short- and long-term actual and time-weighted average environmental concentrations ($PEC_{s,act}$ and $PEC_{s,twa}$) in soil were calculated for the herbicidal active parent compound BAS 635 H (tritosulfuron) and its metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5 after application of the active substance with the maximum annual application rate of 0.05 kg as/ha.

Environmental concentrations were predicted with worst-case field and, if no field data were available, worst-case laboratory half-lives for parent and metabolites. The half-lives for BAS 635 H and its metabolite BH 635-2 and BH 635-4 were derived from field studies conducted in Germany. Degradation rates of BH 635-3 and BH 635-5 were taken from two different laboratory studies. The formation fraction for each metabolite was assumed to be 100 % of the parent compound (worst-case assumption). A correction factor was used to account for the different molecular weights of the metabolites in relation to the active compound (BH 635-2, factor 0.51; BH 635-3, factor 0.70; BH 635-4, factor 0.79; BH 635-5, factor 0.44).

The predicted environmental concentrations in soil were calculated for the maximum annual application amount applied onto cereals (0.05 kg as/ha) considering no interception. Initial PEC_{soil} values were calculated assuming an even distribution to a depth of 5 cm in soil with a bulk density of 1.5 kg/dm^3. Actual and time-weighted average PECs were calculated using the PEC_{ini} values and the worst-case half-lives as described above. Results are shown in Table B.8.3.1-1.

Table B.8.3.1-1: Calculated initial and long-term (after 100 days) PEC_{soil} values of tritosulfuron and metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5

compound	DT_{50} [d]	$PEC_{s,ini}$ [mg/kg]	$PEC_{s,act}$ after 100 days [mg/kg]	$PEC_{s,twa}$ after 100 days [mg/kg]
tritosulfuron	21[1)]	0.067	0.002	0.019
BH 635-2	216[1)]	0.034	0.025	0.029
BH 635-3	600[2,3)]	0.047	0.042	0.044
BH 635-4	600[1,3)]	0.053	0.047	0.050
BH 635-5	98[2)]	0.029	0.014	0.021

1) data from field study
2) data from laboratory study
3) no significant decline observed after 1 year, probably due to continuing formation; therefore half-life assumed to be 600 d

In Table B.8.3.1-2 to Table B.8.3.1-6, the detailed results of the $PEC_{s,act}$ and $PEC_{s,twa}$ calculations are provided for each compound separately.

Table B.8.3.1-2: Calculated actual and time-weighted average concentrations of tritosulfuron in soil

	day after treatment [d]	$PEC_{s,act}$ [mg/kg]	$PEC_{s,twa}$ [mg/kg]
Initial	0	0.067	0.067
Short-term	1	0.065	0.066
	2	0.062	0.065
	3	0.060	0.063
	4	0.058	0.062
Long-term	7	0.053	0.060
	14	0.042	0.053
	21	0.033	0.048
	28	0.026	0.044
	50	0.013	0.033
	100	0.002	0.019

Table B.8.3.1-3: Calculated actual and time-weighted average concentrations of BH 635-2 in soil

	day after treatment [d]	$PEC_{s,act}$ [mg/kg]	$PEC_{s,twa}$ [mg/kg]
Initial	0	0.034	0.034
Short-term	1	0.034	0.034
	2	0.034	0.034
	3	0.034	0.034
	4	0.034	0.034
Long-term	7	0.033	0.034
	14	0.033	0.033
	21	0.032	0.033
	28	0.031	0.033
	50	0.029	0.031
	100	0.025	0.029

Table B.8.3.1-4: Calculated actual and time-weighted average concentrations of BH 635-3 in soil

	day after treatment [d]	$PEC_{s,act}$ [mg/kg]	$PEC_{s,twa}$ [mg/kg]
Initial	0	0.047	0.047
Short-term	1	0.047	0.047
	2	0.047	0.047
	3	0.047	0.047
	4	0.046	0.047
Long-term	7	0.046	0.046
	14	0.046	0.046
	21	0.046	0.046
	28	0.045	0.046
	50	0.044	0.045
	100	0.042	0.044

Table B.8.3.1-5: Calculated actual and time-weighted average concentrations of BH 635-4 in soil

	day after treatment [d]	$PEC_{s,act}$ [mg/kg]	$PEC_{s,twa}$ [mg/kg]
Initial	0	0.053	0.053
Short-term	1	0.053	0.053
	2	0.053	0.053
	3	0.052	0.053
	4	0.052	0.053
Long-term	7	0.052	0.052
	14	0.052	0.052
	21	0.051	0.052
	28	0.051	0.052
	50	0.050	0.051
	100	0.047	0.050

Table B.8.3.1-6: Calculated actual and time-weighted average concentrations of BH 635-5 in soil

	day after treatment [d]	$PEC_{s,act}$ [mg/kg]	$PEC_{s,twa}$ [mg/kg]
Initial	0	0.029	0.029
Short-term	1	0.029	0.029
	2	0.029	0.029
	3	0.029	0.029
	4	0.029	0.029
Long-term	7	0.028	0.029
	14	0.027	0.028
	21	0.025	0.027
	28	0.024	0.027
	50	0.021	0.025
	100	0.014	0.021

Comment of RMS:
The calculations are acceptable. The initial PEC_{soil} values have been used in the assessment of the acute risk to earthworms (see below).

B.8.3.2 Predicted long term concentrations in soil of the soil metabolites of tritosulfuron (635M01, 635M02, 635M03, 635M04) (Dressel, 2003a)

Annex Point: IIA-9.1.3
Author: Dressel, J.
Title: Predicted long term concentrations of the soil metabolites of BAS 635 H (635M01, 635M02, 635M03, 635M04) in soil
Date: 2003-07-18
Doc ID: 2003/1009263; CALC-452
BOD2003-306
GLP: No

Concern on the persistence of the soil metabolites of BAS 635 H (tritosulfuron) was raised by ECCO. An accumulation of the metabolites 635M01 (BH 635-4), 635M02 (BH 635-2), 635M03 (BH 635-3), and 635M04 (BH 635-5) over several years of application would lead to a higher exposure of soil dwelling organisms. Since the metabolites are truly degrading only moderately fast but at the same time show low to moderate adsorption to soil a long term assessment needs to account for both dissipation processes: Soil degradation and displacement of the metabolites.
The assessment was conducted with the simulation model FOCUS PELMO 3.3.2 which is able to simulate the complete degradation pathway of BAS 635 H. An annual application of 0.05 kg as/ha winter cereals was assumed over a calculation period of 26 years using soil and daily weather data of three FOCUS scenarios (Châteaudun, Hamburg, Sevilla). A realistic worst-case combination of parameters was chosen: The fastest possible formation of the metabolites (shortest field half-life of BAS 635 H), average formation fractions (from field studies) and longest field half-lives of the metabolites. In order not to overestimate the displacement of the substances, effective equilibrium Freundlich soil adsorption parameters

($K_{f,OC}$, 1/n) were used, that had been estimated from the adsorption and the desorption branches of the standard soil adsorption/desorption study.
Daily soil concentrations were extracted from the model output for all 5-cm soil layers and the overall average concentration over 26 years as well as the average of the annual peak concentrations were calculated. The concentration in the 0-10 cm soil layer was assumed to be a conservative estimator for long term soil concentration assessment, since the topsoil is regularly mixed by soil tillage.

Simulation model and modelled scenarios

The simulation model FOCUS PELMO 3.3.2 was used in this study. It accounts for soil transformation and the vertical movement of an active substance and up to 8 metabolites. PELMO simulates water transport with a capacity based approach. Solute transport is modelled using the convection-dispersion equation (chromatographic transport). Three FOCUS scenarios were selected as examples for weather conditions throughout Europe: Châteaudun, Hamburg, and Sevilla (see Table B.8.3.2-1). These scenarios were run for the standard period of 26 years with annual application of BAS 635 H. The scenario definitions were slightly modified, in order to produce a daily output of soil concentrations for each 5-cm soil layer. The course of the concentration curve, the average soil concentration over the 26 years as well as the average of the annual maximum concentrations were extracted from the data.

BAS 635 H is intended to be used at an annual rate of 1 × 0.05 kg as/ha in cereals (winter cereals: BBCH 21-39, spring cereals: BBCH 13-39) or maize (in spring: BBCH 13-39). In winter cereals, a pesticide interception by the plant canopy of 50 %, in spring cereals and maize, a crop interception of 25 % can be assumed according to FOCUS. To achieve a realistic worst-case scenario for soil concentrations after application of BAS 635 H, application dates for winter cereals were therefore combined with a crop interception of 25 %. Thus, 0.0375 kg/ha BAS 635 H reach the soil surface during application and contribute to soil residues.

Table B.8.3.2-1: Crop-related model input parameters for BAS 635 H

Input parameter	Unit	Scenario	
Crop	-	Winter cereals	
Application dates	-	Châteaudun:	April, 1st
		Hamburg:	April, 1st
		Sevilla:	March, 10th
Application rate	kg/ha	0.05	
Crop interception	%	25	
Amount reaching soil	kg/ha	0.0375	

Soil degradation of BAS 635 H and formation and degradation of its soil metabolites

The soil metabolisation pathway as implemented in the simulation model PELMO is given in Figure B.8.3.2-1. Regarding the dissipation of an active substance in soil, field dissipation trials are considered to give the most realistic description of the process. However, if it is intended to use dissipation parameters from field studies in FOCUS modelling, it must be ensured that of the possible dissipation pathways (e.g. degradation, volatilisation, photodecomposition, leaching) all pathways except soil degradation are negligible. Then,

half-lives for field formation and degradation can be derived from the course of the formation and dissipation curve. Such assessment had been documented in the monograph for 7 field soil dissipation studies (4 US-studies, 3 EU-studies). Differences in soil temperature and soil moisture were accounted for by normalisation of the transformation rates based on daily average temperature and soil moisture values. This procedure resulted in the standardised field half-lives of BAS 635 H as listed in Table B.8.3.2-2. During the degradation of BAS 635 H, three metabolites are formed directly: 635M01 (BH 635-4), 635M02 (BH 635-2) and 635M04 (BH 635-5) with average normalised molar formation fractions of 17.1 %, 16.5 % and 32.9 %, respectively. Metabolite 635M03 (BH 635-3) is formed by 100 % transformation from 635M01 (BH 635-4). The standardised half-lives of the metabolites are listed below the values of the active substance in Table B.8.3.2-2.

Figure B.8.3.2-1: Metabolisation pathway of BAS 635 H in soil as implemented in the PELMO model

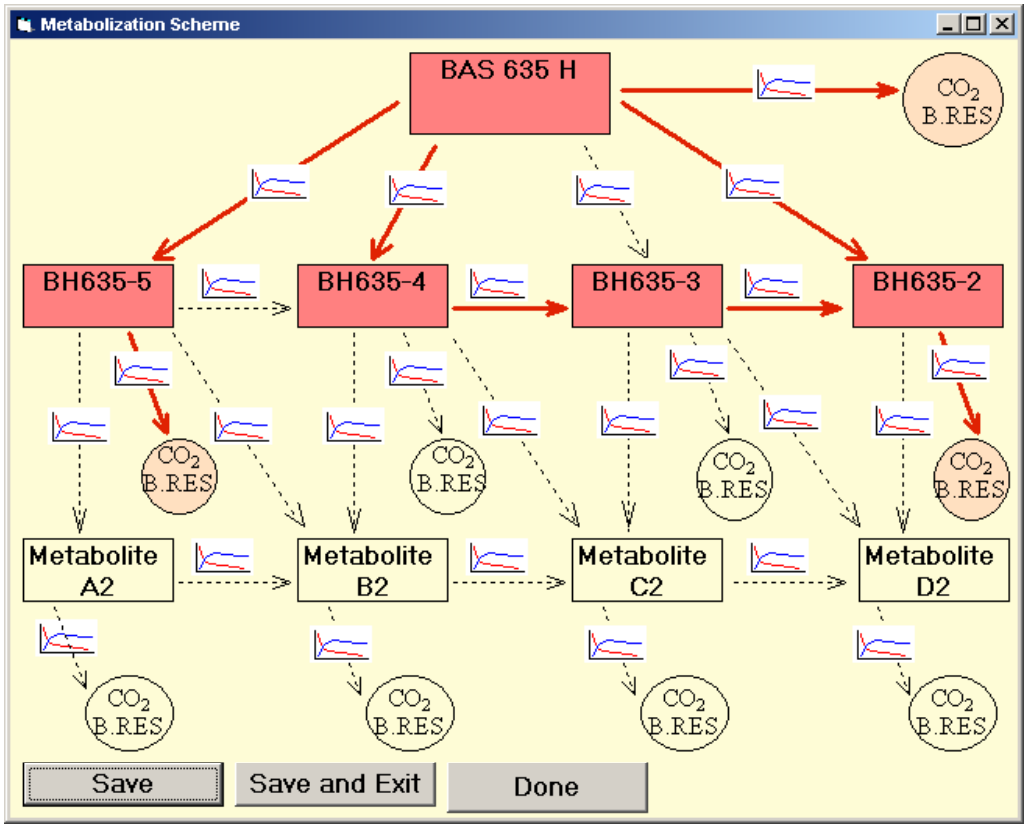


Table B.8.3.2-2: Standardised half-lives (20 °C, pF 2) for tritosulfuron, 635M01, 635M02, 635M03 and 635M04 (AMTT) estimated from different field studies

Compound	half-life [d]							
	South Dakota	Cali-fornia	Indiana	Texas	Man-zanilla	Utrera	Bjärred	best case
tritosulfuron	32.5[1)]	32.7	5.4	2.6	11.0	5.9	8.4	2.6
Compound	South Dakota	Cali-fornia	Indiana	Texas	Man-zanilla	Utrera	Bjärred	worst case
635M01	n.s.[2)]	115.5	47.2	74.5	66.0	n.s.[2)]	18.6	115.5
635M02	n.s.[2)]	126.0	9.5	n.s.[2)]	n.s.[2)]	n.a.[3)]	n.a.[3)]	126.0
635M03	n.s.[2)]	62.4	90.0	57.3	n.s.[2)]	n.s.[2)]	n.s.[2)]	90.0
635M04 (AMTT)	4.1	5.6	23.1	2.5	n.s.[2)]	n.a.[3)]	13.0	23.1

1) The confidence level of the overall (lumped) degradation rate of BAS 635 H in South Dakota, calculated at 94.8 %, was still considered significant, and kept as a high-end half-life value for BAS 635 H degradation in the field.
2) n.s.: non-significant, confidence level of the estimated degradation rate < 95 %
3) n.a.: non-applicable, no significant levels of the compound detected in the field trial

To provide a realistic worst-case assessment of the time course of the soil concentration of the metabolites,

- a combination of rapid formation and slow degradation of the metabolites was considered: the worst case (i.e. longest) soil half-life of each individual metabolite and the best-case (i.e. shortest) soil half-life of BAS 635 H, and
- the average molar formation fractions were considered.

Soil adsorption of BAS 635 H and its soil metabolites

The adsorption of BAS 635 H and its metabolites was reevaluated (Jene, 2002; Jene, 2003) by analysing the difference between adsorption and desorption $K_{f,OC}$ values which indicate an increase of soil adsorption with time. As a result, effective equilibrium $K_{f,OC}$ values were derived. These equilibrium $K_{f,OC}$ values are suitable to be used for long term assessments on a timescale that is considerably longer than the equilibration time in the adsorption batch study. They are considerably higher than those determined from the adsorption batch experiment alone (Table B.8.3.2-3).
Since higher adsorption to soil results in a slower movement of substances to lower soil layers and therefore a higher local concentration, these effective equilibrium adsorption values were used in these PEC_{soil} calculations.
Additionally, it was shown that the adsorption of BAS 635 H and its metabolites does not depend on soil properties, especially the soil pH (Gottesbüren, 2003).

Table B.8.3.2-3: Effective equilibrium sorption parameters of BAS 635 H (tritosulfuron) in 5 different EU soils

Compound	soil OC* range [%]	K_f effective range [L/kg]	$K_{f,oc}$ effective range [L/kg] average [L/kg]	1/n effective range average
BAS 635 H	0.7…3.3	0.202…0.638	7.4…34.1 23.0	0.86…1.00 0.930
BH 635-2	0.5…1.8	0.391…0.912	26.8…83.6 63.7	0.930…0.957 0.942
BH 635-3	0.5…1.8	0.145…0.726	29.0…80.0 50.8	0.942…0.972 0.956
BH 635-4	0.5…1.8	0.521…2.140	45.8…163.0 116.8	0.908…0.976 0.950
BH 635-5	0.7…2.5	0.217…0.513	19.2…50.2 32.0	0.932…0.967 0.954

* OC = organic carbon

FOCUS input parameters for PEC_{soil} calculation

Taking into account the aforementioned data on degradation half-lives and sorption parameters, the input parameters for FOCUS modelling were chosen as listed in Table B.8.3.2-4.

Table B.8.3.2-4: Summary of FOCUS input parameters for BAS 635 H

input parameter	BAS 635 H	635M01	635M02	635M03	635M04
PHYSICO-CHEMICAL PARAMETERS					
molecular weight [g/mol]	445.3	353.3	225.2	310.3	194.1
FORMATION / DEGRADATION PARAMETERS					
formation fraction (from parent)	n.a.[1)]	17.1	16.5	-	32.9
half-life at reference conditions (20 °C; matrix potential -10 kPa) [d]	2.6	90.0	115.5	126.0	23.1
Q_{10}-factor	2.2				
exponent of moisture correction function	0.7				
SORPTION PARAMETERS					
$K_{f,oc}$-value [L/kg]	23.0	116.8	63.7	50.8	32.0
Freundlich exponent 1/n	0.930	0.950	0.942	0.956	0.954
CROP RELATED PARAMETERS					
TSCF (crop uptake)	0.5				

1) n.a. = not applicable

Results of PEC_{soil} calculation

The average of the annual maximum concentrations over the 26 year simulation period and the overall maximum concentration for the soil layers 0-5 cm, 5-10 cm, 0-10 cm, 10-15 cm, 15-20 cm and 25-30 cm are listed in Table B.8.3.2-5 to Table B.8.3.2-8.
It can be concluded that no significant accumulation of all four soil metabolites of BAS 635 H is to be expected since the substances dissipate by degradation and slow movement to lower soil layers.
For a long term assessment of accumulation, a soil layer of 0-5 cm is considered inappropriate due to mixture of the topsoil by soil tillage. Therefore, the soil layer 0-10 cm was chosen to be the target layer of exposure assessment. Displacement of BAS 635 H and its metabolites to lower soil layers is less pronounced in the Sevilla than in the Châteaudun or Hamburg scenario. Thus, the highest soil concentrations of all metabolites can be found in the Sevilla scenario. The average of the annual peak concentrations of 635M01 (BH 635-4), 635M02 (BH 635-2), 635M03 (BH 635-3), and 635M04 (BH 635-5) in the 0-10 cm layer amounted to 0.00387, 0.00251, 0.00144, and 0.00318 mg/kg soil, respectively.

Table B.8.3.2-5: Predicted soil concentrations of 635M01 (BH 635-4) in 3 FOCUS scenarios

Soil layer	Châteaudun		Hamburg		Sevilla	
	Average max. [mg/kg]	Average [mg/kg]	Average max. [mg/kg]	Average [mg/kg]	Average max. [mg/kg]	Average [mg/kg]
0-5 cm	0.00510	0.00139	0.00427	0.00121	0.00696	0.00235
5-10 cm	0.00236	0.00116	0.00209	0.00105	0.00212	0.00105
0-10 cm	0.00340	0.00128	0.00290	0.00113	**0.00387**	**0.00170**
10-15 cm	0.00121	0.00077	0.00116	0.00078	0.00095	0.00053
15-20 cm	0.00064	0.00046	0.00070	0.00054	0.00045	0.00026
25-30 cm	0.00018	0.00015	0.00033	0.00025	0.00013	0.00007

Table B.8.3.2-6: Predicted soil concentrations of 635M02 (BH 635-2) in 3 FOCUS scenarios

Soil layer	Châteaudun		Hamburg		Sevilla	
	Average max. [mg/kg]	Average [mg/kg]	Average max. [mg/kg]	Average [mg/kg]	Average max. [mg/kg]	Average [mg/kg]
0-5 cm	0.00303	0.00078	0.00253	0.00063	0.00436	0.00161
5-10 cm	0.00165	0.00085	0.00140	0.00065	0.00177	0.00102
0-10 cm	0.00209	0.00081	0.00174	0.00064	**0.00251**	**0.00131**
10-15 cm	0.00112	0.00078	0.00095	0.00061	0.00120	0.00081
15-20 cm	0.00085	0.00068	0.00072	0.00054	0.00092	0.00065
25-30 cm	0.00046	0.00041	0.00055	0.00046	0.00054	0.00038

Table B.8.3.2-7: Predicted soil concentrations of 635M03 (BH 635-3) in 3 FOCUS scenarios

Soil layer	Châteaudun		Hamburg		Sevilla	
	Average max. [mg/kg]	Average [mg/kg]	Average max. [mg/kg]	Average [mg/kg]	Average max. [mg/kg]	Average [mg/kg]
0-5 cm	0.00083	0.00038	0.00064	0.00024	0.00188	0.00098
5-10 cm	0.00109	0.00060	0.00076	0.00039	0.00145	0.00080
0-10 cm	0.00090	0.00049	0.00063	0.00031	**0.00144**	**0.00089**
10-15 cm	0.00098	0.00065	0.00075	0.00045	0.00109	0.00069
15-20 cm	0.00081	0.00061	0.00066	0.00047	0.00088	0.00058
25-30 cm	0.00046	0.00039	0.00054	0.00045	0.00051	0.00032

Table B.8.3.2-8: Predicted soil concentrations of 635M04 (BH 635-5) in 3 FOCUS scenarios

Soil layer	Châteaudun		Hamburg		Sevilla	
	Average max. [mg/kg]	Average [mg/kg]	Average max. [mg/kg]	Average [mg/kg]	Average max. [mg/kg]	Average [mg/kg]
0-5 cm	0.00422	0.00049	0.00351	0.00044	0.00599	0.00084
5-10 cm	0.00196	0.00033	0.00173	0.00031	0.00161	0.00027
0-10 cm	0.00274	0.00041	0.00234	0.00038	**0.00318**	**0.00055**
10-15 cm	0.00096	0.00019	0.00093	0.00020	0.00069	0.00013
15-20 cm	0.00050	0.00010	0.00048	0.00011	0.00028	0.00005
25-30 cm	0.00009	0.00003	0.00010	0.00003	0.00002	0.00000

Comment of RMS:

The calculations are acceptable. The maximum average PEC_{soil} for all scenarios have been used in the assessment of the long-term risk to earthworms (see below). The use of 'effective' $K_{f,oc}$ values in $FOCUS_{gw}$ modelling is no standard approach. However, since these 'effective' $K_{f,oc}$ values are greater than $K_{f,oc}$ values from standard laboratory tests, they will result in more retention and higher concentrations of the compounds in upper soil layers. In this context, where calculations actually aim at the concentrations in those upper soil layers, they can thus be regarded as a worst case.

B.8.6 Predicted environmental concentrations in surface water and in ground water (PECsw, PECgw) (Annex IIIA 9.2.1, 9.2.3)

B.8.6.4 Calculation of predicted environmental concentrations for BAS 635 H and metabolites BH 635-2, BH 635-4 and BH 635-5 in static surface waters (PECsw) and sediment (PECsed) after drift entry (Dressel, 2002a; Dressel, 2002b; Dressel, 2003c, Dressel, 2003d)

Annex Point: IIA-9.2.3
Author: Dressel, J.
Title: Calculation of predicted environmental concentrations for BAS 635 H and metabolites BH 635-2, BH 635-4 and BH 635-5 in static surface waters (PECsw) and sediment (PECsed) after drift entry
Date: 2004-16-05
Doc ID: BASF 2002/1000213
WAS2002-96
GLP: No

Annex Point: IIA-9.2.3
Author: Dressel, J.
Title: First Amendment to Final Report: Calculation of Predicted Environmental Concentations for BAS 635 H and metabolites BH 635-2, BH 635-4 and BH 635-5 in static surface waters (PECsw) and sediment (PECsed) after drift entry
Date: 2002-01-30
Doc ID: BASF 2002/1004269
WAS2005-2
GLP: No

Annex Point: IIA-9.2.3
Author: Dressel, J.
Title: Second Amendment to Final Report: Calculation of Predicted Environmental Concentations for BAS 635 H and metabolites BH 635-2, BH 635-4 and BH 635-5 in static surface waters (PECsw) and sediment (PECsed) after drift entry
Date: 2003-01-30
Doc ID: 2003-1001039; CALC315
WAS2005-4
GLP: No

Annex Point: IIA-9.2.3
Author: Dressel, J.
Title: Third Amendment to Final Report: Calculation of Predicted Environmental Concentations for BAS 635 H and metabolites BH 635-2, BH 635-4 and BH 635-5 in static surface waters (PECsw) and sediment (PECsed) after drift entry
Date: 2003-07-17
Doc ID: 2003/1009268; CALC-315
WAS2005-3
GLP: No

Predicted environmental concentration (PEC) calculations are needed to estimate exposure in ecotoxicology. Modelling was conducted to calculate initial, short-term and long-term actual and time-weighted average concentrations ($PEC_{SW,act}$ and $PEC_{SW,twa}$) of BAS 635 H and its metabolites BH 635-2, BH 635-4 and BH 635-5 in static surface water bodies. The entry route spray drift was assessed for the prediction of the environmental concentrations in static surface water. The initial PEC-values in surface water were calculated for a 30 cm deep water layer. According to good agriculture practice BAS 635 H is intended to be applied with a single application per year at a rate of 0.05 kg as/ha onto cereals and maize in spring.

B.8.6.4.1 Method of calculation

$PEC_{sw,ini}$ via spray drift

BAS635-H. Buffer zone widths *B* of 1 m, 3 m and 5 m were taken into account for the calculation of the initial concentrations. Respective spray drift fractions of 2.77 %, 0.95 % and 0.57 % of the active substance onto surface water bodies (90^{th} percentile drift percentage) valid for the application scenario to field crops were used for calculation. The value for 3 m is not reported in the EU Guidance Document on Aquatic Ecotoxicology, but was calculated with the same underlying equation (Drift percentage = $2.7705 \times B^{-09787}$ with *B* representing the width of bufferzone in [m]).

Metabolites. The maximum concentrations of BH 635-2 and BH 635-4 in the water phase observed during the water sediment study with sediments from two different origins (see monograph) were taken into account for the calculation of the initial concentrations of the metabolites. The highest observed concentrations of BH 635-2 and BH 635-4 in system A and B were 14.6 % TAR and 28.1 % TAR (Table B.8.6.4-1 and Table B.8.6.4-2), respectively.

Table B.8.6.4-1: BH 635-2 residues in the water phase of the water-sediment study

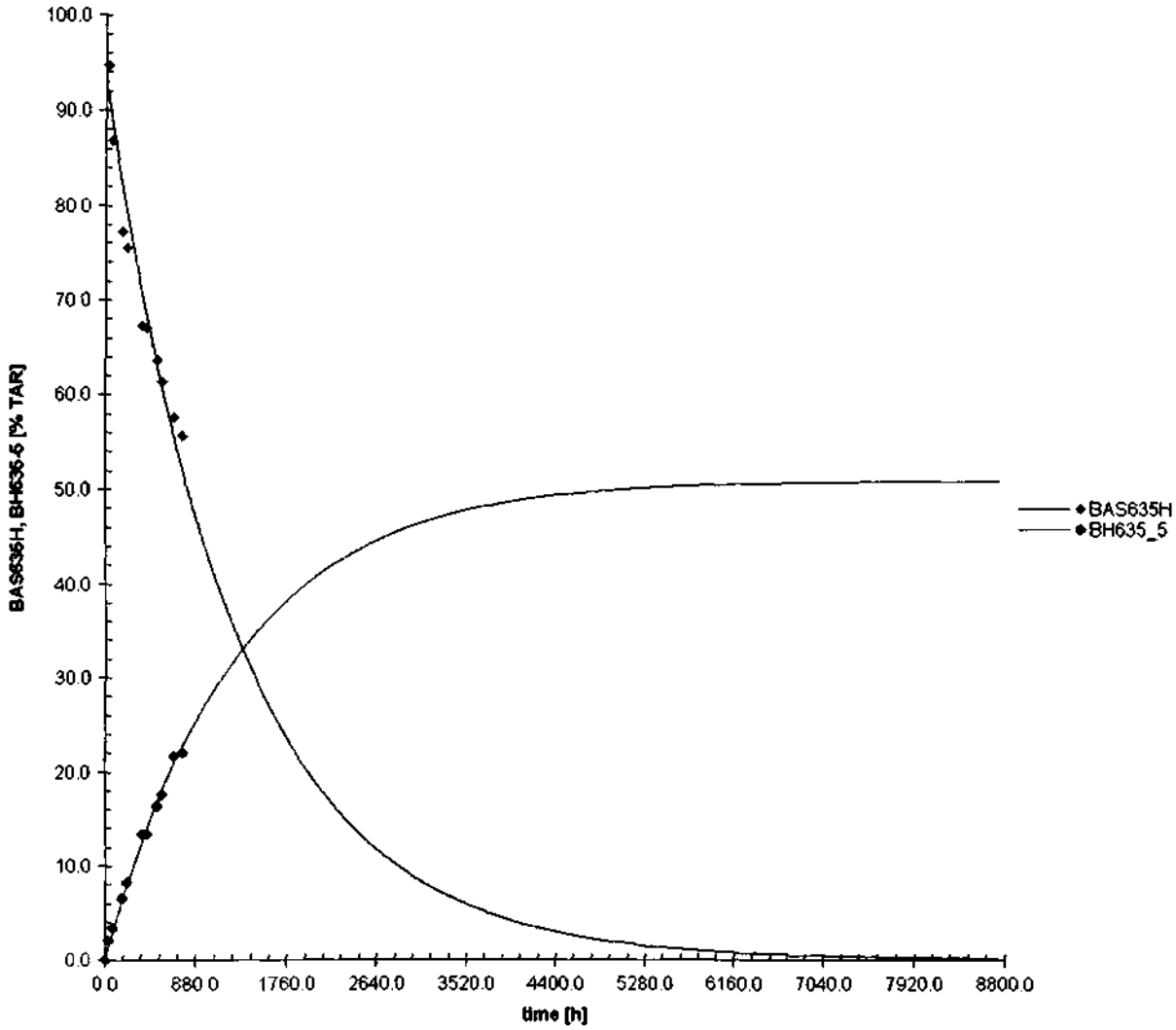
T[d]	fraction of phenyl-[^{14}C]-BH 635-2 [% TAR]		fraction of triazine-[^{14}C]-BH 635-2 [% TAR]	
	sediment A	sediment B	sediment A	sediment B
1			BH 635-2 not detectable with triazine label	
2		1.7		
7		5.8		
14	7.1	14.6 (max.)		
28	9.3	8.3		
63	13.0	6.3		
100	7.5	8.2		

Table B.8.6.4-2: BH 635-4 residues in the water phase of the water-sediment study

T[d]	fraction of [^{14}C]-BH 635-4 [% TAR]			fraction of [^{14}C]-BH 635-4 [% TAR]		
	phenyl-label sediment A	triazine-label sediment A	average sediment A	phenyl-label sediment B	triazine-label sediment B	average sediment B
1						
2						
7						
14					3.3	1.6
28	3.1	6.5	4.8	11.3	13.2	12.3
63	18.2	16.6	17.4	18.6	14.8	16.7
100	24.4	26.0	25.2	27.0	29.3	28.1

BH 635-5 was not detected in both water sediment systems, but could be postulated, as it is known to be a hydrolysis product of BAS 635 H under acidic conditions. To calculate a worst case $PEC_{sw,ini}$, a kinetic evaluation (parameter estimation) of the hydrolysis study with the software tool ModelMaker v4.0 was conducted, considering parallel fluxes from BAS 635 H to BH 635-5 and from BAS 635 H to all other metabolites. For the formation of BH 635-5, a 1st oder rate $k = 0.01025 \pm 0.00071$ d^{-1} was obtained with an estimated initial BAS 635 H-concentration of 93.92 ± 1.04 %. As BH 635-5 is known to be hydrolytically stable, a theoretical maximum formed fraction of BH 635-5 was calculated by extrapolating the curves to 1 a (8760 h) yielding 50.9 % (Figure B.8.6.4-1). Since at that time the residual BAS 635 H was estimated to be only 0.1 % of TAR, an asymptotic hydrolytic formation of BH 635-5 from BAS 635 H of 51 % was assumed. This procedure to estimate this maximum fraction from an extrapolation far beyond the study duration is deemed justified, because the process described is abiotic and expected to continue in the same way as during the study.

Figure B.8.6.4-1: Extrapolated curves describing hydrolysis of BAS 635 H and formation of BH 635-5 over 1 year yielding an estimated theoretical maximum molar formation of 51 %



For calculation of the initial concentration $PEC_{sw,ini}$ of the metabolites, a metabolite mass fraction was calculated by multiplying the molar fraction with the quotient of metabolite molar mass and active substance molar mass. The resulting mass fraction factors $frac_{mass,metabolite}$ are 0.0738, 0.223 and 0.222 for BH 635-2, BH 635-4 and, BH 635-5, respectively (the corresponding factor for the active substance itself is 1). The predicted environmental initial concentrations of the parent compound BAS 635 H and its metabolites BH 635-2, BH 635-4 and BH 635-5 in static surface water caused by spray drift were calculated with the following equation.

$$PEC_{ini} = \frac{a \times dr}{V_{sw} \times 100} \times frac_{mass,metabolite}$$

where:

a	=	application rate (50 µg/m^2)
dr	=	spray drift [%]
V_{sw}	=	water volume per m^2 (300 L/m^3)
$frac_{mass,metabolite}$	=	mass fraction of metabolite, related to active substance

Actual Predicted Environmental Concentrations ($PEC_{sw,act}$)

BAS 635-H. For the calculation of $PEC_{sw,act}$-values for BAS 635 H, an exponential decline of the concentrations in water phase corresponding with the worst case half-life of BAS 635 H in water is assumed. The water sediment study provides a half-life of BAS 635 H in the water phase of 67 d according to Timme, Frehse and Laska (first order kinetics). This comprises both processing occuring in the study, (i) degradation in the water and (ii) partitioning onto the sediment.

Metabolites. As a worst case assumption, a degradation of the metabolites BH 635-2, BH 635-4 and BH 635-5 in surface water was not taken into account for the calculation of the actual and the time-weighted average PECs

The $PEC_{sw,act}$-values for BAS 635 H were calculated using the following equation.

$$PEC_{sw,act}(t) = PEC_{sw,ini} \times e^{-k\_water \times t} = PEC_{sw,ini} \times e^{-\frac{t \times \ln 2}{half-life\_water}}$$

where:

$PEC_{sw,ini}$	=	initial PECsw [µg/L]
k_water	=	1st order dissipation rate in water [1/d]
half-life_water	=	half-life in water (67 d)
t	=	time [d]

Time-weighted Predicted Environmental Concentrations ($PEC_{sw,twa}$)

The $PEC_{sw,twa}$-values for BAS 635 H were calculated using the following equation.

$$PEC_{sw,twa}(t) = PEC_{sw,ini} \times \frac{half-life\_water}{t \times \ln 2} \times \left(1 - e^{-\frac{t \times \ln 2}{half-life\_water}}\right)$$

where:

$PEC_{sw,ini}$	=	initial PECsw [µg/L]
half-life_water	=	half-life in water (67 d)
t	=	time [d]

Calculation of PEC_{sed}

The concentrations in sediment were calculated using the measured residue-percentages of the parent compound and its metabolite in the sediments of the water/sediment study (2 labels, 2 sediment systems). Only BAS 635 H (Table B.8.6.4-3) and BH 635-4 (Table B.8.6.4-4) were detected in the sediment phase with maximum concentrations of 12.5 % and 35.2 %, respectively.

Table B.8.6.4-3: BAS 635 H residues in the sediment phase of the water-sediment study

T[d]	fraction of phenyl-[^{14}C]-BAS 635 H [% TAR]		fraction of triazine-[^{14}C]-BAS 635 H [% TAR]	
	sediment A	sediment B	sediment A	sediment B
1	5.2	9.8	5.1	9.1
2	6.2	12.0	5.8	10.1
7	9.7	10.5	10.9	10.9
14	10.9	11.1	9.3	12.4
28	10.3	12.5 (= max)	10.4	11.0
63	7.0	6.5	6.5	11.1
100	6.7	1.2	6.5	3.9

Table B.8.6.4-4: BH 635-4 residues in the sediment phase of the water-sediment study

T[d]	fraction of phenyl-[^{14}C]-BH 635-4 [% TAR]		fraction of triazine-[^{14}C]-BH 635-4 [% TAR]	
	sediment A	sediment B	sediment A	sediment B
1	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0
7	0.6	2.8	0.0	2.6
14	1.5	9.6	1.6	9.4
28	4.0	14.8	4.6	16.6
63	10.9	24.6	10.3	23.5
100	10.7	31.6	12.8	35.2 (= max)

The actual concentrations in sediment after single application were calculated using the following equation. No molecular-weight correction factor was applied for BH 635-4, therefore the respective calculated concentrations can be regarded worst case.

$$PEC_{sed}(t) = \frac{PEC_{sw,ini} \times V_{sw} \times P_{sed}(t)}{V_{sed} \times bd_{sed} \times 100}$$

where:

PEC_{sed}	=	PEC_{sed} at time *t* [µg/g wet sediment]
$PEC_{sw,ini}$	=	initial PECsw [µg/L]
V_{sw}	=	water volume (300 L)
$P_{sed}(t)$	=	portion of the substance in sediment at time *t* (from water/sediment study) [%]
V_{sed}	=	volume of sediment for a proposed depth of the sediment layer of either 2 cm (20 L) or 5 cm (50 L)
$bd_{sed}(t)$	=	bulk density of the wet sediment consisting of 80 vol. % water and 20 vol. % of dry sediment (1300 g/L)
t	=	time [d]

For BH 635-5, which was not detected in the sediment, the constructed case for the calculation of $PEC_{sw,ini}$ is extended by a calculation of an instantaneous partitioning between the water and the sediment phase based on the average K_{oc}-value of 20.8 L/kg.. The sediment

with the highest organic carbon content (4.7 %, sediment B) was chosen for this estimation. The depth $DEPTH_{water}$ of the assumed system is 30 cm and the thickness of the sediment is assumed to be 2 cm. The sorbed fraction was calculated as follows.

$$\text{fraction}_{\text{dissolved}} = \frac{DEPTH_{water}}{DEPTH_{water} + DEPTH_{sed} \times \text{bd}_{\text{sed}} \times K_{\text{OC}} \times \frac{\text{OC}_{\text{sed}}}{100}}$$

$$\text{fraction}_{\text{sorbed}} = 1 - \text{fraction}_{\text{dissolved}}$$

The sorbed fraction was calculated to be 7.81 %. Since no degradation of BH 635-5 in the sediment was assumed, the PEC_{sed} was calculated according to the equation shown above with $P_{sed}(t)=7.81$.

B.8.6.4.2 Results

Calculated $PEC_{sw,ini}$

The initial PEC_{sw}-values ($PEC_{sw,ini}$) for BAS 635 H and its metabolites BH 635-2, BH 635-4 and BH 635-5 after entry via spray drift are given in Table B.8.6.4-5 for buffer zone widths of 1 m, 3 m and 5 m. In Table B.8.6.4-6, the resulting $PEC_{sw,ini}$ was compared to the $PEC_{sw,ini}$-values resulting from overspray and runoff taken from Platz (2001) "Calculation of predicted environmental concentrations (PEC_{sw}) for BAS 635 H and metabolites BH 635-2, BH 635-4 and BH 635-5 in static surface waters" (see monograph). The overspray scenario is given for reference but is not considered a relevant scenario under Good Agricultural Practice. For all substances except BH 635-4, the loading by runoff represented the relevant worst case. For BH 635-4, the drift entry of BAS 635 H and subsequent transformation to BH 635-4 yielded higher $PEC_{sw,ini}$ than entry by runoff and therefore represented the worst case. The worst case results were taken to the calculation of the $PEC_{sw,act}$.

Table B.8.6.4-5: $PEC_{sw,ini}$-values for BAS 635 H, BH 635-2, BH 635-4 and BH 635-5 after spray drift loadings with 1 to 5 m buffer distance

Entry route	Spray drift loading "1 m buffer zone" $PEC_{sw,ini}$ [µg/L]	Spray drift loading "3 m buffer zone" $PEC_{sw,ini}$ [µg/L]	Spray drift loading "5 m buffer zone" $PEC_{sw,ini}$ [µg/L]
BAS 635 H	0.461	0.158	0.095
BH 635-2	0.034	0.012	0.007
BH 635-4	0.103	0.035	0.021
BH 635-5	0.103	0.035	0.021

Table B.8.6.4-6: $PEC_{sw,ini}$-values for BAS 635 H, BH 635-2, BH 635-4 and BH 635-5 after different loadings

Entry route	Spray drift loading "overspray" $PEC_{sw,initial}$ [µg/L]	Spray drift loading "1 m buffer zone" $PEC_{sw,initial}$ [µg/L]	Runoff loading "no buffer zone" $PEC_{sw,initial}$ [µg/L]
BAS 635 H	16.7	0.461	0.655
BH 635-2	1.2	0.034	0.055
BH 635-4	3.7	0.103	0.083
BH 635-5	3.7	0.103	0.039

Calculated $PEC_{sw,act}$ and $PEC_{sw,twa}$

The actual and the time weighted average concentrations of BAS 635 H in surface water ($PEC_{sw,act}$ and $PEC_{sw,twa}$) after entry via spray drift were calculated as described above. The actual and time weighted average concentrations for the minimum buffer zone are given in Table B.8.6.4-7.

Table B.8.6.4-7: $PEC_{sw,act}$ and $PEC_{sw,ftwa}$ for BAS 635 H, BH 635-2, BH 635-4 and BH 635-5

Time [d]		BAS 635 H (runoff "no buffer")		BH 635-2 (runoff)	BH 635-4 (drift 1 m buffer)	BH 635-5 (runoff)
		$PEC_{sw.act}$ [µg/L]	$PEC_{sw.twa}$ [µg/L]	$PEC_{sw.act}$, $PEC_{sw.twa}$ [µg/L]	$PEC_{sw.act}$, $PEC_{sw.twa}$ [µg/L]	$PEC_{sw.act}$, $PEC_{sw.twa}$ [µg/L]
Initial	0	0.655	-	0.055	0.103	0.039
Short-term	1	0.648	0.651	0.055	0.103	0.039
	2	0.641	0.648	0.055	0.103	0.039
	3	0.634	0.644	0.055	0.103	0.039
	4	0.628	0.641	0.055	0.103	0.039
Long-term	7	0.609	0.631	0.055	0.103	0.039
	14	0.566	0.609	0.055	0.103	0.039
	21	0.527	0.588	0.055	0.103	0.039
	28	0.490	0.568	0.055	0.103	0.039
	42	0.424	0.531	0.055	0.103	0.039
	100	0.233	0.408	0.055	0.103	0.039

As no degradation is considered for PEC calculation of BH 635-2, BH 635-4 and BH 635-5, the actual and time weighted average concentrations in surface water are identical with the respective initial concentrations.

Calculated PEC_{sed}

The predicted concentrations of BAS 635 H and BH 635-4 in sediment after entry of BAS 635 H into the water phase by overspray, spray drift and runoff considering minimum buffer zones are given in Table B.8.6.4-8 and Table B.8.6.4-9 for a sediment depth V_{sed} of 2 cm and in Table B.8.6.4-10 and Table B.8.6.4-11 for a sediment depth V_{sed} of 5 cm.

Table B.8.6.4-8: PEC_{sed} for BAS 635 H for a sediment depth of 2 cm (loading of water body by overspray and drift in 1 m buffer distance using the 90th percentile drift factor)

time [d]	Scenarios		
	overspray [mg/kg]	drift: 1 m buffer [mg/kgl	runoff (no buffer) [mg/kg]
1	0.019	0.00052	0.00074
2	0.023	0.00064	0.00091
7	0.020	0.00056	0.00079
14	0.021	0.00059	0.00084
28	0.024 (=max)	0.00066 (=max)	0.00094 (=max)
63	0.013	0.00035	0.00049
100	0.002	0.00006	0.00009

Table B.8.6.4-9: PEC_{sed} for BH 635-4 for a sediment depth of 2 cm (loading of water body with BAS 635 H by overspray and drift using 90th percentile drift factor)

time [d]	Scenarios		
	overspray [mg/kg]	drift: 1 m buffer [mg/kg]	runoff (no buffer) [mg/kg]
1	0.00000	0.00000	0.00000
2	0.00000	0.00000	0.00000
7	0.00397	0.00011	0.00016
14	0.01437	0.00040	0.00056
28	0.02538	0.00070	0.00100
63	0.03593	0.00099	0.00141
100	0.05381 (=max)	0.00149 (=max)	0.00211 (=max)

Table B.8.6.4-10: PEC_{sed} for BAS 635 H for a sediment depth of 5 cm (loading of water body by overspray and drift in 1 m buffer distance using the 90th percentile drift factor)

time [d]	Scenarios		
	overspray [mg/kg]	drift: 1 m buffer [mg/kg]	runoff (no buffer) [mg/kg]
1	0.00754	0.00021	0.00030
2	0.00923	0.00026	0.00036
7	0.00808	0.00022	0.00032
14	0.00854	0.00024	0.00034
28	0.00962 (=max)	0.00027 (=max)	0.00038 (=max)
63	0.00500	0.00014	0.00020
100	0.00092	0.00003	0.00004

Table B.8.6.4-11: PEC_{sed} for BH 635-4 for a sediment depth of 5 cm (loading of water body with BAS 635 H by overspray and drift using 90th percentile drift factor)

time [d]	Scenarios		
	Overspray [mg/kg]	drift: 1 m buffer [mg/kg]	runoff (no buffer) [mg/kg]
1	0.00000	0.00000	0.00000
2	0.00000	0.00000	0.00000
7	0.00159	0.00004	0.00006
14	0.00574	0.00016	0.00023
28	0.01013	0.00028	0.00040
63	0.01434	0.00040	0.00056
100	0.02148 (=max)	0.00059 (=max)	0.00084 (=max)

Comment of RMS:
The calculations are acceptable.

B.8.6.5 Calculation of predicted environmental concentrations in groundwater (PECgw) of BAS 635 H and its metabolites for UK under special considering of sorption dependencies

Annex Point: IIA-9.2.1
Author: Gottesbüren, B..
Title: Calculation of predicted environmental concentrations in groundwater (PEC_{gw}) of BAS 635 H and its metabolites for UK under special considering of sorption dependencies; study CALC-365
Date: 2002-12-02
Doc ID: 2002/1011916
WAS2003-115
GLP: No

Abstract

Simulations with regard to the leaching behaviour of the herbicidal substance BAS 635 H after early spring application in winter cereals were performed with the environmental fate model FOCUS-PELMO 3.3.2

The leaching assessment of BAS 635 H and four soil metabolites was conducted based on average field dissipation half-lives and metabolite formation fractions, which were obtained from four American and three European field trials as well as from two lysimeter studies. All half-lives were standardised to the FOCUS reference conditions for soil temperature and moisture (20 °C and field capacity). Finally, a relevant standardised half life of 15.1 days was considered for the parent compound.
A previous evaluation of the laboratory sorption studies with a kinetic sorption model resulted in effective $K_{f,oc}$-values ranging from 7.4-64.3 mL/g. Analysis of the sorption data in relation to soil properties revealed a negative correlation between the effective $K_{f,oc}$-values and the pH-value of the soils. The choice of appropriate sorption parameters was made according to guidance given by FOCUS and *the choice of input parameter is made in relation to the pH of the soils in the scenarios* which are relevant for the UK. From the FOCUS scenarios relevant

for the UK, Kremsmünster and Châteaudun have pH-values above 7. For these scenarios, the lowest effective $K_{f,oc}$-value of BAS 635 H – as measured in a soil with high pH – was used. For the FOCUS scenarios Hamburg and Okehampton, the lowest $K_{f,oc}$-value of all soils with pH below 7 was considered. Average laboratory adsorption parameters were taken for the sorption coefficients of the metabolites.
Early spring application was assumed on 1st of February onto winter cereals with an application rate of 50 g/ha. Analysis of the relevant crop growth stages in the UK showed that the dominant majority of the winter cereal crops will have reached a BBCH stage of at least 20 (tillering) in January and February. Therefore, a crop interception rate of 50 % has to be assumed, resulting in an amount of 25 g/ha reaching the soil surface.

Simulations were carried out for four FOCUS scenarios that were shown to be either directly representative for areas (scenarios) in the UK or that have been shown to be more vulnerable and thus be able to cover the leaching risk of the whole agricultural area above minor and major aquifers in the UK. The 80th percentile annual leaching concentrations are shown in Table B.8.6.5-1.

Table B.8.6.5-1: 80th percentile annual leachate concentrations for BAS 635 H and its metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5 after early spring application of BAS 635 H in winter cereals (0.05 kg/ha with 50 % interception on 1st February)

Scenario	BAS 635 H [µg/L]	BH 635-2 [µg/L]	BH 635-3 [µg/L]	BH 635-4 [µg/L]	BH 635-5 [µg/L]
Châteaudun	0.017	0.205	0.138	0.023	0.003
Hamburg	0.030	0.219	0.170	0.035	0.008
Kremsmünster	0.086	0.230	0.168	0.077	0.016
Okehampton	0.034	0.193	0.152	0.052	0.008

Considering the sorption dependency of BAS 635 H (tritosulfuron) according to the recommendations of FOCUS, the simulation results for the scenarios relevant for UK conditions demonstrate that the leaching of unacceptable amounts of BAS 635 H after early spring application in winter cereals is unlikely.

Comment of RMS:
This report was prepared for supporting a national authorisation in the UK and additionally submitted in the EU process. The selection of input parameters for FOCUS modelling is partly based on reports and assessments documented in this addendum. The half-life of tritosulfuron of 15.1 days was derived from field and lysimeter studies as described by Jene (2002) – see B.8.2.2. The calculation of metabolite half-lives and partial degradation followed the approach already described in the monograph. With respect to sorption of tritosulfuron, 'effective' coefficients according to Jene (2002) – see B.8.2.2 – were used, whereas the coefficients for the metabolites were the same as described in the monograph. However, the selection of input parameters for modelling is still based on the assumption of a pH-dependency of tritosulfuron sorption to soil, which is in contrast to the newer findings of Richter (2003) and Zirnstein (2003) and also to the assessment of Gottesbüren (2003) – see B.8.2.3. Therefore, the report is considered outdated and only the abstract is documented for reasons of completeness. An assessment of the leaching to groundwater based on all currently available information can be found under B.8.6.6.

B.8.6.6 Evaluation of the soil degradation, soil adsorption and leaching potential of BAS 635 H and its metabolites 635M01, 635M02, 635M03, and 635M04 (Dressel, 2003b; Dressel, 2004)

Soil adsorption of tritosulfuron and its metabolites

Annex Point:	IIA-9.2.1
Author:	Dressel, J.
Title:	Evaluation of the soil degradation, soil adsorption and leaching potential of BAS 635 H and its metabolites 635M01, 635M02, 635M03, and 635M04
Date:	2003-12-16
Doc ID:	BASF Doc ID 2003/1018144 BOD2004-13
GLP:	Yes

Annex Point:	IIA-9.2.1
Author:	Dressel, J.
Title:	Evaluation of the soil degradation, soil adsorption and leaching potential of BAS 635 H and its metabolites 635M01, 635M02, 635M03, and 635M04 -1. Amendment to Final Report-
Date:	2004-16-05
Doc ID:	BASF 2004/1009160 BOD2004-488
GLP:	Yes

pH-dependence of soil adsorption

It could be shown in a detailed analysis that neither tritosulfuron nor its metabolites show a pH-dependence of adsorption to soil (Gottesbüren, 2003). The apparent pH-dependence of the K_{oc}-value was a cross correlation since the organic carbon (OC) content of the soils of the adsorption experiments was correlated to the soil pH. Additional experimental evidence for tritosulfuron and metabolite 635M02 for five additional soils (Richter, 2003; Zirnstein, 2003) also supported the fact that soil adsorption does not depend on soil pH. It was already stated in the Evaluation Table that no pH-dependence of soil adsorption can be derived from the data presented so far. Therefore additional leaching studies were not carried out.

Kinetic sorption of tritosulfuron and its metabolites to soil

Two studies have been conducted to derive long-term sorption parameters for tritosulfuron and its soil metabolites. For tritosulfuron (Jene, 2001), the following available data were evaluated:

- An inverse modelling approach with 5 individual lysimeters,
- an approach to calculate effective sorption parameters from the adsorption and the desorption branch of the batch equilibrium adsorption/desorption studies according to Streck et al. (1997), and
- the depth distribution in field studies.

All approaches showed, that, owing to sorption increase with time, considerably higher sorption values are relevant for realistic long term assessments than those obtained in the laboratory batch studies.

For the metabolites (Jene, 2003), the batch equilibrium adsorption/desorption studies were re-evaluated according to Streck et al. (1997). An increase of soil adsorption was shown, but it was less pronounced than for tritosulfuron.

PEC_{soil} with worst case DT_{50} values

With the original dossier, a PEC_{soil} report was submitted that used standardised field half-lives. For this addendum, a second study was submitted that used worst case half-lives for parent and metabolites that were not corrected for soil temperature or soil moisture (Hauck, 2002). Field half-lives were used where available, otherwise laboratory values were considered. The used DT_{50} values are shown in Table B.8.6.6-1. The worst-case half-life of 635M03 of 737 d was not considered, since a mistake in the concentration calculation was made within the study (a change of the specific radioactivity in the triazine label due to the degradation process was not accounted for). This mistake only affected 635M03. The values are used for the risk assessment in combination with worst-case assumptions for crop interception (0 %) and formation fraction of metabolites (100 %).

Table B.8.6.6-1: Worst-case DT_{50} values for BAS 635 H – tritosulfuron and its metabolites

compound	range of DT_{50} field [d]	range of DT_{50} laboratory [d]	worst-case DT_{50} used for PEC_{soil} [d]
BAS 635 H	15…21	-	21
635M01	30…336	-	600
635M02	36…216	-	216
635M03	-	32…347	600
635M04	-	98	98

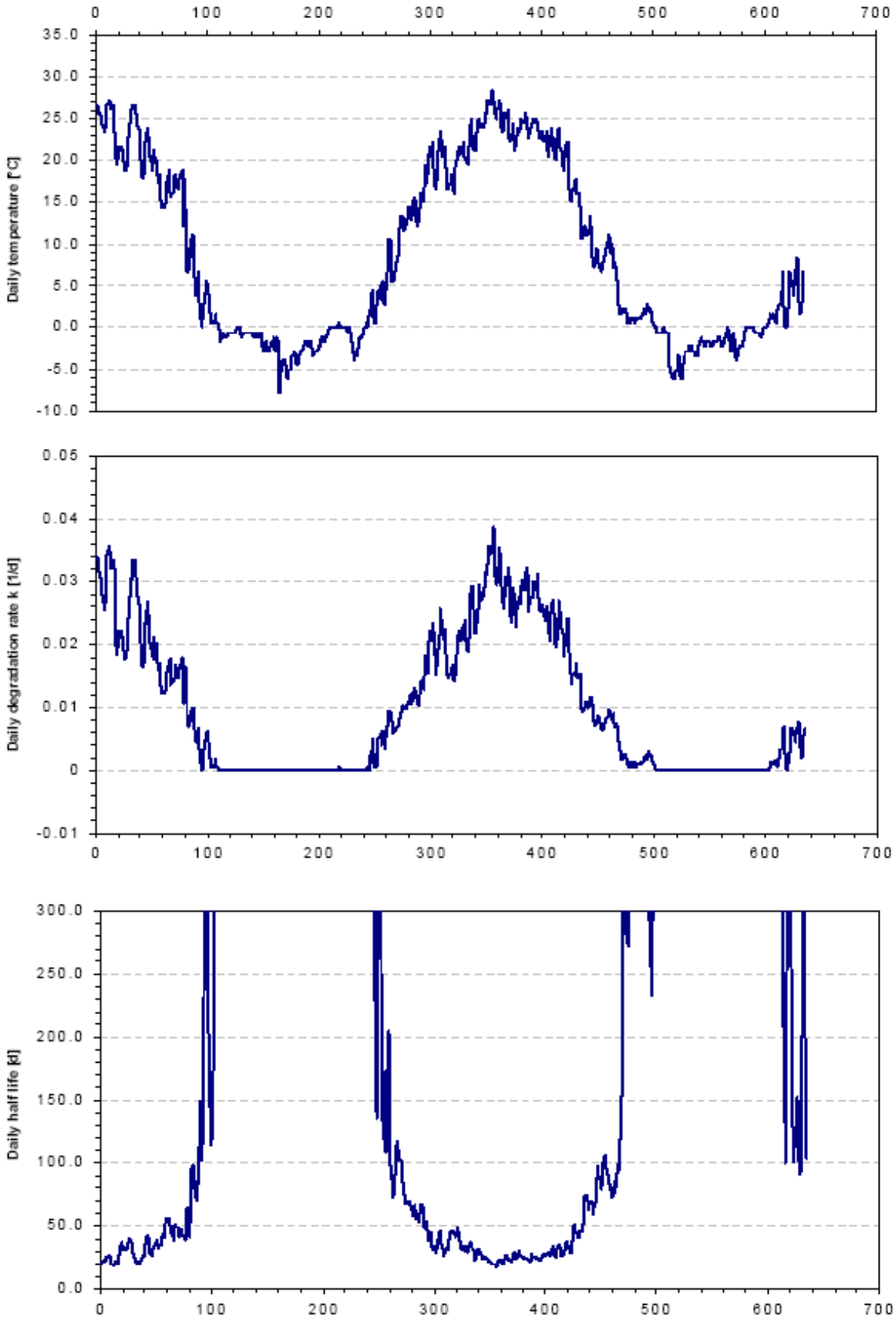
Soil degradation of tritosulfuron

Justification for standardisation steps used in the evaluation of field dissipation studies

In the notifier's original dossier, the degradation of tritosulfuron in aerobic soil was extensively studied in the laboratory (6 different soils) and in the field (10 locations). The risk assessment for leaching of tritosulfuron and its metabolites to groundwater was based on field half-lives that were obtained from a parameter estimation procedure that accounted for fluctuations in soil temperature and soil moisture during the trial.
According to the common principles set out by the FOCUS groundwater group, an actual daily degradation rate of each compound is calculated in all models from the half-life at reference conditions (20 °C, soil moisture at pF2), the simulated soil temperature, and the simulated soil moisture. The dependency of the soil degradation rate on the soil temperature is either expressed by the Arrhenius equation or the well known Q_{10}-approach, which are equivalent. The dependency of the soil degradation rate on soil moisture is described according to the Walker equation. A clear prerequisite to using these dependencies is the estimation of half-lives that are standardised/normalised to reference conditions.
Since soil temperature and soil moisture vary in the field, the correction of the degradation rate k (half-life = ln(2)/k) was conducted applying specifically derived correction factors on the uncorrected half-lives for each single day of the experiment, as described in the report of the FOCUS groundwater group. The effect of fluctuating temperature on the actual

degradation rate k can be observed in Figure B.8.6.6-1. Low temperatures result in slow degradation (low degradation rates k = long half-lives) with no degradation below 0°C. The additional correction for soil moisture is not shown here as it works likewise. Each of the estimated parameters was evaluated statistically by a t-test if it contributed significantly to the description of the data. Inappropriate values were discarded from averaging as it is routinely done for laboratory half-lives. Thus, parameter estimation according to this procedure followed the same principles as they are used by the FOCUS models during simulation runs.

Figure B.8.6.6-1: Example plot of daily soil temperature, the corresponding daily degradation rate k of tritosulfuron, and the corresponding half-life (if k=0, i.e. temperature below 0 °C, the half-life is not defined) of the parameter estimation for trial site South Dakota



Specific questions of ECCO 137

- More information was requested on the climate and field history of the EU field trial sites.

The notifier has submitted respective information in his document 2003/1018144 (Dressel, 2003b). **Since this document has been distributed to all Member States and no revised risk assessment follows from the data, the RMS considers it unnecessary to document the numerous graphs and tables in this addendum.**

- The comparability of the US field trial sites to EU conditions was questioned.

According to Table B.8.6.6-2, two corresponding EU sites could be identified for each US trial site that have similar temperature and rainfall conditions. For the South Dakota site, corresponding sites could only be identified for the vegetation period. A direct comparison of the US sites and the EU sites was not deemed necessary, since it could be shown that the conditions of the US trial sites do prevail somewhere in the EU and the trials can therefore be used as if they were conducted within the EU.

Table B.8.6.6-2: Yearly average temperature and precipitation of weather station of US trial sites and corresponding sites in the EU

Study code	US Site yearly average temp. accumulated natural rainfall	Corresponding EU site 1 yearly average temp. accumulated natural rainfall	Corresponding EU site 2 yearly average temp. accumulated natural rainfall
RCN97010	California (USA) 16.3 °C 188 mm	Almeria (Spain) 18.4 °C 215 mm	Heraklion (Greece) 18.4 °C 501 mm
RCN 97012	Texas (USA) 20.3 °C 548 mm	Trapani (Italy) 17.5 °C 489 mm	Athens (Greece) 17.7 °C 377 mm
RCN 97011	Indiana (USA) 12.7 °C 536 mm	Marseille (France) 14.5 °C 534 mm	Karlsruhe (Germany) 10.3 °C 770mm
	US Site APR-OCT average temp. accumulated natural rainfall	Corresponding EU site 1 APR-OCT average temp. accumulated natural rainfall	Corresponding EU site 2 APR-OCT average temp. accumulated natural rainfall
RCN 97009	South Dakota (USA) 16.2 °C 260mm	Valladolid (Spain) 16.4 °C 228 mm	Paris (France) 14.6 °C 388 mm

- The meeting preferred to see the raw non-standardised field DT_{50} values presented transparently, together with step by step calculations, so that evaluators could consider the reliability of the DT_{50} values at each stage.

However, as explained above, no raw non-standardised DT_{50} values exist in this parameter estimation procedure. It is not one DT_{50} value per trial that has been corrected for soil temperature and moisture, but daily actual transformation rates. These daily actual transformation rates have a precisely defined relation to the transformation rate at reference conditions. This relation is solely a function of soil temperature and soil moisture via the Arrhenius equation and the Walker equation. The transformation rate at reference conditions is the parameter that is actually subject to optimisation. Thus, unfortunately, the procedure cannot be broken down to smaller steps from a raw half-life to the standardised half-life to be

easier accessible during evaluation. The example in Figure B.8.6.6-1 shows the lumped intermediate daily degradation rates for tritosulfuron and their dependence on the soil temperature. These intermediate data are produced internally during the model runs for each of the up to 8 first order transformation rates per trial.
The mentioned parameter estimation study has been published (Dressel & Beigel, 2001) and evaluated by the current FOCUS workgroup on degradation kinetics. It serves as one example how field half-lives should be estimated from field data. If the prerequisites are fulfilled that a field dissipation trial can be used for parameter estimation, FOCUS kinetics considers it useful to normalise these data using a reference temperature and moisture condition in order to permit the broadest possible use of the field dissipation data.

- Use and robustness of standardised field data over laboratory degradation data as input for groundwater modelling were questioned.

The doubts concerning the robustness of standardised field half-lives could be attributed to the robustness of the parameter estimation procedure, to the quality of the raw data, or to possible mistakes of the authors that cannot be easily found by evaluators. The parameter estimation procedure is identical to the parameter estimation procedure for laboratory studies using a compartment model or other fitting procedures. For each transformation, one transformation parameter is fitted. The step that makes the actual procedure more laborious is the introduction of background calculations using temperature and moisture. In these background calculations, no additional fitting is done, only well accepted fixed functional relationships between temperature/moisture and transformation velocity are solved, as the FOCUS groundwater simulation models do likewise. The statistical evaluation (t-test) of each parameter ensured that unreliable transformation parameters were not considered for further assessments. The parameter estimation procedure is therefore as robust as that used for laboratory studies; there are only more calculation steps involved.
Since the input data are not readily available and the overall model is more laborious to set up, an evaluation of the study is more difficult than an evaluation of laboratory studies. Moreover, due to many more input data, a more complex model etc., the procedure may be more prone to operator errors. The notifier offered assistance to evaluators by providing any necessary assistance (e.g. raw data), thereby taking advantage of his quality assurance procedure in place (independent modeller check). **The RMS considers the presented data and calculations reliable. There is deemed no need for further assessment.**
The quality of the field raw data is in many cases inferior to laboratory data, due to more scattering of the datapoints. The final statistical evaluation in the estimation procedure indicated that parameters estimated from scattered data were not reliable. Thus, unreliable parameters were eliminated.
The notifier chose to use higher tier data, i.e field data, instead of laboratory data, because leaching assessments based on laboratory data did not reflect the result of the experimental leaching studies (lysimeters). These initial calculations were not presented in the original dossier.

- Strong reluctance was expressed to agree to the standardisation of the field dissipation data in preference over laboratory data. There was noted to be an order of magnitude difference between the laboratory and field DT_{50} results of ca. 98 versus 9.7 days, respectively, at 20 °C.

Laboratory studies to elucidate soil metabolism and degradation of tritosulfuron were conducted and are summarised in Table B.8.6.6-3. One additional study on the soil degradation of metabolite 635M04 in three soils was conducted to provide a reliable average value from laboratory studies (Hein, 2003). An overview of laboratory half-lives of tritosulfuron and metabolites standardised to reference conditions is given in Table B.8.6.6-4.

The differences between average standardised laboratory half-lives and average standardised field half-lives is much less pronounced than expected by the ECCO 137 meeting.

Table B.8.6.6-3: Rates of degradation as obtained from laboratory studies as listed in the list of endpoints of the EU-monograph of 20.09.2003

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

Method of calculation	ModelMaker 3.0.3/3.0.4 (Cherwell Scientific Publishing Limited); TOPFIT pharmacokinetic analysis; Timme and Frehse, 1st order kinetics, DT_{50} of metabolites calculated from studies with tritosulfuron
Laboratory studies (range or median, with n value, with r^2 value)	DT_{50lab} (20 °C, aerobic) in days:

soil	as	635M01	635M02	635M03	635M04
Li35b	31/32	110/184	96	347/737	98
Lufa2.2	16	65	37	203	nc
US-soil	19	59	44	32	nc
Bruch	38	23	28	nc	nc
Canad.	(124)	44	nc	nc	nc
Speyer	20	115	nc	nc	nc
mean	26	86	51	330	-
r^2 (low)	0.970	0.886	0.900	0.893	0.962
r^2 (high)	0.997	0.979	0.951	0.977	-

	tritosulfuron: DT_{90lab} (20°C, aerobic): 53 - 125 d (409 d)
	tritosulfuron: DT_{50lab} (10°C, aerobic, calc.): 42 - 271 d
	tritosulfuron: DT_{50lab} (20°C, anaerobic): 61 - 82 d
	degradation in the saturated zone: not relevant

Table B.8.6.6-4: Laboratory soil half-lives of tritosulfuron and its metabolites, their standardisation according to FOCUS (all studies conducted at 20 °C and 40 % MWC), and the comparison to arithmetic mean standardised field half-lives

Soil	Soil type	OC [%]	pH	Soil moisture [%]	Tritosulfuron		635M01		635M02		635M03		635M04	
					DT_{50} (study) [d]	DT_{50} (FOCUS) [d]	DT_{50} (study) [d]	DT_{50} (FOCUS) [d]	DT_{50} (study) [d]	DT_{50} (FOCUS) [d]	DT_{50} (study) [d]	DT_{50} (FOCUS) [d]	DT_{50} (study) [d]	DT_{50} (FOCUS) [d]
Lufa 2.2	sandy loam	1.8	5.8	16.4	16.0	14.4	65.0	58.6	37.0	33.4	203.0	183.1		
US	sandy loam	0.6	4.9	11.6	19.0	13.5	59.0	41.8	44.0	31.1	32.0	22.7		
Bruch West	sandy loam	2	7.6	12.4	38.0	28.2	23.0	17.1	28.0	20.8		n.c.		
Canadian soil	sandy loam	3.9	8.1	19.6	(124.0)	n.c.	44.0	44.0	n.c.	n.c.		n.c.		
Li35B (phenyl)	sandy loam	0.7	6.3	12.4	31.0	23.0	110.0	81.6	96.0	71.2	347.0	257.4		
LJ35B (triazin)	sandy loam	0.9	6.5	13.2	32.0	24.8	184.0	142.6	n.c.	n.c.	(737.0)	n.c.	98.0	75.9
Speyerer Wald	loamy sand	0.8	5.4	9.6	20.0	15.4	115.1	88.4	n.c.	n.c.	n.c.	n.c.		
Birkenheide	sandy loam	0.6	5.8	8.7									35.3	20.4
Mussbach	loam	0.6	7.4	10.5									53.9	29.3
Lufa 6S	clay	2.0	6.8	12.8									94.5	37.6
Arithmetic mean					**26.0**	**19.9**	**85.7**	**67.7**	**51.3**	**39.1**	**194.0**	**115.8**	**70.4**	**40.8**
Arithmetic mean field half-lives						**14.1**		**69.9**		**64.4**		**67.7**		**9.7**

To account for the doubts concerning the use of standardised field half-lives in risk assessment, particularly with respect to metabolite half-lives, leaching to groundwater was re-evaluated. Since the half-life estimation for the parent compound showed to be more reliable (no deselection of half-life estimations due to bad statistics), yielded a very plausible average standardised half-life and, moreover, was validated with three independent field trials, the standardised field half-life of tritosulfuron is considered well supported. An additional calculation of PEC_{gw} according to FOCUS was conducted with FOCUS-PELMO v3.3.2, considering, on the one hand, the average FOCUS-standardised field half-life for tritosulfuron and, on the other hand, metabolite formation fractions and FOCUS-standardised half-lives derived from the laboratory studies for the metabolites. The main input parameters are given in Table B.8.6.6-5 and the metabolisation pattern with transformation rates k is depicted in Figure B.8.6.6-2. The results are shown in Table B.8.6.6-6 (winter cereals), Table B.8.6.6-7 (spring cereals) and Table B.8.6.6-8 (maize).

Table B.8.6.6-5: Summary of the main input parameters used for the FOCUS simulations

Compound	Molecular weight	K_{oc}	1/n	Formation fraction	Formation rate	Half-life	Transformation rate to CO_2
tritosulfuron	445.3	7.4	0.913			14.1	0.0092
635M01	353.3	89.0	0.923	0.759	0.0373	67.7	0.0004
635M02	225.2	40.6	0.957	From M01: 0.719 From M03: 0.580	From M01: 0.0074 From M03: 0.0035	39.1	0.0177
635M03	310.3	30.1	0.912	0.240	0.0019	115.8	0.0025
636M04	194.1	20.8	0.935	0.055	0.0026	40.8	0.0170

Figure B.8.6.6-2: Metabolisation scheme following the kinetic evaluation of the laboratory soil degradation studies. The figures on the flux-arrows represent the values of the first order rate constants. For the metabolites, laboratory half-lives and formation fractions were used, for tritosulfuron (BAS 635 H), the lumped field half-life was considered.

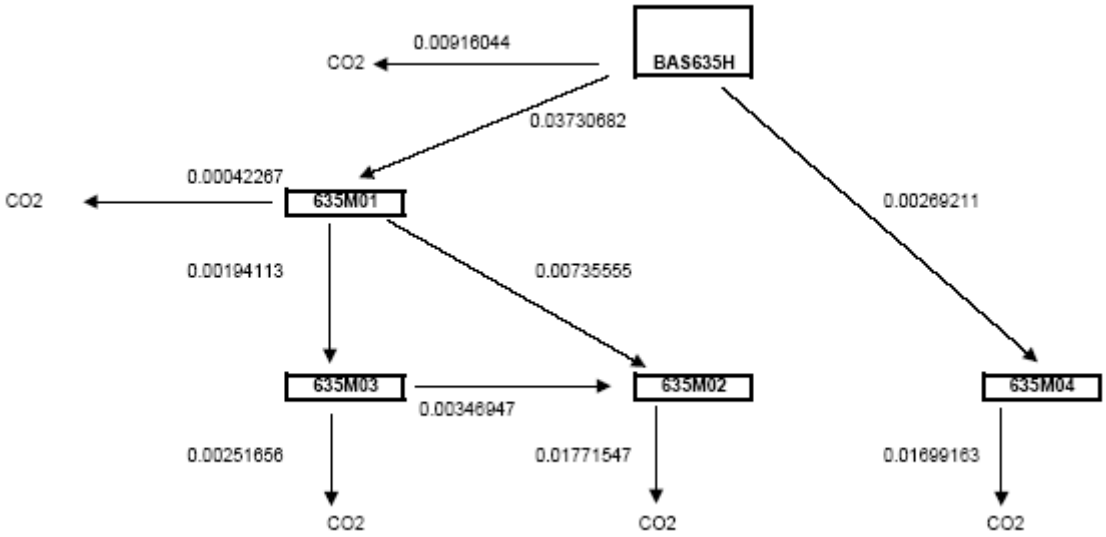


Table B.8.6.6-6: Predicted 80th percentile concentration at 1 m depth following an application of 0.050 kg/ha of tritosulfuron on winter cereals three weeks after emergence (worst case of recommended range BBCH 20-39), resulting in a loading of 0.025 kg/ha after 50 % interception (FOCUS recommendation for BBCH 10-19). Simulations performed with FOCUS-PELMO v3.3.2.

Location	Application date [dd-mm]	Tritosulfuron [ug/L]	635M01 [ug/L]	635M02 [ug/L]	635M03 [ug/L]	635M04 [ug/L]
Châteaudun	01-04	0.008	0.062	0.145	0.193	0.007
Hamburg	01-04	0.066	0.224	0.286	0.239	0.017
Jokioinen	01-06	0.082	0.108	0.226	0.236	0.016
Kremsmiinster	01-04	0.079	0.313	0.258	0.224	0.021
Okehampton	01-04	0.089	0.338	0.263	0.203	0.020
Piacenza	01-04	0.074	0.373	0.293	0.274	0.018
Porto	10-03	0.006	0.009	0.052	0.070	0.002
Sevilla	10-03	0.001	0.001	0.004	0.015	<0.001
Thiva	10-03	0.001	0.010	0.072	0.144	0.002

Table B.8.6.6-7: Predicted 80th percentile concentration at 1 m depth following an application of 0.050 kg/ha of tritosulfuron on spring cereals three weeks after emergence (worst case of recommended range BBCH 13-39), resulting in a loading of 0.0375 kg/ha after 25 % interception (FOCUS recommendation for BBCH 10-19). Simulations performed with FOCUS-PELMO v3.3.2.

Location	Application date [dd-mm]	Tritosulfuron [ug/L]	635M01 [ug/L]	635M02 [ug/L]	635M03 [ug/L]	635M04 [ug/L]
Châteaudun	31-03	0.004	0.041	0.136	0.228	0.006
Hamburg	22-04	0.062	0.259	0.381	0.373	0.026
Jokioinen	01-06	0.143	0.103	0.259	0.336	0.019
Kremsmünster	22-04	0.075	0.367	0.353	0.337	0.024
Okehampton	22-04	0.099	0.346	0.374	0.320	0.029
Porto	31-03	0.002	0.003	0.039	0.075	0.002

Table B.8.6.6-8: Predicted 80th percentile concentration at 1 m depth following an application of 0.050 kg/ha of tritosulfuron on maize three weeks after emergence (worst case of recommended range BBCH 13-39), resulting in a loading of 0.0375 kg/ha after 25 % interception (FOCUS recommendation for BBCH 10-19). Simulations performed with FOCUS-PELMO vS.3.2.

Location	Application date [dd-mm]	Tritosulfuron [ug/L]	635M01 [ug/L]	635M02 [ug/L]	635M03 [ug/L]	635M04 [ug/L]
Châteaudun	22-05	0.016	0.082	0.183	0.253	0.010
Hamburg	26-05	0.084	0.267	0.393	0.381	0.029
Kremsmünster	26-05	0.050	0.186	0.282	0.313	0.018
Okehampton	15-06	0.083	0.274	0.363	0.342	0.025
Piacenza	05-06	0.062	0.449	0.373	0.290	0.024
Porto	22-05	0.001	0.001	0.017	0.049	0.001
Sevilla	28-03	<0.001	<0.001	<0.001	0.001	<0.001
Thiva	11-05	<0.001	0.001	0.033	0.093	<0.001

Conclusions

- Soil adsorption of tritosulfuron and its metabolites is not pH-dependent.
- Soil adsorption of tritosulfuron showed kinetic behavior, i.e. an increase of soil adsorption with time.
- The derivation of standardised field dissipation half-lives with a subsequent statistical evaluation of the parameters is a suitable tool to estimate degradation parameters that reflect the real field situation and meet the needs of FOCUS groundwater simulation models. However, since the ECCO 137 meeting was reluctant to accept this procedure, especially for derivation of metabolite half-lives, new $FOCUS_{gw}$ calculations were conducted with formation fractions and half-lives of the metabolites derived from laboratory studies, but the well supported and validated average standardised field half life of the parent compound tritosulfuron. The key results are:
  - 635M01, 635M02, and 635M03 were predicted at concentrations > 0.1 µg/L but < 0.75 µg/L in the groundwater in 1 m depth, whereas 635M04 never exceeded 0.1 µg/L.
  - Several safe uses with respect to leaching into groundwater could be identified concerning the parent compound and its metabolite of toxicological relevance 635M04. Also uses could be identified where neither tritosulfuron nor any of its metabolites exceeded 0.1 µg/L.

Comment of RMS:

The reasoning of the notifier with respect to non-dependency of sorption to pH is agreed by the RMS. The time-dependency of sorption parameters needs not be further discussed at this stage, since no "effective" $K_{f,oc}$ values were used in the presented $FOCUS_{gw}$ calculations. The input parameters are accepted and the calculation results are plausible. The RMS is of the opinion that the questions and open points from the ECCO 137 meeting have been sufficiently addressed.

B.8.10 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
IIA-7.1.1.1.1	Hein, W.	2003	Determination of the degradation rate of [triazine-2,4-^{14}C] AMTT in three different soils under aerobic conditions BASF Doc ID 2003/1001038 BOD2004-16		
IIA-7.1.2	Altfelder, S., Streck, T and Richter, J.	2000	Nonsingular sorption of organic compounds in soil: The role of slow kinetics. J. Environ. Qual. 29: 917-925.		

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
IIA-7.1.2	Gottesbüren, B.	2003	Evaluation of the sorption of BAS 635 H - Tritosulfuron and metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5 considering the dependency of sorption to soil parameters and sorption kinetics 2003/1005456 BOD2003-300		
IIA-7.1.2	Jene, B.	2002	Estimation of effective relevant sorption values of BAS 635 H in soils 2001/1015001 BOD2003-170		
IIA-7.1.2	Jene, B.	2003	Kinetic evaluation of the adsorption behaviour of the metabolites of BAS 635 H (tritosulfuron): BH 635-2, BH 635-3, BH 635-4, and BH 635-5 2003/1000991 BOD2003-171		
IIA-7.1.2	Richter, T.	2003	Study for the adsorption/desorption determination of BAS 635 H on 5 European soils BASF Doc ID 2003/1005441 BOD2004-14		
IIA-7.1.2	Streck, T. et al.	1997	FITHYST 3: Parameter estimation program for hysteretic sorption isotherms Department of Geoecology, Technical University of Braunschweig, Germany		
IIA-7.1.2	Streck, T., Poletika, N.N., Jury, W.A. and Farmer, W.J	1995	Description of simazin transport with rate-limited, two stage, linear and non-linear sorption. Water Resour. Res. 31 (4): 811-822.		
IIA-7.1.2	Streck, T. and Richter, J.	1999	Field-scale study of chlortoluron movement in a sandy soil over winter: II. Modelling. J. Environ. Qual. 28: 1824-1831.		
IIA-7.1.2	Zirnstein, M.	2003	Adsorption/desorption-study of BAS 635 H metabolite BH 635-2 (reg. no. 292564) on five European soils BASF Doc ID 2003/1005442 BOD2004-57		
IIA-7.1.3.3	Staudenmaier, H.	2003	Non-identified radioactivity in lysimeter leachates of lysimeters treated with BAS 635 H 2003/1009267; Li 740 BOD2003-301		
IIIA-9.1.3	Dressel, J.	2003a	Predicted long term concentrations of the soil metabolites of BAS 635 H (635M01, 635M02, 635M03, 635M04) in soil 2003/1009263; CALC-452 BOD2003-306		

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
IIIA-9.1.3	Dressel, J. and Beigel, C.	2001	Estimation of standardised transformation rates of a pesticide and its four soil metabolites from field dissipation studies BCPC symposium proceedings No.78: Pesticide behaviour in soils and water, 119-126.		
IIIA-9.1.3	Hauck, T.	2002	Calculation of predicted environmental concentrations for BAS 635 H (tritosulfuron) and metabolites BH 635-2, BH 635-3, BH 635-4 and BH 635-5 in soil using worst case half-lives 2002/1000216; CALC-316 BOD2003-302		
IIIA-9.2.1 (assigned to IIIA- 9.1.3 by notifier)	Dressel, J.	2003b	Evaluation of the soil degradation, soil adsorption and leaching potential of BAS 635 H and its metabolites 635M01, 635M02, 635M03, and 635M04 BASF Doc ID 2003/1018144 BOD2004-13		
IIIA-9.2.1 (assigned to IIIA- 9.1.3 by notifier)	Dressel J.	2004	Evaluation of the soil degradation, soil adsorption and leaching potential of BAS 635 H and its metabolites 635M01, 635M02, 635M03, and 635M04 - 1. Amendment to Final Report – BASF 2004/1009160 BOD2004-488		
IIIA-9.2.1	Gottesbüren, B.	2002	Calculation of predicted environmental concentrations in groundwater (PECgw) of BAS 635 H and its metabolites for UK under special considering of sorption dependencies 2002/1011916; CALC-365 WAS2003-115		
IIIA-9.2.3	Dressel, J.	2002a	Calculation of predicted environmental concentrations for BAS 635 H and metabolites BH 635-2, BH 635-4 and BH 635-5 in static surface waters (PECsw) and sediment (PECsed) after drift entry 2002/1000213; CALC315 WAS2002-96		
IIIA-9.2.3	Dressel, J.	2002b	First amendment to Final Report: Calculation of Predicted Environmental Concentations for BAS 635 H and metabolites BH 635-2, BH 635-4 and BH 635-5 in static surface waters (PECsw) and sediment (PECsed) after drift entry 2002/1004269; CALC315 WAS2005-2		

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
IIIA-9.2.3	Dressel, J.	2003c	Second Amendment to Final Report: Calculation of predicted environmental concentrations for BAS 635 H and metabolites BH 635-2, BH 635-4 and BH 635-5 in static surface waters (PECsw) and sediment (PECsed) after drift entry 2003-1001039; CALC315 WAS2005-4		
IIIA-9.2.3	Dressel, J.	2003d	Third Amendment to Final Report: Calculation of Predicted Environmental Concentrations for BAS 635 H and metabolites BH 635-2, BH 635-4 and BH 635-5 in static surface waters (PEC_{sw}) and sediment (PEC_{sed}) after drift entry 2003/1009268; CALC-315 WAS2005-3		

B.9 Ecotoxicology

B.9.2 Effects on aquatic organisms (Annex IIA 8.2; Annex IIIA 10.2)

B.9.2.1 Toxicity data

B.9.2.1.1 Aquatic plants

Annex Point:	IIA-8.2.8
Author:	Junker, M.
Title:	Effect of BH 635-4 on the Growth of *Lemna gibba*
Date:	
Doc ID:	2003/1012053 WAT2004-804
Guidelines:	OECD – guidelines for testing of chemicals, draft guideline: "*Lemna* sp., Growth Inhibition Test", Oct. 2000 ASTM E1415-91, "Standard Guide for Conducting Static Toxicity Tests with Lemna gibba G3", OPPTS 850.4400 (draft, April, 1996)
GLP:	Yes

Material and methods:

BH 635-4
batch 2059-011, purity: 96.4 %
control, 3.13, 6.25, 12.5, 25, 50 and 100 mg/L (nominally).

Lemna gibba G3
from in-house culture, culture conditions comparable to test conditions, regular renewal of cultures, inocula were taken from cultures that were 7-10 days old.

400 mL glass beakers, filled with 160 mL 20x-AAP medium and covered by a clear glass plate; pH of nutrient solution 7.5 at test initiation, temperature 24-26 °C; continuous light, ca. 7.94 klux.
Static test over 7 days; 6 concentrations each with 3 replicates plus a control with 6 replicates. Two plants with four fronds and one plant with three fronds were added impartially to each vessel under axenic conditions giving a total number of 11 fronds at test initiation. Assessment of growth and other effects on days 2, 5 and at test termination on day 7. The biomass based on the dry weight was determined at test beginning from a sample of the inoculum culture and at test termination with the plant material from each test concentration and control.

Standard procedures, analysis of variance, Bonferroni test

Findings:

Analytical verification of test item concentrations was conducted at all tested concentrations. Measured concentrations of BH 635-4 ranged from 85-86 % of nominal at test initiation and from 85-87 % of nominal at the end. The following biological results are based on the nominal concentrations.

Table B.9.2.1-1: Effect of BH 635-4 on the growth of Lemna gibba:

Concentration [mg/L]	% Inhibition after 7 days - growth rate	% Inhibition after 7 days - frond number	% Inhibition after 7 days - dry weight
3.13	1.0	2.8	-6.9
6.25	-2.9	-8.2	-10.9
12.5	-2.4	-8.2	-17.9
25	2.5	6.9	-5.9
50	-5.3	-16.2	- 29.3
100	-3.7	-10.8	-24.9

Growth rate based on frond number: E_rC_{50}(7day) > 100 mg/L
Biomass based on frond number: E_bC_{50}(7day) > 100 mg/L
Biomass based on dry weight: E_bC_{50}(7day) > 100 mg/L

NOEC = 100 mg/L

Valid: Yes

Conclusions:

No concentration related effects on dry weight or appearance of the fronds were observed during the course of the study. Since even the highest concentration had no significant adverse effect on the respective observed test parameter, all EC_{50} were determined to be greater 100 mg/L and the NOEC = 100 mg/L.

B.9.2.2 Risk assessment

As compared to the toxicity of the active substance tritosulfuron towards *Lemna* with an E_rC_{50}(7day) = 0.0476 mg/L, an E_bC_{50}(7day) = 0.0255 mg/L and a NOEC (based on growth) of 0.0075 mg/L, the toxicity of the metabolite BH 635-4 is smaller by several orders of magnitude. The additional risk to higher aquatic plants can therefore be considered negligible.

Comment of RMS:
The RMS agrees to the presented risk assessment with its conclusion on a neglibigle risk to higher aquatic plants from AMTT.

B.9.5 Effects on other terrestrial vertebrates (Annex IIIA 10.3)

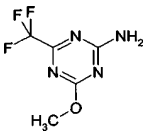
B.9.5.2 Risk assessment for wild mammals

Annex Point: IIA-8.2.8
Author: Welter, K.
Title: AMTT (Reg. No. 231 700, BH 635-5) Risk assessment for terrestrial vertebrates other than birds - In support of the provisional registration in the United Kingdom
Date:
Doc ID: 2002/1011551
AVS2003-225
GLP: No

Metabolites

An assessment of the risk to wild mammals resulting from AMTT was provided from the notifier in reaction to a concern raised in the UK national process for a provisional authorisation. The assessment is based on the use pattern in the UK, i.e. an early application on 1st February with 1 × 50 g as/ha.

Identity of AMTT:



CAS registry number: 5311-05-7
EINECS No. not assigned
BASF Reg. No. 231700
Empirical formula: $C_5H_5F_3N_4O$
Molar mass: 194.12
Chemical name: 2-amino-4-methoxy-6-(trifluoromethyl)-l,3,5-triazine
Synonym: AMTT or BH 635-5 or 635M04
Maximum concentration in the technical concentrate is 0.2 %.

Toxicity of BAS 635 H and AMTT:

AMTT was an impurity in the tox batch N24 that caused severe effects in the long-term rat studies and in the 2-generation study in rats. It is a metabolite in soil and water, and is also found in cereal plants. The oral LD_{50} for AMTT was found to be > 200 and < 2000 mg/kg bw for male and female rats.

Two-generation reproduction toxicity study with BAS 635 H, batch N24 containing 2.45 % AMTT:

- NOAEL for fertility is 3,500 ppm/2,100 ppm (about 369/206 mg/kg bw/d) for the F0 parental rats and 2,100 ppm (about 206 mg/kg bw/d) for the F1 parental animals.
- NOAEL for reproductive performance is 100 ppm (about 10 mg/kg bw/d) for the F0 parental females, but a NOAEL was not achieved for the F1 parental animals.

- NOAEL for general toxicity is 100 ppm (about 10 mg/kg bw/d) for the F0 and F1 parental animals.
- NOAEL for developmental toxicity is 100 ppm (about 10 mg/kg bw/d) for the F1a/F1b progeny, but a NOAEL was not achieved for the F2 progeny.

A supplementary study investigating dose levels of 25 and 50 ppm was performed to demonstrate a clear NOAEL for reproductive performance and developmental toxicity:
- NOAEL for reproductive performance is 50 ppm (about 5.1 mg/kg bw/d) for the F0 parental females and 25 ppm (about 2.6 mg/kg bw/d) for the F1 parental females.
- NOAEL for developmental toxicity is 50 ppm (about 5.1 mg/kg bw/d) for the F1a/F1b progeny and 25 ppm (about 2.6 mg/kg bw/d) for the F2 progeny.

Since it was clear that AMTT was responsible for most of the effects seen, the 2-generation reproduction toxicity study was repeated with a batch containing minor quantities of AMTT.

Two-generation reproduction toxicity study with BAS 635 H, batch N34 containing 0.02 % AMTT:
- NOAEL for reproductive performance and fertility is 3,600 ppm (about 388 mg/kg bw/d) for the F0 and F1 parental rats.
- NOAEL for general toxicity/systemic effects of the test substance is 3,600 ppm (about 394 mg/kg bw/d) for the F1 parental males and 600 ppm (about 65 mg/kg bw/d) for the F0 parental males and females and F1 parental females.
- NOAEL for developmental toxicity is 3,600 ppm (about 387 mg/kg bw/d) for the F1a/F1b and F2 progeny.

In a supplementary pre/postnatal screening study the oral application of AMTT induced severe maternal and developmental toxicity at 20 mg/kg bw/d and at 50 mg/kg bw/d. Based on these results, AMTT is seen responsible for the effects observed in the 2-generation study with BAS 635 H containing 2.45 % AMTT. Thus the NOAEL for reproductive performance and developmental toxicity of this study (25 ppm or 2.6 mg/kg bw/d) can fully be attributed to AMTT. The NOAEL for reproductive performance and developmental toxicity of AMTT calculates to be 1.23 ppm (0.125 mg/kg bw/d).

Comment of RMS:
The NOAEL for AMTT is 0.6 ppm (0.06 mg/kg bw/d) rather than 1.23 ppm (0.125 mg/kg bw/d). The NOAEL for BAS 635 H, batch N24 with an AMTT content of 2.45 % was 25 ppm (0.6 ppm). Since the toxicity can be attributed to AMTT alone, the respective NOAEL value for this compound calculates as follows: 2.45 % × 25 ppm = 0.6 ppm. The long-term risk assessment below has been recalculated accordingly by the RMS.

Spray Solution Droplets:

A potentially relevant route of exposure is via uptake of fresh spray solution droplets from plants. These droplets would dry off within a few hours after application; hence the exposure of small mammals would be an acute one. The oral LD_{50} for AMTT was found to be > 200 and < 2000 mg/kg bw for male and female rats.
AMTT is an impurity in the technical grade active substance (maximum 0.2 %) and a degradation product occurring upon storage of BAS 635 H (below 0.2 %) and the spray solution. Thus the concentration of AMTT in ready to use spray is a function of these factors. As a worst case, it is assumed that a formulation being stored for two years is used to prepare

the spray, and that the spray is allowed to stand for 48 hours before being applied. This would result in maximum 0.366 % AMTT, which corresponds to 0.924 mg AMTT/L.
Assuming a small mammal (10 g body weight) with a daily water consume of 30 % of its body weight, this animal would consume 3 mL of water or 0.0028 mg AMTT. Using the acute oral LD_{50} for the rat of > 200 and < 2000 mg AMTT/kg bw, the estimated median lethal dose for a 10 g mammal would be > 2 and < 20 mg AMTT. **The TER_a spray droplet for AMTT is thus [> 2 and < 20] / 0.0028 = [> 714 and < 7,140].** This exceeds the Annex VI trigger value of 10 and indicates an acceptable acute risk to small mammals from consuming spray droplets from plants.

Herbivorous Diet:

The most relevant route of exposure is the oral route via contaminated food. From the residue section, data for AMTT in cereal plants without root can be taken. At 0 days after treatment, between < 0.001 and 0.05332 mg AMTT/kg were found. After 8-30 days, only seven out of 46 plant samples contained AMTT between 0.00107 and 0.00285 mg/kg. The maximum initial AMTT residue in cereal plants is 0.05332 mg/kg, which would cover AMTT forming in the formulation, spray tank and on the plant.

Assuming a small herbivorous mammal (10 g body weight) with a daily food consumption of 30 % of its body weight, this animal would consume 3 g of plant material or 0.00016 mg AMTT. Using the acute oral LD_{50} for the rat of > 200 and < 2000 mg AMTT/kg bw, the estimated median lethal dose for a 10 g mammal would be > 2 and < 20 mg AMTT.
The TER_a herbivorous diet AMTT is thus [> 2 and < 20] / 0.00016 = [> 12,500 and < 125,000]. This exceeds the Annex VI trigger value of 10 and indicates an acceptable acute risk to small mammals from consuming plants containing residues of AMTT.

The NOAEL for reproductive performance and developmental toxicity of AMTT is calculated to be 0.6 ppm (0.06 mg/kg bw/d).
The TER_{lt} herbivorous diet for AMTT is thus 0.6 / 0.05332 = 12. This exceeds the Annex VI trigger value of 5 and indicates an acceptable risk to small herbivorous mammals on the long-term scale. This is still considered to represent a worst case because,

- BAS 635 H is only applied during a restricted time period in spring (up to BBCH 39).
- AMTT residues are not persistent in plants, but rapidly decline.
- at 8-30 days after application, only seven out of 46 plant samples contained AMTT between 0.00107 and 0.00285 mg/kg, which is a factor of 19 below the maximum initial value.
- AMTT does not accumulate in rats, but is effectively excreted.
- The log P_{ow} of AMTT is 1.13, which further confirms that there is not potential for an accumulation.

Earthworm Diet:

A potential additional route of exposure is via uptake of earthworms containing soil with residues of AMTT. The half-life of AMTT in soil at 5, 15 and 20 °C is 75, 34 and 9.7 days, respectively. With an assumed early application on 1^{st} February, the degradation in soil is assumed to be slow first, but with increasing temperature in spring it will progressively increase in speed. It can thus be concluded that the half-life will be below 75 days, which would be the value for a soil temperature of constantly 5 °C. Also, at the time of application, small earthworm eating mammals would not be present in the cereal field due to insufficient shelter. These animals are assumed to expand their feeding range to the cereal field not earlier

then at the end of tillering (BBCH 39) when the crop cover provides sufficient shelter. In conclusion, the exposure of small earthworm eating mammals to AMTT is assumed to be of no relevance. Nevertheless, although AMTT is instable in soil and BAS 635 H is applied far earlier than the area may be colonised by earthworm feeders, as a worst-case it is assumed that an acute exposure might be possible.

The species being in focus concerning this route of exposure would be the common shrew *(Sorex araneus).* The majority of prey items are obtained from subterranean layers (Churchfield, 1990). The diet consists predominantly of invertebrates (arthropods and their larvae, earthworms) and it can be assumed that it comprises 50 % arthropods (25 % on soil surface, 25 % endogeic) and 50 % earthworms. However, the common shrew is known to occur in hedges bordering arable land (Kotzageorgis & Mason, 1997), but not in arable fields. For the purpose of this assessment, it is assumed that shrews will take up 10 % of their diet in the borderline between hedges and cereal field.

The log P_{ow} of AMTT is 1.13, which confirms that there is no potential for an accumulation in the food chain. Therefore, the concentration in earthworms is assumed to be depending on the amount of soil in their gastrointestinal tract. The initial PEC for the top 5 cm of soil after one application in cereals at 50 g BAS 635 H/ha and with the worst-case assumption of no crop interception is 0.006 mg AMTT/kg soil. Assuming further that a 100 mg earthworm contains 30 mg of that contaminated soil, it would contain 0.0001 ng AMTT (equivalent to 0.001 mg AMTT/kg worm).

Comment of RMS:
The concentration of AMTT in worms is calculated as follows:
0.006 mg AMTT/kg soil × (30 mg soil/100 mg worm) = 0.0018 mg AMTT/kg worm
Thus, the AMTT content in one single 100-mg earthworm is in fact 0.00018 ng.

Assuming that 50 % of the daily food intake of a common shrew [body weight 10g (Hausser et al., 1990), daily food intake based on fresh weight assumed to be 100 % of body weight (Hawkins & Jewell, 1962), hence 10 g] consists of earthworms and that 10 % of this diet is contaminated with AMTT, then this shrew would consume at maximum one contaminated worm containing 0.0001 mg AMTT per day. Using the acute LD_{50} for rats of > 200 and < 2000 mg AMTT/kg bw, the estimated median lethal dose for a 10 g common shrew would be > 2 and < 20 mg AMTT. Comparison of this number with the predicted consumption level of 0.0001 mg AMTT gives a **TER_a earthworm of > 20,000 and < 200,000**. This is in excess of the Annex VI trigger of 10 and indicates an acceptable acute risk to earthworm-eating birds. This scenario is still considered to represent a worst case, because maximum residues from the day of application were used, and the crop at BBCH 20-39 (tillering) would intercept 50 % of the spray according to FOCUS.

Comment of RMS:
The notifier's calculations are not comprehensible. A simpler assessment for the common shrews is proposed, based on the worst case diet of 100 % contaminated earthworms. Considering a food uptake of 10 g, this would be equivalent to 0.010 kg worm × 0.0018 mg AMTT/kg worm = 0.000018 mg AMTT. Using the acute LD_{50} for rats of > 200 and < 2000 mg AMTT/kg bw, the estimated median lethal dose for a 10 g common shrew would be > 2 and < 20 mg AMTT. The resulting TER_a earthworm for the predicted consumption level of 0.000018 mg AMTT then calculates as > 111,000 and < 1,111,000.

Conclusions:

The risk assessments provided for small mammals concerning their potential exposure via the sprayed solution and the food sources was based on the following toxicological data:

- The oral LD_{50} for AMTT was found to be > 200 and < 2000 mg/kg bw for male and female rats.
- The NOAEL for reproductive performance and developmental toxicity of two-generation reproduction toxicity study with BAS 635 H (batch N24 containing 2.45 % AMTT) was 25 ppm or 2.6 mg/kg bw/d and can fully be attributed to AMTT. The NOAEL for reproductive performance and developmental toxicity of AMTT calculates to be 0.6 ppm (0.06 mg/kg bw/d).

The risk assessments for small mammals concerning a potential exposure via the sprayed solution and food sources resulted in the following TER values:

- **TER_a spray droplets > 714 and < 7,140**
- **TER_a herbivorous diet > 12,500 and < 125,000**
- **TER_{lt} herbivorous diet = 12**
- **TER_a earthworm diet > 111,000 and < 1,111,000.**

These TERs are clearly in excess of the Annex VI trigger values of 10 and 5 and indicate that the risk to small mammals arising from potential uptake of AMTT is acceptable.

Comment of RMS:

Taking into account the corrections and amendments made by the RMS, the risk to mammals from AMTT appears acceptable. However, the notifier's assessment above has not been done according to the principles of the EU "Guidance Document on Risk Assessment for Birds and Mammals" (SANCO/4145/2000 – final). Since this was requested during the peer review process, the RMS has performed an own risk assessment according to SANCO/4145. This assessment is presented in the following.

Introduction

The compound AMTT occurs as an impurity in technical tritosulfuron and also as metabolite 635M04. In the toxicological studies, AMTT demonstrated significantly higher toxicity against mammals than the active substance tritosulfuron. The risk to small mammals in the field therefore needs to be addressed individually.
The oral LD_{50} for AMTT ist > 200 und < 2000 mg/kg bw (rat). The NOAEL from the 2-generation study (rat) ist 0.06 mg/kg bw/d (equivalent to a concentration in food of 0.6 mg/kg)

Spray solution droplets

Potential route of exposure: Uptake of fresh spray solution droplets from plants during water uptake from dew.
Exposure concentration: Formulation stored over two years and spray solution over 48 h, results in AMTT percentage of 0.366 %. A spray solution with 50 g as/150 L thus contains 1.22 mg AMTT/L.
Exposure: Small mammal, 10 g bw, water uptake 30 % of bw, equivalent to 300 mL water/kg bw and, consequently, 0.366 mg AMTT/kg bw (absolute amounts: 3 mL water, equivalent to 0.00366 mg AMTT).
Risik calculation: **TER = (> 200 mg/kg KG) / (0.366 mg/kg KG) = (> 546)**

The TER value for the exposure route via spray solution droplets is significantly above the Annex VI trigger of 10 for the protection of wild mammals from acute exposure.

Herbivorous diet

Potential route of exposure: Uptake of AMTT residues on plants during feeding.
Exposure concentration: Maximum AMTT concentration on plants as obtained from residue trials in plants is 0.05332 mg AMTT/kg and includes AMTT residues originating from the spray solution as well as from metabolism. The RUD is calculated as quotient from plant residue concentration divided by active substance application rate and equals 1.07.
Exposure: small herbivorous mammal (FIR/bw = 1.392)

ETE_a = (FIR/bw) × RUD × AWM × AV × PT × PD = 0.075 mg/kg bw/d

ETE_{lt} = (FIR/bw) × RUD × f_{twa} × AWM × AV × PT × PD = 0.039 mg/kg bw/d

Risk calculation:
TER_a = (> 200 mg/kg KG) / (0.075 mg/kg KG) = (> 2686)
TER_{lt} = (0.06 mg/kg KG/d) / (0.039 mg/kg KG/d) = 1.5

For refining the long-term risk assessment, the mean was calculated from the 46 analytically determined concentrations of AMTT in cereal plants from supervised residue trials. This mean amounts to 0.0087 mg AMTT/kg, which is equivalent to an RUD of 0.24
Cereal and maize plants in later growing stages are no appropriate food sources for small herbivorous mammals. Therefore, long-term exposure over several weeks actually does not take place. It can further be assumed that voles (indicator species for small herbivorous mammals) do not cover their whole food demand on the treated area, but also utilise the vegetation from field border structures. This is taken in account by setting the factor PT to 0.5.
Considering both refinement options, the reduced value for the actual daily uptake of AMTT calculates as ETE = 0.008 mg/kg bw/d.
Refined risk calculation:
TER_{lt} = (0.06 mg/kg bw/d) / (0.008 mg/kg bw/d) = 7.2

The TER value for acute exposure via herbivorous diet is significantly above the Annex VI trigger of 10. The TER value for long-term exposure surpasses the Annex VI trigger of 5 after refinement of the risk assessment (mean residue concentrations, PT).

Earthworm diet

Potential route of exposure: Uptake of earthworms contaminated with AMTT
Exposure concentration: No accumulation (log P_{OW} 1.13), therefore the content of AMTT in earthworms is assumed to equal the content on the soil (30 mg) in the digestive tract of an earthworm (100 mg). The actual concentration is:
0.006 mg AMTT/kg soil × (30 mg soil/100 mg worm) = 0.0018 mg AMTT/kg worm,
equivalent to an RUD of 0.036.
Exposure: A shrew (*Sorex araneus*) with a daily food uptake of 10 g, consisting to 100 % of earthworms. The ETE is 0.002 mg/kg bw/d
Risk calculation:
TER_a = (> 200 mg/kg KG) / (0.002 mg/kg KG) = (> 111,111)
TER_{lt} = (0.06 mg/kg KG/d) / (0.002 mg/kg KG/d) = 33

Both the TER values for acute exposure as well as for uptake over a longer period of time are above the Annex VI trigger values of 10 and 5, respectively.

Conclusions:

TER values calculated according to the EU Guidance document SANCO/4145 demonstrate an acceptable risk to mammals from uptake of AMTT via different exposure routes.

B.9.8 Effects on earthworms (Annex IIA 8.4; Annex IIIA 10.6.1)

B.9.8.3 Sublethal effects (Annex IIA 8.4.2; Annex IIIA 10.6.1.2)

Annex Point:	IIA-8.4.2
Author:	Lührs, U.
Title:	Effects of BH 635-2 on Reproduction and Growth of Earthworms *Eisenia fetida* in Artificial Soil
Date:	2002-10-29
Doc ID:	2002/1012746 ARW2003-150
Guidelines:	ISO 11268-2; BBA VI, Nr. 2-2
GLP:	Yes

Material and methods:

BH 635-2; Metabolite of BAS 635 H
Lot no. 00831-201; Reg. No. 292 564; content of as: 98.2 %

Test species
Earthworms (*Eisenia fetida*), from in-house culture IBACON (Rossdorf, Germany), adult worms with clitellum, weight 330-530 mg, ca. 10 months old

Test conditions
Artificial soil according to OECD 207; pH 5.7-5.8 (initial), 5.7 (end of test); water content 31.7-33.5 % (initial), 35.3-38.1 % (end of test)
Temperature 17-23 °C; illumination 16 h light/8 h dark

Different concentrations of the test item were mixed homogenously in the soil, which was filled into plastic boxes before the earthworms were introduced on top of the soil. 2 concentrations, 4 replicates per concentration with 10 worms each. Assessment of worm mortality, behavioural effects and measurement of weight change was carried out after 28-d exposure of adult worms in treated artificial soil. After additional 28 days, determination of offspring number was conducted.

Test concentrations
Control, 0.02 mg and 0.05 mg BH 635-2 per kg dry soil. The test concentrations reflect approximately 2-fold PEC_{ini} and 5-fold PEC_{ini}, respectively. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

Endpoints
Mortality, weight change, feeding activity and behavioural effects of earthworms after exposure over 28 days; reproduction rate within the 28 days of exposure (assessed 56 days after application).
Standard procedures, Fisher exact test, Dunnett test

Findings:

At a concentration of 0.02 mg/kg dry soil, one worm died within the 4 weeks of exposure, which was not significantly different from the control (Fisher exact test, $\alpha = 0.05$).
No effects on body weight, reproduction and feeding activity of the earthworms could be observed up to the concentration of 0.05 mg BH 635-2 per kg dry soil, the highest concentration tested.

Table B.9.8.3-1: Effects on on Reproduction and Growth of Earthworms *Eisenia fetida* of BH 635-2

endpoint	control	0.02 mg BH 635-2 per kg dry soil	0.05 mg BH 635-2 per kg dry soil
mortality [%][1)]	0.0 ± 0.0	2.5 n.s.[2)] ± 5.0	0.0 ± 0.0
Body weight change [%][1)]	38.6 ± 4.7	44.3 n.s.[3)] ± 10.2	41.2 n.s.[3)] ± 4.9
reproduction [# of juveniles][1)] [% of control]	411 ± 57	425 n.s.[3)] ± 52 103.2	460 n.s.[3)] ± 18 111.9
amount of food added [g][1)]	25.0 ± 0.0	25.0 ± 0.0	25.0 ± 0.0

1) mean ± standard deviation from 4 replicates
n.s. = not significantly different from control
2) Fisher exact test, $\alpha = 0.05$
3) Dunnett test, $\alpha = 0.05$

The most recent toxic standard test with carbendazim showed statistically significant effects on reproduction at a concentration of 1.28 mg/kg dry soil; the EC_{50} for reproduction was calculated as 1.46 mg/kg dry soil.

Valid: Yes

Conclusions:

In this study, the no observed effect concentration (NOEC) of BH 635-2 for mortality, growth, reproduction and feeding activity of the earthworm *Eisenia fetida* was found as 0.05 mg/kg dry soil, i.e. the highest tested concentration (> 5-fold PEC_{ini}).

Annex Point:	IIA-8.4.2
Author:	Lührs, U.
Title:	Effects of BH 635-3 on Reproduction and Growth of Earthworms *Eisenia fetida* in Artificial Soil
Date:	2002-10-29
Doc ID:	2002/1012745 ARW2003-149
Guidelines:	ISO 11268-2; BBA VI, Nr. 2-2
GLP:	Yes

Material and methods:

BH 635-3; Metabolite of BAS 635 H
Lot no. 01185-269; Reg. No. 335 182: content of as: 99.2 %;

Test species
Earthworms (*Eisenia fetida*), from in-house culture IBACON (Rossdorf, Germany), adult worms with clitellum, weight 300-496 mg, 8-9 months old

Test conditions
Artificial soil according to OECD 207; pH 6.1-6.2 (initial), 5.7-5.8 (end of test); water content 32.1-32.7 % (initial), 34.6-37.2 % (end of test)
Temperature 17-23 °C; illumination 16 h light/8 h dark

Different concentrations of the test item were mixed homogenously in the soil, which was filled into plastic boxes before the earthworms were introduced on top of the soil. 2 concentrations, 4 replicates per concentration with 10 worms each. Assessment of worm mortality, behavioural effects and measurement of weight change was carried out after 28-d exposure of adult worms in treated artificial soil. After additional 28 days, determination of offspring number was conducted.

Test concentrations
Control, 0.014 mg and 0.035 mg BH 635-3 per kg dry soil. The test concentrations reflect approximately 2-fold PEC_{ini} and 5-fold PEC_{ini}, respectively. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

Endpoints
Mortality, weight change, feeding activity and behavioural effects of earthworms after exposure over 28 days; reproduction rate within the 28 days of exposure (assessed 56 days after application).
Standard procedures, Fisher exact test, Dunnett test

Findings:

No effects on body weight, reproduction and feeding activity of the earthworms could be observed up to the concentration of 0.035 mg BH 635-3 per kg dry soil, the highest concentration tested.

Table B.9.8.3-2: Effects on on Reproduction and Growth of Earthworms *Eisenia fetida* of BH 635-3

endpoint	control	0.014 mg BH 635-3 per kg dry soil	0.035 mg BH 635-3 per kg dry soil
mortality [%][1)]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Body weight change [%][1)]	67.1 ± 4.6	71.1 n.s.[2)] ± 9.6	60.3 n.s.[2)] ± 10.0
reproduction [# of juveniles][1)] [% of control]	449 ± 63	395 n.s.[2)] ± 21 87.9	470 n.s.[2)] ± 70 104.6
amount of food added [g][1)]	25.0 ± 0.0	24.9 ± 0.3	25.0 ± 0.0

1) mean ± standard deviation from 4 replicates
n.s. = not significantly different from control
2) Dunnett test, $\alpha = 0.05$

The most recent toxic standard test with carbendazim showed statistically significant effects on reproduction at a concentration of 1.28 mg/kg dry soil; the EC_{50} for reproduction was calculated as 1.46 mg/kg dry soil.

Valid: Yes

Conclusions:

In this study, the no observed effect concentration (NOEC) of BH 635-3 for mortality, growth, reproduction and feeding activity of the earthworm *Eisenia fetida* was found as 0.035 mg/kg dry soil, i.e. the highest tested concentration (> 5-fold PEC_{ini}).

Annex Point: IIA-8.4.2
Author: Lührs, U.
Title: Effects of BH 635-4 on Reproduction and Growth of Earthworms *Eisenia fetida* in Artificial Soil
Date: 2002-10-29
Doc ID: 2002/1012744
ARW2003-148
Guidelines: ISO 11268-2; BBA VI, Nr. 2-2
GLP: Yes

Material and methods:

BH 635-4; Metabolite of BAS 635 H
Lot no. 01185-088; Reg. No. 335 184: content of as: 97.0 %

Test species
Earthworms (*Eisenia fetida*), from in-house culture IBACON (Rossdorf, Germany), adult worms with clitellum, weight 300-483 mg, 8-9 months old

Test conditions
Artificial soil according to OECD 207; pH 6.0-6.2 (initial), 5.7-5.8 (end of test); water content 31.3-33.7 % (initial), 35.2-37.5 % (end of test)
Temperature 17-23 °C; illumination 16 h light/8 h dark

Different concentrations of the test item were mixed homogenously in the soil, which was filled into plastic boxes before the earthworms were introduced on top of the soil. 2 concentrations, 4 replicates per concentration with 10 worms each. Assessment of worm mortality, behavioural effects and measurement of weight change was carried out after 28-d exposure of adult worms in treated artificial soil. After additional 28 days, determination of offspring number was conducted.

Test concentrations
Control, 0.065 mg and 0.16 mg BH 635-4 per kg dry soil. The test concentrations reflect approximately 2-fold PEC_{ini} and 5-fold PEC_{ini}, respectively. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

Endpoints
Mortality, weight change, feeding activity and behavioural effects of earthworms after exposure over 28 days; reproduction rate within the 28 days of exposure (assessed 56 days after application).
Standard procedures, Fisher exact test, Dunnett test

Findings:

No effects on body weight, reproduction and feeding activity of the earthworms could be observed up to the concentration of 0.16 mg BH 635-4 per kg dry soil, the highest concentration tested.

Table B.9.8.3-3: Effects on on Reproduction and Growth of Earthworms *Eisenia fetida* of BH 635-4

endpoint	control	0.065 mg BH 635-4 per kg dry soil	0.16 mg BH 635-4 per kg dry soil
mortality [%][1)]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Body weight change [%][1)]	67.1 ± 4.6	60.0 n.s.[2)] ± 11.8	68.5 n.s.[2)] ± 6.2
reproduction [# of juveniles][1)] [% of control]	 449 ± 63	 428 n.s.[2)] ± 64 95.3	 423 n.s.[2)] ± 60 94.1
amount of food added [g][1)]	25.0 ± 0.0	24.9 ± 0.3	25.0 ± 0.0

[1)] mean ± standard deviation from 4 replicates
n.s. = not significantly different from control
[2)] Dunnett test, $\alpha = 0.05$

The most recent toxic standard test with carbendazim showed statistically significant effects on reproduction at a concentration of 1.28 mg/kg dry soil; the EC_{50} for reproduction was calculated as 1.46 mg/kg dry soil.

Valid: Yes

Conclusions:

In this study, the no observed effect concentration (NOEC) of BH 635-4 for mortality, growth, reproduction and feeding activity of the earthworm *Eisenia fetida* was found as 0.16 mg/kg dry soil, i.e. the highest tested concentration (> 5-fold PEC_{ini}).

Annex Point:	IIA-8.4.2
Author:	Lührs, U.
Title:	Effects of BH 635-5 on Reproduction and Growth of Earthworms *Eisenia fetida* in Artificial Soil
Date:	2002-10-29
Doc ID:	2002/1012743 ARW2003-147
Guidelines:	ISO 11268-2; BBA VI, Nr. 2-2
GLP:	Yes

Material and methods:

BH 635-5; Metabolite of BAS 635 H
Lot no. 00831-237; Reg. No. 231 700: content of as: 99.9 %

Test species
Earthworms (*Eisenia fetida*), from in-house culture IBACON (Rossdorf, Germany), adult worms with clitellum, weight 330-530 mg, 10 months old

Test conditions
Artificial soil according to OECD 207; pH 5.7 (initial), 5.7-5.8 (end of test); water content 32.9-33.5 % (initial), 35.7-38.1 % (end of test)
Temperature 18-22 °C; illumination 16 h light/8 h dark

Different concentrations of the test item were mixed homogenously in the soil, which was filled into plastic boxes before the earthworms were introduced on top of the soil. 2 concentrations, 4 replicates per concentration with 10 worms each. Assessment of worm mortality, behavioural effects and measurement of weight change was carried out after 28-d exposure of adult worms in treated artificial soil. After additional 28 days, determination of offspring number was conducted.

Test concentrations
Control, 0.006 mg and 0.015 mg BH 635-5 per kg dry soil. The test concentrations reflect approximately 2-fold PEC_{ini} and 5-fold PEC_{ini}, respectively. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

Endpoints
Mortality, weight change, feeding activity and behavioural effects of earthworms after exposure over 28 days; reproduction rate within the 28 days of exposure (assessed 56 days after application).
Standard procedures, Fisher exact test, Dunnett test

Findings:

No effects on body weight, reproduction and feeding activity of the earthworms could be observed up to the concentration of 0.015 mg BH 635-5 per kg dry soil, the highest concentration tested.

Table B.9.8.3-4: Effects on on Reproduction and Growth of Earthworms *Eisenia fetida* of BH 635-5

endpoint	control	0.065 mg BH 635-5 per kg dry soil	0.16 mg BH 635-5 per kg dry soil
mortality [%][1)]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Body weight change [%][1)]	38.6 ± 4.7	46.6 n.s.[2)] ± 9.2	43.3 n.s.[2)] ± 12.3
reproduction [# of juveniles][1)] [% of control]	411 ± 57	455 n.s.[2)] ± 35 110.6	439 n.s.[2)] ± 23 106.8
amount of food added [g][1)]	25.0 ± 0.0	25.0 ± 0.0	25.0 ± 0.0

1) mean ± standard deviation from 4 replicates
n.s. = not significantly different from control
2) Dunnett test, $\alpha = 0.05$

The most recent toxic standard test with carbendazim showed statistically significant effects on reproduction at a concentration of 1.28 mg/kg dry soil; the EC_{50} for reproduction was calculated as 1.46 mg/kg dry soil.

Valid: Yes

Conclusion:

In this study, the no observed effect concentration (NOEC) of BH 635-4 for mortality, growth, reproduction and feeding activity of the earthworm *Eisenia fetida* was found as 0.015 mg/kg dry soil, i.e. the highest tested concentration (> 5-fold PEC_{ini}).

B.9.8.4 Acute and long-term risk assessment

B.9.8.4.1 Acute risk

To assess the acute risk to earthworms from the metabolites BH 635-2, -3, -4 and -5, the LC_{50} values for acute toxicity from the monograph were taken and compared to the worst-case $PEC_{s,ini}$ values calculated according to Hauck (2002, see Chapter B.8.3.1), as demonstrated in Table B.9.8.4-1.

Table B.9.8.4-1: Calculation of TER values for the acute effects of tritosulfuron metabolites BH 635-2, BH 635-3, BH 635-4, and BH 635-5 on earthworms

compound	LC_{50} [mg/kg dry soil]	$PEC_{s,ini}$ [mg/kg dry soil][1)]	TER
BH 635-2	> 1,000	0.034	> 29,000
BH 635-3	> 1,000	0.047	> 21,000
BH 635-4	> 1,000	0.053	> 18,000
BH 635-5	671	0.029	23,138

1) based on tritosulfuron application of 0.05 kg/ha and calculated with mol. weight correction factors: BH 635-2 – 0.5057; BH 635-3 – 0.6967; BH 635-4 – 0.7934; BH 635-5 – 0.4359

The calculated TER values are farabove the Annex VI trigger of 10. The acute risk to earthworms arising from the tested tritosulfuron soil metabolites can therefore be considered negligible.

B.9.8.4.2 Long-term risk

In the studies with the metabolites BH 635-2, -3, -4 and -5, no effects on mortality, growth, reproduction and feeding activity of the earthworm *Eisenia fetida* were found at the highest tested rates, respectively. NOECs, initial PECs in soil and resulting TER values are shown in Table B.9.8.4-2. All PEC_{ini} values were calculated applying molecular weight correction factors. Besides the worst case values for 100 % metabolite formation, the table also lists the values based on actual metabolite formation rates as documented in the monograph (rounded up to the next multiple of 5 %). The latter were used in the assessment. Since the tested rates were chosen individually for each metabolite to represent approximately the respective 5-fold PEC_{ini} in soil, all TER values are equal or slightly above the Annex VI trigger of 5, indicating an acceptable risk to earthworms.

Table B.9.8.4-2: Calculation of TER values for the long-term effects of tritosulfuron metabolites BH 635-2, BH 635-3, BH 635-4, and BH 635-5 on earthworms (based on initial PEC_{soil})

compound	NOEC [mg/kg dry soil]	PEC_{ini}, soil [mg/kg dry soil][1)]		TER
		100 % formation	actual formation[2)]	actual formation[2)]
BH 635-2	0.05	0.034	0.0084	6.0
BH 635-3	0.035	0.047	0.0070	5.0
BH 635-4	0.16	0.053	0.0317	5.0
BH 635-5	0.015	0.029	0.0029	5.2

1) based on tritosulfuron application of 0.05 kg/ha and calculated with mol. weight correction factors: BH 635-2 – 0.5057; BH 635-3 – 0.6967; BH 635-4 – 0.7934; BH 635-5 – 0.4359

2) actual formation rates: BH 635-2 – 25 %; BH 635-3 – 15 %; BH 635-4 – 60 %; BH 635-5 – 10 %

In addition to that assessment based on initial PECs, the risk to earthworms was also assessed with respect to potential accumulation of metabolites in soil, i.e. based on long-term PECs calculated with FOCUS PELMO 3.3.2 for the scenarios Châteaudun, Hamburg and Sevilla (Dressel, 2003a). The resulting TER values are presented in Table B.9.8.4-3.

Table B.9.8.4-3: Calculation of TER values for the long-term effects of tritosulfuron metabolites BH 635-2, BH 635-3, BH 635-4, and BH 635-5 on earthworms (based on annual average maximum PECs during 26 years of application of tritosulfuron)

compound	NOEC [mg/kg dry soil]	FOCUS scenario	PEC_{soil} (average max.) [mg/kg dry soil]	TER
BH 635-2	0.05	Châteaudun	0.00209	23.9
		Hamburg	0.00174	28.7
		Sevilla	0.00251	19.9
BH 635-3	0.035	Châteaudun	0.00090	38.9
		Hamburg	0.00063	55.6
		Sevilla	0.00144	24.3
BH 635-4	0.16	Châteaudun	0.00340	47.1
		Hamburg	0.00290	55.2
		Sevilla	0.00387	41.3
BH 635-5	0.015	Châteaudun	0.00274	5.5
		Hamburg	0.00234	6.4
		Sevilla	0.00318	4.7

All TER values except the TER for BH 635-5 in the Sevilla scenario are above the Annex VI trigger of 5. However, the Sevilla TER for BH 635-5 amounts to 4.7, which is only slightly below 5. Since the calculation of the annual maximum average PECs has been done on the basis of rather conservative assumptions, the long-term risk for earthworms arising from potential accumulation of tritosulfuron metabolites in soil is considered acceptable in the overall view.

Comment of RMS:
The RMS agrees to the presented risk assessments with their conclusions on an acceptable risk to earthworms from tritosulfuron metabolites on the short as well as the long time-scale.

B.9.10 Effects on soil non-target micro-organisms (Annex IIA 8.5; Annex IIIA 10.7)

B.9.10.4 Laboratory testing

Annex Point: IIA-8.5
Author: Koelzer, U.
Title: Effects of Reg. No. 292 564 (BH 635-2; metabolite of BAS 635 H) on the activity of the soil microflora, carbon transformation test
Date: 2004-26-04
Doc ID: BASF 2004/1004410
BMF2004-68
Guidelines: OECD 217
GLP: Yes

Material and methods:

BH 635-2; Metabolite of BAS 635 H
Lot no. 00831-201; Reg. No. 292 564: content of as: 98.2 %

Biologically active agricultural soil
Silty sand, 0.88 % C_{org}, pH 6.03
Soil moisture 45 % of its maximum water holding capacity. Soil samples were incubated at 20 °C ± 2 °C while stored in glass bottles.

Determination of carbon transformation in soil after addition of glucose. Comparison of test item treated soil with a non-treated soil. 3 replicates per treatment and concentration. An OxiTop® system was used to determine the O_2-consumption over a period of maximum 24 hours at different sampling intervals. Sampling scheme: 0, 7, 14, and 28 days after treatment, subsamples were withdrawn from the bulk batches exposed and subjected to the measurement.

Test concentrations
Control, 0.0682 mg BH 635-2 per kg dry soil (1x-rate) and 0.336 mg BH 635-2 per kg dry soil (5x-rate). Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

Reference item
Dinoterb was applied at a rate of 13.3 mg/kg in a separate study within one year before start of experimental phase of this study.

Endpoints
Effects on O_2-consumption after 28 days of exposure
Calculation of mean values per treatment, standard deviation and coefficient of variance.

Findings:

No adverse effects of BH 635-2 on carbon transformation could be observed in the soil at both tested concentrations after 28 days.

Table B.9.10.4-1: Effects on carbon transformation in soil 28 days after treatment with BH 635-2

Days after application	Control	0.0682 mg BH 635-2 per kg dry soil		0.336 mg BH 635-2 per kg dry soil	
	mg O_2/h.kg dry weight[1)]	mg O_2/h.kg dry weight[1)]	Deviation from control [%][1)]	mg O_2/h.kg dry weight[1)]	Deviation from control [%][1)]
0	3.44	2.90*	-15.9*	3.04	-11.6
7	2.71	2.86	+5.49	2.07	-23.9
14	3.06	2.99	-2.29	2.82	-7.81
28	2.49	2.48	-0.224	2.37	-4.80

1) + = % stimulation; - = % inhibition
* Value was determined with only two instead of three individual values due to a defective measuring device

In a separate study the reference item dinoterb produced in the soil the expected level of effect (-47.8 % deviation from control).

Valid: Yes

Conclusions:

Based on the results of this study, BH 635-2 caused no short-term and long-term effects on carbon transformation in a field soil tested up to a concentration of 0.336 mg BAS 635-2 per kg dry soil (5x-rate). The difference in soil microflora activity as compared to the control is < 25 %.

Annex Point:	IIA-8.5
Author:	Koelzer, U.
Title:	Effects of Reg. No.. 335 182 (BH 635-3; metabolite of BAS 635 H) on the activity of the soil microflora, carbon transformation test
Date:	2004-26-04
Doc ID:	BASF 2004/1004411 BMF2004-69
Guidelines:	OECD 217
GLP:	Yes

Material and methods:

BH 635-3; Metabolite of BAS 635 H
Lot no. 01185-269; Reg. No. 335 182: content of as: 99.2 %;

Biologically active agricultural soil
Silty sand, 0.88 % C_{org}, pH 6.03
Soil moisture 45 % of its maximum water holding capacity. Soil samples were incubated at 20 °C ± 2 °C while stored in glass bottles.

Determination of carbon transformation in soil after addition of glucose. Comparison of test item treated soil with a non-treated soil. 3 replicates per treatment and concentration. An OxiTop® system was used to determine the O_2-consumption over a period of maximum 24 hours at different sampling intervals. Sampling scheme: 0, 7, 14, and 28 days after treatment, subsamples were withdrawn from the bulk batches exposed and subjected to the measurement.

Test concentrations
Control, 0.0675 mg BH 635-3 per kg dry soil (1x-rate) and 0.333 mg BH 635-3 per kg dry soil (5x-rate). Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

Reference item
Dinoterb was applied at a rate of 13.3 mg/kg in a separate study within one year before start of experimental phase of this study.

Endpoints
Effects on O_2-consumption after 28 days of exposure
Calculation of mean values per treatment, standard deviation and coefficient of variance.

Findings:

No adverse effects of BH 635-3 on carbon transformation could be observed in the soil at both tested concentrations after 28 days.

Table B.9.10.4-2: Effects on carbon transformation in soil 28 days after treatment with BH 635-3

Days after application	Control	0.0675 mg BH 635-3 per kg dry soil		0.333 mg BH 635-3 per kg dry soil	
	mg O_2/h.kg dry weight[1)]	mg O_2/h.kg dry weight[1)]	Deviation from control [%][1)]	mg O_2/h.kg dry weight[1)]	Deviation from control [%][1)]
0	3.46	3.10	-10.5	3.11	-9.93
7	2.72	2.61	-4.35	2.89*	+6.17*
14	3.07	2.75	-10.5	2.87	-6.56
28	2.50	2.41	-3.69	2.49	-0.250

1) + = % stimulation; - = % inhibition
* Value was determined with only two instead of three individual values due to a defective measuring device

In a separate study the reference item dinoterb produced in the soil the expected level of effect (-47.8 % deviation from control).

Valid: Yes

Conclusions:

Based on the results of this study, BH 635-3 caused no short-term and long-term effects on carbon transformation in a field soil tested up to a concentration of 0.333 mg BAS 635-3 per kg dry soil (5x-rate). The difference in soil microflora activity as compared to the control is < 25 %.

Annex Point: IIA-8.5
Author: Koelzer, U.
Title: Effects of Reg. No. 335 184 (BH 635-4; metabolite of BAS 635 H) on the activity of the soil microflora, carbon transformation test
Date: 2004-26-04
Doc ID: BASF 2004/1004412
BMF2004-70
Guidelines: OECD 217
GLP: Yes

Material and methods:

<u>BH 635-4; Metabolite of BAS 635 H</u>
Batch no. 2059-011; Reg. No. 335 184: content of as: 96.4 %

<u>Biologically active agricultural soil</u>
Silty sand, 0.88 % C_{org}, pH 6.03
Soil moisture 45 % of its maximum water holding capacity. Soil samples were incubated at 20 °C ± 2 °C while stored in glass bottles.

Determination of carbon transformation in soil after addition of glucose. Comparison of test item treated soil with a non-treated soil. 3 replicates per treatment and concentration. An OxiTop® system was used to determine the O_2-consumption over a period of maximum 24 hours at different sampling intervals. Sampling scheme: 0, 7, 14, and 28 days after treatment, subsamples were withdrawn from the bulk batches exposed and subjected to the measurement.

Test concentrations
Control, 0.0695 mg BH 635-4 per kg dry soil (1x-rate) and 0.342 mg BH 635-4 per kg dry soil (5x-rate). Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

Reference item
Dinoterb was applied at a rate of 13.3 mg/kg in a separate study within one year before start of experimental phase of this study.

Endpoints
Effects on O_2-consumption after 28 days of exposure
Calculation of mean values per treatment, standard deviation and coefficient of variance.

Findings:

No adverse effects of BH 635-4 on carbon transformation could be observed in the soil at both tested concentrations after 28 days.

Table B.9.10.4-3: Effects on carbon transformation in soil 28 days after treatment with BH 635-4

Days after application	Control	0.0695 mg BH 635-4 per kg dry soil		0.342 mg BH 635-4 per kg dry soil	
	mg O_2/h.kg dry weight[1)]	mg O_2/h.kg dry weight[1)]	Deviation from control [%][1)]	mg O_2/h.kg dry weight[1)]	Deviation from control [%][1)]
0	3.15	2.82*	-10.4	2.68	-14.8
7	2.84*	2.84	0.0215	2.38	-16.3
14	2.54	2.54	-0.112	2.46	-3.07
28	2.48	2.28	-7.80	2.19	-11.8

1) + = % stimulation; - = % inhibition
* Value was determined with only two instead of three individual values due to a defective measuring device

In a separate study the reference item dinoterb produced in the soil the expected level of effect (-47.8 % deviation from control).

Valid: Yes

Conclusions:

Based on the results of this study, BH 635-4 caused no short-term and long-term effects on carbon transformation in a field soil tested up to a concentration of 0.342 mg BAS 635-4 per kg dry soil (5x-rate). The difference in soil microflora activity as compared to the control is < 25 %.

Annex Point:	IIA-8.5
Author:	Koelzer, U.
Title:	Effects of Reg. No. 231 700 (BH 635-5; metabolite of BAS 635 H) on the activity of the soil microflora, carbon transformation test
Date:	2004-26-04
Doc ID:	BASF 2004/1004413 BMF2004-71
Guidelines:	OECD 217
GLP:	Yes

Material and methods:

BH 635-5; Metabolite of BAS 635 H
Lot no. 00831-237; Reg. No. 231 700: content of as: 99.9 %

Biologically active agricultural soil
Silty sand, 0.88 % C_{org}, pH 6.03
Soil moisture 45 % of its maximum water holding capacity. Soil samples were incubated at 20 °C ± 2 °C while stored in glass bottles.

Determination of carbon transformation in soil after addition of glucose. Comparison of test item treated soil with a non-treated soil. 3 replicates per treatment and concentration. An OxiTop® system was used to determine the O_2-consumption over a period of maximum 24 hours at different sampling intervals. Sampling scheme: 0, 7, 14, and 28 days after treatment, subsamples were withdrawn from the bulk batches exposed and subjected to the measurement.

Test concentrations
Control, 0.067 mg BH 635-5 per kg dry soil (1x-rate) and 0.33 mg BH 635-5 per kg dry soil (5x-rate). Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

Reference item
Dinoterb was applied at a rate of 13.3 mg/kg in a separate study within one year before start of experimental phase of this study.

Endpoints
Effects on O_2-consumption after 28 days of exposure
Calculation of mean values per treatment, standard deviation and coefficient of variance.

Findings:

No adverse effects of BH 635-5 on carbon transformation could be observed in the soil at both tested concentrations after 28 days.

Table B.9.10.4-4: Effects on carbon transformation in soil 28 days after treatment with BH 635-5

Days after application	Control	0.067 mg BH 635-5 per kg dry soil		0.33 mg BH 635-5 per kg dry soil	
	mg O_2/h.kg dry weight[1)]	mg O_2/h.kg dry weight[1)]	Deviation from control [%][1)]	mg O_2/h.kg dry weight[1)]	Deviation from control [%][1)]
0	3.14	2.64	-15.9	2.72*	-13.5
7	2.84*	2.70*	-4.82	2.69	-5.04
14	2.54	2.51	-0.919	2.56	0.967
28	2.48	2.24	-9.49	2.23	-9.99

1) + = % stimulation; - = % inhibition

* Value was determined with only two instead of three individual values due to a defective measuring device

In a separate study the reference item dinoterb produced in the soil the expected level of effect (-47.8 % deviation from control).

Valid: Yes

Conclusions:

Based on the results of this study, BH 635-5 caused no short-term and long-term effects on carbon transformation in a field soil tested up to a concentration of 0.33 mg BAS 635-5 per kg dry soil (5x-rate). The difference in soil microflora activity as compared to the control is < 25 %.

B.9.10.5 Carbon mineralisation risk assessment

In the studies with the metabolites BH 635-2, -3, -4 and -5, no effects (deviation from control > 25 %) on carbon mineralisation in soil were observed at the highest tested rates between 0.330 and 0.342 mg/kg dry soil, respectively. These tested rates represent a worst case, since they correspond directly to the 5-fold application rate of the active substance (0.05 kg as/ha, equivalent to 0.067 mg/kg soil, multiplication by 5 yields 0.333 mg/kg soil). No molecular weight correction factors or actual metabolite formation rates were applied. Therefore, the risk to carbon mineralisation in soil from the tested tritosulfuron metabolites is considered negligible.

Comment of RMS:

The RMS agrees to the presented risk assessment with its conclusion on a negligible risk to carbon mineralisation in soil from tritosulfuron metabolites.

B.9.13 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
IIA-8.2.8	Junker, M.	2003	Effect of BH 635-4 on the Growth of *Lemna gibba* 2003/1012053 WAT2004-804		
IIA-8.4.2	Lührs, U.	2002a	Effects of BH 635-5 on Reproduction and Growth of Earthworms *Eisenia fetida* in Artificial Soil 2002/1012743 ARW2003-147		
IIA-8.4.2	Lührs, U.	2002b	Effects of BH 635-4 on Reproduction and Growth of Earthworms *Eisenia fetida* in Artificial Soil 2002/1012744 ARW2003-148		
IIA-8.4.2	Lührs, U.	2002c	Effects of BH 635-3 on Reproduction and Growth of Earthworms *Eisenia fetida* in Artificial Soil 2002/1012745 ARW2003-149		
IIA-8.4.2	Lührs, U.	2002d	Effects of BH 635-2 on Reproduction and Growth of Earthworms *Eisenia fetida* in Artificial Soil 2002/1012746 ARW2003-150		
IIA-8.5	Koelzer, U.	2004a	Effects of Reg. No. 292 564 (BH 635-2; metabolite of BAS 635 H) on the activity of the soil microflora, carbon transformation test BASF 2004/1004410 BMF2004-68		
IIA-8.5	Koelzer, U.	2004b	Effects of Reg. No.. 335 182 (BH 635-3; metabolite of BAS 635 H) on the activity of the soil microflora, carbon transformation test BASF 2004/1004411 BMF2004-69		
IIA-8.5	Koelzer, U.	2004c	Effects of Reg. No. 335 184 (BH 635-4; metabolite of BAS 635 H) on the activity of the soil microflora, carbon transformation test BASF 2004/1004412 BMF2004-70		
IIA-8.5	Koelzer, U.	2004d	Effects of Reg. No. 231 700 (BH 635-5; metabolite of BAS 635 H) on the activity of the soil microflora, carbon transformation test BASF 2004/1004413 BMF2004-71		

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
IIIA-10.3	Churchfield, S.	1990	The natural history of shrews. Christopher Helm, London		
IIIA-10.3	Hausser, J., Hutterer, R. and Vogel, P.	1990	*Sorex araneus* Linnaeus 1758 – Waldspitzmaus. In: Niethammer, J. & Krapp, F. (Eds.) Handbuch der Säugetiere Europas. Bd 3 Insectivora –Primates pp. 237-277		
IIIA-10.3	Hawkins, A.E. and Jewell, P.A.	1962	Food consumption and energy requirements of captive British shrews and the mole. Proc. Zool. Soc. London 138: 137-167		
IIIA-10.3	Kotzageorgis, G.C. and Mason, C.F.	1997	Small mammal populations in relation to hedgerow structure in an arable landscape. Journal of Zoology (London) **242** (3): 425-434		
IIIA-10.3	Welter, K.	2002	AMTT (Reg. No. 231 700, BH 635-5) Risk assessment for terrestrial vertebrates other than birds - In support of the provisional registration in the United Kingdom 2002/1011551 AVS2003-225; TOX2003-1943		

Addendum 4

to the Draft Assessment Report

of 20 August 2002

(relating to Volume 4)

Tritosulfuron

confidential

04 February 2005

Rapporteur Member State: Germany

Confidential information available at RMS.

Addendum 5

to the Draft Assessment Report

of 20 August 2002

(relating to Volume 3)

Tritosulfuron

12 August 2005

Rapporteur Member State: Germany

Contents

B.7 Residue data 1

B.7.3 Definition of the residue 1
B.7.15 Estimates of potential and actual dietary exposure through diet and other means 2
B.7.17 References relied on: 8

B.7 Residue data

B.7.3 Definition of the residue

B.7.3.1 Plants

The following conclusions can be drawn from all previous ^{14}C-studies in plants including the new wheat metabolism study:

- Unchanged tritosulfuron was detected in almost all matrices of the plant metabolism studies as major and predominant component. It was also found in a majority of the matrices investigated during the rotational crop study (exception: wheat). In most of the samples, tritosulfuron is present in amounts significantly greater than 10 % TRR and 0.05 mg/kg. Compared with parent all other metabolites are present in considerably lower amounts.
- As major degradation pathway in maize, wheat, carrot and lettuce (both succeeding crops), hydroxylation of the phenyl ring system followed by conjugation (635M13) or cleavage (635M06) was found.
- The metabolite 635M01, which is formed by cleavage of the triazine ring system was detected in all studies.
- The cleavage reaction of the sulfonyl urea bridge does not play an important role in plants. Metabolites 635M02 (TBSA) and 635M04 (AMTT) were only detected in minor amounts in wheat. Both metabolites were not found in maize. In the edible portions of the confined rotational crop study, they also occurred in trace amounts (about 0.001 mg/kg). Significant hydrolytical cleavage of tritosulfuron was only observed in the hydrolysis study performed at exaggerated temperatures. Tritosulfuron was almost quantitatively cleaved to TBSA and AMTT.

Due to all findings of the hydrolysis investigations, plant metabolism studies, supervised residue trials, rotational crop studies, and recent toxicological studies on TBSA the following definitions of the relevant residues in plant matrices are proposed:

Risk assessment: tritosulfuron and AMTT, expressed as AMTT
Monitoring purpose: tritosulfuron.

B.7.15 Estimates of potential and actual dietary exposure through diet and other means (Annex IIA 6.9; Annex IIIA 8.8)

LONG TERM CONSUMER RISK ASSESSMENT

Tritosulfuron in primary crops

The results of the re-calculation of the consumer risk based on the ADI = 0.06 mg/kg bw*d are given in the end point list. The TMDI calculation leads to the very low values of 0.61 % ADI (German model) or 0.35 % (WHO model). This result differs insignificantly from the former assessment given in the DAR.
Based on the detected residues of <0.002 mg/kg tritosulfuron and AMTT in grain from directly treated cereal plants and the effective mixing of this crop there is no acute risk to the consumer to be expected.

Metabolite AMTT in succeeding crops

The metabolite AMTT (635M04) is a main soil metabolite and can be formed through hydrolysis under basic conditions or at elevated temperature. Investigations on residues in rotational crops (Veit, P., 2001; RIP2001-625 and Schulz, H., 2005; RIP2005-997) have shown that AMTT residues are detectable under practical conditions. The recent study of Schulz was conducted during the years 2003 and 2004. Finally reported are the residue results of 2003 in rotational plants (spinach, carrots, cauliflower, wheat). Most results from 2004 are also reported preliminary because the final report of the 2004 part is still pending. A summary and conclusion of the entier study was not prepared yet for this addendum. The total results of this investigation reveal only detectable residues in spinach at a level of up to 0.003 mg AMTT/kg. Tritosulfuron was found in some samples of up to 0.0011 mg/kg and TBSA was never detected in any sample of any commodity.

Therefore, the following risk assessment is based on the results of the first study (Veit, 2001) which is reported in the DAR.

The evaluation of the toxicity of AMTT leads to the very low end points of ADI = 0.0001 mg/kg bw*d and ARfD = 0.0001 mg/kg bw and gives reason of high toxicological concern. Therefore, a separate consumer risk assessment on AMTT residues in rotational crops is necessary which is given as follows:

To cover the worst case scenario all residue values of tritosulfuron have to be converted to AMTT equivalents by the factor of 0.43 (M-AMTT / M-tritosulfuron = 194 g/mol / 445.3 g/mol = 0.43).

Because HPLC radio detection was used in the analysis method residues of AMTT could not be quantified in the phenyl labelled study. Since AMTT and ist counterpart metabolite TBSA (635M02) are formed in equal amounts after cleavage of the sulfonyl urea bridge the TBSA residues are used for the calculation taking the conversion factor of 0.86 (M-AMTT / M-TBSA = 194 g/mol / 225 g/mol = 0.86).

Calculation of total residues is as follows:

Phenyl label:
AMTT-equivalents [mg/kg] = 0,43 * tritosulfuron [mg/kg] + 0,86 * 635M02 [mg/kg]

Triazine label:
AMTT-equivalents [mg/kg] = 0,43 * tritosulfuron [mg/kg] + AMTT [mg/kg]

The results are given in the following tables:

Table B.7.15-3: Summary of calculated AMTT-equivalents after treatment with phenyl labelled tritosulfuron

Matrix / DAP* [d]	Residues [mg/kg]		
	Tritosulfuron	TBSA	AMTT equiv.
30 d rotation			
Carrot green plant / 111	0.005	0.001	0.003
Carrot green plant / 133	0.005	<0.001	0.003
Carrot root / 133	0.001	<0.001	0.001
Bean plant / 75	0.005	<0.001	0.003
Lettuce plant / 29	0.005	0	0.002
Wheat straw / 141	0	0.015	0.013
Wheat grain / 141	0	0	0.000
120 d rotation			
Carrot green plant / 110	0.016	0	0.007
Carrot green plant / 125	0.007	0.005	0.007
Bean plant / 81	0.007	0	0.003
Lettuce head / 29	0.008	<0.001	0.004
Lettuce head (B) / 61	0.006	0.003	0.005
Wheat straw / 127	<0.001	0.02	0.018
365 d rotation			
Carrot green plant / 99	0	0.003	0.003
Carrot green plant / 127	<0.001	0.005	0.005
Carrot root / 99	<0.001	<0.001	0.001
Bean plant / 83	0.001	0.001	0.001
Lettuce plant / 31	0	0.002	0.002
Wheat straw / 129	0	0.035	0.030
Wheat grain / 129	0	<0.001	<0.001

*) DAP = Days After Planting

Table B.7.15-3: Summary of calculated AMTT-equivalents after treatment with triazine labelled tritosulfuron

Matrix / DAP* [d]	Residues [mg/kg]		
	Tritosulfuron	AMTT	AMTT equiv.
30 d rotation			
Carrot green plant / 36	0.013	0.005	0.011
Carrot green plant / 110	0.004	0.002	0.004
Bean plant / 28	0.028	0.011	0.023
Bean plant / 55	0.006	0.001	0.004
Bean plant / 74	0.009	0.002	0.006
Lettuce plant 28	0.003	<0.001	0.002
Wheat green plant / 61	0	0.013	0.013
Wheat straw / 140	0.008	0.029	0.032
Wheat grain / 140	0	<0.001	<0.001
120 d rotation			
Carrot green plant / 109	0.007	0.006	0.009
Carrot green plant / 124	0	0.001	0.001
Bean plant / 56	0.015	0.003	0.009
Bean plant / 80	0.016	0.002	0.009
Lettuce head (A) 60	0.006	0	0.003
Lettuce head (B) 60	0.007	0	0.003
Wheat straw / 126	<0.001	0.004	0.004
365 d rotation			
Carrot green plant / 98	0	0	0.000
Bean plant / 82	0	0.001	0.001
Wheat green plant / 47	0	0	0.000
Wheat straw / 129	0	<0.001	<0.001

*) DAP = Days After Planting

The results of the these trials show that under practical conditions (application rate of 60 g as/ha) residues above 0.001 mg/kg but below the proposed MRL of 0.01 mg/kg can be expected in rotational crops even after a rotation period of up to one year.

In the following NEDI calculation the expected residue levels of all relevant annual succeeding crops are included. It was assumed that fruiting vegetables, leafy vegetables and fresh herbs are eaten raw:

Table B.7.15-4: NEDI calculation of tritosulfuron and AMTT (expressed as AMTT equivalents)

Substance:	**AMTT**				
ADI (mg/kg bw/d):	**0.0001**				
Average food consumption [g/d] for a 4 – 6 years old girl (German model)					
Food	**raw**	**processed**	**total**	**MRL [mg/kg]**	**Intake [mg/kg bw*d]**
Strawberries	1.6	3.2	4.8	0.01	0.00000356
Root and tuber vegetables	3.3	11.1	14.4	0.01	0.00001067
Bulb vegetables	2.9	8.0	10.9	0.01	0.00000807
Fruiting vegetables	18.9	13.1	32.0	0.001	0.00000237
Brassica vegetables	2.5	22.7	25.2	0.01	0.00001867
Leafy vegetables and fresh herbs	4.8	3.6	8.4	0.001	0.00000062
Legume vegetables	0.1	7.8	7.9	0.01	0.00000585
Stem vegetables	0.3	6.2	6.5	0.01	0.00000481
Pulses		1.5	1.5	0.01	0.00000111
Oilseeds	0.3	11.0	11.3	0.01	0.00000837
Potatoes		71.1	71.1	0.01	0.00005267
Cereals	0.2	107.8	108.0	0.001	0.00000800
Sugar beets		0.3	0.3	0.01	0.00000022
Total intake [mg/kg bw*d]	0.000125				
NEDI [% of ADI]	**125**				

In total, this result shows that residues of AMTT in rotational crops represent a possible chronic risk to consumers. However, the main contribution to the intake of residues is due to root and tuber vegetables, brassica vegetables and potatoes in particular.
Therefore, a separate calculation excluding potatoes as succeeding crops to treated cereals is carried out:

Table B.7.15-5: NEDI calculation of tritosulfuron and AMTT (expressed as AMTT equivalents) excluding potatoes

Substance:	**AMTT**				
ADI (mg/kg bw/d):	**0.0001**				
Average food consumption [g/d] of a 4 – 6 years old girl (German model)					
Food	**raw**	**processed**	**total**	**MRL [mg/kg]**	**Intake [mg/kg bw*d]**
Strawberries	1.6	3.2	4.8	0.01	0.00000356
Root and tuber vegetables	3.3	11.1	14.4	0.01	0.00001067
Bulb vegetables	2.9	8.0	10.9	0.01	0.00000807
Fruiting vegetables	18.9	13.1	32.0	0.001	0.00000237
Brassica vegetables	2.5	22.7	25.2	0.01	0.00001867
Leafy vegetables and fresh herbs	4.8	3.6	8.4	0.001	0.00000062
Legume vegetables	0.1	7.8	7.9	0.01	0.00000585
Stem vegetables	0.3	6.2	6.5	0.01	0.00000481
Pulses		1.5	1.5	0.01	0.00000111
Oilseeds	0.3	11.0	11.3	0.01	0.00000837
Cereals	0.2	107.8	108.0	0.001	0.00000800
Sugar beets		0.3	0.3	0.01	0.00000022
Total intake [mg/kg bw*d]	0.000073				
NEDI [% of ADI]	**73**				

Taking into account that potatoes in particular are excluded form agricultural rotations after treatment of primary crops with tritosulfuron the exhaustion of the ADI is only 73 %.

SHORT TERM CONSUMER RISK ASSESSMENT

Because of the low acute toxicity of tritosulfuron no ARfD was allocated. An acute risk to the consumer caused from the intake of residues in food produced under normal agricultural conditions can be excluded.

For AMTT an ARfD of 0.0001 mg/kg bw was derived from a two generation rat study. This metabolite can be contained in rotational crops taken up from the soil or it can be formed by hydrolysis during cooking of tritosulfuron residues containing raw food commodities. It has been shown in the chapter before that in potato tubers (as a main succeeding crop) AMTT residues between 0.001 and 0.01 mg/kg are to be expected. Therefore, the acute risk for toddlers is assessed using the new UK and German (VELS) intake models (NESTI calculations) for comparison. Based on the results of the rotational crop studies the highest residue level HR = 0.003 mg/kg as a more realistic residue level to be expected in succeeding crops was chosen in the calculations. Results of these calculations show that besides potatoes further crops as carrots and some brassica vegetables as potential rotational main crops appear to be of concern. The following table contains the results of the corresponding calculations:

Table B.7.15-6: NESTI Calculation for commodities of concern (raw or cooked) using highest expected residue of HR = 0.003 mg AMTT/kg

Substance: AMTT ARfD (mg/kg bw): 0.0001								
Commodity	Portion size [g]	Unit weight (g)	Proc. factor	Variability factor	HR [mg/kg]	Intake [mg/kg bw]	% ARfD	Case
Portion size and unit-weight for UK toddlers of the age of 1.5 to 4.5 years (97.5th percentile)								
Potatoes (cooked)	246	216	1.0	7	0.003	0.0003	**262.1**	2b
Carrots (raw)	90	80	1.0	7	0.003	0.0001	**117.9**	
Broccoli (raw)	61	74	1.0	7	0.003	0.0001	87.9	
Cauliflower (raw)	96	780	1.0	7	0.003	0.0001	99.4	
Kohl Rabi (raw)	-	227	1.0	7	0.003	-	-	
Portion size and unit-weight for German toddlers of the age of 2 to <5 years (95 – 97.5th percentile)								
Potatoes (cooked)	90	219	1.0	7	0.003	0.0001	**140.9**	2a/2b
Carrots (raw)	119	62	1.0	7	0.003	0.0001	91.2	2a/2b
Carrots (juice)	-	693	1.0	1	0.003	0.0001	**128.7**	3
Broccoli (raw)	41	347	1.0	5	0.003	0.00004	38.1	2a/2b
Broccoli (cooked)	115	347	1.0	5	0.003	0.0001	**106.9**	2a/2b
Cauliflower (raw)	10	879	1.0	5	0.003	0.00001	9.0	2a/2b
Cauliflower (cooked)	121	879	1.0	5	0.003	0.0001	**112.5**	2a/2b
Kohl Rabi (raw)	162	265	1.0	5	0.003	0.0001	**150.3**	2a/2b
Kohl Rabi (cooked)	106	351	1.0	5	0.003	0.0001	98.8	2a/2b

The assessment based on the new German model shows that AMTT residues in processed potatoes, carrots, broccoli, cauliflower and kohl rabi reaching a realistic level of 0.003 mg/kg could represent an acute risk to certain consumers (toddlers). Partial confirmation is given applying the UK calculation model.

CONCLUSION

The submitted residue data of tritosulfuron and AMTT in cereal grain reveal that after PHI of 107 – 140 days no total residues above 0.002 mg/kg occur. It is therefore expected that in practice the proposed MRL of 0.01 mg tritosulfuron/kg will not be exceeded. Based on this residue situation in grain from directly treated cereal plants and the effective mixing of this crop no acute risk to the consumer is expected.

Rotational crop studies reveal uptake of soil residues of the metabolite AMTT. This metabolite has been found to be of high toxicological concern resulting in the proposed very low acute reference dose of 0.0001 mg/kg bw. Application of different calculation models to assess the consumer risk (toddlers) lead to unacceptable intake values of certain crops (raw or processed).

B.7.17 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
AIIA-6.1	Bross,M., Mackenroth, C.	2004	Metabolism of 14C-Tritosulfuron (14C-BAS 635 H) in Wheat. REPORT NO. BASF DocID 2004/1010546 GLP, unpublished RIP2004-1274	Y	BAS
AIIA-6.1	Bross,M.	2004	Tritosulfuron (BAS 635 H): Summary of the plant metabolism studies including a proposal for the definition of the relevant residue REPORT NO. BASF DocID 2004/1015920 unpublished RIP2004-1275	Y	BAS
AIIA-6.6	Schulz, H.	2005	Study on the residue behaviour of tritosulfuron (BAS 635 H) in rotational crops: Spinach, carrots, cauliflower and spring wheat after application to the soil of BAS 635 H and the adjuvant Citowett (BAS 152 00 S) under field conditions in Denmark, Germany, Great Britain and Spain, 2003 GLP, unpublished BASF DocID 2005/1007590 RIP2005-997	Y	BAS
AIIIA-7.2	Stadler, R.	2004	Hydrolysis Stability of BAS 635 00 H in Spray Tank under Field Conditions – Determination od AMTT (635M04, BH 635-5, Reg.No. 231 700) REPORT NO. BASF DocID 2004/1016478 GLP, unpublished PHY2004-636 TOX2004-1952	N	BAS

Codes of owner

BAS: BASF Aktiengesellschaft

Addendum 6

to the Draft Assessment Report

of 20 August 2002

(relating to Volume 3)

Tritosulfuron

5 January 2006

Rapporteur Member State: Germany

Contents

B.7 Residue data 2

B.7.9 Residues in succeeding and rotational crops (Annex IIA 6.6; Annex III 8.5) 2
B.7.17 References relied on 17

B.7 Residue data

B.7.9 Residues in succeeding and rotational crops (Annex IIA 6.6; Annex III 8.5)

An additional two years field rotational crop study has been performed in 2003 and 2004 to determine residues of tritosulfuron (BAS 635 H) and its metabolite AMTT (635M04) in representative succeeding crops. In general, the study design of both study parts were identical.

The following crops were selected as representative crops for specific crop groups:

Wheat:	cereals
Carrots:	root and tuber vegetables
Spinach:	leafy vegetables
Cauliflower:	brassica vegetables

The study design of the field parts were set up in a way which also provides an indication whether residues are accumulating in plants when tritosulfuron containing formulations are annually applied to the same field plot at the maximum label rate.

The residue levels present at a replant interval of approximately 30 days were investigated regarded as suitable for simulating emergency replanting after crop failure. Additionally, the confined rotational crop study demonstrates that at 30 DAT the tritosulfuron and AMTT residue levels were higher or comparable to other intervals (120 DAT, 365 DAT) tested in the previous confined rotational crop study.

Report:	Schulz H., 2005 Study on the Residue Behaviour of Tritosulfuron (BAS 635 H) in Rotational Crops: Spinach, Carrots, Cauliflower and Spring Wheat after Application to the Soil of BAS 635 00 H and the Adjuvant Citowett (BAS 152 00 S) under Field Conditions in Denmark, Germany, Great Britain and Spain, 2003 SGS INSTITUT FRESENIUS GmbH, Im Maisel 14,D-65232 Taunusstein, Germany Fed.Rep. unpublished BASF DocID 2005/1007584 and BASF DocID 2005/1007590 (Amendment No. 1)
Guidelines:	Guideline for the generation of data concerning residues as provided in Annex II, part A, Section 6 and Annex III, part A, section 8 of Directive 91/414/EEC concerning the placing of plant protection products on the market: 1607/VI/97 rev. 2, 10.06.99. European Community Guideline 7029/VI/95 - rev.5, 22/07/97: General recommendations for the design preparation and realization of residue trials European Community Guideline 7524/VI/95 - rev. 2, 22/07/1997: Testing of Plant Protection Products in Rotational Crops. European Community Guideline 7525/VI/95 - rev. 7, 12/06/2001: Comparability, extrapolation, group tolerances and data requirements for setting MRLs. SANCO/3029/99 rev. 4 SANCO/825/00 rev. 7

GLP:	Yes (laboratory certified by the Hessische Ministerium für Umwelt, ländlichen Raum und Verbraucherschutz, Wiesbaden)
Acceptability:	The study is considered to be acceptable.

Report:	Schulz H., 2005 Study on the Residue Behaviour of Tritosulfuron (BAS 635 H) in Rotational Crops: Spinach, Carrots, Cauliflower and Spring Wheat after Application to the Soil of BAS 635 00 H and the Adjuvant Dash HC (BAS 9047 0 S) under Field Conditions in Denmark, Germany, Great Britain and Spain, 2004 SGS INSTITUT FRESENIUS GmbH, Im Maisel 14,D-65232 Taunusstein, Germany Fed.Rep. BASF DocID: 2005/1025537
Guidelines:	Guideline for the generation of data concerning residues as provided in Annex II, part A, Section 6 and Annex III, part A, section 8 of Directive 91/414/EEC concerning the placing of plant protection products on the market: 1607/VI/97 rev. 2, 10.06.99. European Community Guideline 7029/VI/95 - rev.5, 22/07/97: General recommendations for the design preparation and realization of residue trials European Community Guideline 7524/VI/95 - rev. 2, 22/07/1997: Testing of Plant Protection Products in Rotational Crops. European Community Guideline 7525/VI/95 - rev. 7, 12/06/2001: Comparability, extrapolation, group tolerances and data requirements for setting MRLs. SANCO/3029/99 rev. 4 SANCO/825/00 rev. 7
GLP:	Yes (laboratory certified by the Hessische Ministerium für Umwelt, ländlichen Raum und Verbraucherschutz, Wiesbaden)
Acceptability:	The study is considered to be acceptable.

MATERIAL AND METHODS

During the growing seasons of 2003 and 2004, four field trials each were conducted as rotational crop study with spinach, carrots, cauliflower and spring wheat. BAS 635 00 H, a WG formulation of tritosulfuron was spray-applied once at a rate of 70 g/ha of formulated product on bare soil, corresponding to 50 g/ha of tritosulfuron together with the adjuvants Citowett (2003, BAS 152 00 S) and Dash HC (2004, BAS 9047 0 S). The application took place approximately 29 – 35 days before planting of the crops. According to commercial agricultural practice, the bare soil was prepared before planting by harrowing and tilling.

Soil specimens were taken on the application day, as well as 29 – 35 days after treatment, when spinach, carrots, cauliflower and spring wheat were sown/planted, and again during sampling event when the spinach, carrots and cauliflower had reached the growth stages 41 and 49 and spring wheat 30 – 33. The last sampling of soil specimens was carried out at crop maturity (BBCH 49 for spinach, carrots, cauliflower and BBCH 89 for spring wheat). On each sampling date and for each soil specimen, 10 cores at a depth of 20 cm were taken. The soil specimens of 2003 and 2004 were analysed for tritosulfuron and its metabolites 635M01, 635M02 (TBSA),

635M03, and 635M04 (AMTT) using BASF method D9907. The method quantifies the residues with a limit of quantitation of 0.001 mg/kg for each analyte.

Samples of spinach, carrots and cauliflower were taken at the growth stages 41 and 49 (crop maturity), and spring wheat at the growth stages 30 – 33, 61 – 65 and 89. The plant specimens were analysed for tritosulfuron and its metabolites TBSA and AMTT using BASF method 555/0. The method quantifies the residues with a limit of quantitation of 0.001 mg/kg each for edible portions and 0.01 mg/kg for cereal straw and forage.

The suitability of both methods was proven by fortification experiments and in separate validation studies.

FINDINGS OF THE STUDY PERFORMED IN 2003

In the soil specimens of 2003, the residues of tritosulfuron ranged from 0.01 to 0.0164 mg/kg immediately after application. They decreased to 0.0027 – 0.0086 mg/kg at the time of the sowing/planting of the crops (DAT 29 – 30). The decline continued during the sampling stages of the crops to a final range of < 0.001 to 0.0035 mg/kg. Contrary to the decline of the parent compound in soil, an increase of the residues of the metabolites TBSA and AMTT was observed. Initially, no residues above the LOQ were found immediately after application, but subsequently at the time of the two crop samplings, the residues of TBSA increased to a range of < 0.001 to 0.0019 mg/kg (mainly in the german trials); and the residues of 635M01 in particular started to increase already after DAT 29 – 30 to a range of < 0.001 to 0.0024 mg/kg and remained at that level (< 0.001 to 0.0032 mg/kg) until the sampling dates of the crops. The residues of the metabolites 635M03 and AMTT were below the LOQ immediately after application and remained below the LOQ throughout the sampling stages, apart from the soil specimens at sampling "cauliflower GS 49" with a 635M03 residue concentration of < 0.001 to 0.0011 mg/kg, and the soil specimens at the sowing of spinach with a AMTT residue concentration of < 0.001 to 0.0011 mg/kg.

No enrichment of tritosulfuron, TBSA and AMTT was observed in the edible portions of cauliflower and carrots as well as in wheat grain. In spinach leaves, AMTT residues were obtained between 0.001 and 0.002 mg/kg, whereas no residues of tritosulfuron and TBSA above the LOQ were found. In the non-edible portions of the crops, no tritosulfuron and TBSA residues above the LOQ were obtained. AMTT was found in the "plant without root" specimens ranging from < 0.001 to 0.002 mg/kg. In carrot leaves and in wheat ears, residues of AMTT amounting to 0.005 and 0.006 mg/kg respectively were observed. No AMTT residues above the LOQ were found in straw. Except of spinach, tritosulfuron and AMTT occurred in amounts below the LOQs of 0.001 mg/kg. Individual results obtained are summarised in Table B. 7-1 Residues in rotational crops and soil (2003 trials in Germany) to Table B. 7-4 Residues in rotational crops and soil (2003 trials in UK)
:

Table B. 7-1 Residues in rotational crops and soil (2003 trials in Germany)

Country Year	Replant Interval (DAT)	Sub-plot	Succeeding Crop	Matrix	Sampling Timing Growth stage	Sampling Timing DAT	Residue Concentration mg/kg tritosulfuron	TBSA	635M03	635M01	AMTT
Germany		1		soil	at application	0	0.0072	< 0.001	< 0.001	< 0.001	< 0.001
2003		2		soil	at application	0	0.01	< 0.001	< 0.001	< 0.001	< 0.001
ACK/05/03		3		soil	at application	0	0.0098	< 0.001	< 0.001	< 0.001	< 0.001
		4		soil	at application	0	0.0093	< 0.001	< 0.001	< 0.001	< 0.001
	29	1		soil	at planting	29	0.007	< 0.001	< 0.001	< 0.001	< 0.001
	29	2		soil	at planting	29	0.0086	< 0.001	< 0.001	< 0.001	< 0.001
	29	3		soil	at planting	29	0.0077	< 0.001	< 0.001	< 0.001	< 0.001
	29	4		soil	at planting	29	0.0069	< 0.001	< 0.001	< 0.001	< 0.001
		1	spinach	plant	41 BBCH *	---	---	---	---	---	---
		2	carrots	plant	41 BBCH *	---	---	---	---	---	---
		3	cauliflower	plant	41 BBCH	69	< 0.001	< 0.001	---	---	0.001
		4	spring wheat	shoots	33 BBCH	65	< 0.01	< 0.01	---	---	< 0.01
		1		soil	41 BBCH *	58	0.0024	0.0015	< 0.001	0.0017	< 0.001
		2		soil	41 BBCH *	79	0.0018	0.0019	< 0.001	0.0021	< 0.001
		3		soil	41 BBCH	69	0.0022	0.0014	< 0.001	0.0021	< 0.001
		4		soil	33 BBCH	65	0.0032	0.0016	< 0.001	0.0019	< 0.001
		1	spinach	leaves	49 BBCH	69	**< 0.001**	< 0.001	---	---	**0.001**
		2	carrots	plants	49 BBCH	154	< 0.001	< 0.001	---	---	0.0016
		2	carrots	roots	49 BBCH	154	< 0.001	< 0.001	---	---	< 0.001
		3	cauliflower	inflorescences	49 BBCH	96	< 0.001	< 0.001	---	---	< 0.001
		4	spring wheat	ear, panicle	61 BBCH	84	< 0.001	< 0.001	---	---	0.006
		1		soil	49 BBCH	69	0.0025	0.0017	< 0.001	0.0018	< 0.001
		2		soil	49 BBCH	154	< 0.001	0.0018	< 0.001	0.0018	< 0.001
		3		soil	49 BBCH	96	< 0.001	0.0013	< 0.001	0.0017	< 0.001
		4	spring wheat	grain	89 BBCH	117	< 0.001	< 0.001	---	---	< 0.001
		4	spring wheat	straw	89 BBCH	117	< 0.01	< 0.01	---	---	< 0.01
		4		soil	89 BBCH	117	< 0.001	0.0019	< 0.001	0.0018	< 0.001

* was not taken because of crop damage

Table B. 7-2 Residues in rotational crops and soil (2003 trials in Denmark)

Country Year	Replant Interval (DAT)	Sub-Plot	Succeeding Crop	Matrix	Sampling Timing		Residue Concentration mg/kg				
					Growth stage	DAT	tritosulfuron	TBSA	635M03	635M01	AMTT
Denmark		1		soil	at application	0	0.0128	< 0.001	< 0.001	< 0.001	< 0.001
2003		2		soil	at application	0	0.0104	< 0.001	< 0.001	< 0.001	< 0.001
ALB/04/03		3		soil	at application	0	0.0063	< 0.001	< 0.001	< 0.001	< 0.001
		4		soil	at application	0	0.0103	< 0.001	< 0.001	< 0.001	< 0.001
	29	1		soil	at planting	29	0.0048	0.0011	< 0.001	0.0024	< 0.001
	29	2		soil	at planting	29	0.0036	< 0.001	< 0.001	0.0024	< 0.001
	29	3		soil	at planting	29	0.0027	< 0.001	< 0.001	0.0019	< 0.001
	29	4		soil	at planting	29	0.0029	< 0.001	< 0.001	0.0017	< 0.001
		1	spinach	plant	41 BBCH	83	0.001	< 0.001	---	---	0.002
		2	carrots	plant	41 BBCH	92	< 0.001	< 0.001	---	---	< 0.001
		3	cauliflower	plant	41 BBCH	92	< 0.001	< 0.001	---	---	< 0.001
		4	spring wheat	shoots	33 BBCH	77	< 0.01	< 0.01	---	---	< 0.01
		1		soil	41 BBCH	83	< 0.001	< 0.001	< 0.001	0.0022	< 0.001
		2		soil	41 BBCH	92	< 0.001	< 0.001	< 0.001	0.0025	< 0.001
		3		soil	41 BBCH	92	< 0.001	< 0.001	< 0.001	0.0026	< 0.001
		4		soil	33 BBCH	77	< 0.001	< 0.001	< 0.001	0.0022	< 0.001
		1	spinach	leaves	49 BBCH	92	**0.001**	< 0.001	---	---	**0.002**
		2	carrots	plants	49 BBCH	114	< 0.001	< 0.001	---	---	< 0.001
		2	carrots	roots	49 BBCH	114	< 0.001	< 0.001	---	---	< 0.001
		3	cauliflower	inflorescences	49 BBCH	128	< 0.001	< 0.001	---	---	< 0.001
		4	spring wheat	ear, panicle	61 BBCH	83	< 0.001	< 0.001	---	---	< 0.001
		1		soil	49 BBCH	92	< 0.001	< 0.001	< 0.001	0.0025	< 0.001
		2		soil	49 BBCH	114	< 0.001	< 0.001	< 0.001	0.0024	< 0.001
		3		soil	49 BBCH	128	< 0.001	< 0.001	< 0.001	0.0019	< 0.001
		4	spring wheat	grain	89 BBCH	127	< 0.001	< 0.001	---	---	< 0.001
		4	spring wheat	straw	89 BBCH	127	< 0.01	< 0.01	---	---	< 0.01
		4		soil	89 BBCH	127	< 0.001	< 0.001	< 0.001	0.0019	< 0.001

Table B. 7-3 Residues in rotational crops and soil (2003 trials in Spain)

Country Year	Replant Interval (DAT)	Sub-Plot	Succeeding Crop	Matrix	Sampling Timing		Residue Concentration mg/kg				
					Growth stage	DAT	tritosulfuron	TBSA	635M03	635M01	AMTT
Spain		1		soil	at application	0	0.0154	< 0.001	< 0.001	< 0.001	< 0.001
2003		2		soil	at application	0	0.0133	< 0.001	< 0.001	< 0.001	< 0.001
ALO/05/03		3		soil	at application	0	0.0164	< 0.001	< 0.001	< 0.001	< 0.001
		4		soil	at application	0	0.0125	< 0.001	< 0.001	< 0.001	< 0.001
	30	1		soil	at planting	30	0.0057	< 0.001	< 0.001	< 0.001	0.0011
	30	2		soil	at planting	30	0.0062	< 0.001	< 0.001	< 0.001	< 0.001
	30	3		soil	at planting	30	0.0043	< 0.001	< 0.001	< 0.001	< 0.001
	30	4		soil	at planting	30	0.0034	< 0.001	< 0.001	< 0.001	< 0.001
		1	spinach	plant	41 BBCH	84	< 0.001	< 0.001	---	---	< 0.001
		2	carrots	plant	41 BBCH	88	< 0.001	< 0.001	---	---	0.001
		3	cauliflower	plant	41 BBCH	121	< 0.001	< 0.001	---	---	< 0.001
		4	spring wheat	shoots	33 BBCH	64	< 0.01	< 0.01	---	---	< 0.01
		1		soil	41 BBCH	84	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		2		soil	41 BBCH	88	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		3		soil	41 BBCH	121	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		4		soil	33 BBCH	64	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		1	spinach	leaves	49 BBCH	94	**< 0.001**	< 0.001	---	---	**0.002**
		2	carrots	plants	49 BBCH	108	< 0.001	< 0.001	---	---	0.0045
		2	carrots	roots	49 BBCH	108	< 0.001	< 0.001	---	---	< 0.001
		3	cauliflower	inflorescences	49 BBCH	143	< 0.001	< 0.001	---	---	< 0.001
		4	spring wheat	ear, panicle	65 BBCH	78	< 0.001	< 0.001	---	---	< 0.001
		1		soil	49 BBCH	94	0.0016	< 0.001	< 0.001	< 0.001	< 0.001
		2		soil	49 BBCH	108	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		3		soil	49 BBCH	143	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		4		soil	65 BBCH	78	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		4	spring wheat	grain	89 BBCH	101	< 0.001	< 0.001	---	---	< 0.001
		4	spring wheat	straw	89 BBCH	101	< 0.01	< 0.01	---	---	< 0.01

Table B. 7-4 Residues in rotational crops and soil (2003 trials in UK)

Country Year	Replant Interval (DAT)	Sub-Plot	Succeeding Crop	Matrix	Sampling Timing		Residue Concentration mg/kg				
					Growth stage	DAT	tritosulfuron	TBSA	635M03	635M01	AMTT
UK		1		soil	at application	0	0.0116	< 0.001	< 0.001	< 0.001	< 0.001
2003		2		soil	at application	0	0.0103	< 0.001	< 0.001	< 0.001	< 0.001
OAT/07/03		3		soil	at application	0	0.01	< 0.001	< 0.001	< 0.001	< 0.001
		4		soil	at application	0	0.0144	< 0.001	< 0.001	< 0.001	< 0.001
	29	1		soil	at planting	29	0.0084	< 0.001	< 0.001	0.0023	< 0.001
	29	2		soil	at planting	29	0.0062	< 0.001	< 0.001	0.0015	< 0.001
	29	3		soil	at planting	29	0.0061	< 0.001	< 0.001	0.0017	< 0.001
	29	4		soil	at planting	29	0.0077	< 0.001	< 0.001	0.0017	< 0.001
		1	spinach	plant	41 BBCH	88	< 0.001	< 0.001	---	---	< 0.001
		2	carrots	plant	41 BBCH	120	< 0.001	< 0.001	---	---	< 0.001
		3	cauliflower	plant	41 BBCH	135	< 0.001	< 0.001	---	---	< 0.001
		4	spring wheat	shoots	30 BBCH	67	< 0.01	< 0.01	---	---	< 0.01
		1		soil	41 BBCH	88	< 0.001	< 0.001	< 0.001	0.0015	< 0.001
		2		soil	41 BBCH	120	< 0.001	< 0.001	< 0.001	0.0019	< 0.001
		3		soil	41 BBCH	135	< 0.001	< 0.001	< 0.001	0.0019	< 0.001
		4		soil	33 BBCH	67	0.0036	< 0.001	< 0.001	0.0031	< 0.001
		1	spinach	leaves	49 BBCH	98	**< 0.001**	< 0.001	---	---	**0.002**
		2	carrots	plants	49 BBCH	151	< 0.001	< 0.001	---	---	< 0.001
		2	carrots	roots	49 BBCH	151	< 0.001	< 0.001	---	---	< 0.001
		3	cauliflower	inflorescences	49 BBCH	151	< 0.001	< 0.001	---	---	< 0.001
		4	spring wheat	ear, panicle	65 BBCH	91	< 0.001	< 0.001	---	---	< 0.001
		1		soil	49 BBCH	98	0.0013	< 0.001	< 0.001	0.0019	< 0.001
		2		soil	49 BBCH	151	< 0.001	< 0.001	< 0.001	0.001	< 0.001
		3		soil	49 BBCH	151	< 0.001	< 0.001	0.0011	0.0017	< 0.001
		4		soil	65 BBCH	91	0.0035	< 0.001	< 0.001	0.0032	< 0.001
		4	spring wheat	grain	89 BBCH	117	< 0.001	< 0.001	---	---	< 0.001
		4	spring wheat	straw	89 BBCH	117	< 0.01	< 0.01	---	---	< 0.01

FINDINGS OF THE STUDY PERFORMED IN 2004

The results available from the field program 2004 confirm the findings of the 2003 study. The residue levels were in the same range as in 2003. In all edible portions no residues or residues slightly above the LOQ were found.

In the plant matrices of cauliflower, wheat and carrot, which are destined for human consumption, no residues of tritosulfuron, TBSA and AMTT were measured. In spinach leaves, parent residues slightly above the LOQ (0.001 mg/kg) were present. The AMTT residues detected were in the same range as detected in 2003 with maximum amounts of 0.002 mg/kg (see Table B. 7-5 Residues in rotational crops and soil (2004 trials in Germany)

Table B. 7-5 Residues in rotational crops and soil (2004 trials in Germany)

Country Year	Replant Interval (DAT)	Sub-Plot	Succeeding Crop	Matrix	Sampling Timing		Residue Concentration mg/kg				
					Growth stage	DAT	tritosulfuron	TBSA	635M03	635M01	AMTT
Germany 2004 ACK/05/04		1		soil	im. pre-treatment	0	< 0.001	0.0015	< 0.001	0.0012	< 0.001
		2		soil	im. pre-treatment	0	< 0.001	0.0011	< 0.001	0.0011	< 0.001
		3		soil	im. pre-treatment	0	< 0.001	0.0010	< 0.001	0.0010	< 0.001
		4		soil	im. pre-treatment	0	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		1		soil	at application	0	0.0062	0.0013	< 0.001	0.0013	< 0.001
		2		soil	at application	0	0.0073	0.0010	< 0.001	0.0011	< 0.001
		3		soil	at application	0	0.0077	0.0010	< 0.001	0.0012	< 0.001
		4		soil	at application	0	0.0072	0.0011	< 0.001	0.0011	< 0.001
	29	1		soil	at planting	29	0.0027	0.0020	< 0.001	0.0030	< 0.001
	29	2		soil	at planting	29	0.0035	0.0016	< 0.001	0.0024	< 0.001
	29	3		soil	at planting	29	0.0033	0.0016	< 0.001	0.0025	< 0.001
	29	4		soil	at planting	29	0.0021	0.0018	< 0.001	0.0030	< 0.001
		1	carrots	plant w/o root	41 BBCH	99	< 0.001	< 0.001	---	---	< 0.001
		3	spring wheat	plant w/o root	31 BBCH	69	< 0.01	< 0.01	---	---	< 0.01
		4	spinach	plant w/o root	41 BBCH	69	0.0011	< 0.001	---	---	0.0020
		1		soil	41 BBCH	99	< 0.001	0.0021	< 0.001	0.0038	< 0.001
		2		soil	49 BBCH	78	< 0.001	0.0014	< 0.001	0.0027	< 0.001
		3		soil	31 BBCH	69	0.0013	0.0015	< 0.001	0.0032	< 0.001
		4		soil	41 BBCH	69	0.0011	0.0016	< 0.001	0.0028	< 0.001
		1	carrots	tops	49 BBCH	146	< 0.001	< 0.001	---	---	< 0.001
		1	carrots	roots	49 BBCH	146	< 0.001	< 0.001	---	---	< 0.001
		2	cauliflower	inflorescences	49 BBCH	99	< 0.001	< 0.001	---	---	< 0.001
		3	spring wheat	shoots	65 BBCH	99	< 0.01	< 0.01	---	---	< 0.01
		4	spinach	leaves	49 BBCH	78	**< 0.001**	< 0.001	---	---	**0.002**
		1		soil	49 BBCH	146	< 0.001	0.0016	< 0.001	0.0031	< 0.001
		2		soil	49 BBCH	99	< 0.001	0.0016	< 0.001	0.0028	< 0.001
		4		soil	49 BBCH	78	< 0.001	0.0013	< 0.001	0.0020	< 0.001
		3	spring wheat	grain	89 BBCH	146	< 0.001	< 0.001	---	---	< 0.001
		3	spring wheat	straw	89 BBCH	146	< 0.01	< 0.01	---	---	< 0.01
		3		soil	89 BBCH	146	< 0.001	0.0011	< 0.001	0.0025	< 0.001

Table B. 7-6 Residues in rotational crops and soil (2004 trials in Denmark

Country Year	Replant Interval (DAT)	Sub-Plot	Succeeding Crop	Matrix	Sampling Timing		Residue Concentration mg/kg				
					Growth stage	DAT	tritosulfuron	TBSA	635M03	635M01	AMTT
Denmark 2004 ALB/04/04		1		soil	im. pre-treatment	0	< 0.001	< 0.001	< 0.001	0.0014	< 0.001
		2		soil	im. pre-treatment	0	< 0.001	< 0.001	< 0.001	0.0010	< 0.001
		3		soil	im. pre-treatment	0	< 0.001	< 0.001	< 0.001	0.0012	< 0.001
		4		soil	im. pre-treatment	0	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		1		soil	at application	0	0.0037	< 0.001	< 0.001	0.0014	< 0.001
		2		soil	at application	0	0.0062	< 0.001	< 0.001	0.0011	< 0.001
		3		soil	at application	0	0.0064	< 0.001	< 0.001	0.0015	< 0.001
		4		soil	at application	0	0.0012	< 0.001	< 0.001	0.0013	< 0.001
	29	1		soil	at planting	29	0.0037	< 0.001	< 0.001	0.0023	< 0.001
	29	2		soil	at planting	29	0.0059	< 0.001	< 0.001	0.0031	< 0.001
	29	3		soil	at planting	29	0.0060	< 0.001	< 0.001	0.0026	< 0.001
	29	4		soil	at planting	29	0.0064	< 0.001	< 0.001	0.0023	< 0.001
		1	carrots	plant w/o root	41 BBCH	99	< 0.001	< 0.001	---	---	< 0.001
		2	cauliflower	plant w/o root	41 BBCH	114	< 0.001	< 0.001	---	---	< 0.001
		3	spring wheat	plant w/o root	31 BBCH	71	< 0.01	< 0.01	---	---	< 0.01
		4	spinach	plant w/o root	41 BBCH	87	< 0.001	< 0.001	---	---	0.0024
		1		soil	41 BBCH	99	0.0012	< 0.001	< 0.001	0.0026	< 0.001
		2		soil	41 BBCH	114	< 0.001	0.0012	< 0.001	0.0037	< 0.001
		3		soil	31 BBCH	71	0.0015	0.0011	< 0.001	0.0034	< 0.001
		4		soil	41 BBCH	87	0.0010	< 0.001	< 0.001	0.0025	< 0.001
		1	carrots	tops	49 BBCH	121	< 0.001	< 0.001	---	---	< 0.001
		1	carrots	roots	49 BBCH	121	< 0.001	< 0.001	---	---	< 0.001
		2	cauliflower	inflorescences	49 BBCH	121	< 0.001	< 0.001	---	---	< 0.001
		3	spring wheat	shoots	61 BBCH	99	< 0.01	< 0.01	---	---	< 0.01
		4	spinach	leaves	49 BBCH	99	**0.0011**	< 0.001	---	---	**0.0021**
		1		soil	49 BBCH	121	< 0.001	< 0.001	< 0.001	0.0028	< 0.001
		2		soil	49 BBCH	121	< 0.001	< 0.001	< 0.001	0.0034	< 0.001
		4		soil	49 BBCH	99	< 0.001	< 0.001	< 0.001	0.0032	< 0.001
		3	spring wheat	grain	89 BBCH	150	< 0.001	< 0.001	---	---	< 0.001
		3	spring wheat	straw	89 BBCH	150	< 0.01	< 0.01	---	---	< 0.01
		3		soil	89 BBCH	150	< 0.001	< 0.001	< 0.001	0.0032	< 0.001

Table B. 7-7 Residues in rotational crops and soil (2004 trials in Spain)

Country Year	Replant Interval (DAT)	Sub-Plot	Succeeding Crop	Matrix	Sampling Timing		Residue Concentration mg/kg				
					Growth stage	DAT	tritosulfuron	TBSA	635M03	635M01	AMTT
Spain		1		soil	im. pre-treatment	0	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
2004		2		soil	im. pre-treatment	0	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
ALO/05/04		3		soil	im. pre-treatment	0	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		4		soil	im. pre-treatment	0	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		1		soil	at application	0	0.0093	< 0.001	< 0.001	< 0.001	< 0.001
		2		soil	at application	0	0.0088	< 0.001	< 0.001	< 0.001	< 0.001
		3		soil	at application	0	0.0047	< 0.001	< 0.001	< 0.001	< 0.001
		4		soil	at application	0	0.0038	< 0.001	< 0.001	< 0.001	< 0.001
	30	1		soil	at planting	30	0.0035	< 0.001	< 0.001	0.0012	< 0.001
	30	2		soil	at planting	30	0.0049	< 0.001	< 0.001	0.0011	< 0.001
	30	3		soil	at planting	30	0.0050	< 0.001	< 0.001	0.0016	< 0.001
	30	4		soil	at planting	30	0.0040	< 0.001	< 0.001	0.0015	< 0.001
		1	carrots	plant w/o root	41 BBCH	93	< 0.001	< 0.001	---	---	0.0017
		2	cauliflower	plant w/o root	41 BBCH	104	< 0.001	< 0.001	---	---	< 0.001
		3	spring wheat	plant w/o root	33 BBCH	71	< 0.01	< 0.01	---	---	< 0.01
		4	spinach	plant w/o root	41 BBCH	71	< 0.001	< 0.001	---	---	0.0032
		1		soil	41 BBCH	93	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		2		soil	41 BBCH	104	0.0017	< 0.001	< 0.001	0.0010	< 0.001
		3		soil	33 BBCH	71	0.0013	< 0.001	< 0.001	< 0.001	< 0.001
		4		soil	41 BBCH	71	0.0019	< 0.001	< 0.001	0.0012	< 0.001
		1	carrots	tops	49 BBCH	114	0.001	< 0.001	---	---	< 0.001
		1	carrots	roots	49 BBCH	114	< 0.001	< 0.001	---	---	< 0.001
		2	cauliflower	inflorescences	49 BBCH	114	< 0.001	< 0.001	---	---	< 0.001
		3	spring wheat	shoots	61 BBCH	83	< 0.01	< 0.01	---	---	< 0.01
		4	spinach	leaves	49 BBCH	83	**< 0.001**	< 0.001	---	---	**0.0017**
		1		soil	49 BBCH	114	0.0024	< 0.001	< 0.001	0.0013	< 0.001
		2		soil	49 BBCH	114	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		4		soil	49 BBCH	83	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		3	spring wheat	grain	89 BBCH	114	< 0.001	< 0.001	---	---	< 0.001
		3	spring wheat	straw	89 BBCH	114	< 0.01	< 0.01	---	---	< 0.01
		3		soil	89 BBCH	114	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table B. 7-8 Residues in rotational crops and soil (2004 trials in UK)

Country Year	Replant Interval (DAT)	Sub-Plot	Succeeding Crop	Matrix	Sampling Timing		Residue Concentration mg/kg				
					Growth stage	DAT	tritosulfuron	TBSA	635M03	635M01	AMTT
UK 2004 OAT/07/04		1		soil	im. pre-treatment	0	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		2		soil	im. pre-treatment	0	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		3		soil	im. pre-treatment	0	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		4		soil	im. pre-treatment	0	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		1		soil	at application	0	0.0096	< 0.001	< 0.001	< 0.001	< 0.001
		2		soil	at application	0	0.0140	< 0.001	< 0.001	< 0.001	< 0.001
		3		soil	at application	0	0.0110	< 0.001	< 0.001	< 0.001	< 0.001
		4		soil	at application	0	0.0100	< 0.001	< 0.001	< 0.001	< 0.001
	35	1		soil	at planting	30	0.0048	< 0.001	< 0.001	0.0025	< 0.001
	35	2		soil	at planting	30	0.0048	< 0.001	< 0.001	0.0019	< 0.001
	35	3		soil	at planting	30	0.0054	< 0.001	< 0.001	0.0026	< 0.001
	35	4		soil	at planting	30	0.0061	< 0.001	< 0.001	0.0021	< 0.001
		1	carrots	plant w/o root	41 BBCH	110	0.0014	< 0.001	---	---	< 0.001
		2	cauliflower	plant w/o root	41 BBCH	111	< 0.001	< 0.001	---	---	< 0.001
		3	spring wheat*	plant w/o root*	33 BBCH*	84	---	---	---	---	---
		4	spinach	plant w/o root	41 BBCH	74	0.0021	< 0.001	---	---	0.0029
		1		soil	41 BBCH	110	< 0.001	< 0.001	0.0013	0.0024	< 0.001
		2		soil	41 BBCH	111	0.0013	< 0.001	0.0012	0.0022	< 0.001
		3		soil	33 BBCH	84	0.0021	0.0010	0.0013	0.0030	< 0.001
		4		soil	41 BBCH	74	0.0034	0.0011	0.0010	0.0030	< 0.001
		1	carrots	tops	49 BBCH	137	0.0016	< 0.001	---	---	< 0.001
		1	carrots	roots	49 BBCH	137	< 0.001	< 0.001	---	---	< 0.001
		2	cauliflower	inflorescences	49 BBCH	127	< 0.001	< 0.001	---	---	< 0.001
		3	spring wheat	shoots	61 BBCH	110	< 0.01	< 0.01	---	---	< 0.01
		4	spinach	leaves	49 BBCH	90	**0.0011**	< 0.001	---	---	**0.0017**
		1		soil	49 BBCH	137	< 0.001	< 0.001	0.0015	0.0020	< 0.001
		2		soil	49 BBCH	127	< 0.001	< 0.001	0.0013	0.0019	< 0.001
		4		soil	49 BBCH	90	0.0016	< 0.001	0.001	0.0024	< 0.001
		3	spring wheat	grain	89 BBCH	162	< 0.001	< 0.001	---	---	< 0.001
		3	spring wheat	straw	89 BBCH	162	< 0.01	< 0.01	---	---	< 0.01
		3		soil	89 BBCH	162	< 0.001	< 0.001	0.0014	0.0017	< 0.001

*not sampled due to poor crop

RESULTS AND DISCUSSION

It appears that out of the selected rotational crops only spinach plants take up AMTT and some tritosulfuron residues from soil. Since only spinach leaves are intended for human consumption the corresponding values are used for evaluation. Tritosulfuron residue levels measured are converted to AMTT data by multiplication with the stoichiometric factor of 0.43. Only the parent molecule and AMTT itself can contribute to the total AMTT exposure. Despite the transformation factor measured was lower, a quantitative conversion (100 %) was assumed in the calculation. Table B. 7-9 shows that the contribution of parent molecule to the total AMTT content is low.

Table B. 7-9: Total AMTT residues in spinach leaves

Country / Year / Trial No.	Tritosulfuron – measured [mg/kg]	AMTT – measured [mg/kg]	Total AMTT – calculated [mg/kg]
DK / 2004 / ALB/04/04	0.0011	0.0021	0.0025
DK / 2003 / ALB/04/03	0.001	0.002	0.0024
UK / 2004 / OAT/07/04	0.0011	0.0017	0.0022
ES / 2004 / ALO/05/04	< 0.001	0.0017	0.0017
DE / 2004 / ACK/05/04	< 0.001	0.002	0.002
UK / 2003 / OAT/07/03	< 0.001	0.002	0.002
ES / 2003 / ALO/05/03	< 0.001	0.002	0.002
DE / 2003 / ACK/05/03	< 0.001	0.001	0.001

CONSUMER RISK ASSESSMENT

Tritosulfuron in primary and succeeding crops

Referring to the DAR and to the addendum 5 to the DAR no chronic risk to consumers was identified based on the residue data from directly treated cereals with tritosulfuron or possible residues which might be taken up by the plants from soil during the vegetation period or by succeeding crops.

Metabolite AMTT in succeeding crops

Based on the results of the confined rotational crop study conducted under green house conditions (reported in the DAR) acute and chronic risks to toddlers were assessed concerning consumption of food produced from certain succeeding crops after treatment of a primary crop.

Short and long term risk from AMTT residues based on the results of a new two years field rotational crop study

In the two years field rotational crop study including carrots, cauliflower, spinach, and wheat determinable residues are only found in spinach representing leafy vegetables. Related to the residue range of 0.001 – 0.0025 mg/kg found in spinach leaves 0.003 mg/kg as a highest residue (HR) is applied for leaf vegetables and fresh herbs in the risk calculations. Separately, lettuce is included at the same residue level because this crop represents a worst case out of leafy vegetables. For all other crops the residue level of LOQ = 0.001 mg/kg is used.

Applying the German consumption model for children of age 2 - < 5 years [VELS-model, in Bundesgesundheitsblatt 48, 84-98 (2005)] the following maximum intake figures of AMTT as well as their contribution to the ARfD are calculated (see Table B. 7-10). As a result the

highest exhaustion of the ARfD is 48 % in case of lettuce from which no acute health risk can be derived.

Table B. 7-10 Maximum intake of AMTT residues from rotational crops / acute consumer risk

Crop	HR [mg/kg]	Intake [mg/kg bw]	% ARfD
Carrots	0.001	3.0×10^{-5}	30.4
Cauliflower	0.001	3.0×10^{-6}	3.0
Spinach	0.003	2.7×10^{-5}	27.2
Lettuce	0.003	4.8×10^{-5}	48.2
Wheat	0.001	2.0×10^{-6}	2.0

Concerning the chronic risk from consumption of possible residues in succeeding crops the NEDI accounts for 37 % of the ADI. Based on this no long term consumer risk is expected (see Table B. 7-11).

Table B. 7-11 NEDI calculation (German VELS model) of tritosulfuron + AMTT (as sum of AMTT equivalents)

ADI of AMTT [mg/kg bw]	**0.0001**		
Body weight (bw) of a 2 - <5 years old child [kg]	**16.15**		
Total intake [mg/kg bw]	**3.7×10^{-5}**		
% ADI	**37**		
Crop	**Consumption**	**HR**	**Intake**
	[g/d]	**[mg/kg]**	**[mg/kg bw]**
Fruits	372.1	0.001	2.3×10^{-5}
Root and tuber vegetables	18.1	0.001	1.1×10^{-6}
Bulb vegetables	3.1	0.001	1.9×10^{-7}
Fruiting vegetables	35.8	0.001	2.2×10^{-6}
Brassica vegetables	7.9	0.001	4.9×10^{-7}
Leaf vegetables and fresh herbs	5.5	0.003	1.0×10^{-6}
Legume vegetables	4.5	0.001	2.8×10^{-7}
Stem vegetables	1.8	0.001	1.1×10^{-7}
Fungi	1.3	0.001	8.0×10^{-8}
Pulses	0.1	0.001	6.2×10^{-9}
Oil seed	3.1	0.001	1.9×10^{-7}
Potatoes	41.4	0.001	2.6×10^{-6}
Tea	0.1	0.001	6.2×10^{-9}
Tea like products	0.7	0.001	4.3×10^{-8}
Cereals	89.9	0.001	5.6×10^{-6}
Spices	0.6	0.001	3.7×10^{-8}

CONCLUSION

Two different types of studies are available, in which the uptake and translocation behaviour of tritosulfuron and AMTT residues from soil into plants were investigated. The results of the

confined rotational crop study reported in the DAR reveal critical consumer risk in particular from certain crops to toddlers.

In order to investigate the residue behaviour in root and tuber vegetables, brassica vegetables, leafy vegetables, and cereals under more realistic conditions field rotational crop studies were performed on carrots, cauliflower, spinach, and wheat. The test compound was applied annually at the maximum label rate on the same test plots at four different sites located in the Northern and Southern part of Europe. The representative crops were sown or planted 29 - 35 days after application. In general, the data indicate that tritosulfuron residues are only taken up to a very limited extent from soil into the edible parts of plants. In carrot roots, wheat grain and cauliflower heads, no residues of tritosulfuron or its soil metabolite AMTT were found above the LOQ of the analysis method used. Only in spinach leaves, residues at or slightly above the LOQ were detected (tritosulfuron: 0.001 mg/kg, AMTT: $\leq$ 0.0021 mg/kg). The residue levels in the non-edible parts or unripe plants were somewhat higher; in all cases they were significantly below 0.01 mg/kg. No TBSA residues were found in any of the crop samples investigated. The residue levels found were comparable over two years indicating that no accumulation of residues occurred when tritosulfuron containing formulations are annually applied. The acute consumer risk assessment takes into account all crops tested in the study and lettuce representing a worst case crop in the group of leafy vegetables.

As a result, following the intake of possible AMTT residues via consumption of products of rotational crops the new assessment does not reveal an acute or chronic health risk to toddlers and therefore to other kind of consumers.

B.7.17 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner
AIIA-6.6	Schulz, H.	2005	Study on the residue behaviour of tritosulfuron (BAS 635 H) in rotational crops: Spinach, carrots, cauliflower and spring wheat after application to the soil of BAS 635 H and the adjuvant Citowett (BAS 152 00 S) under field conditions in Denmark, Germany, Great Britain and Spain, 2003 GLP, unpublished BASF DocID 2005/1007590 RIP2005-997	Y	BAS
AIIA-6.6	Schulz, H.	2005	Study on the Residue Behaviour of Tritosulfuron (BAS 635 H) in Rotational Crops: Spinach, Carrots, Cauliflower and Spring Wheat after Application to the Soil of BAS 635 00 H and the Adjuvant Dash HC (BAS 9047 0 S) under Field Conditions in Denmark, Germany, Great Britain and Spain, 2004 GLP, unpublished BASF DocID: 2005/1025537 RIP2005-2082	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft

Addendum 7

to the Draft Assessment Report

of 20 August 2002

(relating to Volume 1 and Volume 3)

Tritosulfuron

Vol. 1,	**Level 2.3 (Impact on human and animal health)**
Vol. 3,	**B.2 (Physical and chemical properties)**
	B.6 (Toxicology and metabolism)

6 December 2007

Rapporteur Member State: Germany

Preface

Based on the comments of the notifier and the other Member States to the DAR in the Peer Review and further studies submitted by the notifier after the completion of the DAR the following issues are addressed in this Addendum:

- Physical and chemical properties of the active substance
- Toxicology and metabolism, Further toxicological studies
  (in order to give a concise overview this addendum compiles parts of Chapter B.6.8 from Vol. 3 of the original DAR (20.08.2002), the respective parts from Addendum 3 (04.02.2005) as well as the evaluation of new studies submitted)

New text with respect to previous versions is highlighted in yellow.

Contents

To VOLUME 1: 3

2 Reasoned statement of the overall conclusions 3
2.3 Impact on human and animal health 3
2.3.1 Effects having relevance to human and animal health arising from exposure to the active substance or to impurities contained in the active substance or to their transformation products 3
2.3.1.9 Further toxicological studies 3
2.8 Appendices 13
2.8.2 Appendix II: Specific terms and abbreviations 13

To VOLUME 3: 14

B.2 Physical and chemical properties 14

B.2.1 Physical and chemical properties of the active substance (Annex IIA 2) 14
B.2.3 References relied on 15

B.6 Toxicology and metabolism 16

B.6.8 Further toxicological studies (Annex IIA 5.8) 17
B.6.8.1 Toxicity studies of metabolites 17
B.6.8.1.1 635M02 17
B.6.8.1.1.1 First mutagenicity study 22
B.6.8.1.1.2 Second mutagenicity study 23
B.6.8.1.1.3 Re-evaluation of the third mutagenicity study 25
B.6.8.1.1.4 Fourth mutagenicity study 29
B.6.8.1.1.5 Fifth mutagenicity study 30
B.6.8.1.1.6 First acute oral toxicity study 31
B.6.8.1.1.7 Second acute oral toxicity study 32
B.6.8.1.1.8 Third acute oral toxicity study 33
B.6.8.1.1.9 Range finding study: 28-day subacute study with 635 M02 (TBSA) 35
B.6.8.1.1.10 Second 28-day subacute study with 635 M02 (TBSA) 41
B.6.8.1.1.11 First 1-generation study with 635 M02 (TBSA) 44
B.6.8.1.1.12 Second 1-generation study with 635 M02 (TBSA) 48
B.6.8.1.1.13 TBSA: Assessment of Reproduction Toxicity Potential 53
B.6.8.1.2 635M03 54
B.6.8.1.2.1 First mutagenicity study 55
B.6.8.1.2.2 Second mutagenicity study 57
B.6.8.1.2.3 Third mutagenicity study 58
B.6.8.1.2.4 Acute oral toxicity study 60
B.6.8.1.2.5 Subchronic toxicity study 61

B.6.8.1.3 635M01 62
B.6.8.1.3.1 First mutagenicity study 63
B.6.8.1.3.2 Second mutagenicity study 64
B.6.8.1.3.3 Third mutagenicity study 65
B.6.8.1.3.4 Acute oral toxicicty study 66
B.6.8.1.3.5 28-day toxicity study in Wistar rats 67
B.6.8.1.4 635M17 69
B.6.8.1.4.1 First mutagenicity study 69
B.6.8.1.4.2 Second mutagenicity study 70
B.6.8.1.4.3 Third mutagenicity study 72
B.6.8.1.4.4 Acute oral toxicity study 73
B.6.8.2 Supplementary studies – AMTT (635M04) 74
B.6.8.2.1 Study of the biokinetics and metabolism in rats 77
B.6.8.2.2 Study on the acute oral toxicity 79
B.6.8.2.3 Subchronic toxicity study 80
B.6.8.2.4 First mutagenicity study 82
B.6.8.2.5 Second mutagenicity study 83
B.6.8.2.6 Third mutagenicity study 84
B.6.8.2.7 Fourth mutagenicity study 87
B.6.8.2.8 Pre-/postnatal screening toxicity study in Wistar rats 89
B.6.8.2.9 Study of a possible bond of AMTT and Tritosulfuron to the estrogen receptor 90
B.6.15 References relied on 92

To VOLUME 1:

2 Reasoned statement of the overall conclusions

2.3 Impact on human and animal health

2.3.1 Effects having relevance to human and animal health arising from exposure to the active substance or to impurities contained in the active substance or to their transformation products

2.3.1.9 Further toxicological studies

635M02 (Reg. No. 292 564; BH 635-2, TBSA) is a soil metabolite and was detected in the rat metabolism study (< 1 % in urine/feces). In the first data package submitted, it was tested in three mutagenicity assays as well as in two acute oral tests. When it became obvious that this metabolite exceeds the groundwater threshold of 0.1 µg/L by leaching the toxicological relevance had to be addressed in greater detail.
In an additional acute oral toxicity study in rats, TBSA of high purity (99.9 %) was tested in order to rule out the possibility that the acute oral toxicity seen in a previous study with a less pure batch of the test material was due to the presence of an impurity [see Wiemann C. and Hellwig J. 1999(a); BASF RegDoc# 1999/10099].
In an additional 28-day toxicity study no NOAEL could be derived and - beside others - effects on the female reproductive organs were observed. Therefore, a second 28-day study conducted with lower doses of TBSA was submitted.
The objective of the 1-generation studies was to determine the possible adverse effects of TBSA on the integrity and performance of the male and female reproductive systems since effects were noted in the first 28-day study.

For an overview of the study results see table below.

The metabolite TBSA was found to be not mutagenic in the *Salmonella typhimurium/ Escherichia coli* reverse mutation assay and not mutagenic *in vitro* in the CHO/HPRT mutation assay.

Based mainly on comments of the UK, at ECCO 136 it was concluded that 635M02 may have some chromosome-damaging (clastogenic) potential under *in vitro* conditions in V79 cells with S-9 mix. Therefore in the endpoint list the *in vitro* chromosome aberration assay was considered positive. The re-evaluation of the study led to the conclusion that 635M02 has no clastogenic potential. The justification is given in the assessment of the study results (see Vol. 3). The metabolite TBSA was found to be not mutagenic *in vivo* in the Micronucleus assay in bone marrow cells of the rat and not mutagenic *in vivo* in the UDS assay in the rat.
Three acute oral studies were conducted in total. One study resulted in an oral LD50 of 1000 mg/kg bw, one in an oral LD50 of > 2000 mg/kg bw. The new study was conducted with the test substance of high purity (99.9 %). The acute oral LD50 was found to be greater than 300 mg/kg and less than 2000 mg/kg body weight in rats. The median lethal dose lies between 500 and 1000 mg/kg bw. Thus, it can be considered that the metabolite 635M02 is of higher oral toxicity than the parent compound tritosulfuron (N12).

In addition, two 28-day repeated dose toxicity study in rats were submitted after the completion of the DAR. In the first study effects on the body weight (decreases), in the functional observation battery (reduced rearing and overall motor activity, tremor), on hematological (decreased red blood cell parameters) and clinico-chemical examinations (increased γ–glutamyltransferase activities, increased bilirubin and cholesterol levels), on urinalysis (increased number of degenerated renal tubular epithelial cells and transitional epithelial cells as well as higher number of granular casts and epithelial cell casts) and on organ weights (higher kidney, liver and spleen weights and lower ovary and uterine weights) were noted. The histopathological examinations showed that the ovaries of high-dose group females had a significantly decreased number of corpora lutea. The differential ovarian follicle count (DOFC) indicated that the number of clearly discernible antral follicles was reduced to 60 % of the control value at 5000 ppm. An increased number of females with slight or moderate atrophy of the uterus (mucosa and musculature) in the mid and high dose groups was observed. In the spleen, increased incidences of hemosiderin deposition and congested blood vessels were observed in mid and/or high dose groups. A NOAEL could not be derived from this study, based on effects on red blood cells, organ weights and ovaries at the lowest dose of 200 ppm tested (corresponding to daily intakes of 18.6 and 20.0 mg/kg bw/d for males and females, respectively).

Therefore, a subsequent 28-day oral toxicity study was conducted using three lower dose levels (0, 50, 100 and 150 ppm, corresponding to approximately 5.0, 10.0 and 14.7 mg/kg bw/d; see BASF RegDoc# 2003/1004049). In this study an increased α2u-globulin accumulation in the kidneys of male rats were noted in all dose-groups. This finding was considered to represent a sex and species specific effect, without relevance to human males. Thus, the NOAEL was set at 150 ppm (14.7 mg/kg bw/d).

To possibly exclude effects of 635M02 on fertility and reproduction two 1-generation studies were conducted with similar dose levels (0, 200 and 400 ppm; corresponding to approximately 12.8/16.2 and 25.3/32.4 mg/kg bw/d in males/females). In the first study, the male/female fertility index was slightly reduced in the high dose of 400 ppm (70 % versus 90 % and 100 % in the control and low dose), with 3/10 non-pregnant females in the 400 ppm group. One female of the 400 ppm group showed a severely reduced number of corpora lutea and a massive follicle degeneration in both ovaries. No further effect on female reproduction, delivery and litter/pup data was noted.

Cholesterol concentrations were increased in males and females at 400 ppm. High dose females showed lower magnesium levels. The mean absolute and relative liver, kidney and spleen weights were significantly increased in high dose F_0 parental males, when compared with controls. In high dose F_0 parental females, the relative spleen weight was significantly increased.

Under the conditions of this study the NOAEL for reproductive toxicity is 200 ppm (corresponding to approximately 12.8 mg/kg bw/d and 16.2 mg/kg bw/d in males and females respectively). The NOAEL for general toxicity is below 200 ppm based on lower body weight and organ weight changes at this dose level.

In the second 1-generation study, one female of the high dose group (400 ppm) was fertile (proved by 6 implantation sites in utero at terminal sacrifice) but did not deliver pups. There was no further substance-related effect on female reproduction, delivery and litter data. The DOFC revealed no changes in the number of primordial and/or growing follicles between the control and treated groups.

The F1 pups body weights and body weight gains were minimally lower in the 400 ppm group at all investigation points, although without statistical significance and with values lying mainly within the historical control values. In the presence of maternal toxicity at this dose level, these changes were not considered to represent a reproductive adverse effect.

Under the conditions of this study the NOAEL for reproductive toxicity is 200 ppm (corresponding to approximately 17.6 mg/kg bw/d and 17.3 mg/kg bw/d in males and females respectively) based on postimplantation loss noted in one female at the next higher dose of 400 ppm (corresponding to approximately 35.0 mg/kg bw/d and 33.1 mg/kg bw/d in males and females respectively).
The NOAEL for general toxicity is below 200 ppm based on lower body weight and clinico-chemical findings (higher white blood cell count in males, higher cholesterol concentrations in males and females, lower magnesium concentrations in females) at this dose level.

Table 2.3-9: Summary of toxicity studies of metabolite 635M02 (Reg.-No. 292 564)

Study/strains/species	Test material/conditions	Results
Acute oral toxicity in rats Wistar rats (Chbb:THOM (SPF)) 5 male / 5 female 500, 2000 and 5000 mg/kg bw	Batch No. 00831-201, purity: 98.2 % Test substance preparation in 0.5 % Tylose CB 30.000 in aqua bidest	LD_{50}: 1000 mg/kg bw Mortality at ≥ 2000 mg/kg bw (delayed after 3 to 4 days), main signs of toxicity: dyspnea, apathy, excitation, abdominal and lateral position, staggering, ataxia, atonia, paresis, narcotic-like state, pain and corneal reflex absent, tremor, spastic gait, erythema, exsiccosis, salivation, lacrimation, discoloured urine, squatting posture and red clammy snout and eyelid.
Acute oral toxicity of TBSA in rats Wistar rats (Chbb:THOM (SPF)) 3 male / 3 female 200 and 2000 mg/kg bw	Batch no. 26778/99; 26778/101, purity: > 98.5 % Test substance preparation in olive oil DAB 10	LD_{50}:> 2000 mg/kg bw No mortality Main signs of toxicity: dyspnea, apathy, staggering, exsiccosis, red discoloured urine until day 5 post dosing
Acute oral toxicity of TBSA in rats Wistar rats (CrlGlxBrlHan:WI) 3 x 3 female 2000 and 300 mg/kg bw	Batch no: 2059-029, purity: 99.9 % Test substance preparation in 0.5 % CMC-solution (cleaned sodium carboxymethylcellulose) in double distilled water	Median lethal dose: around 500 mg/kg bw All animals at 2000 mg/kg bw were found dead on study day 3 No mortality at 300 mg/kg bw. Main signs of toxicity: dyspnoea, abdominal or lateral position, staggering, smeared fur, lacrimation and red smeared fur in the anogenital area
Salmonella typhimurium/ Escherichia coli reverse mutation assay S. typhimurium strains TA 100, TA 1535, TA 1537, TA 98 and E. coli strain WP2 uvrA	Batch No. 00831-201, purity: 98.2 %	Not mutagenic
Gene mutation assay in Chinese hamster ovary (CHO) V79 cells *in vitro* (V79 / HPRT)	Batch No. 00831-201, purity: 98.2 %	Not mutagenic
In vitro chromosome aberration assay in V79 cells	Batch No. 00831-201, purity: 98.2 %	Not mutagenic
Micronucleus assay in bone marrow cells of the rat	Batch No. 00831-201, purity: 98.2 %	Not mutagenic
In vivo UDS assay in the rat	Batch No. 00831-201, purity: 98.2 %	Not mutagenic

Study/strains/species	Test material/conditions	Results
28-day oral toxicity study in rats Wistar rats (CrlGlxBrlHan:WI) 5 males / 5 females 0; 200; 1000 and 5000 ppm (18.6/20.0, 90.7/96.1, 417.3/414.2 mg/kg bw/d for males and females)	Batch no. 25887 Fass 2; purity: 98.4 %	No NOAEL LOAEL 200 ppm: ↑ liver wt.,↑ kidney wt. (males),↓ RBC (f), α_{2u}–globulin accumulation (male kidneys), hyperplastic ovarian stroma cells 1000 ppm: ↓ bw (females), ↓ overall motor activity, ↓ RBC, ↑ bilirubin, ↑ cholesterol, cells and casts in urine sediment (males), ↑ liver wt., ↑ kidney wt. (males), ↑ spleen wt., α_{2u}–globulin accumulation (male kidneys), hepatocellular hypertrophy (males), hyperplastic ovarian stroma cells, atrophy of the uterus (mucosa and musculature), hemosiderin deposition (spleen) 5000 ppm: tremor, piloerection from day 14 onwards, ↓ bw, ↓ rearing, ↓ overall motor activity, ↓ RBC, ↑ γ–glutamyltransferase activities, ↑ bilirubin, ↑ cholesterol, cells and casts in urine sediment (males), ↑ liver wt., ↑ kidney wt. (males), ↓ ovary and uterus wt., ↑ spleen wt., α_{2u}–globulin accumulation (male kidneys), chronic progressive nephropathy (male), hepatocellular hypertrophy, ↓ number of corpora lutea (ovary), hyperplastic ovarian stroma cells, atrophy of the uterus (mucosa and musculature), hemosiderin deposition (spleen)
28-day oral toxicity study in rats Wistar rats (CrlGlxBrlHan:WI) 5 male / 5 female 0, 50, 100 and 150 ppm (4.8/5.0; 9.0/10.0; 13.9/14.7 mg/kg bw/d in males and females)	Batch no. 25887 Fass 2; purity: 98.4 %.	NOAEL: 150 ppm All dose groups: $\alpha_{2\mu}$-globulin accumulation in the kidney (males)
1-generation study in rats (range finding) Wistar rats (CrlGlxBrlHan:WI) 10 male / 10 female 5 male / 5 female as satellite groups 0; 200 and 400 ppm (12.9/16.2; 25.3/32.4 mg/kg bw/d in males and females) premating phase: 4 weeks	Batch no. 25887 Fass 2; purity: 98.4 %	NOAEL (reproduction): 200 ppm NOAEL (general toxicity): ≤ 200 ppm 200 ppm: ↓ bw (f), ↑ liver wt.(f) 400 ppm: ↓ bw (f), ↓ male/female fertility index, severely reduced number of corpora lutea/ massive follicle degeneration in the ovaries (1 female), ↑ cholesterol, ↓ magnesium (f), ↑ kidney wt. (m); ↑ spleen wt., ↑ liver wt., ↑ thyroid wt. (f)
1-generation study in rats (main study) Wistar rats (CrlGlxBrlHan:WI) 10 male / 10 female 5 male / 5 female as satellite groups 0; 200 and 400 ppm (17.7/20.7; 35.0/40.9 mg/kg bw/d in males and females) premating phase: 10 weeks	Batch no. 25887 Fass 2; purity: 98.4 %.	NOAEL (reproduction): 200 ppm NOAEL (general toxicity): ≤ 200 ppm 200 ppm: ↓ bw (f), ↑ WBC (m), ↑ cholesterol, ↓ magnesium (f) 400 ppm: ↓ bw (f), postimplantation loss (1 female), ↑ WBC (m), ↓ RBC, Hb, ,HCT, ↑ Reticulocytes, ↑ cholesterol, ↓ magnesium (f), ↑ kidney wt. (m), ↑ liver wt., ↑ spleen wt.

Statement on the toxicological relevance of 635M02 (TBSA):

TBSA toxicity and fertility study data were reviewed to establish whether it meets the 2001/59/EC criteria for classification as toxic to reproduction category 2 or 3.

Uterine atrophy, reduced ovary weight and reduced corpora lutea were seen at the markedly toxic dose of 5000 ppm in the first 28-day subacute study (range finding, high dose), most likely secondary to the marked reduction in body weight at this dose (mean - 20% versus controls). Chapin et al (1993) reported that 30% body weight reduction in rats (by feed restriction) decreased the number of corpora lutea and ovary weights, but did not affect male reproductive organs (Chapin R. E., Gulati D.K., Barnes L. H, Teague J.L. The effects of feed restriction on reproductive function in Sprague-Dawley rats. Fundam. Appl. Toxicol. 1993 Jan; 20(1):23-9).

Hyperplasia of the ovarian stromal cells was also noted in the first 28-day subacute study (range finding, high dose), minimal in 3 animals at 200 ppm, slight in all animals at 1000 ppm, and slight to moderate in all animals at 5000 ppm. The etiology and biological significance of this finding are unclear. No such effect was observed in the second 28-day subacute study at 50, 100 or 150 ppm, or in the two 1-generation reproduction studies at 200 or 400 ppm, with treatment periods extending from the begin of the 4- or 10-week premating period through mating, gestation and lactation. Furthermore, it could be demonstrated that, the control females in the second 1-generation study had similar or even higher degrees of hyperplasia than the 5000 ppm females in the first 28-day subacute study, but this level of hyperplasia was regarded as normal for animals of this age (the 1-generation study females were older than those in the 28-day subacute study. They had also been pregnant. See also B.6.8.1.1.1. FitzGerald R. E., 2007 TBSA: Assessment of Reproduction Toxicity Potential (Photo documentation „Hyperplasia of the ovarian stroma cells“ recorded after TBSA treatment)).

Differential ovarian follicle count (DOFC) were affected in the first 28-day subacute study only at 5000 ppm, not at 1000 or 200 ppm, nor in the second 28-day subacute study at up to the highest concentration of 150 ppm. In the first 1-generation study, 1 of 15 rats had massive degeneration at 400 ppm but there were no other findings in DOFC. In the second 1-generation reproduction study (longer treatment duration than in the first 1-generation study), there were no effects on DOFC in 15 rats/dose at 200 or 400 ppm.

Fertility index was slightly reduced at 400 ppm in the first 1-generation study. Three of 10 positively mated females were not pregnant at 400 ppm versus 1 of 10 control females. Infertility in one of these 400 ppm females (no.121) was due to a severely reduced follicle and corpus luteum count. There was no notable pathology in the other 2 non-pregnant females or their mating partners. Comparison of fertility indices in the two 1-generation studies indicates that the difference in the first 1-generation study between controls and 400 ppm animals was due to chance, because in the second 1-generation study, with a much longer pre-mating dosing period (10 versus 4 weeks), all 10 of 10 animals of the 400 ppm group were pregnant, while 2 of 10 controls were not. These data show that there was no effect of treatment on fertility even at maternally toxic doses.

No substance related effects on testis were seen in the 1-generation studies. Total sperm quantity and quality in 400 ppm males were also unaffected by treatment in the 1-generation studies. The data show that treatment had no effect on male reproduction organs.

There was a non-significantly reduced pup weight on day 1 postpartum at 400 ppm in the second 1-generation study. F1-pups were well within the range of historical control data. If at all, these weight changes occurred in the presence of maternal toxicity. Therefore, no relevance is attributed.

A minimal non-significant increase in post-implantation loss at 400 ppm in the second 1-generation study was due to female no. 122, with 6 implantation sites but no pups delivered

(total litter loss). Historical control data show that total litter loss in single control group animals does occur spontaneously.
In summary, based on detailed examination of all reproduction parameters, TBSA had no effect on mating, fertility, gestation and livebirth indices or reproductive organ pathology indicative of potential reproductive toxicity. TBSA had no primary effect on developmental toxicity. In conclusion, based on the available repeat dose and reproduction toxicity study data, TBSA should clearly not be classified as toxic to reproduction.

The acute oral toxicity of TBSA is approximately 4 times higher than the parent compound tritosulfuron (TBSA: LD_{50} approximately 1000 mg/kg bw versus tritosulfuron: LD_{50} 4700 mg/kg bw).
The results of the toxicity studies (28-day repeat-dose and 1-generation studies) in rats mainly indicated the ovaries (reduced ovary weight, decreased number of corpora lutea) and the uterus (atrophy of mucosa and musculature) as target organs. Repeated high doses of TBSA (5000 ppm corresponding to approximately 400 mg/kg bw/d) affected all female rats, whereas at lower doses (400 ppm corresponding to approximately 30 mg/kg bw/d) only single females were affected. Effects on the uterus (atrophy of mucosa and musculature as noted in the 28-day study at ≥ 1000 ppm, 96.1 mg/kg bw/d) were not reported in the 1-generation studies.
The following is therefore proposed in accordance with the latest classification and labelling guidance under Directive 67/548/EEC (i.e. in the 18th ATP published as Directive 93/21/EEC):

TBSA:	Hazard symbol:	Xn	
	Indication of danger:	Harmful	
	Risk phrase:	R 22	Harmful if swallowed.

In conclusion, according to the criteria given in the EU guidance document on relevant metabolites in groundwater, the metabolite has not to be considered as toxicologically relevant. However, because the acute oral toxicity of TBSA suggests a moderate toxicity that is markedly higher than exhibited by the active ingredient and the effects observed mainly in the female reproductive organs (ovaries and uterus) after repeated high dose, a groundwater concentration of 0.75 µg/L must not be exceeded.

635M03 (Reg. No. 335 182; BH 635-3) is a soil metabolite. It was detected in the rat metabolism study as a transient metabolite. It was tested in three mutagenicity assays as well as in an acute oral test and a 90-day feeding study. 635M03 was found to be not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay, not mutagenic *in vitro* in the CHO/HPRT mutation assay and not chromosome-damaging (clastogenic) under *in vitro* conditions in V79 cells. An acute oral toxicity study revealed an LD_{50} of > 5000 mg/kg bw. The no observed adverse effect level in a 90-day dietary rat study was 15000 ppm (equal to 1187 mg/kg bw). Thus, the metabolite 635M03 is not of toxicological relevance in ground-water.

Table 2.3-10: Summary of toxicity studies of metabolite 635M03 (Reg.-No. 335 182)

Study/strains/species	Test material/conditions	Results
Acute oral toxicity in rats Wistar rats (Chbb:THOM (SPF))	Batch No. 00831-274, Purity: 98.7 %; Batch 01185-044, purity 99.4 %.	LD_{50}: >5000 mg/kg bw
Subchronic toxicity study in Wistar rats [CRL:WI(GLX/BRL/HAN)IGS BR]. Administration in the diet for 3 months	Batch No. 01185-269, Purity: 99.2 %.	No substance related effects NOAEL: 15000 ppm (1187 mg/kg bw/d)
Salmonella typhimurium/Escherichia coli reverse mutation assay *S. typhimurium* strains TA 100, TA 1535, TA 1537, TA 98 and *E. coli* strain WP2 uvrA	Batch No. 00831-274, Purity: 98.7 %.	Not mutagenic
Gene mutation assay in Chinese hamster V79 cells *in vitro* (V79 / HPRT)	Batch No. 01185-085, Purity: 99.8 %.	Not mutagenic
In vitro chromosome aberration assay in Chinese hamster V79 cells	Batch No. 01185-085, Purity: 99.8 %.	Not mutagenic

635M01 (Reg.-No. 335 184; BH 635-4) is a soil metabolite. It was detected in the rat metabolism study (urine, faeces, bile). Nevertheless, it was tested in three mutagenicity assays as well as in an acute oral test. It was found to be not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay, not mutagenic *in vitro* in the CHO/HPRT mutation assay and not chromosome-damaging (clastogenic) under *in vitro* conditions in V79 cells. An acute oral toxicity study revealed an LD_{50} of > 5000 mg/kg bw. A 28-day dietary toxicity study in rats was submitted after the completion of the DAR and revealed a NOAEL of 3900 ppm, the highest dose tested. There were no signs of toxicity. Thus, the metabolite 635M01 is not of toxicological relevance in groundwater.

Table 2.3-11: Summary of toxicity studies of metabolite Reg. 635M01

Study/strains/species	Test material/conditions	Results
Acute oral toxicity in rats Wistar rats (Chbb:THOM (SPF))	Batch no. 01185-088, purity: 97.0 %	LD_{50}: > 5000 mg/kg bw
Salmonella typhimurium/Escherichia coli reverse mutation assay S. typhimurium strains TA 100, TA 1535, TA 1537, TA 98 and E. coli strain WP2 uvrA	Batch no. 00831-277, purity: 97.9 %	Not mutagenic
Gene mutation assay in Chinese hamster V79 cells *in vitro* (V79 / HPRT) Chinese hamster ovary (CHO) cells	Batch no. 01185-088, purity: 97.0 %	Not mutagenic
In vitro chromosome aberration assay in V79 cells (cells derived Chinese hamster)	Batch no. 01185-088, purity: 97.0 %	Not mutagenic
28-d oral (diet) toxicity study in Wistar rats (CrlGlxBrlHan:WI) 10 male / 10 female 0; 430; 1300 and 3900 ppm (corresponding to approximately 38, 115 and 344 mg/kg bw/d)	Batch no. 2059-011, purity: 96.4 %.	NOAEL: 3900 ppm No signs of toxicity

635M17 (Reg. No. 373 906) is a plant metabolite. It was detected in the rat metabolism study in minor quantities. It was tested in three mutagenicity assays as well as in an acute oral test. It was found to be not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay, not mutagenic *in vitro* in the CHO/HPRT mutation assay and not chromosome-damaging (clastogenic) *in vivo*. There were no indications of any impairment of chromosome distribution in the course of mitosis. An acute oral toxicity study revealed an LD_{50} of > 2000 mg/kg bw. Thus, the metabolite 635M017 is not of toxicological relevance in groundwater.

Table 2.3-12: Summary of toxicity studies of metabolite 635M17 (Reg.-No. 373 906)

Study/strains/species	Test material/ conditions	Results
Acute oral toxicity study in Wistar rats Crl: WI(GLX/BRL/HAN)IGS BR (SPF)	Batch No. 01742-22, Purity: 98.3 %.	LD_{50}: >2000 mg/kg bw
Salmonella typhimurium/Escherichia coli reverse mutation assay (standard plate test and preincubation test) *S. typhimurium* strains TA 100, TA 1535, TA 1537, TA 98 and *E. coli* strain WP2 uvrA	Batch No. 01742-22, Purity: 98.3 %.	Not mutagenic
In vitro gene mutation test in Chinese hamster ovary (CHO) cells (HPRT Locus Assay)	Batch No. 01742-22, Purity: 98.3 %.	Not mutagenic
Cytogenetic study *in vivo* in the mouse micronucleus test after two intraperitoneal administrations (NMRI mice)	Batch No. 01742-22, Purity: 98.3 %.	Not mutagenic

AMTT (635M04) was an impurity in the batch no. N24 (2.45 %) which caused severe effects in the long-term rat studies and in the two-generation reproduction toxicity study in rats. AMTT is also a metabolite in the rat, soil and water. A separate metabolism study was conducted with AMTT, it was tested in an acute oral test, as well as in three mutagenicity tests. Furthermore, in order to prove that the effects seen in the 2-generation study using tritosulfuron batch no. N24 was due to high AMTT content, it was tested in a pre/postnatal toxicity study. In order to understand the mechanism by which AMTT exerts its effects it was subjected to two additional studies: a subchronic toxicity study with estrus cycle determination as well as hormone analysis and determination of the binding capacity to the estrogen receptor.

AMTT does not accumulate in rats, but is effectively excreted. The major metabolite AHTT is generated by demethylation and is detected as different tautomeric structures. The oral LD_{50} was found to be > 200 < 2000 mg/kg bw. Estrus cycle determination, hormone analysis as well as PCNA resp. BrdU and TUNEL–stain analysis of mammary glands and a density calculation of estrogen (Eα)– and progesterone receptors in uterus and vagina revealed no treatment-related changes in a subchronic toxicity study.

AMTT is not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay, not mutagenic *in vitro* in the CHO/HPRT mutation assay and did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the mouse and therefore is considered to be non-mutagenic in this micronucleus assay. After the completion of the DAR, an additional study was submitted in which AMTT was assessed for its potential to induce structural chromosomal aberrations (clastogenic activity) and/or changes in the number of chromosomes (aneugenic activity) in V79 cells *in vitro* both in the presence and in the absence of a metabolising system. AMTT is not considered to be a clastogenic or an aneugenic agent under in-vitro conditions in V79 cells. Altogether, AMTT has no genotoxic potential under the conditions of the *in vitro* and *in vivo* studies conducted.

The oral application of AMTT induced severe maternal and developmental toxicity at 20 mg/kg bw/day and at 50 mg/kg bw/day in a pre/postnatal screening study. Therefore,

AMTT might be responsible for the effects observed in the 2-generation study with tritosulfuron containing high levels of AMTT, with respect to pup mortality. In the presence of endogenous estrogens, the bonding capacity of tritosulfuron and AMTT to the estrogen receptor is regarded as extremely low. A biological effect of the substances, the activation of the receptor-mediated gene expression, is extremely unlikely.

Based on the submitted data for tritosulfuron with a high content of AMTT (i.e. 2.45 %), AMTT was considered to possess carcinogenic and reproduction disturbing properties. In conclusion AMTT should be considered as toxicologically relevant metabolite in groundwater.

Based on the 2-generation study in rats conducted with batch no. N24 with a calculated level of AMTT of 0.06 mg/kg bw/d and applying a safety factor of 500, the following reference values were derived for AMTT:

ADI: 0.0001 mg/kg bw
AOEL (syst.): 0.0001 mg/kg bw/d
ARfD: 0.0001 mg/kg bw

Table 2.3-13: Summary of supplementary studies with AMTT (635M04) (CAS-Nr. 5311-05-07)

Study/strains/species	Test material/ conditions	Results
Study of the biokinetics and metabolism in Wistar rats (Chbb:THOM (SPF))	14C-AMTT; Batch no. 687-1008, chemical purity > 98 %, radiochemical purity: > 95 %.	Rapid excretion, major metabolite: AHTT
Study on the acute oral toxicity of AMTT in Wistar rats (Chbb:THOM (SPF))	Batch no. 27 939/16, purity: 92.3 % - 94.2 %.	LD_{50}: > 200 < 2000 mg/kg bw
Subchronic toxicity study with estrus cycle determination and hormone analysis in female Wistar rats (Chbb:THOM (SPF)) Administration in the diet up to 32 weeks 0, 40, 120 ppm	Batch no. 01185-097, purity: 99.9 %	LOAEL: 40 ppm (3.6 mg/kg bw/d): Estrus cycle determination, hormone analysis, PCNA resp. BrdU and TUNEL–stain analysis of mammary glands and a density calculation of estrogen (Eα)– and progesterone receptors in uterus and vagina revealed no treatment-related changes.
Ames Salmonella/mammalian-microsome mutagenicity test and Escherichia coli / mammalian microsome reverse mutation assay (standard plate test and preincubation test) S. typhimurium strains TA 100, TA 1535, TA 1537, TA 98 and E. coli strain WP2 uvrA	Batch no. 27 939/16, purity: 92.3 % - 94.2 %.	Not mutagenic
Gene mutation assay in Chinese hamster ovary (CHO) V79 cells *in vitro* (V79/HPRT)	Batch no. 01185-097, purity: 99.9 %.	Not mutagenic
In vitro chromosome aberration assay in V79 cells	Batch No. 00831-237, purity: 99.9 %	Not mutagenic
Micronucleus assay in bone marrow cells of the mouse (NMRI) after a single	Batch no. 27939-141 CP031929,	Not mutagenic

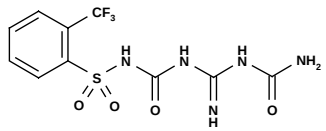
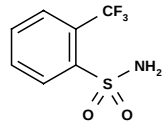
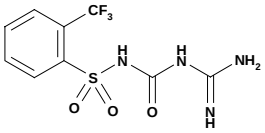
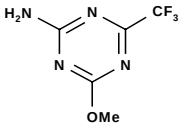
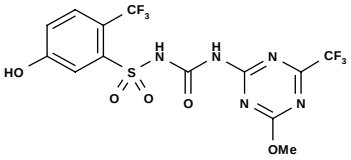
Study/strains/species	Test material/ conditions	Results
intraperitoneal administration	purity: 99.8 %.	
AMTT and BisSH - Pre-/ postnatal screening toxicity study in Wistar rats (Chbb: THOM (SPF)) – Oral administration (gavage)	Batch no. CP031929, purity: 99.8 %, BisSH; batch no. CP 031930, purity: 99.7 %	Severe maternal and developmental toxicity at 20 mg/kg bw/d and at 50 mg/kg bw/d AMTT
Study of a possible bond of AMTT and tritosulfuron to the estrogen receptor in the cytosol from the endometrial RUCA-I adenocarcinoma cell line	Endometrial RUCA-I-adenocarcinoma cell line of the rats	Extremely low bonding capacity of tritosulfuron and AMTT to the estrogen receptor in the presence of endogenous estrogens

2.8 Appendices

2.8.2 Appendix II: Specific terms and abbreviations

Different names were used for the metabolites of tritosulfuron (BAS 635 H) in the additional studies submitted by the notifier and assessed in this addendum. For the readers' convenience, an overview of names, structural formulae and occurrences of the metabolites referred to in this addendum has been extracted from Volume 1 of the draft assessment report and is presented as Table 2.8.2-1.

Table 2.8.2-1: List of metabolites of tritosulfuron discussed in Addendum 3 (excerpt from Volume 1 of the draft assessment report)

Code	Structure	Chemical Name [CAS]/or IUPAC Name	Trivial Name, Codes used	Found in matrix
635M01		1-(carbamoylamidino)-3-(2-trifluoromethyl-benzenesulfonyl) urea	335 184 (BH 635-4)	rat, maize, rotat. crops, soil, water, sediment
635M02		2-trifluoromethyl-benzenesulfonamide	TBSA, 292 564 (BH 635-2)	rat, goat, hen, rotat. crops, soil, water, sediment
635M03		1-amidino-3-(2-trifluoromethyl-benzenesulfonyl) urea	335 182 (BH 635-3)	rotat. crops, soil, water, sediment
635M04		2-amino-4-methoxy-6-(trifluoromethyl)-1,3,5-triazine	AMTT, 231 700 (BH 635-5)	rat, goat, hen, rotat. crops, soil, water
635M17		1-[(4-methoxy-6-trifluoromethyl)-1,3,5-triazin-2-yl]-3-(5-hydroxy-2-trifluoromethyl-benzenesulfonyl) urea	373 906 (BH 635-16)	rat, maize, rotat. crops, water

To VOLUME 3:

B.2 Physical and chemical properties

B.2.1 Physical and chemical properties of the active substance (Annex IIA 2)

Section (Annex point)	Study	Purity (w/w)	Method	GLP	Results	Comment / Conclusion	Reference
B.2.1.6 (IIA 2.6)	Solubility in water of purified active substance	PAS (99.8 %)	EEC A 6 (column elution method)	**Y**	38.6 mg/L pH 4.7 (deionized water) 78.3 g/L pH 10.2 0.94 mg/L pH 1.7 all at 20 °C	Acceptable	Daum, 2001 (CHE2001-500)
B.2.1.7 (IIA 2.7)	Solubility in organic solvent of the active substance as manufactured	TAS (95.6 %)	US-EPA Subdivision D Reference No. 63-8	Y	**Solvent — Solubility (20 °C)** n-Heptane — insoluble Toluene — 4.2 g/L Dichloromethane — 25 g/L Methanol — 23 g/L Acetone — 250 g/L Ethyl acetate — 83 g/L Acetonitrile — 90 g/L 1-Octanol — 13 g/L 2-Propanol — 3.3 g/L olive oil — 0.46 g/L	Acceptable	Türk, 1994 (CHE2001-501)
B.2.1.9.3 (IIA 2.9)	Quantum yield of direct photodegradation	PAS (99.8 %)	OECD 112	Y	$\Phi < 1.05 \cdot 10^{-4}$ (pH 5) $\Phi < 2.23 \cdot 10^{-4}$ (pH 7)	Acceptable	Scharf, 1998 (LUF2001-186)

B.2.3 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner
AIIA-2.6	Daum	2001	Determination of the solubility in water of Tritosulfuron (BAS 635 H, Reg.-No. 271272). 2001/1001015 GLP, unpublished CHE2001-500	Y	BAS
AIIA-2.7	Türk	1994	Determination of the solubility of Reg.-No. 271272 technical active ingredient (TAI) in organic solvents at 20 °C. 94/11101 GLP, unpublished CHE2001-501	Y	BAS
AIIA-2.9	Scharf	1998	Aqueous photolysis of BAS 635 H at pH 5 and pH 7. 1998/10981 GLP, unpublished LUF2001-186	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft

B.6 Toxicology and metabolism

Introduction

With regard to the conclusions drawn at ECCO-Meeting 136 and the studies submitted by the notifier after the DAR had been completed, the following main issues were addressed in Addendum 3 (4 February 2005). Text passages highlighted in yellow indicate amended or revised evaluations with respect to Addendum 3 or the orginial DAR respectively. The changes were considered necessary due to the submission of 3 further studies:

Annex IIA, point 5.8: Additional toxicological studies with TBSA (metabolite 635M02)

Acute oral toxicity study in rats
28-day repeated dose toxicity study in rats (range finding study)
28-day repeated dose toxicity study in rats (main study)
Micronucleus assay in bone marrow cells of the rat
In vivo UDS assay in the rat
1-generation toxicity study (range finding study)
1-generation toxicity study (main study)
Re-evaluation of the *in vitro* chromosome aberration assay in V79 cells
Assessment of Reproduction Toxicity Potential (Photo documentation „Hyperplasia of the ovarian stroma cells“ recorded after TBSA treatment)
Statement on the toxicological relevance of 635M02 (TBSA)

Annex IIA, point 5.8: Additional toxicological studies with AMTT (metabolite 635M04)

In vitro chromosome aberration assay with Reg. No. 231700 (metabolite of BAS 635 H) in V79 cells
Statement on the toxicological relevance of 635M04

Annex IIA, point 5.8: Additional toxicological studies with metabolite 635M01

Reg. No. 335 184 (metabolite of BAS 635 H) - Subacute toxicity study in Wistar rats administration in the diet for 4 weeks
Statement on the toxicological relevance of 635M01

B.6.8 Further toxicological studies (Annex IIA 5.8)

B.6.8.1 Toxicity studies of metabolites

B.6.8.1.1 635M02

635M02 (Reg. No. 292 564; BH 635-2; TBSA) is a soil metabolite and was detected in the rat metabolism study (< 1 % in urine/feces). In the first data package submitted, it was tested in three mutagenicity assays as well as in two acute oral tests. When it became obvious that this metabolite exceeds the groundwater threshold of 0.1 µg/L by leaching the toxicological relevance had to be addressed in greater detail.
In an additional acute oral toxicity study in rats, TBSA of high purity (99.9 %) was tested in order to rule out the possibility that the acute oral toxicity seen in a previous study with a less pure batch of the test material was due to the presence of an impurity [see Wiemann C. and Hellwig J. 1999(a); BASF RegDoc# 1999/10099].
In an additional 28-day toxicity study no NOAEL could be derived and - beside others - effects on the female reproductive organs were observed. Therefore, a second 28-day study conducted with lower doses of TBSA was submitted.
The objective of the 1-generation studies was to determine the possible adverse effects of TBSA on the integrity and performance of the male and female reproductive systems since effects were noted in the first 28-day study.
For an overview of the study results see table below.

The metabolite TBSA was found to be not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay and not mutagenic *in vitro* in the CHO/HPRT mutation assay.
Based mainly on comments of the UK, at ECCO 136 it was concluded that 635M02 may have some chromosome-damaging (clastogenic) potential under *in vitro* conditions in V79 cells with S-9 mix. Therefore in the endpoint list the *in vitro* chromosome aberration assay was considered positive. The re-evaluation of the study led to the conclusion that 635M02 has no clastogenic potential. The justification is given in the assessment of the study results (see Vol. 3). The metabolite TBSA was found to be not mutagenic *in vivo* in the Micronucleus assay in bone marrow cells of the rat and not mutagenic *in vivo* in the UDS assay in the rat.
Three acute oral studies were conducted in total. One study resulted in an oral LD50 of 1000 mg/kg bw, one in an oral LD50 of > 2000 mg/kg bw. The new study was conducted with the test substance of high purity (99.9 %). The acute oral LD50 was found to be greater than 300 mg/kg and less than 2000 mg/kg body weight in rats. The median lethal dose lies between 500 and 1000 mg/kg bw. Thus, it can be considered that the metabolite 635M02 is of higher oral toxicity than the parent compound tritosulfuron (N12).
In addition, two 28-day repeated dose toxicity study in rats were submitted after the completion of the DAR. In the first study effects on the body weight (decreases), in the functional observation battery (reduced rearing and overall motor activity, tremor), on hematological (decreased red blood cell parameters) and clinico-chemical examinations (increased γ–glutamyltransferase activities, increased bilirubin and cholesterol levels), on urinalysis (increased number of degenerated renal tubular epithelial cells and transitional epithelial cells as well as higher number of granular casts and epithelial cell casts) and on organ weights (higher kidney, liver and spleen weights and lower ovary and uterine weights) were noted. The histopathological examinations showed that the ovaries of high-dose group

females had a significantly decreased number of corpora lutea. The differential ovarian follicle count (DOFC) indicated that the number of clearly discernible antral follicles was reduced to 60 % of the control value at 5000 ppm. An increased number of females with slight or moderate atrophy of the uterus (mucosa and musculature) in the mid and high dose groups. In the spleen, increased incidences of hemosiderin deposition and congested blood vessels were observed in mid and/or high dose groups. A NOAEL could not be derived from this study, based on effects on red blood cells, organ weights and ovaries at the lowest dose of 200 ppm tested (corresponding to daily intakes of 18.6 and 20.0 mg/kg bw/d for males and females, respectively).

Therefore, a subsequent 28-day oral toxicity study was conducted using three lower dose levels (0, 50, 100 and 150 ppm, corresponding to approximately 5.0, 10.0 and 14.7 mg/kg bw/d; see BASF RegDoc# 2003/1004049). In this study an increased α2u-globulin accumulation in the kidneys of male rats were noted in all dose-groups. This finding was considered to represent a sex and species specific effect, without relevance to human males. Thus, the NOAEL was set at 150 ppm (14.7 mg/kg bw/d).

To possibly exclude effects of 635M02 on fertility and reproduction two 1-generation studies were conducted with similar dose levels (0, 200 and 400 ppm; corresponding to approximately 12.8/16.2 and 25.3/32.4 mg/kg bw/d in males/females). In the first study, the male/female fertility index was slightly reduced in the high dose of 400 ppm (70 % versus 90 % and 100 % in the control and low dose), with 3/10 non-pregnant females in the 400 ppm group. One female of the 400 ppm group showed a severely reduced number of corpora lutea and a massive follicle degeneration in both ovaries. No further effect on female reproduction, delivery and litter/pup data was noted.

Cholesterol concentrations were increased in males and females at 400 ppm. High dose females showed lower magnesium levels. The mean absolute and relative liver, kidney and spleen weights were significantly increased in high dose F_0 parental males, when compared with controls. In high dose F_0 parental females, the relative spleen weight was significantly increased.

Under the conditions of this study the NOAEL for reproductive toxicity is 200 ppm (corresponding to approximately 12.8 mg/kg bw/d and 16.2 mg/kg bw/d in males and females respectively). The NOAEL for general toxicity is below 200 ppm based on lower body weight and organ weight changes at this dose level.

In the second 1-generation study, one female of the high dose group (400 ppm) was fertile (proved by 6 implantation sites in utero at terminal sacrifice) but did not deliver pups. There was no further substance-related effect on female reproduction, delivery and litter data. The DOFC revealed no changes in the number of primordial and/or growing follicles between the control and treated groups.

The F1 pups body weights and body weight gains were minimally lower in the 400 ppm group at all investigation points, although without statistical significance and with values lying mainly within the historical control values. In the presence of maternal toxicity at this dose level, these changes were not considered to represent a reproductive adverse effect.

Under the conditions of this study the NOAEL for reproductive toxicity is 200 ppm (corresponding to approximately 17.6 mg/kg bw/d and 17.3 mg/kg bw/d in males and females respectively) based on postimplantation loss noted in one female at the next higher dose of 400 ppm (corresponding to approximately 35.0 mg/kg bw/d and 33.1 mg/kg bw/d in males and females respectively).

The NOAEL for general toxicity is below 200 ppm based on lower body weight and clinico-chemical findings (higher white blood cell count in males, higher cholesterol concentrations in males and females, lower magnesium concentrations in females) at this dose level.

Table B.6.8-1: Summary of toxicity studies of metabolite 635M02 (Reg.-No. 292 564)

Study/strains/species	Test material/conditions	Results
Acute oral toxicity in rats Wistar rats (Chbb:THOM (SPF)) 5 male / 5 female 500, 2000 and 5000 mg/kg bw	Batch No. 00831-201, purity: 98.2 % Test substance preparation in 0.5 % Tylose CB 30.000 in aqua bidest	LD_{50}: 1000 mg/kg bw Mortality at 2000 mg/kg bw (delayed after 3 to 4 days), main signs of toxicity: dyspnea, apathy, excitation, abdominal and lateral position, staggering, ataxia, atonia, paresis, narcotic-like state, pain and corneal reflex absent, tremor, spastic gait, erythema, exsiccosis, salivation, lacrimation, discoloured urine, squatting posture and red clammy snout and eyelid.
Acute oral toxicity of TBSA in rats Wistar rats (Chbb:THOM (SPF)) 3 male / 3 female 200 and 2000 mg/kg bw	Batch no. 26778/99; 26778/101, purity: > 98.5 % Test substance preparation in olive oil DAB 10	LD_{50}:> 2000 mg/kg bw No mortality Main signs of toxicity: dyspnea, apathy, staggering, exsiccosis, red discoloured urine until day 5 post dosing
Acute oral toxicity of TBSA in rats Wistar rats (CrlGlxBrlHan:WI) 3 x 3 female 2000 and 300 mg/kg bw	Batch no: 2059-029, purity: 99.9 % Test substance preparation in 0.5 % CMC-solution (cleaned sodium carboxymethylcellulose) in double distilled water	Median lethal dose: around 500 mg/kg bw All animals at 2000 mg/kg bw were found dead on study day 3 No mortality at 300 mg/kg bw. Main signs of toxicity: dyspnoea, abdominal or lateral position, staggering, smeared fur, lacrimation and red smeared fur in the anogenital area
Salmonella typhimurium/ Escherichia coli reverse mutation assay S. typhimurium strains TA 100, TA 1535, TA 1537, TA 98 and E. coli strain WP2 uvrA	Batch No. 00831-201, purity: 98.2 %	Not mutagenic
Gene mutation assay in Chinese hamster ovary (CHO) V79 cells *in vitro* (V79 / HPRT)	Batch No. 00831-201, purity: 98.2 %	Not mutagenic
In vitro chromosome aberration assay in V79 cells	Batch No. 00831-201, purity: 98.2 %	Not mutagenic
Micronucleus assay in bone marrow cells of the rat	Batch No. 00831-201, purity: 98.2 %	Not mutagenic
In vivo UDS assay in the rat	Batch No. 00831-201, purity: 98.2 %	Not mutagenic

Study/strains/species	Test material/conditions	Results
28-day oral toxicity study in rats Wistar rats (CrlGlxBrlHan:WI) 5 males / 5 females 0; 200; 1000 and 5000 ppm (18.6/20.0, 90.7/96.1, 417.3/414.2 mg/kg bw/d for males and females)	Batch no. 25887 Fass 2; purity: 98.4 %	No NOAEL LOAEL 200 ppm: ↑ liver wt.,↑ kidney wt. (males),↓ RBC (f), α_{2u}–globulin accumulation (male kidneys), hyperplastic ovarian stroma cells 1000 ppm: ↓ bw (females), ↓ overall motor activity, ↓ RBC, ↑ bilirubin, ↑ cholesterol, cells and casts in urine sediment (males), ↑ liver wt., ↑ kidney wt. (males), ↑ spleen wt., α_{2u}–globulin accumulation (male kidneys), hepatocellular hypertrophy (males), hyperplastic ovarian stroma cells, atrophy of the uterus (mucosa and musculature), hemosiderin deposition (spleen) 5000 ppm: tremor, piloerection from day 14 onwards, ↓ bw, ↓ rearing, ↓ overall motor activity, ↓ RBC, ↑ γ–glutamyltransferase activities, ↑ bilirubin, ↑ cholesterol, cells and casts in urine sediment (males), ↑ liver wt., ↑ kidney wt. (males), ↓ ovary and uterus wt., ↑ spleen wt., α_{2u}–globulin accumulation (male kidneys), chronic progressive nephropathy (male), hepatocellular hypertrophy, ↓ number of corpora lutea (ovary), hyperplastic ovarian stroma cells, atrophy of the uterus (mucosa and musculature), hemosiderin deposition (spleen)
28-day oral toxicity study in rats Wistar rats (CrlGlxBrlHan:WI) 5 male / 5 female 0, 50, 100 and 150 ppm (4.8/5.0; 9.0/10.0; 13.9/14.7 mg/kg bw/d in males and females)	Batch no. 25887 Fass 2; purity: 98.4 %.	NOAEL: 150 ppm All dose groups: $\alpha_{2\mu}$-globulin accumulation in the kidney (males)
1-generation study in rats (range finding) Wistar rats (CrlGlxBrlHan:WI) 10 male / 10 female 5 male / 5 female as satellite groups 0; 200 and 400 ppm (12.9/16.2; 25.3/32.4 mg/kg bw/d in males and females) premating phase: 4 weeks	Batch no. 25887 Fass 2; purity: 98.4 %	NOAEL (reproduction): 200 ppm NOAEL (general toxicity): ≤ 200 ppm 200 ppm: ↓ bw (f), ↑ liver wt.(f) 400 ppm: ↓ bw (f), ↓ male/female fertility index, severely reduced number of corpora lutea/ massive follicle degeneration in the ovaries (1 female), ↑ cholesterol, ↓ magnesium (f), ↑ kidney wt. (m); ↑ spleen wt., ↑ liver wt., ↑ thyroid wt. (f)
1-generation study in rats (main study) Wistar rats (CrlGlxBrlHan:WI) 10 male / 10 female 5 male / 5 female as satellite groups 0; 200 and 400 ppm (17.7/20.7; 35.0/40.9 mg/kg bw/d in males and females) premating phase: 10 weeks	Batch no. 25887 Fass 2; purity: 98.4 %.	NOAEL (reproduction): 200 ppm NOAEL (general toxicity): ≤ 200 ppm 200 ppm: ↓ bw (f), ↑ WBC (m), ↑ cholesterol, ↓ magnesium (f) 400 ppm: ↓ bw (f), postimplantation loss (1 female), ↑ WBC (m), ↓ RBC, Hb, ,HCT, ↑ Reticulocytes, ↑ cholesterol, ↓ magnesium (f), ↑ kidney wt. (m), ↑ liver wt., ↑ spleen wt.

Statement on the toxicological relevance of 635M02 (TBSA)

TBSA toxicity and fertility study data were reviewed to establish whether it meets the 2001/59/EC criteria for classification as toxic to reproduction category 2 or 3.

Uterine atrophy, reduced ovary weight and reduced corpora lutea were seen at the markedly toxic dose of 5000 ppm in the first 28-day subacute study (range finding, high dose), most likely secondary to the marked reduction in body weight at this dose (mean - 20% versus controls). Chapin et al (1993) reported that 30% body weight reduction in rats (by feed restriction) decreased the number of corpora lutea and ovary weights, but did not affect male reproductive organs (Chapin R. E., Gulati D.K., Barnes L. H, Teague J.L. The effects of feed restriction on reproductive function in Sprague-Dawley rats. Fundam Appl Toxicol. 1993 Jan;20(1):23-9).

Hyperplasia of the ovarian stromal cells was also noted in the first 28-day subacute study, minimal in 3 animals at 200 ppm, slight in all animals at 1000 ppm, and slight to moderate in all animals at 5000 ppm. The etiology and biological significance of this finding are unclear; no such effect was observed in the second 28-day subacute study at 50, 100 or 150 ppm, or in the two 1-generation reproduction studies at 200 or 400 ppm, with treatment periods extending from the begin of the 4- or 10-week premating period through mating, gestation and lactation. Furthermore, it could be demonstrated that, the control females in the second 1-generation study had similar or even higher degrees of hyperplasia than the 5000 ppm females in the first 28-day subacute study, but this level of hyperplasia was regarded as normal for animals of this age (the 1-generation study females were older than those in the first 28-day subacute study; they had also been pregnant; see also B.6.8.1.1.1. FitzGerald R. E., 2007 TBSA: Assessment of Reproduction Toxicity Potential (Photo documentation „Hyperplasia of the ovarian stroma cells“ recorded after TBSA treatment)).

Differential ovarian follicle count (DOFC) were affected in the first 28-day subacute study only at 5000 ppm, not at 1000 or 200 ppm, nor in the second 28-day subacute study at up to the highest concentration of 150 ppm. In the first 1-generation study, 1 of 15 rats had massive degeneration at 400 ppm but there were no other findings in DOFC. In the second 1-generation reproduction study (longer treatment duration than in the first 1-generation study), there were no effects on DOFC in 15 rats/dose at 200 or 400 ppm.

Fertility index was slightly reduced at 400 ppm in the first 1-generation study. Three of 10 positively mated females were not pregnant at 400 ppm versus 1 of 10 control females. Infertility in one of these 400 ppm females (no.121) was due to a severely reduced follicle and corpus luteum count. There was no notable pathology in the other 2 non-pregnant females or their mating partners. Comparison of fertility indices in the two 1-generation studies indicates that the difference in the first 1-generation study between controls and 400 ppm animals was due to chance, because in the second 1-generation study, with a much longer pre-mating dosing period (10 versus 4 weeks), all 10 of 10 animals of the 400 ppm group were pregnant, while 2 of 10 controls were not. These data show that there was no effect of treatment on fertility even at maternally toxic doses.

No substance related effects on testis were seen in the 1-generation studies. Total sperm quantity and quality in 400 ppm males were also unaffected by treatment in the 1-generation studies. These data show that treatment had no effect on male reproduction organs.

There was a non-significantly reduced pup weight on day 1 postpartum at 400 ppm in the second 1-generation study. F1-pups were well within the range of historical control data. If at all, these weight changes occurred in the presence of maternal toxicity. Therefore, no relevance is attributed.

A minimal non-significant increase in post-implantation loss at 400 ppm in the second 1-generation study was due to female no. 122, with 6 implantation sites but no pups delivered (total litter loss). Historical control data show that total litter loss in single control group animals does occur spontaneously.

In summary, based on detailed examination of all reproduction parameters, TBSA had no effect on mating, fertility, gestation and livebirth indices or reproductive organ pathology indicative of potential reproductive toxicity. TBSA had no primary effect on developmental toxicity. In conclusion, based on the available repeat dose and reproduction toxicity study data, TBSA should clearly not be classified as toxic to reproduction.

The acute oral toxicity of TBSA is approximately 4 times higher than the parent compound tritosulfuron (TBSA: LD_{50} approximately 1000 mg/kg bw versus tritosulfuron: LD_{50} 4700 mg/kg bw).
The results of the toxicity studies (28-day repeat-dose and 1-generation studies) in rats mainly indicated the ovaries (reduced ovary weight, decreased number of corpora lutea) and the uterus (atrophy of mucosa and musculature) as target organs. Repeated high doses of TBSA (5000 ppm corresponding to approximately 400 mg/kg bw/d) affected all female rats, whereas at lower doses (400 ppm corresponding to approximately 30 mg/kg bw/d) only single females were affected. Effects on the uterus (atrophy of mucosa and musculature as noted in the 28-day study at ≥ 1000 ppm, 96.1 mg/kg bw/d) were not reported in the 1-generation studies.
The following is therefore proposed in accordance with the latest classification and labelling guidance under Directive 67/548/EEC (i.e. in the 18th ATP published as Directive 93/21/EEC):

TBSA:	Hazard symbol:	Xn	
	Indication of danger:	Harmful	
	Risk phrase:	R 22	Harmful if swallowed.

In conclusion, according to the criteria given in the EU guidance document on relevant metabolites in groundwater, the metabolite has not to be considered as toxicologically relevant. However, because the acute oral toxicity of TBSA suggests a moderate toxicity that is markedly higher than exhibited by the active ingredient and the effects observed mainly in the female reproductive organs (ovaries and uterus) after repeated high dose, a groundwater concentration of 0.75 µg/L must not be exceeded.

B.6.8.1.1.1 First mutagenicity study

Report: Engelhardt G., Hoffmann H. D., 1999
Report: Salmonella typhimurium/Escherichia coli reverse mutation assay (standard plate test and preincubation test) with Reg.-No. 292 564; BH 635-2
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 1999/11412

GLP: Yes (Laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 471, EEC 92/69 B 13, EEC 92/69 B 14
Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: 635M02 (Reg.-No. 292 564; BH 635-2); batch no. 00831-201, purity: 98.2%.
Test system: Salmonella typhimurium strains TA 100, TA 1535, TA 1537 and TA 98 and Escherichia coli strain WP2 uvrA

635M02 was tested for its mutagenic potential based on the ability to induce back mutations in selected loci of several bacterial strains in the Ames reverse mutation assay. The Salmonella typhimurium strains TA 100, TA 1535, TA 1537 and TA 98 and Escherichia coli strain WP2 uvrA were exposed to the test substance dissolved in DMSO. The study consisted of a standard plate test (with doses ranging from 20 to 5,000 µg/plate) and preincubation test (with doses ranging from 20 to 5,000 µg/plate) both with and without metabolic activation (Aroclor induced Sprague-Dawley rat liver S-9 mix). Three plates were used per dose for each strain and test condition.

For control purposes and to demonstrate the sensitivity of the test system, negative controls (with and without S9 mix) and positive controls (2-aminoanthracene - with S9 mix; N-methyl-N'-nitro-N-nitrosoguanidine, 4-nitro-o-phenylendiamine, 9-aminoacridine and 4-nitroquinoline-N-oxide - all without S9 mix) were tested.
A substance is considered positive in this test if the following criteria are met:
- there is at least a doubling of the negative control mutation rate with or without S-9 mix,
- there is a dose-response relationship, and
- the results are reproducible.

Findings:
The stability of the test substance throughout the study period was guaranteed. The stability of 635M02 in the vehicle DMSO and in water over a period of 4 hours has been determined analytically. No test substance precipitation was observed. A weak bacteriotoxic effect was noticed only in the preincubation test from about 500 µg – 2,500 µg/plate. The mean number of revertant colonies was not increased in any strain either with or without S-9 activation. Expected increases in revertant colonies were obtained with the positive controls.

Conclusion:
According to the results of the study, 635M02 is not mutagenic in the Salmonella typhimurium/Escherichia coli reverse mutation assay.

B.6.8.1.1.2 Second mutagenicity study

Report:	Wollny H.-E. et al., 1999 Gene mutation assay in Chinese hamster V79 cells *in vitro* (V79 / HPRT) with Reg.-No. 292 564; BH 635-2 RCC Cytotest Cell Research GmbH, Roßdorf, Germany Fed. Rep. unpublished BASF RegDoc# 1999/11691
GLP:	Yes (Laboratory certified by Hessisches Ministerium fuer Umwelt, Energie, Jugend, Familie und Gesundheit, Wiesbaden)
Guideline:	EEC 87/302, OECD 476

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: 635M02 (Reg.-No. 292 564; BH 635-2); batch no. 00831-201, purity: 98.2 %.
Test system: Chinese hamster ovary (CHO) cells
635M02 was tested for its ability to induce gene mutations at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in Chinese hamster ovary (CHO) cells *in vitro*. Two independent experiments were carried out, using two parallel cultures each either with or without the addition of Aroclor 1254-induced Sprague-Dawley rat liver S-9 mix (exogenous metabolic activation). In an initial range-finding test, precipitation of the test substance occurred at doses > 300 µg/ml. Concentrations above 1,200 µg/ml led to non applicable inhomogenous suspensions. No changes in osmolarity and pH-values were observed.
The test substance was evaluated at the following doses in the 1st experiment (4 hours exposure): Without and with S-9 mix 0; 75.0; 150.0; 300.0; 600.0; and 1200.0 µg/ml.
A 2nd experiment for confirmation was performed using the following doses (4 hours exposure): Without and with S-9 mix: 0; 75.0; 150.0; 300.0; 600.0; and 1200.0 µg/ml.
After an attachment period of 20 - 24 hours, a treatment period of 4 hours both with and without metabolic activation and an expression phase of about 7 - 9 days and a selection period of about 1 week, the colonies of each test group were stained with 10 % methylene blue in 0.01 % KOH solution and counted. For control purposes and to demonstrate the sensitivity of the test system, negative controls and two appropriate positive control chemicals 7,12-dimethylbenz(a)anthracene (with S9 mix) and Ethylmethane sulfonate (without S9 mix) were tested.
The criteria for a positive response are:

- Increases of the mutation frequencies, that are three times above the spontaneous mutation frequency at least at one of the concentrations in the experiment.
- Evidence of reproducibility of any increase in mutant frequencies.
- Evidence of a dose-response relationship, even if a threefold increase of the mutant frequency is not observed.

Findings:
The stability of 635M02 in the vehicle DMSO and in water has been analytically confirmed. No cytotoxicity occurred up to the maximal concentration of 1200 µg/ml. The negative controls (untreated and vehicle controls) gave mutant frequencies within the range expected for the CHO cell line. Appropriate positive control chemicals led to the expected increase in the frequencies of forward mutations.
The test substance did not cause a relevant increase in the mutant frequencies either without S-9 mix or after adding a metabolising system in both main experiments performed independently of each other. Two isolated increases exceeding the threshold of three times the corresponding solvent control were observed in experiment II, culture I at 75.0 µg/ml (without S-9 mix) and at 1200 µg/ml (with S-9 mix). These increases were judged as biologically irrelevant since they are based upon statistical fluctuations at such low absolute numbers. The corresponding solvent control is close to the lower border of historical solvent controls. Furthermore the effect was not reproduced in the parallel culture under identical conditions and the absolute values of colonies remained well within the range of our historical negative controls.
Thus it can be stated that in this mutagenity assay and under the experimental conditions reported the test substance did not induce gene mutations at the HPRT locus in V 79 cells.

Conclusion:
Under the experimental conditions of this assay, 635M02 is considered to be non-mutagenic *in vitro* in the CHO/HPRT mutation assay.

B.6.8.1.1.3 Re-evaluation of the third mutagenicity study

Report: Engelhardt G., Hoffmann H. D., 1999
Report: *In vitro* chromosome aberration assay with Reg.-No. 292 564; BH 635-2 in V79 cells
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 1999/11684

GLP: Yes (Laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 473, EEC 92/69 B 10

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: 635M02 (Reg.-No. 292 564; BH 635-2; batch no. 00831-201, purity: 98.2 %.
Test system: V79 cells

635M02 (BH 635-2; Reg.-No. 292 564) was assessed for its potential to induce structural chromosomal aberrations in V79 cells *in vitro* both in the presence and in the absence of a metabolising system (S-9 mix of Aroclor 1254-induced Sprague-Dawley rat liver). According to an initial range-finding cytotoxicity test the following doses were evaluated.
1st experiment:
- 4 hours exposure, 18 hours harvest time, with and without S-9 mix:
  0; 575; 1,150; and 2,300 µg/ml,

2nd experiment:
- 18 hours exposure, 18 hours harvest time, without S-9 mix:
  0; 287.5; 575; and 1150 µg/ml
- 18 hours exposure, 28 hours harvest time, without S-9 mix:
  0; 2300 µg/ml
- 4 hours exposure, 28 hours harvest time, with S-9 mix:
  0; 575; 1150; and 2300 µg/ml.

The cell cycle of the untreated V79 cells is about 13 - 14 hours under the selected culture conditions. Thus, the selected 1st sampling time of 18 hours was within the 1 - 1.5 x the normal cell cycle time, as recommended by the OECD Guideline No. 473. The later sampling time of 28 hours was chosen to cover a possible cell cycle delay. About 2 - 3 hours prior to harvesting the cells, colcemid was added to arrest cells in a metaphase-like stage of mitosis (c-metaphases). After preparation of the chromosomes and staining with Giemsa, 100 metaphases for each culture in the case of the test substance and vehicle controls, or 50 or 100 cells for each culture in the case of the concurrent positive controls, were analysed for chromosomal aberrations. The criteria for a positive response are:

- A dose-related and reproducible significant increase in the number of structural chromosomal aberrations.
- The proportion of aberrations exceeded both the concurrent negative control range and the negative historical control range.

A test substance is generally considered non clastogenic in this test system if:

- There was no significant increase in the number of chromosomally damaged cells at any dose above concurrent control frequencies.
- The aberration frequencies were within the historical control range.

Findings:

The stability of the test substance throughout the study period was verified by reanalysis. Homogeneity of the test substance was achieved by mixing. The stability of 635M02 in the vehicle DMSO and in water each over a period of 4 hours has been determined analytically. Test substance precipitation occurred at concentrations of 575 µg/ml and higher. According to the results of the determination of the mitotic index, no suppression of the mitotic activity was observed under any of the experimental conditions. Cell count indicated a slight growth inhibition at 1150 µg/ml (18 hours exposure, 18 hours harvest) and 2300 µg/ml (4 hours exposure, 18 hours harvest and 18 hours exposure, 28 hours harvest) both without metabolic activation. Cell attachment was occasionally slightly reduced. Osmolarity and pH values were not influenced by test substance treatment. The negative controls (vehicle controls) gave frequencies of aberrations within the range expected for the V79 cell line. Both of the positive control chemicals, EMS (ethyl methane sulfonate) and cyclophosphamide, led to the expected increase in the number of cells containing structural chromosomal aberrations.

At 1150 and 2300 µg/mL (with S-9 mix), in the presence of precipitation, there were some significant dose-related increases in the number of aberrations. The results including gaps (gaps are not considered an indication of clastogenicity) and excluding gaps were still within the upper range of the historical control data.

Table B.6.8.1-1: Summary of metaphases with chromosome aberrations – 1st experiment

Dose (µg/mL)	No of Metaphases	Including gaps		Excluding gaps		Exchanges	
		No	%	No	%	No	%
1st experiment (4 hours exposure, 18 hours harvest time, without S-9 mix)							
DMSO	200	6	3.0	1	0.5	0	0.0
575	200	14	7.0	7	3.5	2	1.0
1150	200	14	7.0	5	2.5	4	2.0
2300	200	8	4.0	3	1.5	1	0.5
EMS 350	100	28	28.0**	24	24.0**	12	12.0**

Dose (µg/mL)	No of Metaphases	Including gaps	Excluding gaps	Exchanges

		No	%	No	%	No	%
1st experiment (4 hours exposure, 18 hours harvest time, with S-9 mix)							
DMSO	200	6	3.0	2	1.0	1.0	0.5
575	200	4	2.0	1	0.5	1.0	0.5
1150	200	18	9.0*	7	3.5	2	1.0
2300	200	21	10.5**	10	5.0	5	2.5
CPP 0.5	100	21**	21.0**	21**	21.0**	18	18.0**

Table B.6.8.1-2: Individual data

Dose (µg/mL)	**No of Metaphases**	**Including gaps**		**Excluding gaps**		**Exchanges**	
		No	%	No	%	No	%
1st experiment (4 hours exposure, 18 hours harvest time, without S-9 mix)							
2300	100	3	3.0	1	1.0	0	0.0
2300	100	5	5.0	2	2.0	1	1.0
EMS 350	50	16	32.0	12	24.0	8	16.0
EMS 350	50	12	24.0	12	24.0	4	8.0

Dose (µg/mL)	**No of Metaphases**	**Including gaps**		**Excluding gaps**		**Exchanges**	
		No	%	No	%	No	%
1st experiment (4 hours exposure, 18 hours harvest time, with S-9 mix)							
2300	100	11	11.0	5	5.0	2	2.0
2300	100	10	10.0	5	5.0	3	3.0
CPP 0.5	50	11	22.0	11	22.0	9	18.0
CPP 0.5	50	10	20.0	10	20.0	9	18.0

Table B.6.8.1-3: Summary of metaphases with chromosome aberrations – 2nd experiment

2nd experiment (18 hours exposure, 18 hours harvest time, without S-9 mix)							
DMSO	200	4	2.0	1	0.5	1.0	0.5
287.5	200	14	7.0*	5	2.5	4.0	2.0
575	200	7	3.5	1	0.5	1.0	0.5
1150	200	7	3.5	1	0.5	0	0.0
EMS 350	100	19	19.0**	17	17.0**	16	16.0**

* P<0.05, ** P<0.01

Dose (µg/mL)	**No of Metaphases**	**Including gaps**		**Excluding gaps**		**Exchanges**	
		No	%	No	%	No	%
2nd experiment (4 hours exposure, 28 hours harvest time, with S-9 mix)							
DMSO	200	9	4.5	1	0.5	0	0.0
575	200	8	4.0	5	2.5	3	1.5
1150	200	9	4.5	3	1.5	2	1.0
2300	200	10	5.0	5	2.5	3	1.5
CPP 0.5	100	18	18.0**	14	14.0**	11	11.0**

Table B.6.8.1-4: Individual data

Dose (µg/mL)	No of Metaphases	Including gaps		Excluding gaps		Exchanges	
		No	%	No	%	No	%
2nd experiment (4 hours exposure, 28 hours harvest time, with S-9 mix)							
2300	100	5	5.0	3	3.0	2	2.0
2300	100	5	5.0	2	2.0	1	1.0
CPP 0.5	50	9	18.0	8	16.0	7	14.0
CPP 0.5	50	9	18.0	6	12.0	4	8.0

The results of this study indicate that the increase in aberrations (excluding gaps) at 1150 and 2300 µg/mL (with S-9 mix) occurred in the first experiment only, was not reproducible in the 2nd experiment and the values were still at the upper range of the historical control data (see below).

Table B.6.8.1-5: Historical negative control data

Untreated controls (without S-9 mix)						
Treatment/harvest	4/18 hours			18/18 hours		
No of experiments	46			3		
Aberrations	Incl gaps	Excl gaps	Exch	Incl gaps	Excl gaps	Exch
Mean (%)	4.6	1.8	0.8	2.3	1.0	0.3
Minimum (%)	1.5	0.0	0.0	1.5	0.5	0.0
Maximum (%)	9.0	4.0	3.0	3.5	1.5	0.5

Untreated controls (without S-9 mix)						
Treatment/harvest	4/28 hours			18/28 hours		
No of experiments	15			3		
Aberrations	Incl gaps	Excl gaps	Exch	Incl gaps	Excl gaps	Exch
Mean (%)	4.6	1.8	0.8	5.0	1.3	1.2
Minimum (%)	1.5	0.5	0.0	3.5	0.5	0.0
Maximum (%)	7.5	3.0	1.5	7.0	2.5	2.5

Untreated controls (with S-9 mix)						
Treatment/harvest	4/18 hours			4/28 hours		
No of experiments	46			18		
Aberrations	Incl gaps	Excl gaps	Exch	Incl gaps	Excl gaps	Exch
Mean (%)	4.4	1.7	0.7	4.5	1.6	0.5
Minimum (%)	1.5	0.0	0.0	2.0	0.5	0.0
Maximum (%)	10.5	5.0	2.5	9.0	3.0	1.0

Discussion: Based on the comments of the UK at ECCO 136 it was concluded that a clastogenic potential of the metabolite 635M02 cannot be excluded and this test was considered positive in the endpoint list. The re-evaluation of this study cannot support this conclusion anymore. The slight increases were within the range of the historical control data, were not reproducible in the second experiment and occurred in the presence of precipitation. Thus, the criteria for a positive response are not fulfilled.

Conclusion:
Under the conditions of this study the metabolite 635M02 has no clastogenic potential under *in vitro* conditions in V79 cells.

B.6.8.1.1.4 Fourth mutagenicity study

Report:	N. Honarvar, 2006 Report: Micronucleus Assay in Bone Marrow Cells of the Rat with Reg.-No. 292564 RCC Cytotest Cell Research GmbH, Roßdorf, Germany Fed. Rep. unpublished RCC-Study no. 1055401 BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep. unpublished BASF RegDoc# 06M0599/969032
GLP:	Yes (Laboratory certified by Hess. Ministerium für Umwelt, ländlichen Raum und Verbraucherschutz, Wiesbaden)
Guideline:	OECD 474 (1997), Commission Directive 2000/32/EC, Annex 4C, dated May 19, 2000
Deviations:	None
Acceptability:	The study is considered to be acceptable.

Material and Methods:
Test material: 635M02 (Reg.-No. 292 564; BH 635-2; batch no. 00831-201, purity: 98.2 %.
Test system: Rat (Wistar), age: 6 - 10 weeks

635M02 (BH 635-2; Reg.-No. 292 564) was assessed for its potential to induce to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the rat. The test substance was formulated in 0.5% Carboxy Methyl Cellulose (CMC), which was also used as vehicle control. The volume administered orally was 10 mL/kg bw. 24 h and 48 h after a single administration of the test substance the bone marrow cells were collected for micronuclei analysis. Ten animals (5 males, 5 females) per test group were evaluated for the occurrence of micronuclei. At least 2 000 polychromatic erythrocytes (PCEs) per animal were scored for micronuclei. To describe a cytotoxic effect due to the treatment with the test substance the ratio between polychromatic and total erythrocytes was determined in the same sample and reported as the number of PCEs per 2 000 erythrocytes.
The following dose levels of the test substance were investigated:
24 h preparation interval: 500, 1 000, and 2 000 mg/kg bw.
48 h preparation interval: 2 000 mg/kg bw.

Findings:
The highest dose (2 000 mg/kg bw; maximum guideline-recommended dose) was estimated by a pre-experiment to be suitable. The animals treated with ≥ 500 mg/kg bw expressed toxic reactions (reduction of spontaneous activity, abdominal position, eyelid closure, ruffled fur, apathy).
After treatment with the test substance the number of PCEs was not substantially decreased as compared to the mean value of PCEs of the vehicle control thus indicating that 635M02 did not exert any cytotoxic effects in the bone marrow.
In comparison to the corresponding vehicle controls there was no biologically relevant or statistically significant enhancement in the frequency of the detected micronuclei at any preparation interval after administration of the test substance and with any dose level used.

The mean values of micronuclei observed after treatment with 635M02 were below or the same as the value of the vehicle control group.
20 mg/kg bw cyclophosphamide administered orally was used as positive control which showed a substantial increase of induced micronucleus frequency.

Conclusion:
In conclusion, under the experimental conditions reported, the test substance did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the rat.
Therefore, 635M02is considered to be non-mutagenic in this micronucleus assay.

B.6.8.1.1.5 Fifth mutagenicity study

Report:	N. Honarvar, 2006 Report: *In vivo* Unscheduled DNA Synthesis in Rat Hepatocytes with Reg.-No. 292564 RCC Cytotest Cell Research GmbH, Roßdorf, Germany Fed. Rep. unpublished RCC-Study no. 1055402 BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep. unpublished BASF RegDoc# 80M0599/969031
GLP:	Yes (Laboratory certified by Hess. Ministerium für Umwelt, ländlichen Raum und Verbraucherschutz, Wiesbaden)
Guideline:	OECD 486 (1997), EEC Directive 2000/32, B.39 (2000)
Deviations:	None
Acceptability:	The study is considered to be acceptable.

Material and Methods:
Test material: 635M02 (Reg.-No. 292 564; BH 635-2; batch no. 00831-201, purity: 98.2 %.
Test system: Wistar HanIbm: WIST (SPF), 32 male rats, age: 6 - 10 weeks

635M02 (BH 635-2; Reg.-No. 292 564) was assessed for its potential to induce to induce DNA repair. Repair was measured as unscheduled DNA-synthesis (UDS) by the uptake of ^{3}HTdR into hepatocytes of rats. The test item was formulated in 0.5% CMC (Carboxy Methyl Cellulose), which was used as vehicle control. The volume administered orally was 10 mL/kg bw. After a treatment period of 4 h and 16 hours, respectively, the animals were anaesthetised and sacrificed by liver perfusion. Primary hepatocyte cultures were established and exposed for 4 hours to ^{3}HTdR (methyl-^{3}H-thymidine) which is incorporated if UDS occurs.

The test item was tested at the following dose levels:
4 and 16 hours preparation intervals: 1000 and 2000 mg/kg bw. The highest dose was estimated in a pre-experiment to be the maximum applicable dose, at which clinical signs of toxicity occurred without affecting the survival rates. For each experimental group including the controls, hepatocytes from three treated animals were assessed for the occurrence of UDS. Appropriate reference mutagens [DMH (N,N´-dimethylhydrazinedihydrochloride), 80 mg/kg bw and 2-AAF (2-acetylaminofluorene), 100 mg/kg bw] were used as positive controls.

Findings:
The viability of the hepatocytes was not substantially affected by to the in vivo treatment with the test item. The interindividual variations obtained for the numbers and the viabilities of the isolated hepatocytes are in the range of the historical laboratory control. None of the tested dose levels revealed UDS induction in the hepatocytes of the treated animals as compared to the corresponding vehicle controls. Neither the nuclear grains nor the resulting net grains were distinctly enhanced due to the in vivo treatment of the animals with the test item for 4 hours or 16 hours, respectively. Therefore, the net grain values obtained after treatment with the test item were consistently negative. In addition, no substantial shift to higher values was obtained in the percentage of cells in repair. Treatment with the positive control substances revealed distinct increases in the number of nuclear and net grain counts.

Conclusion:
In conclusion, it can be stated that under the experimental conditions reported, the test item did not induce DNA-damage leading to increased repair synthesis in the hepatocytes of the treated rats. Therefore, Reg.-No. 292564 is considered to be non-genotoxic in this in vivo UDS test system.

B.6.8.1.1.6 First acute oral toxicity study

Report: Kirsch P. et al., 1995
Report on the study on the acute oral toxicity of TBSA in rats
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 1995/11408

GLP: Yes
(Laboratory certified by Ministerium fuer Arbeit, Soziales und Gesundheit, Postfach 3180, 55021 Mainz)

Guideline: EEC 92/69

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: 635M02; batch no. 26778/99; 26778/101, purity: > 98.5 %.
Test animals: Wistar rats
Single administration of a test substance preparation in olive oil DAB 10 by gavage to three male and three female fasted Wistar rats at dose levels of 200 and 2000 mg/kg bw, using an application volume of 5 ml/kg bw. The observation period lasted for up to 14 days.

Findings:
The stability of the test substance was verified by reanalysis. The stability of the test substance in the vehicle olive oil DAB 10 over a period of 4 hours was confirmed by analysis. The correctness of the concentration of the test substance preparation and its homogeneity were analytically proven. There was no mortality in either males or females. Signs of toxicity noted in the 2000 mg/kg bw dose group comprised impaired or poor general state, dyspnoea, apathy, staggering, exsiccosis, red discoloured urine, and smeared fur in the anogenital area.

These symptoms were considered to be unspecific. Animals appeared normal after 6, 7 or 12 days after application. In the 200 mg/kg bw dose group no signs of toxicity were observed. Body weight development appeared to be normal. There were no macroscopic pathological findings in animals sacrificed at the end of the observation period.

Conclusion:
The oral LD_{50} was found to be > 2000 mg/kg bw for male and female rats.

B.6.8.1.1.7 Second acute oral toxicity study

Report: Wiemann C., Hellwig J., 1999
Report: Reg.-No. 292 564; BH 635-2: Acute oral toxicity in rats
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 1999/10099
and
Wiemann C., 1999
Amendment No. 1: Reg.-No. 292 564; BH 635-2 - Acute oral toxicity in rats
BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep.
unpublished
BASF RegDoc# 1999/10381

GLP: Yes (Laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 92/69, OECD 401

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: 635M02 (Reg.-No. 292 564; BH 635-2); batch no. 00831-201, purity: 98.2 %.
Test animals: Wistar rats (Chbb:THOM (SPF))
Single administration of a test substance preparation in 0.5 % Tylose CB 30.000 in aqua bidest. Was given by gavage to five male and five female fasted Wistar rats at dose levels of 500, 2000 and 5000 mg/kg bw, using an application volume of 20 ml/kg bw. The observation period lasted for up to 14 days.

Findings:
The stability of the test substance over the study period was verified by reanalysis. The stability of the test substance in the vehicle over a period of 4 hours was confirmed by analysis. The correctness of the concentration of the test substance preparation and its homogeneity were analytically proven.
All animals of the 5000 mg/kg bw dose group died within 4 days after application. 9 animals (4 males and 5 females) of the 2000 mg/kg bw dose group died within 3 days after application. A delayed mortality (6 days after application) was observed in 1 female rat of the 500 mg/kg bw dose group. Signs of toxicity noted in all dose groups comprised impaired or poor general state, dyspnea, apathy, excitation, abdominal and lateral position, staggering,

ataxia, atonia, paresis, narcotic-like state, pain and corneal reflex absent, tremor, spastic gait, erythema, exsiccosis, salivation, lacrimation, discoloured urine, squatting posture and red clammy snout and eyelid. The surviving animals appeared normal within 9 days after treatment. Body weight development appeared to be normal, except for one male rat of the 2000 mg/kg bw dose group, which showed weight reduction on day 7. Necropsy findings of the animals that died included liquid, bloody or discolored contents, or dilatation of the urinary bladder, erosion/ulcer in the glandular stomach, discoloration of contents or bloody contents of the stomach and/or small intestine, discoloration of the pancreas and kidneys, discoloration or edema in all lobes of the lung, discoloration or prominent lobular pattern of the liver and discharge of the nose. Histological examination revealed fatty infiltration of hepatocytes in the liver, vacuolation of tubular cells, tubular calcification in the medulla area, and degeneration of single tubules of the kidneys, dilatation, mucosal edema and ulceration with dystrophic calcification of the urinary bladder.

Conclusion:
The oral LD_{50} was found to be 1000 mg/kg bw for male and female rats.

B.6.8.1.1.8 Third acute oral toxicity study

Report: Gamer A.O., Leibold E., 2003
TBSA - Acute oral toxicity study in rats
BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep.
Unpublished, BASF RegDoc# 2003/1021650

GLP: Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 423, 96/54/EC, EPA / OPPTS 870.1100

Deviations: None, which can be considered to have influenced the integrity of the study.

Acceptability: The study is considered to be acceptable.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Reg. No. 292 564 (*syn.* TBSA); batch: 2059-029: purity: 99.9 %
Test animals: Female Wistar rats (CrlGlxBrlHan:WI), Source: Charles River Deutschland GmbH, Sulzfeld.
In this acute oral toxicity study in rats, TBSA of high purity (99.9 % pure) was tested in order to rule out the possibility that the acute oral toxicity seen in a previous study with a less pure batch of the test material was due to the presence of an impurity [see Wiemann C. and Hellwig J., 1999(a); BASF RegDoc# 1999/10099].
Single doses of the test material was administered by gavage to groups of three fasted female Wistar rats as a preparation in 0.5 % CMC-solution (cleaned sodium carboxymethylcellulose) in doubly distilled water. The application volume administered was 10 mL/kg body weight. The first group of three females received a dose of 2000 mg/kg bw. Based on the findings in this first group, two further groups of three animals were sequentially treated with a dose of 300 mg/kg bw. Animals were examined for mortality for at least 14 days. Clinical signs and

symptoms were recorded several times on the day of administration, and thereafter at least once each workday for the individual animals throughout the observation period. Individual body weights were determined shortly before administration (day 0), weekly thereafter and at the end of the study. Additionally, at body weights were recorded on the day of death in animals that died starting with study day 1.

Findings:
All animals of the 2000 mg/kg administration group were found dead on study day 3.
No mortality occurred in the 300 mg/kg bw administration groups.
Clinical observation in the 2000 mg/kg bw administration group revealed impaired and poor general state, dyspnoea, abdominal or lateral position, staggering, smeared fur, lacrimation and red smeared fur in the anogenital area. Findings were observed from hour 4 until including study day 2 after administration.
Clinical observation in one 300 mg/kg bw administration group revealed impaired general state, dyspnoea and staggering. Findings were observed from hour 4 until including hour 5 after administration.
No clinical signs and findings were observed in the second 300 mg/kg administration group.
The mean body weights of one 300 mg/kg administration group increased during the first post exposure observation week but did not adequately increase during the second week. This effect is observed at times in the rat strain used, because in the required age range the female animals have already reached the phase of slow growth.
The mean body weights of the second 300 mg/kg administration group increased throughout the study period.
During necropsy the animals that died showed many black erosions/ulcers in the glandular stomach and red diffuse discoloration of the large and small intestine.
No macroscopic pathologic abnormalities were noted in the animals examined at the end of the observation period.

Discussion:
According to OECD Guideline No. 423 the starting dose was 2000 mg/kg bw. All animals died. The next step was 300 mg/kg bw, instead of the suggested dose of 200 mg/kg bw. None of the six animals died. The testing was stopped. According to Annex 1c of the guideline the median lethal dose is around 500 mg/kg bw. Thus, the metabolite 635 M02 is more toxic than the parent compound tritosulfuron. The results of this study confirm the results of the previously conducted study (Wiemann C., Hellwig J., 1999; BASF RegDoc# 1999/10099) where all animals of the 5000 mg/kg bw dose group died within 4 days after application. Nine animals (4 males and 5 females) of the 2000 mg/kg bw dose group died within 3 days after application. A delayed mortality (6 days after application) was observed in 1 female rat of the 500 mg/kg bw dose group.

Conclusion:
Under the conditions of this study the median lethal dose of Reg. No. 292 564 after oral administration was found to be greater than 300 mg/kg and less than 2000 mg/kg body weight in rats. The median lethal dose lies between 500 and 1000 mg/kg bw.

B.6.8.1.1.9 Range finding study: 28-day subacute study with 635 M02 (TBSA)

Report: Kaspers U. et al., 2003
BSA - Subacute toxicity study in Wistar rats administration in the diet for 4 weeks
BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep., Unpublished,
BASF RegDoc# 2003/1004048

GLP: Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 96/54, OECD 407

Deviations: None, which can be considered to have influenced the integrity of the study.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Reg. No. 292 564 (syn. TBSA, 635 M02); batch no. 25887 Fass 2; purity: 98.4 %.
Test animals: Males and female Wistar rats, CrlGlxBrlHan:WI; supplied by Charles River, Sulzfeld, Germany, age: 32-34 days.

Reg. No. 292 564 was administered to groups of 5 male and 5 female Wistar rats in the diet for four weeks at doses of 0; 200; 1000 and 5000 ppm, corresponding to 18.6/20.0, 90.7/96.1, 417.3/414.2 mg/kg bw/d for males and females respectively.
Food consumption and body weight were determined weekly. The animals were examined for clinical signs of toxicity or mortality at least once a day. Detailed clinical examinations were conducted prior to the start of the administration period and weekly thereafter. A functional observational battery (FOB) and measurement of motor activity were performed towards the end of administration. The FOB included evaluation of home cage observations, Open Field examinations, sensorimotor tests/reflexes, quantitative parameters (feces, urine, rearing, grip strength, landing foot splay).
Clinicochemical, hematological examinations and urinalyses were carried out towards the end of the administration period. Finally, all animals were assessed by gross pathology including the determination of organ weights (liver, kidneys, adrenal glands, testes, epididymides, ovaries, uterus, spleen, brain, heart and thymus), followed by histopathological examinations.

Findings:
Sufficient stability over the study period and homogenous distribution of the test substance in the diet was demonstrated. Concentration control analyses showed that actual concentrations in the diet were within a range of 94.2 – 101.4 % of the nominal concentrations.

Mortality
No animals died during the study.

Clinical examinations
One male animal administered 5000 ppm showed slight tremor from day 14 onwards and piloerection from day 5 onwards. In addition, one male animal of this dose group showed piloerection from day 5 to day 13. In four high dose females tremor as well as in three high

dose females piloerection was observed at several days of the study. These observations were assessed as substance-related.

Table B.6.8.1-6: Treatment-related clinical observations

Sex	Males				Females			
Dietary concentration (ppm)	0	200	1000	5000	0	200	1000	5000
Clinical observations								
Tremor, slight or moderate	0	0	0	1 (20 %)	0	0	0	4 (80 %)
Observed how many times	0	0	0	12	0	0	0	50
Mean onset (day)				15				20
Piloerection	0	0	0	2 (40 %)	0	0	0	3 (60 %)
Observed on x days	0	0	0	28				21
Mean onset (day)				5				7

Food consumption and body weight data

Food consumption was significantly decreased in males and females at 5000 ppm throughout the entire study with a maximum of –33.0 % and –43.2 % at the beginning of the study (day 7). This effect was assessed as related to the test compound. Body weight as well as body weight change was statistically significantly reduced in males and females at 5000 ppm from day 7 until the end of the study. In male animals the decreased body weight reached a maximum of –23.5 % on day 28, whereas the highest decrease measured in female rats was -21.5 % on day 21.

Food efficiency

In high-dose group males food efficiency was decreased over the whole study period, statistically significant on day 7, 21, and 28. In females, food efficiency was statistically significantly reduced at 5000 ppm on day 7, only.

Functional observation battery and motor activity assessment

The following treatment related findings were observed:

- Reduced rearing: at 5000 ppm statistically significant effect in both sexes (males: -77.8 %, females: -90.7 %), slightly reduced rearing without statistical significance in females at 1000 ppm.
- Slight to moderate tremor (home cage or open field observations): at 5000 ppm in all females and in three of five males. Some of the involved animals also showed an impairment of coordination with unsteady or shuffling gait.
- Reduced overall motor activity: at 5000 ppm statistically significant effect in males. In females, motor activity was also slightly reduced at 1000 and 5000 ppm when compared to control and low dose level, although without statistical significance (except at interval 5 at 1000 ppm).

No substance-related effects were observed in the sensorimotor/reflex tests at any dose level.

Hematology

At the end of the study statistically significantly decreased red blood cell counts, hemoglobin concentrations and hematocrit values were found in the peripheral blood of the mid dose males and the low and mid dose females. When compared to control values, red blood cell parameters were decreased in high dose animals, although without statistical significance.
Mean corpuscular volume (MCV) was increased in the high dose animals of both sexes and in the mid dose females. Reticulocyte counts were increased in the mid and high dose rats of

either sex. Since most of these changes occurred not dose-related, they were considered only as a minor trend towards lower red blood cell values.

Table B.6.8.1-7: Hematological findings

Test parameter		Dose level (ppm)							
		0		200		1000		5000	
RBC [10^{12}/L]	M	7.87	(100 %)	7.66	(97 %)	7.12**	(90 %)	7.38	(94 %)
	F	7.87	(100 %)	7.04*	(89 %)	6.88*	(87 %)	7.28	(93 %)
Hb [mmol/L]	M	9.3	(100 %)	9.1	(98 %)	8.6**	(92 %)	9.2	(99 %)
	F	9.3	(100 %)	8.6*	(92 %)	8.3*	(89 %)	8.8	(95 %)
Hct [ratio]	M	0.441	(100 %)	0.425	(96 %)	0.404**	(92 %)	0.435	(99 %)
	F	0.430	(100 %)	0.394*	(92 %)	0.388*	(90 %)	0.417	(97 %)
MCV [fl]	M	56.1	(100 %)	55.5	(99 %)	56.8	(101 %)	58.9*	(105 %)
	F	54.6	(100 %)	55.9	(102 %)	56.6*	(104 %)	57.3*	(105 %)
Reticulocytes [%]	M	25±5	(100 %)	29±8	(116 %)	47±6	(188 %)	35±7	(140 %)
	F	21±7	(100 %)	24±6	(114 %)	62±12	(295 %)	43±6	(205 %)

Statistics: * = p< 0.05; ** = p< 0.01 (Kruskal-Wallis + Wilcoxon-test, 2-sided)

Clinicochemistry

Serum enzyme examinations revealed increased γ–glutamyltransferase activities in the high dose females.

Total bilirubin levels were increased in the serum of the mid and high dose animals of either sex. In males of the high dose group increased urea and decreased glucose levels were noted. In the mid and high dose females cholesterol concentrations were increased. All other changes were considered not to be treatment-related.

Table B.6.8.1-8: Clinicochemical findings

Test parameter		Dose level (ppm)							
		0		200		1,000		5,000	
γ GT [nkat/L]	M	0	(100 %)	0	–	0	–	3	–
	F	1	(100 %)	0	(0 %)	0	(0 %)	40*	(4000 %)
Total bilirubin [µmol/L]	M	2.05	(100 %)	2.28	(111 %)	2.56*	(125 %)	3.30*	(161 %)
	F	2.68	(100 %)	2.54	(95 %)	3.69	(138 %)	4.30**	(160 %)
Urea [mmol/L]	M	5.27	(100 %)	5.62	(107 %)	5.86	(111 %)	7.23**	(137 %)
	F	8.61	(100 %)	6.43	(75 %)	7.42	(86 %)	8.00	(93 %)
Glucose [mmol/L]	M	5.73	(100 %)	5.80	(101 %)	5.44	(95 %)	4.14*	(72 %)
	F	4.76	(100 %)	6.12*	(129 %)	4.95	(104 %)	3.90	(82 %)
Inorganic phosphate [mmol/L]	M	2.69	(100 %)	2.63	(98 %)	2.54	(94 %)	2.66	(99 %)
	F	2.96	(100 %)	2.07*	(70 %)	2.17*	(73 %)	2.64	(89 %)
Creatinine [µmol/L]	M	51.1	(100 %)	48.2	(94 %)	47.4	(93 %)	51.8	(101 %)
	F	60.5	(100 %)	52.4	(87 %)	50.2	(83 %)	47.6*	(79 %)
Cholesterol [mmol/L]	M	1.55	(100 %)	1.76	(114 %)	2.66	(172 %)	2.10	(135 %)
	F	1.21	(100 %)	1.35	(112 %)	1.95**	(161 %)	2.48**	(205 %)

Statistics: * = p< 0.05; ** = p< 0.01 (Kruskal-Wallis + Wilcoxon-test, 2-sided)

Urinalyses

Blood was found in the urine of males with a dose-related increase, reaching statistical significance in the high dose.

Males and females of the high dose group produced slightly increased amounts of urine with decreased specific gravity.

Microscopic examination of the urine sediments revealed increased numbers of degenerated renal tubular epithelial cells and transitional epithelial cells as well as higher numbers of granular casts and epithelial cell casts from the low dose males onwards. Leucocytes were recorded in the urine of high dose animals of either sex.

Table B.6.8.1-9: Urinalyses

Test parameter			Dose level (ppm)			
			0	**200**	**1000**	**5000**
Urine volume [mL]	M		3.5	3.1	3.6	4.3
	F		1.5	1.7	2.1	2.4
Specific gravity	M		1060	1088	1077	1058
	F		1092	1098	1081	1062
Urinary blood 1 = 10 ery/µL 2 = 50 ery/µL	M	<=1	5	3	2	0**
		>=2	0	2	3	5
	F	<=1	4	5	5	5
		>=2	1	0	0	0
Renal tubular 1 = few 2 = many	M	<=1	5	5	2	2
		>=2	0	0	3	3
	F	<=1	5	5	5	5
		>=2	0	0	0	0
Transitional epithelial cells 1 = few 2 = many	M	<=1	5	3	1*	0**
		>=2	0	2	4	5
	F	<=1	5	5	5	5
		>=2	0	0	0	0
Casts 1 = few 2 = many	M	<=1	5	3	1*	0**
		>=2	0	2	4	5
	F	<=1	5	5	5	5
		>=2	0	0	0	0
Leucocytes	M	<=1	5	5	5	3
		>=2	0	0	0	2
	F	<=1	5	5	4	4
		>=2	0	0	1	1

Statistics: * = p< 0.05; ** = p< 0.01 (Fisher's Exact-test, 2-sided)

Terminal body weight and organ weight changes

The terminal body weight was decreased in females of the mid dose group and in either sex at the high dose group.

Absolute and relative liver weight was dose-related increased in either sex from the low dose onwards. Likewise, in males the relative kidney weights were increased from the low dose onwards. In high dose females the weight of the ovaries and the uterus was decreased. Spleen weight was increased at 1000 ppm and above in both sexes.

All other changes in absolute and/or relative weights were considered to be a consequence of decreased terminal body weight.

Table B.6.8.1-10: Organ weight changes

Test parameter		Dose level (ppm)							
		0		200		1000		5000	
Terminal body wt [g]	M	251.88	(100 %)	252.72	(100 %)	254.8	(101 %)	193.36**	(77 %)
	F	155.02	(100 %)	158.4	(102 %)	148.86	(96 %)	122.02**	(79 %)
Abs. brain wt	M	1.86	(100 %)	1.888	(102 %)	1.838	(99 %)	1.792	(96 %)
	F	1.754	(100 %)	1.706	(97 %)	1.742	(99 %)	1.616*	(92 %)
Rel. brain wt [%]	M	0.739	(100 %)	0.747	(101 %)	0.723	(98 %)	0.928**	(126 %)
	F	1.137	(100 %)	1.078	(95 %)	1.171	(103 %)	1.325**	(117 %)
Abs. liver wt [g]	M	7.632	(100 %)	8.288	(109 %)	8.954	(117 %)	8.576	(112 %)
	F	4.556	(100 %)	4.968	(109 %)	5.054	(111 %)	5.25	(115 %)
Rel. liver wt [%]	M	3.026	(100 %)	3.279	(108 %)	3.509**	(116 %)	4.423**	(146 %)
	F	2.936	(100 %)	3.131	(107 %)	3.392*	(116 %)	4.302**	(147 %)
Abs. kidney wt [g]	M	1.96	(100 %)	2.182**	(111 %)	2.366*	(121 %)	1.94	(99 %)
	F	1.336	(100 %)	1.362	(102 %)	1.284	(96 %)	1.034**	(77 %)
Rel. kidney wt [%]	M	0.778	(100 %)	0.864**	(111 %)	0.921	(118 %)	1.004**	(129 %)
	F	0.864	(100 %)	0.86	(100 %)	0.864	(100 %)	0.849	(98 %)
Abs. spleen wt [g]	M	0.532	(100 %)	0.578	(109 %)	0.662*	(124 %)	0.700**	(132 %)
	F	0.348	(100 %)	0.42	(121 %)	0.444	(128 %)	0.416	(120 %)
Rel. spleen wt [%]	M	0.211	(100 %)	0.228	(108 %)	0.261	(124 %)	0.363**	(172 %)
	F	0.223	(100 %)	0.265	(119 %)	0.297**	(133 %)	0.341**	(153 %)
Abs. thymus wt [mg]	M	482.8	(100 %)	447	(93 %)	551.2	(114 %)	342	(71 %)
	F	405.4	(100 %)	410.8	(101 %)	368.4	(91 %)	268.0*	(66 %)
Rel. thymus wt. [%]	M	0.193	(100 %)	0.176	(91 %)	0.217	(112 %)	0.177	(92 %)
	F	0.258	(100 %)	0.259	(100 %)	0.246	(95 %)	0.22	(85 %)
Abs. adrenal wt [mg]	M	58.4	(100 %)	61.4	(105 %)	64.2	(110 %)	57.2	(98 %)
	F	70	(100 %)	71.6	(102 %)	68.6	(98 %)	57.0*	(81 %)
Rel. adrenal wt [%]	M	0.023	(100 %)	0.024	(104 %)	0.025	(109 %)	0.03**	(130 %)
	F	0.045	(100 %)	0.045	(100 %)	0.046	(102 %)	0.047	(104 %)
Abs. heart wt [g]	M	0.866	(100 %)	0.91	(105 %)	0.866	(100 %)	0.668**	(77 %)
	F	0.606	(100 %)	0.634	(105 %)	0.57	(94 %)	0.454**	(75 %)
Rel. heart wt [%]	M	0.344	(100 %)	0.36	(105 %)	0.34	(99 %)	0.345	(100 %)
	F	0.392	(100 %)	0.4	(102 %)	0.383	(98 %)	0.372	(95 %)
Abs. testes wt [g]	M	3.102	(100 %)	2.182	(70 %)	2.366	(76 %)	1.94	(63 %)
Rel. testes wt [%]	M	1.233	(100 %)	1.2	(97 %)	1.159	(94 %)	1.643**	(133 %)
Abs. epididymides wt [g]	M	0.682	(100 %)	0.694	(102 %)	0.662	(97 %)	0.634	(93 %)
Rel. epididymides wt [%]	M	0.272	(100 %)	0.275	(101 %)	0.261	(96 %)	0.327**	(120 %)
Abs. ovary wt [mg]	F	76.8	(100 %)	89.4	(116 %)	77.8	(101 %)	41.0**	(53 %)
Rel. ovary wt [%]	F	0.049	(100 %)	0.056	(114 %)	0.052	(106 %)	0.034**	(69 %)
Abs. uterus wt [g]	F	0.438	(100 %)	0.440	(100 %)	0.398	(91 %)	0.250	(57 %)
Rel. uterus wt [%]	F	0.288	(100 %)	0.277	(96 %)	0.266	(92 %)	0.207	(72 %)

Statistics: * = p< 0.05; ** = p< 0.01 (Kruskal-Wallis + Wilcoxon-test, 2-sided)

Gross necropsy

There were no gross lesions noted during necropsy.

Histopathology

α_{2u}–globulin accumulation in the kidneys of male rats was increased in severity with increasing dose groups. Chronic progressive nephropathy was noted in high dose males.
In the liver, minimal-slight liver cell hypertrophy was observed in males at 1000 and at 5000 ppm in either sex.

Histopathological examination of the ovaries of high-dose group females revealed a significantly decreased number of corpora lutea (mean of about 12 CL in the high dose group as compared to a mean of about 23 in the control group), which corresponded to decreased mean absolute and relative ovary weights in this group. A DOFC was performed, which showed that the numbers of primordial and growing follicles as well as the number of the combined primordial plus growing follicles were marginally lower in the animals of the high dose group (-3 %, -12 % or -4 %, respectively) as compared to the control group. The DOFC also indicated that the number of clearly discernible antral follicles was reduced to 60 % of the control value at 5000 ppm. However, this was not significant, and as only one cut level of both ovaries per animal was evaluated, the evidence of this observation was limited – this the more as the number of antral follicles with clearly visible ovum was considerably higher in the low (260 %) and mid dose groups (280 %) than in the control group. In addition, the ovarian stroma cells were hyperplastic in all dose groups, thus, a no adverse effect level for this finding was not obtained. Etiology and pathogenesis of the ovarian weight changes and microscopic findings were not clearly understood.
There was an increased number of females with slight or moderate atrophy of the uterus (mucosa and musculature) in the mid and high dose groups.
In the spleen, increased incidences of hemosiderin deposition and congested blood vessels were observed associated with significantly increased mean absolute (males) and relative spleen weights (males and females) in mid and/or high dose groups. Etiology and pathogenesis of the congested blood vessels remained unresolved. For the latter finding, a clear no adverse effect level was established for both sexes in the low dose group, whereas increased iron pigment accumulation was noted down to the low dose group in females, thus with lack of a no adverse effect level for female rats.

Table B.6.8.1-11: Histopathology findings

	Males				Females			
Dose level (ppm)	0	200	1000	5000	0	200	1000	5000
Liver								
Hypertrophy, central	0/5	0/5	1/5	5/5	0/5	0/5	0/5	5/5
Minimal	0	0	1	2	0	0	0	4
Slight	0	0	0	3	0	0	0	1
Kidney								
Alpha-2µ accumulation,	5/5	5/5	5/5	5/5	0/5	N.D.	N.D.	0/5
Minimal-slight	5	3	2	0	0			0
Moderate-severe	0	2	3	5	0			0
Chronic nephropathy	0/5	0/5	1/5	5/5	2/5	N.D.	N.D.	0/5
Minimal	0	0	0	0	2			0
Moderate	0	0	1	5	0			0
Spleen								
Congested vessels	0/5	0/5	5/5	5/5	0/5	0/5	4/5	5/5
Hemosiderin deposit	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Minimal-slight	5	5	3	1	5	4	0	0
Moderate-severe	0	0	2	4	0	1	5	5
Hematopoiesis, slight	0/5	0/5	1/5	1/5	0/5	0/5	1/5	0/5
Ovaries								
Hyperplasia, stroma					0/5	3/5	5/5	5/5
Minimal					0	3	0	0
Slight					0	0	5	1
Moderate					0	0	0	4
1-20 corpora lutea					1	1	0	5
21-40 corpora lutea					4	4	5	0
Thyroid gland								
Hypertrophy/hyperplasia, slight	0/5	0/5	1/5	1/5	0/5	N.D.	N.D.	0/5
Uterus								
Atrophy					1/5	1/5	3/5	4/5
Slight					1	1	3	0
Severe					0	0	0	4

Conclusion:
In this 28-day oral feed study with Reg. No. 292 564 in Wistar rats, a NOAEL could not be derived, based on lower RBC, effects on organ weights and on ovaries at the lowest dose of 200 ppm tested (corresponding to daily intakes of 18.6 and 20.0 mg/kg bw/d for males and females, respectively). Therefore, a subsequent 28-day oral toxicity study was conducted using three lower dose levels (BASF RegDoc# 2003/1004049).

B.6.8.1.1.10 Second 28-day subacute study with 635 M02 (TBSA)

Report: Kaspers U. et al., 2003
TBSA - Repeated dose oral toxicity study in Wistar rats administration in the diet for 4 weeks.
BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep., Unpublished, BASF RegDoc# 2003/1004049

GLP: Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 96/54, OECD 407

Deviations: In this supplementary study, the methods applied generally complied to OECD 407 requirements. Based on the findings of the previous 4-week investigation with Reg. No. 292 564, the following deviations applied in this supplementary study:
An examination of the sensory reactivity to stimuli of different types was not conducted at the end of the study period. Moreover, assessments of grip strength and motor activity, as well as FOB investigations were not performed.
In addition to guideline requirements, organ weights were determined for ovaries and uterus.
Histopathology was confined to assessment of all gross lesions, spleen, kidneys and ovaries in all control and test groups.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Reg. No. 292 564 (syn. TBSA); batch no. 25887 Fass 2; purity: 98.4 %.
Test animals: Males and female Wistar rats, CrlGlxBrlHan:WI; supplied by Charles River, Sulzfeld, Germany, age: 32-34 days.
This study was performed as a supplementary study to a former sub-acut toxicity study in Wistar rats (BASF RegDoc# 2003/1004048, dose levels**:** 0, 200, 1,000 and 5,000 ppm) to obtain a clear "no observed adverse effect level" (NOAEL) of the test substance.
Reg. No. 292 564 was administered to groups of 5 male and 5 female Wistar rats at dietary concentrations of 0, 50, 100 and 150 ppm, corresponding to 4.8 / 5.0; 9.0 / 10.0; 13.9 / 14.7 mg/kg bw/d in males and females.
Food consumption and body weight were determined weekly. The animals were examined for signs of toxicity or mortality at least once a day. Detailed clinical examinations in an open field were conducted prior to the start of the administration period and weekly thereafter. Clinicochemical, hematological examinations and urinalyses were performed towards the end of the administration period. Finally, animals were assessed by gross pathology, followed by histopathological examinations.

Findings:
Sufficient stability over the study period and homogenous distribution of the test substance in the diet was demonstrated. Concentration control analyses showed that actual concentrations in the diet were within a range of 99.4–107.7 % of the nominal concentrations.
No treatment-related findings were evident from clinical observations, assessment of food consumption, water consumption, body weight data, food efficiency, clinicochemistry, hematology or urinalysis.

Organ weight
In female rats, the mean relative weight of the kidneys was significantly increased in the high dose group (+12.8 %). This was possibly incidental, as no such weight changes were recorded in males. Anyhow, histopathological examinations on the female kidneys were not performed to exclude a treatment-related effect.
The other mean relative weight parameters did not show significant differences when compared with the control group.

Gross necropsy and histopathology

No substance-related effects were revealed upon gross necropsy of the animals under study. Treatment-related microscopic findings were detected in the kidneys of male rats. They consisted of a slightly increased $\alpha_{2\mu}$-globulin accumulation in the epithelia (and occasionally in the tubular lumen) of the proximal tubules of the renal cortex of treated males as compared to the control animals. Detection of $\alpha_{2\mu}$-globulin was based on Mallory's stain. The graded severity ranged from minimal (grade 1) to moderate (grade 3) in treated animals. In the control group, the amount of $\alpha_{2\mu}$-globulin accumulation was minimal or slight, whereas in the low, mid and high dose groups, more animals with higher grades of severity were affected with $\alpha_{2\mu}$-globulin accumulation in a kind of dose related fashion:

Table B.6.8.1-12: Kidney findings in male rats

	Control	50 ppm	100 ppm	150 ppm
$\alpha_{2\mu}$-globulin accumulation	5/5	5/5	5/5	5/5
grade 1 (minimal)	3/5	1/5	2/5	0/5
grade 2 (slight)	2/5	3/5	1/5	2/5
grade 3 (moderate)	0/5	1/5	2/5	3/5
Mean grade	1.4	2.0	2.0	2.6

The $\alpha_{2\mu}$-globulin accumulation did not cause any other microscopic findings in the kidneys. The only other microscopic findings in the kidneys were a unilateral small cortical scar, associated with a cortical cyst in a high dose male rat (No. 17). Both findings were regarded to have developed spontaneously and unrelated to treatment or $\alpha_{2\mu}$-globulin accumulation.
In the spleen of female rats, hemosiderin deposition was noted in all animals of the control and treatment groups, giving no indication of a treatment-related change:

Table B.6.8.1-13: Spleen findings in female rats

	Control	50 ppm	100 ppm	150 ppm
Hemosiderin deposition	5/5	5/5	5/5	5/5
grade 1 (minimal)	4/5	4/5	3/5	3/5
grade 2 (slight)	1/5	1/5	2/5	2/5
Mean grade	1.2	1.2	1.4	1.4

In the ovaries, no microscopic findings were noted up to 150 ppm, the highest dose tested.

Discussion:

In this study no signs of toxicity were observed in female animals at the concentrations tested. Thus, the NOAEL for female rats can be established at 150 ppm (14.7 mg/kg bw/d). In male animals, an increased incidence of $\alpha_{2\mu}$-globulin accumulation in the kidney was found. This finding represents an unique feature of male rats that does not occur in any other mammalian species, especially not in human males. Since an increased incidence of $\alpha_{2\mu}$-globulin accumulation has no relevance for humans, consequently the NOAEL for male rats regarding human risk assessment is also 150 ppm (13.9 mg/kg bw/d).

Conclusion:

Under the conditions of this 4-week dietary study, the NOAEL was 150 ppm, equivalent to 13.9 mg/kg bw/d for male and 14.7 mg/kg bw/d for female rats.

B.6.8.1.1.11 First 1-generation study with 635 M02 (TBSA)

Report: Schneider S. et al., 2004
BSA - One-Generation Reproduction Toxicity Study in Wistar Rats Range finding study (with 4 weeks premating) Continuous Dietary Administration.
BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep., unpublished, BASF RegDoc# 2004/1017198

GLP: Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 415; OECD 416; OPTTS 870.3800

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Reg. No. 292 564 (syn. TBSA); batch no. 25887 Fass 2; purity: 98.4 %.
Test animals: Male and female Wistar rats, CrlGlxBrlHan:WI; supplied by Charles River, Sulzfeld, Germany.
The objective of this range finding study was to determine the possible adverse effects of Reg. No. 292 564 on the integrity and performance of the male and female reproductive systems, including gonadal function, mating behavior, conception, gestation, parturition, lactation and weaning, and on growth and development of offspring from one generation of Wistar rats continuously administered to the test substance in the diet. The study should also provide information about the effects of Reg. No. 292 564 on neonatal morbidity, mortality, possible target organs in the offspring and data on pre- and postnatal developmental toxicity.
Reg. No. 292 564 was administered to groups of 10 male and 10 female healthy young adult Wistar rats (CrlGlxBrlHan:Wi) via the diet at dose levels of 0; 200 and 400 ppm, respectively. In addition, satellite groups each consisting of 5 male and 5 female rats were treated at the same dose levels. The satellite animals were sacrificed after about four weeks. The main groups formed the F_0 parental generation. These animals were allowed to mate at least 33 days after the beginning of treatment to produce a litter (F_1). Mating pairs were from the same treatment group. Treatment of the parental animals continued throughout mating, gestation and the three-week lactation period until about 16 hours before terminal sacrifice. The F_1 pups were raised up until day 4 (culling) or 21 post partum (p.p.). Thereafter, the F_1 weanlings and the F_0 adult animals were sacrificed.
The health status of the F_0 rats and the satellite animals was checked each day, and the F_0 parental animals were examined for their mating and reproductive performance. Food consumption of the F_0 parents and satellite animals was determined regularly during premating (once weekly over a period of 7 days each), and during gestation (days 0-7, 7-14, 14-20) and lactation periods (days 1-4, 4-7, 7-14). In general, body weights of F_0 parents and satellite animals were determined once weekly (each time for a period of 7 days). However, F_0 females were weighed on days 0, 7, 14 and 20 of gestation and on days 1, 4, 7, 14 and 21 of lactation.
Blood samples were taken from 5 satellite animals per sex and group shortly before terminal sacrifice for clinical pathology examinations, which included haematology, clinicochemistry and determination of total triiodothyronine (T_3), total thyroxine (T_4) and thyroid stimulating hormone (TSH).

Sperm head counts and morphology were assessed in all control and high dose satellite males, while sperm motility was examined in the satellite males of all groups at scheduled sacrifice.
All F_1 pups were sexed on the day of birth and were weighed on the subsequent day as well as on day 4 after birth. Their viability was recorded. Standardised litters were weighed on days 4, 7, 14 and 21 post partum. All pups were examined macroscopically at necropsy (including weight determinations of brain, spleen and thymus in one pup/sex/litter).
In the F_0 animals and satellite animals of both genders organ weights (liver, kidneys, adrenal glands, testes, epididymides, cauda epididymis, prostate, seminal vesicle with coagulation gland, ovaries, uterus, spleen, brain, pituitary gland, thyroid gland with parathyroid gland) were determined. Histopathological examinations were performed in F_0 animals and satellite animals of both genders of selected organs (vagina, cervix uteri, uterus, ovaries, oviducts, testes, epididymides, seminal vesicle, coagulation glands, prostate, pituitary, adrenal gland, all gross lesions). Of the ovaries, a DOFC was performed.

Findings:
Sufficient stability over the study period and homogenous distribution of the test substance in the diet was demonstrated. Concentration control analyses showed that actual concentrations in the diet were within a range of 94.5–104.7 % of the nominal concentrations.

Test substance intake
The calculated test substance intakes are presented in the table below:

Table B.6.8.1-14: Test substance intake (mg/kg bw/d)

	200 ppm	400 ppm
F_0 males	12.9	25.3
F_0 females		
Premating	16.2	32.4
Gestation period	16.2	32.6
Lactation period	29.2	60.9
Satellite males	12.8	25.7
Satellite females	16.6	32.2

Body weights, food consumption, food efficiency, water consumption
Body weights gains of in the F_0 parental and satellite females were slightly reduced (about 10 % - 20 %, occasionally attaining statistical significance) compared to control group females at both dose levels tested 200 and 400 ppm.

Male reproduction data
Mating was confirmed for all F0 parental males, which were placed with females to generate F1 pups. Thus the mating index was 100 % in all groups.
The male fertility index was reduced in the 400 ppm group when compared to control and low dose.

Table B.6.8.1-15: Fertility indices of F0 males/females

	0 ppm	200 ppm	400 ppm
Concerning F1 litters	9/10 (90 %)	10/10 (100 %)	7/10 (70 %)

Sperm parameters

Sperm parameters were not influenced by the treatment and values obtained were comparable among control and treatment groups.

Female reproduction data

The female fertility index varied between 70 % and 100 % (see table above), with 3/10 non-pregnant females in the 400 ppm group. One out of these females (No. 121) showed macroscopically a reduced size of the ovaries, which correlates with the severely reduced number of corpora lutea and a massive follicle degeneration in the ovaries. Implantation was not affected by the test substance. The lower number of total implantation sites in the 400 ppm group is due to the fact that 3 females did not become pregnant.
There was no further substance-related effect on female reproduction, delivery and litter data. The viability index (pups surviving days 1 to 4) was 99 %, 97 % and 100 % in the control, 200 and 400 ppm group, the lactation index was 100 % in all groups.

Table B.6.8.1-16: Summary of female reproduction, delivery and litter data

	0 ppm	200 ppm	400 ppm
Females with liveborn (N) Gestation index (%)	9 100	10 100	7 100
With stillborn pups (N)	2	1	0
Pups delivered (mean)	12.2	10.2*	10.6
Liveborn (N) Live birth index (%)	104 95	101 99	74 100
Pups died (N)	0	1	0
pups dead day 1-4	1	3	0
pups dead days 15-21	0	0	0

Laboratory investigations (satellite animals)

There were no treatment-related changes in hematological parameters. Hormone examinations (T3, T4, TSH) revealed slightly higher values in high dose males, although without statistical significance.
Cholesterol concentrations were increased in males and females at 400 ppm. High dose females showed lower magnesium levels.

Table B.6.8.1-17: Laboratory investigations at day 34

	0 ppm	200 ppm	400 ppm
Males			
T3 (nmol/L)	1.25	1.23	1.40
T4 (nmol/L)	43.82	47.60	51.48
TSH (µg/L)	11.12	11.12	14.88
Cholesterol (mmol/L)	1.66	2.17	2.47*
Magnesium (mmol/L)	0.89	0.89	0.88
Females			
T3 (nmol/L)	1.28	1.47	1.38
T4 (nmol/L)	29.73	35.08	35.93
TSH (µg/L)	8.49	9.69	8.76
Cholesterol (mmol/L)	1.68	1.60	2.33*
Magnesium (mmol/L)	0.97	0.93	0.89*

* $p \leq 0.05$ Kruskal-Wallis + Wilcoxon-test

Organ weights

The mean absolute and relative kidney and spleen weights were significantly increased in high dose F_0 parental males, when compared with controls. In high dose F_0 parental females,

the relative spleen weight was significantly increased. The increased kidney and spleen weights are related to the administration of the test substance. Moreover, the relative liver weight was significantly increased in high dose F_0 males. In the F_0 females, the relative liver weights were significantly increased in both treatment groups (200 and 400 ppm). Although the increased liver weights did not show a dose-response relationship, a substance-related effect cannot be ruled out with certainty. The absolute and relative weights of the thyroid glands were significantly increased in high dose F_0 females. However, statistically significantly increased thyroid weights could not be reproduced in male or female animals from the satellite groups of this study, which had been administered the test substance for 4 weeks.
No substance-related weight changes occurred in the male and female satellite rats.

Histopathological examination
One female of the high dose group (F0-Generation) showed a reduced number of corpora lutea and degenerated follicles in the ovaries. None of the satellite animals showed a similar finding. A focal degeneration in the testes was noted in 4 high dose rats versus 1 in the control and none in the low dose (F0-Generation).

Differential ovarian follicle count (DOFC)
One high dose female (No. 121) of the F0 generation group showed a severely reduced number of corpora lutea in the ovaries. This finding correlates with the macroscopically observed reduced organ size. In addition, a massive degeneration of follicles was noted in these ovaries. In the DOFC only one primordial follicle and one growing follicle were counted in this female. The degeneration of follicles caused the infertility in this female. There were no further significant deviations between controls and animals of the high dose.

F1-generation pups/litters

The F1 pups body weight data were unaffected by the treatment.
The absolute and relative spleen weights were higher in male pups of the 400 ppm group although without statistical significance.

Table B.6.8.1-18: Pup organ weight to body weight data

	0 ppm	200 ppm	400 ppm
Body weight day 21 in grams (mean male and female pups)	46.8	44.6	46.2
Absolute spleen weight (grams) of male pups of female pups	 0.185 0.208	 0.187 0.190	 0.229 0.207
Relative spleen weight (organ to bw ratio) of male pups of female pups	 0.396 0.451	 0.403 0.418	 0.484 0.454

Pups necropsy observations
The few changes noted at necropsy were considered not related to the treatment with the test article.

Discussion:
It cannot be concluded with certainty that the impaired fertility in high dose animals (one pair in the control versus 3 pairs in the high dose group) was not substance-related. Historical control data from the laboratory on this endpoint (female fertiliy index) indicate 84 % as the minimum. Thus, 70 % are outside the historical data. In addition, the findings in the ovary of a single high dose female (severely reduced number of corpora lutea, massive degeneration of follicles) may indicate a high sensitivity of single animals. Moreover, effects on the ovaries and uterus were also noted in the 28-day repeated dose study at concentrations of ≥ 200 ppm (20 mg/kg bw/d).

Conclusion:
Under the conditions of this study the NOAEL for reproductive toxicity is 200 ppm (corresponding to approximately 12.8 mg/kg bw/d and 16.2 mg/kg bw/d in males and females respectively) based on impaired fertility and effects on the ovaries noted at the next higher dose of 400 ppm (corresponding to approximately 25.3 mg/kg bw/d and 32.2 mg/kg bw/d in males and females respectively). The NOAEL for general toxicity is below 200 ppm based on lower body weight and organ weight changes at this dose level.

B.6.8.1.1.12 Second 1-generation study with 635 M02 (TBSA)

Report:	Schneider S. et al., 2004 TBSA - One-generation reproduction toxicity study in Wistar rats range finding study (with 10 weeks premating) continuous dietary administration BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep., unpublished BASF RegDoc# 2004/1017197
GLP:	Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)
Guideline:	OECD 415; OECD 416; OPTTS 870.3800
Deviations:	None
Acceptability:	The study is considered to be acceptable.

Material and Methods:
Test material: Reg. No. 292 564 (syn. TBSA); batch no. 25887 Fass 2; purity: 98.4 %.
Test animals: Male and female Wistar rats, CrlGlxBrlHan:WI; supplied by Charles River, Sulzfeld, Germany.
In the following study, Reg. No. 292 564 (635M02; TBSA) was administered to groups of 10 male and 10 female healthy young adult Wistar rats (F_0 parental generation) and 5 male and 5 female satellite animals/group as a constant homogeneous addition to the food at different dietary concentrations (0; 200 and 400 ppm). The satellite animals were sacrificed after about ten weeks. At least 75 days after the beginning of treatment, F_0 rats were mated to produce a litter (F_1). Mating pairs were from the same test concentration group. Treatment of the F_0 parental animals continued throughout mating, gestation and the three-week lactation period until about 16 hours before terminal sacrifice. The F_1 pups were raised up until day 4 (culling) or 21 post partum (p.p.). Thereafter, the F_1 weanlings and the F_0 adult animals were

sacrificed. The examinations and parameters recorded in this study were identical to those of the 4-week premating range-finding study. For details, see the Material and Methods section of BASF DocID 2004/1017198.

Findings:
Sufficient stability over the study period and homogenous distribution of the test substance in the diet was demonstrated. Concentration control analyses showed that actual concentrations in the diet were within a range of 94.5–104.7 % of the nominal concentrations.

Test substance intake
The calculated test substance intakes are presented in the table below:

Table B.6.8.1-19: Test substance intake (mg/kg bw/d)

	200 ppm	400 ppm
F_0 males	17.7	35.0
F_0 females		
Premating	20.7	40.9
Gestation period	17.3	33.1
Lactation period	30.0	62.3
Satellite males	17.6	35.3
Satellite females	20.3	41.2

Clinical findings
No unscheduled mortalities or substance-induced clinical findings were observed.

Body weights, food consumption, food efficiency, water consumption
Mean body weights gains of the treatment-group F_0 females were slightly below the corresponding control values during gestation and lactation periods (up to 17 % - 18 %, statistically not significant). Food consumption of both genders and body weight data of the males (parental and satellite animals) were unaffected by treatment.

Male reproduction data
Mating was confirmed for all F0 parental males, which were placed with females to generate F1 pups. Thus the mating index was 100 % in all groups. The male fertility index was unaffected by the treatment.

Table B.6.8.1-20: Fertility indices of F0 males/females

	0 ppm	200 ppm	400 ppm
Concerning F1 litters	8/10 (80 %)	10/10 (100 %)	10/10 (100 %)

Sperm parameters
Sperm parameters were not influenced by the treatment and values obtained were comparable among control and treatment groups.

Female reproduction data

The female fertility index varied between 80 % and 100 % (see table above), with 2/10 non-pregnant females in the control group. The postimplantation loss appeared slightly increased in the 400 ppm group. This increase was considered to be due to high dose female no. 122, which did not deliver pups but had 6 implantation sites in utero at terminal sacrifice.
There was no further substance-related effect on female reproduction, delivery and litter data. The viability index (pups surviving days 1 to 4) was 100 %, 100 % and 97 % in the control, 200 and 400 ppm group, the lactation index was 100 % in all groups.

A substance-induced effect in male fertility can be excluded, as only two control pairs did not show any indications of fertility. No treatment-related changes occurred in the mean number of homogenization resistent testicular spermatides or caudal epididymal sperms, and the percentages of abnormal and normal sperms in the high dose (400 ppm) males. Sperm motility was not affected by the test substance at any dose level and was substantially similar to the control group.

Table B.6.8.1-21: Summary of female reproduction, delivery and litter data

	0 ppm	200 ppm	400 ppm
Females with liveborn (N) Gestation index (%)	8 100	10 100	9 90
With stillborn pups (N)	1	0	0
Pups delivered (mean)	11.6	10.4	11.7
Liveborn (N) Live birth index (%)	90 97	104 100	105 100
Pups died (N)	0	0	0
pups dead day 1-4	0	0	0
pups dead days 15-21	0	0	0
Implantation sites / Postimplantation loss (N)	96/3	112/8	116/ 11

Laboratory investigations (satellite animals)

Slight increases in white blood cell counts were observed in the 200 ppm and 400 ppm groups satellite males, which were coupled with an increase of lymphocytes, polymorphonuclear neutrophils and eosinophils.
Red blood cell count, hemoglobin and hematocrit was decreased in animals of the 400 ppm group, reaching statistical significance in females only. Reticulocyte count was slightly increased.

Table B.6.8.1-22: Hematological investigations

	0 ppm	200 ppm	400 ppm
Males			
WBC (giga/L)	4.32	5.64**	5.82**
RBC (tera/L)	8.45	8.44	8.20
HGB (mmol/L)	9.2	9.2	8.9
HCT (L/L)	0.422	0.426	0.414
Reti (%)	2.1	2.1	2.6
Females			
WBC (giga/L)	3.81	3.97	3.83
RBC (tera/L)	7.84	7.53	7.18
HGB (mmol/L)	9.2	9.0	8.7**
HCT (L/L)	0.414	0.406	0.395
Reti (%)	2.0	2.3	3.2

* $p \leq 0.05$, ** $p \leq 0.01$ Kruskal-Wallis + Wilcoxon-test

Serum cholesterol was increased in males and females at 200 and 400 ppm, reaching statistical significance in females at 400 ppm only.
Finally, magnesium serum concentrations were slightly decreased in females administered 200 or 400 ppm of the test substance.
Hormone examinations (T3, T4, TSH) revealed slightly lower T4 and TSH values in males at 400 ppm.

Table B.6.8.1-23: Laboratory investigations at day 71

	0 ppm	200 ppm	400 ppm
Males			
T3 (nmol/L)	1.11	1.16	1.18
T4 (nmol/L)	59.57	55.33	50.13*
TSH (µg/L)	10.86	9.65	8.96
Cholesterol (mmol/L)	1.71	1.91	2.17
Magnesium (mmol/L)	0.88	0.87	0.86
Females			
T3 (nmol/L)	1.15	1.06	1.36
T4 (nmol/L)	38.32	36.75	38.17
TSH (µg/L)	6.23	8.10	6.80
Cholesterol (mmol/L)	1.27	1.65	2.04*
Magnesium (mmol/L)	0.98	0.88*	0.89*

* $p \leq 0.05$ Kruskal-Wallis + Wilcoxon-test

Organ weights

The mean relative liver and kidney weights were significantly increased in high dose F_0 parental males, when compared with controls. The relative spleen weight was significantly increased in high dose F_0 parental females and minimally higher in males. The increased liver, kidney and spleen weights in the high dose F_0 parents are related to the administration of the test substance.
In the satellite groups, the mean relative kidney and liver weight was increased in high dose males, when compared with controls. In high dose females, the relative liver weight as well as the absolute and relative spleen weights were significantly increased. The increased liver, kidney and spleen weights in the high dose satellites are related to the administration of the test substance.
The absolute and relative weights of the thyroid glands were significantly increased in treated F_0 parental females. However an increased thyroid weight could not be reproduced in male or

female animals from the satellite groups of this study, which had been administered the test substance for 10 weeks. In F0 males the absolute thyroid weights also appear increased in both treatment groups, although without dose-relation. Altogether these weight changes are considered incidental.
There were no substance-related gross lesions or microscopic findings in any of the organs and tissues examined, neither in the F_0 rats nor in the satellite animals.

Differential ovarian follicle count (DOFC)
The DOFC revealed no changes in the number of primordial and/or growing follicles between the control and treated groups.

F1-generation pups/litters
The F1 pups body weights and body weight gains were minimally lower in the 400 ppm group at all investigation points, although without statistical significance and with values lying mainly within the historical control values. In addition, at the 400 ppm level maternal toxicity was noted. Therefore, these changes were not considered toxicologically relevant.

Organ weights revealed no differences between control and treatment groups.

Table B.6.8.1-24: Pups body weight data

	0 ppm	200 ppm	400 ppm
Body weight in grams (mean male and female pups)			
day 1	6.0	6.1	5.4
day 4 (postculling)	8.8	9.2	8.1
day 7	14.0	14.7	13.0
day 14	28.5	29.4	26.6
day 21	45.5	47.7	42.8

Pups necropsy observations
The few changes noted at necropsy were considered not related to the treatment with the test article.

Discussion:
Under the conditions of this study, there were no indications from the clinical, clinical pathology and from gross and histopathological examinations, that the administration of the test substance at concentrations of 200 or 400 ppm (about 19 or 38 mg/kg bw/d) adversely affected fertility of the F_0 parental animals. The reproductive performance was impaired in one high dose F0 female. At necropsy 6 implantation sites were counted indicating that this female was pregnant and, therefore, fertility was not affected. Anyhow, it cannot be concluded with certainty that the total litter loss in this female was not substance-related.
Estrous cycle data, mating behavior, conception, gestation, parturition, lactation and weaning (F_0 parental rats) as well as sperm parameters (satellite males), sexual organ weights, gross and histopathological findings of the reproductive organs (including differential ovarian follicle counts) were unaffected by treatment with 200 ppm or 400 ppm of the test substance.
F_1-pups of the 400 ppm group had minimally lower body weights when compared to the control and low dose group, but they were well within the range of historical control data. If at all, these weight changes occurred in the presence of maternal toxicity. Therefore, no relevance is attributed. No substance-induced signs of developmental toxicity occurred in the

progeny of the F_0 parents. Pup mortality and survival rate, sex ratio, clinical and necropsy findings and organ weights were unaffected by treatment.

Conclusion:
Under the conditions of this study the NOAEL for reproductive toxicity is 200 ppm (corresponding to approximately 17.6 mg/kg bw/d and 17.3 mg/kg bw/d in males and females respectively) based on postimplantation loss noted in one female at the next higher dose of 400 ppm (corresponding to approximately 35.0 mg/kg bw/d and 40.9 mg/kg bw/d in males and females respectively).
The NOAEL for general toxicity is below 200 ppm based on lower body weight and clinico-chemical findings (higher white blood cell count in males, higher cholesterol concentrations in males and females, lower magnesium concentrations in females) at this dose level.

B.6.8.1.1.13 TBSA: Assessment of Reproduction Toxicity Potential

Report: FitzGerald R. E., 2007
TBSA: Assessment of Reproduction Toxicity Potential
Photo documentation „Hyperplasia of the ovarian stroma cells“ recorded after TBSA treatment (4-week study/ Wistar rats – BASF Proj.No. 30S0499/01034) and “Normal amount of the ovarien stroma cells” (1-generation study/ Wistar rats – BASF Proj.No. 15R0499/01129) prepared by W. Kaufmann
BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep., unpublished
BASF RegDoc# 2007/1005045

GLP: None

Guideline: None

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
In the document a photo documentation about hyperplasia of the ovarian stroma cells recorded after TBSA treatment in the range finding 4-week study with Wistar rats (BASF Proj.No. 30S0499/01034) and the normal amount of the ovarien stroma cells in the second 1-generation study in Wistar rats with 10 weeks premating (BASF Proj.No. 15R0499/01129) was reported.

Findings:
The “ovarian stromal hyperplasia” in the range finding 4-week toxicity study were described as a multifocal, partly nodular increase of luteinized, stromal (theca) cells. The author concluded that according to WHO/ RITA-nomenclature, the histomorphological features correspond to “hyperplasia, tubulostromal” and that this feature was especially prominent at 5000 ppm, where corpora lutea were decreased in number. It was reported that ovarian tubulostromal hyperplasia is known to be age-related in some strains of rats.

The following results were presented also as photos:

Figures 1 to 4 show two control animals of the rang finding 4-week study with no clusters of ovarian stromal cells. All stages of folliculogenesis are visible in the sections (Figures 1 and 3).
Figures 5 and 6 show a grade 3 (moderate) hyperplasia of stroma cells of one high dose female in the 5000 ppm group of the rang finding 4-week study. All stages of folliculogenesis are visible in the sections (Figure 5).
Figures 7 and 8 show a grade 2 (slight) hyperplasia of stroma cells of one mid dose female in the 1000 ppm group of the rang finding 4-week study. All stages of folliculogenesis are visible in the section (Figure 7).
Figures 9 and 10 show a grade 1 (minimal) hyperplasia of stroma cells of one low dose female in the 200 ppm group of the range finding 4-week study. All stages of folliculogenesis are visible in the section (Figure 9).
Figures 11 to 14 show two control animals of the 1-generation study (10-week premating period) with a significant amount of stroma cell clusters in the ovaries. This amount reaches or even exceeds the amount noted in the high dose test animals of the range finding 4-week study with a grade 3 hyperplasia of stroma cells (comparison with Figures 5 and 6).

Conclusion:
The control females in the 1-generation study with 10 weeks premating dosing had similar or even higher degrees of hyperplasia than the 5000 ppm females in the 4-week study. In the 1-generation study (10-week premating period), this level of hyperplasia was regarded as being in the normal range to be expected for test animals of their age.

B.6.8.1.2 635M03

635M03 (Reg.-No. 335 182; BH 635-3) is a soil metabolite. It was detected in the rat metabolism study as a transient metabolite. It was tested in three mutagenicity assays as well as in an acute oral test and a 90-day feeding study. 635M03 was found to be not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay, not mutagenic *in vitro* in the CHO/HPRT mutation assay and not chromosome-damaging (clastogenic) under *in vitro* conditions in V79 cells. An acute oral toxicity study revealed an LD_{50} of > 5000 mg/kg bw. The no observed adverse effect level in a 90-day dietary rat study was 15000 ppm (equal to 1187 mg/kg bw). Thus, the metabolite 635M03 is not of toxicological relevance in groundwater.

Table B.6.8-2: Summary of toxicity studies of metabolite 635M03

Study/strains/species	Test material/conditions	Results
Acute oral toxicity in rats Wistar rats (Chbb:THOM (SPF))	Batch No. 00831-274, purity: 98.7 %; batch 01185-044, purity 99.4 %	$LD_{50:}$ > 5000 mg/kg bw
Subchronic toxicity study in Wistar rats. Administration in the diet for 3 months CRL:WI(GLX/BRL/HAN)IGS BR]	Batch No. 01185-269, purity: 99.2 %	No substance related effects. NOAEL: 15000 ppm (1187 mg/kg bw/d)
Salmonella typhimurium/Escherichia coli reverse mutation assay S. typhimurium strains TA 100, TA 1535, TA 1537, TA 98 and E. coli strain WP2 uvrA	Batch No. 00831-274, purity: 98.7 %	Not mutagenic
Gene mutation assay in Chinese hamster V79 cells *in vitro* (V79 / HPRT)	Batch No. 01185-085, purity: 99.8 %.	Not mutagenic
In vitro chromosome aberration assay in Chinese hamster V79 cells	Batch No. 01185-085, purity: 99.8 %.	Not mutagenic

B.6.8.1.2.1 First mutagenicity study

Report: Engelhardt G., Hoffmann H. D., 1998
Report: Salmonella typhimurium/Escherichia coli reverse mutation assay (standard plate test and preincubation test) with PS 335 182
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 1998/10810
and
Engelhardt G., 1999
Amendment No. 1 to the report: Salmonella typhimurium/Escherichia coli reverse mutation assay (standard plate test and preincubation test) with PS 335 182
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 1999/11629
and
Engelhardt G., 2000
Amendment No. 2 to the report: Salmonella typhimurium/Escherichia coli reverse mutation assay (standard plate test and preincubation test) with PS 335 182
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 2000/1019291
and
Engelhardt G., 2001
Amendment No. 3 to the report: Salmonella typhimurium/Escherichia coli reverse mutation assay (standard plate test and preincubation test) with PS 335 182
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 2001/1007726

GLP: Yes (Laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 471, EEC 92/69 B 13, EEC 92/69 B 14

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: 635M03 (Reg.-No. 335 182; BH 635-3; batch no. 00831-274, purity: 98.7 %).
Test system: Salmonella typhimurium strains TA 100, TA 1535, TA 1537 and TA 98 and Escherichia coli strain WP2 uvrA
BH 635-3 (Reg.-No. 335 182) was tested for its mutagenic potential based on the ability to induce back mutations in selected loci of several bacterial strains in the Ames reverse mutation assay. The Salmonella typhimurium strains TA 100, TA 1535, TA 1537 and TA 98 and Escherichia coli strain WP2 uvrA were exposed to the test substance dissolved in DMSO. The study consisted of a standard plate test (with doses ranging from 20 to 5000 µg/plate) and preincubation test (with doses ranging from 20 to 5000 µg/plate) both with and without metabolic activation (Aroclor induced Sprague-Dawley rat liver S-9 mix). Three plates were used per dose for each strain and test condition.

For control purposes and to demonstrate the sensitivity of the test system, negative controls (with and without S9 mix) and positive controls (2-aminoanthracene - with S9 mix; N-methyl-N'-nitro-N-nitrosoguanidine, 4-nitro-o-phenylendiamine, 9-aminoacridine and 4-nitroquinolineN-oxide - all without S9 mix) were tested.

A substance is considered positive in this test if the following criteria are met:
- there is at least a doubling of the negative control mutation rate with or without S-9 mix,
- there is a dose-response relationship, and
- the results are reproducible.

Findings:
The stability of the test substance throughout the study period was verified by reanalysis. The stability of BH 635-3 in the vehicle DMSO over a period of 4 hours and in water over a period of 4 and 96 hours have been determined analytically. No test substance precipitation was observed. No bacteriotoxic effect was noticed. The mean number of revertant colonies was not increased in any strain either with or without S-9 activation. Expected increases in revertant colonies were obtained with the positive controls.

Conclusion:
According to the results of the study, BH 635-3 (Reg.-No. 335 182) is not mutagenic in the Salmonella typhimurium/Escherichia coli reverse mutation assay.

B.6.8.1.2.2 Second mutagenicity study

Report: Wollny H.-E., 1999
Gene mutation assay in Chinese hamster V79 cells *in vitro* (V79 / HPRT) with Reg.-No. 335 182; BH 635-3
RCC Cytotest Cell Research GmbH, Roßdorf, Germany Fed. Rep.
unpublished
BASF RegDoc# 1999/12026

GLP: Yes (Laboratory certified by Hessisches Ministerium fuer Umwelt, Energie, Jugend, Familie und Gesundheit, Wiesbaden)

Guideline: OECD 476, EEC 87/302

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: 635M03 (Reg.-No. 335 182; BH 635-3; batch no. 01185-085, purity: 99.8 %.
Test system: Chinese hamster ovary (CHO) cells
635M03 (BH 635-3, Reg.-No. 335 182) was tested for its ability to induce gene mutations at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in Chinese hamster ovary (CHO) cells *in vitro*. Two independent experiments were carried out, using two parallel cultures each either with or without the addition of Aroclor 1254-induced Sprague-Dawley rat liver S-9 mix (exogenous metabolic activation). In an initial range-finding test the solubility of the test substance was reduced at doses > 300 µg/ml. No changes in osmolarity and pH-values were observed.
Therefore the test substance was evaluated at the following doses in the 1st experiment (4 hours exposure): Without and with S-9 mix 0: 18.8; 37.5; 75.0; 150.0; and 300.0 µg/ml.
A 2nd experiment for confirmation was performed using the following doses (4 hours exposure): Without and with S-9 mix: 0; 18.8; 37.5; 75.0; 150.0; and 300.0 µg/ml.
After an attachment period of 20 - 24 hours, a treatment period of 4 hours both with and without metabolic activation, an expression phase of about 7 - 9 days and a selection period of about 1 week, the colonies of each test group were stained with 10 % methylene blue in 0.01 % KOH solution and counted. For control purposes and to demonstrate the sensitivity of the test system, negative controls and two appropiate positive control chemicals 7,12-dimethylbenz(a)anthracene (with S9 mix) and Ethylmethane sulfonate (without S9 mix) were tested.
The criteria for a positive response are:
- Increases of the mutation frequencies, that are three times above the spontaneous mutation frequency at least at one of the concentrations in the experiment.
- Evidence of reproducibility of any increase in mutant frequencies.
- Evidence of a dose-response relationship, even if a threefold increase of the mutant frequency is not observed.

Findings:
The stability of 635M03 (BH 635-3; Reg.-No. 335 182) in the vehicle DMSO and in water has been confirmed by analysis. Moderate cytotoxicity was observed by reduced cloning efficiency at doses > 150 µg/ml and > 300 µg/ml (without metabolic activation) in the 1st

experiment in culture I. The negative controls (untreated and vehicle controls) gave mutant frequencies within the range expected for the CHO cell line, except the negative and solvent control in culture I of the first experiment. This deviation was considered to be not of biological relevance since the effect was not observed in the parallel culture and the positive control and the test substance gave results within the expected range. Appropriate positive control chemicals led to the expected increase in the frequencies of forward mutations. The test substance did not cause an increase in the mutant frequencies either without S-9 mix or after adding a metabolising system in both main experiments performed independently of each other.

Conclusion:
Under the experimental conditions of this assay, 635M03 (BH 635-3; Reg.-No. 335 182) is considered to be non-mutagenic *in vitro* in the CHO/HPRT mutation assay.

B.6.8.1.2.3 Third mutagenicity study

Report: Czich A. et al., 1999
In vitro chromosome aberration assay in Chinese hamster V79 cells with Reg.-Nr. 335 182 (BH 635-3)
RCC Cytotest Cell Research GmbH, Roßdorf, Germany Fed. Rep.
unpublished
BASF RegDoc# 1999/11504

GLP: Yes (Laboratory certified by Hessisches Ministerium fuer Umwelt, Energie, Jugend, Familie und Gesundheit, Wiesbaden)

Guideline: OECD 473, EEC 92/69, B 10

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: 635M03 (Reg.-No. 335 182; BH 635-3; batch no. 01185-085, purity: 99.8 %.
Test system: V79 cells
635M03 (BH 635-3; Reg.-No. 335 182) was assessed for its potential to induce structural chromosomal aberrations in V79 cells of the Chinese hamster *in vitro* both in the presence and in the absence of a metabolising system (S-9 mix of Aroclor 1254-induced Sprague-Dawley rat liver).
Test substance precipitation in the pretest occurred at concentrations of ≥ 625 µg/ml with and without S-9 mix. According to the initial range-finding cytotoxicity test and the solubility properties of the test substance the following doses were evaluated.
1st experiment:

- 4 hours exposure, 14 hours recovery time, and 18 hours preparation interval;
  with and without S-9 mix: 0; 312.5; 625.0 and 1250.0 µg/ml,

2nd experiment:

- 18 hours exposure, 18 hours preparation interval,
  without S-9 mix: 0; 156.3; 312.5; and 625.0 µg/ml

- 18 hours exposure, 10 hours recovery time, and 28 hours preparation interval, without S-9 mix: 0; 1250.0 µg/ml
- 4 hours exposure, 24 hours recovery time, and 28 hours preparation interval, with S-9 mix: 0; 312.5; 625.0 and 1250.0 µg/ml

The cell cycle of the untreated V79 cells is about 13 - 14 hours under the selected culture conditions. Thus, the selected 1st sampling time of 18 hours was within the 1 - 1.5 x the normal cell cycle time, as recommended by the OECD Guideline No. 473. The later sampling time of 28 hours was chosen to cover a possible cell cycle delay. About 2 - 3 hours prior to harvesting the cells, colcemid was added to arrest cells in a metaphase-like stage of mitosis (c-metaphases). After preparation of the chromosomes and staining with Giemsa, 100 metaphases for each culture were analysed for chromosomal aberrations. For each experiment two cultures were used.
The criteria for a positive response are:

- The number of induced structural chromosomal aberrations are not in the range of the historical control data.
- Either a concentration-related or a significant increase of the number of structural chromosome aberrations are observed.

A test substance is generally considered non clastogenic in this test system if:

- There was no significant increase in the number of chromosomally damaged cells at any dose above concurrent control frequencies.
- The aberration frequencies were within the historical control range.

Findings:
The stability of the test substance throughout the study period was guaranteed. The stability of 635M03 (BH 635-3; Reg.-No. 335 182) in the vehicle DMSO and in water over a period of 4 hours was determined analytically. Test substance precipitation occurred at concentrations of 1250 µg/ml. In the 1st experiment no signs of toxicity were noticed up to the highest evaluated concentrations. Reduced mitotic indices were observed in the 2nd experiment after 18 hours of treatment without S-9 mix at the highest evaluated concentrations. Osmolarity and pH values were not influenced by test substance treatment. Both of the positive control chemicals, EMS (ethylmethane sulfonate) and cyclophosphamide, led to a significant increase ($p < 0,05$) in the number of cells with structural chromosomal aberrations. The test substance did not cause any biologically relevant or statistically significant increase in the number of cells carrying structural chromosomal aberrations in both independent experiments. No increase in the frequencies of polyploid metaphases was found after treatment with the test substance as compared to the frequencies of the control.

Conclusion:
635M03 (BH 635-3; Reg.-No. 335 182) is not a chromosome-damaging (clastogenic) agent under *in vitro* conditions in V79 cells.

B.6.8.1.2.4 Acute oral toxicity study

Report: Wiemann C., Hellwig J., 1998
Report: Reg.-No. 335 182 (BH 635-3): Acute oral toxicity in rats
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 1998/10843
and
Wiemann C., 1999
Amendment No. 1: Reg.-No. 335 182 (BH 635-3) - Acute oral toxicity in rats
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 1999/11221

GLP: Yes (Laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 92/69, OECD 401

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: 635M03 (Reg.-No. 335 182; BH 635-3); batch no. 00831-274, purity: 98.7 %; batch no. 01185-044, purity 99.4 %.
Test animals: Wistar rats (Chbb:THOM (SPF))
Single administration of the test substance preparation in 0.5 % aqueous Tylose CB 30.000 by gavage to five male and five female fasted Wistar rats at dose levels of 2000 and 5000 mg/kg bw, using an application volume of 20 ml/kg bw. The observation period lasted for up to 14 days.

Findings:
The stability of the test substance was guaranteed. The stability of the test substance in the vehicle for a period of 4 hours was confirmed by analysis. The correctness of the concentration and its homogeneity were analytically confirmed. There was no mortality in either males or females. No signs of toxicity were noticed. Body weight development was normal. There were no macroscopic pathological findings in animals sacrificed at the end of the observation period.

Conclusion:
The oral LD_{50} was found to be > 5000 mg/kg bw for male and female rats.

B.6.8.1.2.5 Subchronic toxicity study

Report: Mellert W. et al., 2001
Report: Reg.-No. 335 182 (BH 635-3) – Subchronic toxicity study in Wistar rats. Administration in the diet for 3 months
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 2001/1006072

GLP: Yes (Laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 87/302, OECD 408, EPA/OPPTS 870.3100

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: 635M03 (Reg.-No. 335 182; BH 635-3); batch no. 01185-269, purity: 99.2 %.
Test animals: Wistar rats CRL:WI(GLX/BRL/HAN)IGS BR]
635M03 (BH 635-3) was administered to groups of 10 male and 10 female Wistar rats at dietary concentrations of 0; 1000; 5000; and 15000 ppm for 3 months. Food consumption, water consumption (from day 49 onward) and body weights were determined each week. The animals were examined for signs of toxicity or mortality at least once a day. Detailed clinical examinations in an open field were conducted prior to the administration period and weekly during the administration period. A functional observational battery (FOB) and motor activity measurement (MA) was carried out at the end of dosing. Ophthalmological examinations were carried out prior to the start and towards the end of dosing. Urinalysis, clinico-chemical and hematological examinations were carried out at the end of the administration period. All animals were subjected to gross-pathological assessment, followed by histopathological examinations.

Findings:
The stability of the test substance during the study period was demonstrated. The stability and homogeneous distribution of the test substance in the diet were confirmed by analysis. The correctness of the concentrations were analytically proven.

Table B.6.8.1-25: Test substance intake (mg/kg bw)

Dietary dose level (ppm)	Males	Females
1000	79	83
5000	392	478
15000	1187	1440

There were no mortalities or clinical signs of toxicity in any of the dose groups. Findings during FOB were assessed not to be test substance related and during MA there were no substance related effects noticed. There were no effects on body weight gain or food consumption in any of the dose groups. Clinical-chemical and hematological examinations did not show any treatment related changes in the parameters measured at any dose level either in males or females.

Urinalysis revealed increased numbers of crystals of unknown origin in 4 of 10 male and 8 of 10 females of the highest test group. In the mid dose group 9 of 10 female rats showed also an increased number of crystals. This were the only test substance related findings. They are considered to be of no pathological relevance, since the formation of crystals occurs when chemicals constituents become saturated and undergo altered solubilities when urine is stored at cooler temperatures. Therefore the formation of crystals is regarded as an artifact of the system of collection.
There were no test substance related effects seen in ophthalmoscopy. Organ weight determination did not show any relation to the treatment. Neither gross macroscopical nor microscopical examinations detected any test substance related changes in the organs. In conclusion no substance related adverse effects were observed in this study.

Conclusion:
The no observed adverse effect level in this 90-day dietary rat study was 15000 ppm (equal to 1187 mg/kg bw).

B.6.8.1.3 635M01

635M01 (Reg.-No. 335 184; BH 635-4) is a soil metabolite. It was detected in the rat metabolism study (urine, faeces, bile). Nevertheless, it was tested in three mutagenicity assays as well as in an acute oral test. It was found to be not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay, not mutagenic *in vitro* in the CHO/HPRT mutation assay and not chromosome-damaging (clastogenic) under *in vitro* conditions in V79 cells. An acute oral toxicity study revealed an LD_{50} of > 5000 mg/kg bw. A 28-day dietary toxicity study in rats was submitted after the completion of the DAR. In the 28-day dietary study, no signs of toxicity were observed in treated Wistar rats up to 4300 ppm (corresponding to daily intakes of 334.4 and 352.6 mg/kg bw, respectively), which was the highest dose level tested. Thus, the metabolite 635M01 is not of toxicological relevance in groundwater.

Table B.6.8-3: Summary of toxicity studies of metabolite Reg. 635M01

Study/strains/species	Test material/conditions	Results
Acute oral toxicity in rats Wistar rats (Chbb:THOM (SPF))	Batch no. 01185-088, purity: 97.0 %	LD_{50}: > 5000 mg/kg bw
Salmonella typhimurium/Escherichia coli reverse mutation assay S. typhimurium strains TA 100, TA 1535, TA 1537, TA 98 and E. coli strain WP2 uvrA	Batch no. 00831-277, purity: 97.9 %	Not mutagenic
Gene mutation assay in Chinese hamster V79 cells *in vitro* (V79 / HPRT) Chinese hamster ovary (CHO) cells	Batch no. 01185-088, purity: 97.0 %	Not mutagenic
In vitro chromosome aberration assay in V79 cells (cells derived Chinese hamster)	Batch no. 01185-088, purity: 97.0 %	Not mutagenic
28-d oral (diet) toxicity study in Wistar rats (CrlGlxBrlHan:WI) 10 male / 10 female 0; 430; 1300 and 3900 ppm (corresponding to approximately 38, 115 and 344 mg/kg bw/d)	Batch no. 2059-011, purity: 96.4 %.	NOAEL: 3900 ppm No signs of toxicity

B.6.8.1.3.1 First mutagenicity study

Report:	Engelhardt G., Hoffmann H. D., 1998 Report: Salmonella typhimurium/Escherichia coli reverse mutation assay (standard plate test and preincubation test) with Reg.-No. 335 184 (BH 635-4) BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep. unpublished BASF RegDoc# 1998/11635
GLP:	Yes (Laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)
Guideline:	OECD 471, EEC 92/69 B 13, EEC 92/69 B 14
Deviations:	None
Acceptability:	The study is considered to be acceptable.

Material and Methods:
Test material: 635M01 (Reg.-No. 335 184; BH 635-4); batch no. 00831-277, purity: 97.9 %.
Test system: Salmonella typhimurium strains TA 100, TA 1535, TA 1537 and TA 98 and Escherichia coli strain WP2 uvrA
635M01 was tested for its mutagenic potential based on the ability to induce back mutations in selected loci of several bacterial strains in the Ames reverse mutation assay. The Salmonella typhimurium strains TA 100, TA 1535, TA 1537 and TA 98 and Escherichia coli strain WP2 uvrA were exposed to the test substance dissolved in DMSO. The study consisted of a standard plate test (with doses ranging from 20 to 5000 µg/plate) and preincubation test (with doses ranging from 20 to 5000 µg/plate) both with and without metabolic activation (Aroclor induced Sprague-Dawley rat liver S-9 mix). Three plates were used per dose for each strain and test condition.

For control purposes and to demonstrate the sensitivity of the test system, negative controls (with and without S9 mix) and positive controls (2-aminoanthracene - with S9 mix; N-methyl-N'-nitro-N-nitrosoguanidine, 4-nitro-o-phenylendiamine, 9-aminoacridine, and 4-nitroquinoline-N-oxide - all without S9 mix) were tested.
A substance is considered positive in this test if the following criteria are met:
- there is at least a doubling of the negative control mutation rate with or without S-9 mix, there is a dose-response relationship, and
- the results are reproducible.

Findings:
The stability of the test substance throughout the study period was guaranteed. The stability of 635M01 (Reg.-No. 335 184) in the vehicle DMSO over a period of 4 hours and in water over a period of 4 and 96 hours has been determined analytically. Test substance precipitation was observed at 5000 µg/plate. A slight bacteriotoxic effect was noticed at about 5000 µg/plate. The mean number of revertant colonies was not increased in any strain either with or without S-9 activation. Expected increases in revertant colonies were obtained with the positive controls.

Conclusion:
According to the results of the study, 635M01 (Reg.-No. 335 184) is not mutagenic in the Salmonella typhimurium/Escherichia coli reverse mutation assay.

B.6.8.1.3.2 Second mutagenicity study

Report: Wollny H.-E. et al., 1999
Gene mutation assay in Chinese hamster V79 cells *in vitro* (V79 / HPRT) with Reg.-No. 335 184; BH 635-4
RCC Cytotest Cell Research GmbH, Roßdorf, Germany Fed. Rep.
unpublished
BASF RegDoc# 1999/12016

GLP: Yes (Laboratory certified by Hessisches Ministerium fuer Umwelt, Energie, Jugend, Familie und Gesundheit, Wiesbaden)

Guideline: OECD 476, EEC 87/302

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: 635M01 (Reg.-No. 335 184; BH 635-4); batch no. 01185-088, purity: 97.0 %.
Test system: Chinese hamster ovary (CHO) cells

635M01 was tested for its ability to induce gene mutations at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in Chinese hamster ovary (CHO) cells *in vitro*. Two independent experiments were carried out, using two parallel cultures each either with or without the addition of Aroclor 1254-induced Sprague-Dawley rat liver S-9 mix (exogenous metabolic activation). In an initial range-finding solubility of the test substance was reduced at doses > 300 µg/ml. No changes in osmolarity and pH-values were observed. Due to the solubility of the test substance in DMSO the following doses were evaluated in the 1st experiment (4 hours exposure): Without and with S-9 mix: 0; 56.3; 112.5; 225.0; 450.0 and 900.0 µg/ml. A 2nd experiment for confirmation was performed using the following doses (4 hours exposure): Without and with S-9 mix: 0; 56.3; 112.5; 225.0; 450.0 and 900.0 µg/ml.
After an attachment period of 20 - 24 hours, a treatment period of 4 hours both with and without metabolic activation, an expression phase of about 7 - 9 days and a selection period of about 1 week, the colonies of each test group were stained with 10 % methylene blue in 0.01 % KOH solution and counted. For control purposes and to demonstrate the sensitivity of the test system, negative controls and two appropiate positive control chemicals 7,12-dimethylbenz(a)anthracene (with S9 mix) and Ethylmethane sulfonate (without S9 mix) were tested.
The criteria for a positive response are:

- Increases of the mutation frequencies, that are three times above the spontaneous mutation frequency at least at one of the concentrations in the experiment.
- Evidence of reproducibility of any increase in mutant frequencies.
- Evidence of a dose-response relationship, even if a threefold increase of the mutant frequency is not observed.

Findings:
The stability of 635M01 in the vehicle DMSO and in water was analytically confirmed. No cytotoxicity was observed at the maximum concentration of 900 µg/ml. Test substance precipitation occurred at > 450 µg/ml (1st experiment) and > 225 µg/ml (2nd experiment) in the absence and at > 900 µg/ml in the presence of metabolic activation throughout the experiments. The negative controls (untreated and vehicle controls) gave mutant frequencies within the range expected for the CHO cell line. Appropriate positive control chemicals led to the expected increase in the frequencies of forward mutations. The test substance did not cause an increase in the mutant frequencies either without S-9 mix or after adding a metabolising system in both main experiments performed independently of each other.

Conclusion:
Under the experimental conditions of this assay, 635M01 is considered to be non-mutagenic *in vitro* in the CHO/HPRT mutation assay.

B.6.8.1.3.3 Third mutagenicity study

Report:	Engelhardt G., Hoffmann H. D., 1999 Report: *In vitro* chromosome aberration assay with Reg.-Nr. 335 184 (BH 635-4) in V79 cells BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep. unpublished BASF RegDoc# 1999/11685
GLP:	Yes (Laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)
Guideline:	OECD 473, EEC 92/69 B 10
Deviations:	None
Acceptability:	The study is considered to be acceptable.

Material and Methods:
Test material: 635M01 (Reg.-No. 335 184; BH 635-4); batch no. 01185-088, purity: 97.0 %.
Test system: V79 cells (Chinese hamster derived)

635M01 was assessed for its potential to induce structural chromosomal aberrations in V79 cells *in vitro* both in the presence and in the absence of a metabolising system (S-9 mix of Aroclor 1254-induced Sprague-Dawley rat liver). According to an initial range-finding cytotoxicity test and the solubility of the test substance the following doses were evaluated.
1st experiment:
- 4 hours exposure, 18 hours harvest time, with and without S-9 mix:
  0; 500; 1000; and 2000 µg/ml,

2nd experiment:
- 18 hours exposure, 18 hours harvest time, without S-9 mix:
  0; 500; 1000; and 2000 µg/ml,
- 18 hours exposure, 28 hours harvest time, without S-9 mix:
  0; 2000 µg/ml

- 4 hours exposure, 28 hours harvest time, with S-9 mix:
  0; 500; 1000; and 2000 µg/ml.

The cell cycle of the untreated V79 cells is about 13 - 14 hours under the selected culture conditions. Thus, the selected 1st sampling time of 18 hours was within the 1 - 1.5 x the normal cell cycle time, as recommended by the OECD Guideline No. 473. The later sampling time of 28 hours was chosen to cover a possible cell cycle delay. About 2 - 3 hours prior to harvesting the cells, colcemid was added to arrest cells in a metaphase-like stage of mitosis (c-metaphases). After preparation of the chromosomes and staining with Giemsa, 100 metaphases for each culture in the case of the test substance and vehicle controls, or 50 cells for each culture in the case of the concurrent positive controls, were analysed for chromosomal aberrations.

The criteria for a positive response are:
- A dose-related and reproducible significant increase in the number of structural chromosomal aberrations.
- The proportion of aberrations exceeded both the concurrent negative control range and the negative historical control range.
  A test substance is generally considered non clastogenic in this test system if:
- There was no significant increase in the number of chromosomally damaged cells at any dose above concurrent control frequencies.
- The aberration frequencies were within the historical control range.

Findings:
The stability of the test substance throughout the study period was verified by reanalysis. Homogeneity of the test substance was achieved by mixing and was verified by analysis. The stability of a comparable batch in the vehicle DMSO over a period of 4 hours and in water for 96 hours has been determined analytically. Test substance precipitation occurred at concentrations of 1000 µg/ml and higher. According to the results of the determination of the mitotic index, no suppression of the mitotic activity was observed under any of the experimental conditions. No growth inhibition was noticed by cell count. Cell attachment was slightly reduced from about 1000 µg/ml. Osmolarity and pH values were not influenced by test substance treatment. The negative controls (vehicle controls) gave frequencies of aberrations within the range expected for the V79 cell line. Both of the positive control chemicals, EMS (ethyl methane sulfonate) and cyclophosphamide, led to the expected increase in the number of cells containing structural chromosomal aberrations.

On the basis from the results of the present study, the test substance did not cause any increase in the number of structurally aberrant metaphases incl. and excl. gaps at both sampling times either without S-9 mix or after adding a metabolising system in two experiments performed independently of each other. No increase in the frequency of cells containing numerical aberrations was demonstrated either.

Conclusion:
635M01 is not a chromosome-damaging (clastogenic) agent under *in vitro* conditions in V79 cells.

B.6.8.1.3.4 Acute oral toxicicty study

Report: Wiemann C., Hellwig J., 1999
Report: Reg.-No. 335 184 (BH 635-4) - Acute oral toxicity in rats

BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 1999/10213

GLP: Yes (Laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 92/69, OECD 401

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: 635M01 (Reg.-No. 335 184; BH 635-4); batch no. 01185-088, purity: 97.0 %.
Test animals: Wistar rats (Chbb:THOM (SPF))

Single administration of a aqueous test substance preparation by gavage to five male and five female fasted Wistar rats at dose levels of 5000 mg/kg bw, using an application volume of 20 ml/kg bw respectively. The observation period lasted for up to 14 days.

Findings:
The stability of the test substance was guaranteed for the duration of the study. The homogeneity of the test substance was confirmed by analysis. The stability of the test substance in aqua bidest for a time period of 96 hours was confirmed by analysis. The correctness of the concentration and the homogeneity of the test substance preparation were analytically confirmed. There was no mortality in either males or females. Signs of toxicity were not noted. Body weight development appeared to be normal. There were no macroscopic pathological findings in animals sacrificed at the end of the observation period.

Conclusion:
The oral LD_{50} was found to be > 5000 mg/kg bw for male and female rats.

B.6.8.1.3.5 28-day toxicity study in Wistar rats

Report: Kaspers U. et al., 2003
Reg. No. 335 184 (metabolite of BAS 635 H) - Subacute toxicity study in Wistar rats administration in the diet for 4 weeks
BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep., unpublished

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: EEC 96/54, OECD 407

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Reg. No. 335 184; batch no. 2059-011, purity: 96.4 %.
Test animals: Male and female Wistar rats (CrlGlxBrlHan:WI), supplied by Charles River, Germany. Age: 33-35 days.
Reg. No. 335 184 (635M01) was administered for 4 weeks to groups of 10 male and 10 female Wistar rats at dietary concentrations of 0; 430; 1300 and 3900 ppm (corresponding to approximately 38, 115 and 344 mg/kg bw/d). Food consumption and body weight were determined weekly. The animals were examined for signs of toxicity or mortality at least once a day. Detailed clinical examinations in an open field were conducted prior to the start of the administration period and weekly thereafter. A functional observational battery (FOB) and measurement of motor activity was carried out at the end of the study The FOB included evaluation of home cage observations, Open Field examinations, sensimotor tests/reflexes, quantitative parameters (feces, rearing, grip strength, landing foot splay). Ophthalmologic examinations were performed in all animals before and in control and high dose animals at the end of the administration period. Clinicochemical, hematological examinations and urinalyses were performed towards the end of the administration period. Finally, animals were assessed by gross pathology, followed by histopathological examinations.

Findings:
Sufficient stability over the study period and homogenous distribution of the test substance in the diet was demonstrated. Concentration control analyses showed that actual concentrations in the diet were within a range of 95.0–109.9 % of the nominal concentrations.

Mortality
No animal died during the study.

Clinical examinations
No substance-related effects were observed.

Food consumption, water consumption and body weight data
No substance-related effects were obtained.

Food efficiency
Food efficiency was statistically significantly reduced in male rats at 430 ppm on day 14. This isolated finding in low dose males was considered incidental and not related to the treatment.

Functional observation battery
No treatment-related effects were evident in the FOB evaluations. Regarding the overall motor activity (summation of all intervals) no substance-related findings were observed in both sexes.
Single intervals were statistically significantly increased in animals administered 1300 ppm (in males at interval 1 and in females at interval 4). These two isolated findings were assessed as fortuitous and not related to the treatment with the test compound, due to the lack of a dose-response relationship connected with no influence on the overall motor activity.

Opthalmologic examinations
No effects that could be related to treatment were observed.

Hematology, clinicochemistry and urinalyses
Substance-related effects were not evident at any dose level in either males or females.

Organ weight changes
Absolute and relative spleen weights were statistically significantly increased in low-dose group females (+16.4 % and 15.7 %, respectively). In the absence of a dose-response relationship, this finding is judged to be incidental. All other weight parameters did not show significant differences when compared to the control group.

Gross lesions and histopathology
No treatment-related findings were observed.

Conclusion:
In conclusion, no signs of toxicity were observed in treated animals at any of the dietary concentrations tested. Thus, the NOAEL was 3900 ppm for both sexes (corresponding to daily intakes of 334.4 and 352.6 mg/kg bw/d in males and females, respectively).

B.6.8.1.4 635M17

635M17 (Reg. No. 373 906) is a plant metabolite. It was detected in the rat metabolism study in minor quantities. It was tested in three mutagenicity assays as well as in an acute oral test. It was found to be not mutagenic in the Salmonella typhimurium/Escherichia coli reverse mutation assay, not mutagenic *in vitro* in the CHO/HPRT mutation assay and not chromosome-damaging (clastogenic) *in vivo*. There were no indications of any impairment of chromosome distribution in the course of mitosis. An acute oral toxicity study revealed an LD_{50} of > 2000 mg/kg bw. Thus, the metabolite 635M017 is not of toxicological relevance in groundwater.

Table B.6.8-4: Summary of toxicity studies of metabolite 635M17

Study/strains/species	Test material/ conditions	Results
Acute oral toxicity study in Wistar rats Crl: WI(GLX/BRL/HAN)IGS BR (SPF)	Batch No. 01742-22, purity: 98.3%	LD_{50}: >2000 mg/kg bw
Salmonella typhimurium/Escherichia coli reverse mutation assay (standard plate test and preincubation test) S. typhimurium strains TA 100, TA 1535, TA 1537, TA 98 and E. coli strain WP2 uvrA	Batch No. 01742-22, purity: 98.3%.	Not mutagenic
In vitro gene mutation test in Chinese hamster ovary (CHO) cells (HPRT Locus Assay)	Batch No. 01742-22, purity: 98.3%	Not mutagenic
Cytogenetic study *in vivo* in the mouse micronucleus test after two intraperitoneal administrations (NMRI mice)	Batch No. 01742-22, purity: 98.3%.	Not mutagenic

B.6.8.1.4.1 First mutagenicity study

Report: Engelhardt G., Hoffmann H. D. 2000
Report: Salmonella typhimurium/Escherichia coli reverse mutation assay (standard plate test and preincubation test) with Reg.-No. 373 906
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 2001/1006087

GLP: Yes (Laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 471, EEC 2000/32 B 13, EEC 2000/32 B 14

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: 635M17 (Reg. No. 373 906); batch no. 01742-22, purity: 98.3%
Test system: Salmonella typhimurium strains TA 100, TA 1535, TA 1537 and TA 98 and Escherichia coli strain WP2 uvrA

635M17 (Reg. No. 373 906) was tested for its mutagenic potential based on the ability to induce back mutations in selected loci of several bacterial strains in the Ames reverse mutation assay. The Salmonella typhimurium strains TA 100, TA 1535, TA 1537 and TA 98 and Escherichia coli strain WP2 uvrA were exposed to the test substance dissolved in acetone. The study consisted of a standard plate test (with doses ranging from 20 to 5,000 µg/plate) and preincubation test (with doses ranging from 4 to 2,500 µg/plate) both with and without metabolic activation (Aroclor induced Sprague-Dawley rat liver S-9 mix). Three plates were used per dose for each strain and test condition.

For control purposes and to demonstrate the sensitivity of the test system, negative controls (with and without S9 mix) and positive controls (2-aminoanthracene - with S9 mix; N-methyl-N'-nitro-N-nitrosoguanidine, 4-nitro-o-phenylendiamine, 9-aminoacridine and 4-nitroquino-line-N-oxide - all without S9 mix) were tested.
A substance is considered positive in this test if the following criteria are met:
- there is at least a doubling of the negative control mutation rate with or without S-9 mix,
- there is a dose-response relationship, and
- the results are reproducible.

Findings:
The stability of the test substance throughout the study period was verified by reanalysis. The stability of Reg. No. 373 906 in the vehicle acetone and in water over a period of 4 hours has been determined analytically. No test substance precipitation was observed. A slight bacteriotoxic effect was noticed from about 500 – 2500 µg/plate depending on the strain and test conditions. The mean number of revertant colonies was not increased in any strain either with or without S-9 activation. Expected increases in revertant colonies were obtained with the positive controls.

Conclusion:
According to the results of the study, 635M17 is not mutagenic in the Salmonella typhimurium/Escherichia coli reverse mutation assay.

B.6.8.1.4.2 Second mutagenicity study

Report: Engelhardt G., Hoffmann H. D. 2000
Report: *In vitro* gene mutation test with Reg.-No. 373 906 in CHO

cells (HPRT Locus Assay)
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 2001/1006073

GLP: Yes (Laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 476, EEC 2000/32 B 17

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: 635M17 (Reg. No. 373 906); batch no. 01742-22, purity: 98.3%.
Test system: Chinese hamster ovary (CHO) cells

Reg. No. 373 906 was tested for its ability to induce gene mutations at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in Chinese hamster ovary (CHO) cells *in vitro*. Two independent experiments were carried out, using two parallel cultures each either with or without the addition of Aroclor 1254-induced Sprague-Dawley rat liver S-9 mix (exogenous metabolic activation).

In an initial range-finding test cytotoxicity was observed by reduced cloning efficiency at doses > 1000 µg/ml (with and without metabolic activation). Precipitation of the test substance occurred at doses > 1000 µg/ml. No changes in osmolarity and pH-values were observed.
Thus, the test substance was evaluated at the following doses in the 1st experiment (4 hours exposure): Without S-9 mix: 0; 31.25; 62.5; 125; 250; 500; and 1000 µg/ml
With S-9 mix: 0; 62.5; 125; 250; 500; 1000; and 2000 µg/ml
A 2nd experiment for confirmation was performed using the following doses (4 hours exposure): Without and with S-9 mix: 0; 62.5; 125; 250; 500; 1000; and 2000 µg/ml.

After an attachment period of 20 - 24 hours, a treatment period of 4 hours both with and without metabolic activation, an expression phase of about 7 - 9 days and a selection period of about 1 week, the colonies of each test group were fixed with methanol, stained with Giemsa, and counted. For control purposes and to demonstrate the sensitivity of the test system, negative controls and two appropiate positive control chemicals methylcholanthrene (with S9 mix) and Ethyl methane sulfonate (without S9 mix) were tested.

The criteria for a positive response are:
- Increases of the corrected mutation frequencies above the concurrent negative control values and above 15 mutants per 106 clonable cells and/or the evidence of a dose response relationship in the increase in mutant frequencies.
- Evidence of reproducibility of any increase in mutant frequencies.
- A statistically significant increase in mutant frequencies and the existence of a dose-response relationship.

Findings:
The stability of the test substance throughout the study period was determined by reanalysis. Its homogeneity was guaranteed. The stability of Reg. No. 373 906 in the vehicle acetone and in water over a period of 4 hours was verified by analysis. Cytotoxicity was observed at > 1000 µg/ml (with and without metabolic activation). The negative controls gave mutant frequencies within the range expected for the CHO cell line. Both of the positive control chemicals led to the expected increase in the frequencies of forward mutations. The test substance did not cause any increase in the mutant frequencies either without S-9 mix or after adding a metabolising system in both main experiments performed independently of each other.

Conclusion:
Under the experimental conditions of this assay, 635M17 (Reg. No. 373 906) is considered to be non-mutagenic *in vitro* in the CHO/HPRT mutation assay.

B.6.8.1.4.3 Third mutagenicity study

Report:	Engelhardt G., Hoffmann H. D. 2000 Report: Cytogenetic study *in vivo* with Reg. No. 373 906 in the mouse micronucleus test after two intraperitoneal administrations BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep. unpublished BASF RegDoc# 2000/1018736
GLP:	Yes (Laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)
Guideline:	OECD 474, EEC 2000/32 B 12
Deviations:	None
Acceptability:	The study is considered to be acceptable.

Material and Methods:
Test material: 635M17 (Reg. No. 373 906); batch no. 01742-22, purity: 98.3%.
Test animals: NMRI mice

635M17 was tested for clastogenicity and for the ability to induce spindle poison effects in NMRI mice using the micronucleus test method. For this purpose the test substance, suspended in an aqueous 0.5% CMC (carboxymethyl cellulose) formulation, was administered twice intraperitoneally with an 24-hour interval between administration, to male animals at dose levels of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight in a volume of 10 ml/kg body weight in each case.

As a negative control the vehicle, 0.5 % CMC was applicated to male mice by the same route. As a positive control, 20 mg cyclophosphamide (CPP)/kg body weight or 0.15 mg vincristine sulphate (VCR)/kg body weight, both, dissolved in purified water, were administered to male and female animals once intraperitoneally each in a volume of 10 ml/kg body weight.

The animals were sacrificed and the bone marrow of the two femora was prepared 24 hours after the second administration. After staining of the preparations, 2,000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The normocytes with and without micronuclei occurring per 2000 polychromatic erythrocytes were also registered.

The test chemical is to be considered positive in this assay if the following criteria are met:
- A dose-related and significant increase in the number of micronucleated polychromatic erythrocytes.
- The proportion of cells containing micronuclei exceed both, the values of the concurrent negative control range and the negative historical control range.

Findings:
The stability of the test substance was verified by reanalysis. Homogeneity of the test substance was achieved by mixing. The stability of Reg. No. 373 906 in water over a period of 4 hours was analytically confirmed. The homogeneity of the test substance in the vehicle was guaranteed by constant stirring before removal and administration and by analytical determination of 3 individual samples of each concentration. Animals which were administered the vehicle or the positive control substances cyclophosphamide or vincristine did not show any clinical signs of toxicity. The administration of the test substance led to evident signs of toxicity as there were piloerection and squatting posture in all dose groups. All animals recovered within of 2 days after treatment at latest. The negative control gave frequencies of micronucleated polychromatic erythrocytes within the historical control range. Both of the positive control chemicals, cyclophosphamide for clastogenicity and vincristine for aneugenic effects, led to the expected increase in the rate of polychromatic erythrocytes containing small or large micronuclei. An inhibition of erythropoiesis determined from the ratio of polychromatic to normocromatic erythrocytes induced by the treatment of mice with Reg. No. 373 906 was detected at a dose of 400 mg kg body weight.
According to the results of the present study, the two intraperitoneal administrations of Reg. No. 373 906 did not lead to any increase in the number of polychromatic erythrocytes containing either small or large micronuclei. The rate of micronuclei was always in the same range as that of the concurrent negative control in all dose groups and within the range of the historical control data.

Conclusion:
The test substance 635M17 (Reg. No. 373 906) has no chromosome-damaging (clastogenic) effect, and there were no indications of any impairment of chromosome distribution in the course of mitosis.

B.6.8.1.4.4 Acute oral toxicity study

Report:	Gamer A. O., Hoffmann H. D. 2000 Report: Reg. No. 373 906 – Acute oral toxicity study in Wistar rats BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep. unpublished BASF RegDoc# 2001/1006074
GLP:	Yes (Laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 423, EEC 96/54

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: 635M17 (Reg. No. 373 906); batch no. 01742-22, purity: 98.3%.
Test animals: Wistar rats (SPF) Crl: WI(GLX/BRL/HAN)IGS BR

Single administration of the test substance preparation in 0.5% aqueous Tylose® by gavage to three male and three female fasted Wistar rats at dose levels of 2000 mg/kg bw, using an application volume of 10 ml/kg bw. The observation period lasted for up to 14 days.

Findings:
The stability and homogeneity of the test substance was guaranteed. The stability of the test substance in distilled water for a period of 4 hours was confirmed by analysis. The correctness of the concentration of the test substance preparation and its homogeneity were analytically confirmed. There was no mortality in either males or females. No signs of toxicity were noticed. Body weight development was normal during the study period. There were no macroscopic pathological findings in animals sacrificed at the end of the observation period.

Conclusion:
The oral LD_{50} was found to be > 2000 mg/kg bw for male and female rats.

B.6.8.2 Supplementary studies – AMTT (635M04)

AMTT was an impurity in the batch no. N24 (2.45 %) which caused severe effects in the long-term rat studies and in the two-generation reproduction toxicity study in rats. AMTT is also a metabolite in the rat, soil and water. A separate metabolism study was conducted with AMTT, it was tested in an acute oral test, as well as in three mutagenicity tests. Furthermore, in order to prove that the effects seen in the 2-generation study using tritosulfuron batch no. N24 was due to high AMTT content, it was tested in a pre/postnatal toxicity study. In order to understand the mechanism by which AMTT exerts its effects it was subjected to two additional studies: a subchronic toxicity study with estrus cycle determination as well as hormone analysis and determination of the binding capacity to the estrogen receptor.
AMTT does not accumulate in rats, but is effectively excreted. The major metabolite AHTT is generated by demethylation and is detected as different tautomeric structures. The oral LD_{50} was found to be > 200 < 2000 mg/kg bw. Estrus cycle determination, hormone analysis as well as PCNA resp. BrdU and TUNEL–stain analysis of mammary glands and a density calculation of estrogen (Eα)– and progesterone receptors in uterus and vagina revealed no treatment-related changes in a subchronic toxicity study. AMTT is not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay, not mutagenic *in vitro* in the CHO/HPRT mutation assay and did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the mouse and therefore is considered to be non-mutagenic in this micronucleus assay. After the completion of the DAR, an additional study was submitted in which AMTT was assessed for its potential to induce structural chromosomal aberrations (clastogenic activity) and/or changes in the number of chromosomes

(aneugenic activity) in V79 cells *in vitro* both in the presence and in the absence of a metabolising system. AMTT is not considered to be a clastogenic or an aneugenic agent under in-vitro conditions in V79 cells. Altogether, AMTT has no genotoxic potential under the conditions of the *in vitro* and *in vivo* studies conducted.
The oral application of AMTT induced severe maternal and developmental toxicity at 20 mg/kg bw/day and at 50 mg/kg bw/day in a pre/postnatal screening study. Therefore, AMTT might be responsible for the effects observed in the 2-generation study with tritosulfuron containing high levels of AMTT, with respect to pup mortality. In the presence of endogenous estrogens, the bonding capacity of tritosulfuron and AMTT to the estrogen receptor is regarded as extremely low. A biological effect of the substances, the activation of the receptor-mediated gene expression, is extremely unlikely.
Based on the submitted data for tritosulfuron with a high content of AMTT (i.e. 2.45 %), AMTT was considered to possess carcinogenic and reproduction disturbing properties. In conclusion AMTT should be be considered as toxicologically relevant metabolite in groundwater.

Based on the 2-generation study in rats conducted with batch no. N24 with a calculated level of AMTT of 0.06 mg/kg bw/d and applying a safety factor of 500, the following reference values were derived for AMTT:

ADI:	0.0001 mg/kg bw
AOEL (syst.):	0.0001 mg/kg bw/d
ARfD:	0.0001 mg/kg bw

Table B.6.8-5: Summary of supplementary studies with AMTT (635M04) (CAS-Nr. 5311-05-07)

Study/strains/species	Test material/ conditions	Results
Study of the biokinetics and metabolism in Wistar rats (Chbb:THOM (SPF))	14C-AMTT; Batch no. 687-1008, chemical purity > 98 %, radiochemical purity: > 95 %.	Rapid exceretion, major metabolite: AHTT
Study on the acute oral toxicity of AMTT in Wistar rats (Chbb:THOM (SPF))	Batch no. 27 939/16, purity: 92.3 % - 94.2 %.	LD_{50}: > 200 < 2000 mg/kg bw
Subchronic toxicity study with estrus cycle determination and hormone analysis in female Wistar rats (Chbb:THOM (SPF)) Administration in the diet up to 32 weeks 0, 40, 120 ppm	Batch no. 01185-097, purity: 99.9 %	LOAEL: 40 ppm (3.6 mg/kg bw/d): Estrus cycle determination, hormone analysis, PCNA resp. BrdU and TUNEL–stain analysis of mammary glands and a density calculation of estrogen (Eα)– and progesterone receptors in uterus and vagina revealed no treatment-related changes.
Ames Salmonella/mammalian-microsome mutagenicity test and Escherichia coli / mammalian microsome reverse mutation assay (standard plate test and preincubation test) S. typhimurium strains TA 100, TA 1535, TA 1537, TA 98 and E. coli strain WP2 uvrA	Batch no. 27 939/16, purity: 92.3 % - 94.2 %.	Not mutagenic
Gene mutation assay in Chinese hamster ovary (CHO) V79 cells *in vitro* (V79/HPRT)	Batch no. 01185-097, purity: 99.9 %.	Not mutagenic
In vitro chromosome aberration assay in V79 cells	Batch No. 00831-237, purity: 99.9 %	Not mutagenic
Micronucleus assay in bone marrow cells of the mouse (NMRI) after a single intraperitoneal administration	Batch no. 27939-141 CP031929, purity: 99.8 %.	Not mutagenic
AMTT and BisSH - Pre-/ postnatal screening toxicity study in Wistar rats (Chbb: THOM (SPF)) – Oral administration (gavage)	Batch no. CP031929, purity: 99.8 %, BisSH; batch no. CP 031930, purity: 99.7 %	Severe maternal and developmental toxicity at 20 mg/kg bw/d and at 50 mg/kg bw/d AMTT
Study of a possible bond of AMTT and tritosulfuron to the estrogen receptor in the cytosol from the endometrial RUCA-I adenocarcinoma cell line	Endometrial RUCA-I-adenocarcinoma cell line of the rats	Extremely low bonding capacity of tritosulfuron and AMTT to the estrogen receptor in the presence of endogenous estrogens

B.6.8.2.1 Study of the biokinetics and metabolism in rats

Report: Leibold E. et al., 2001
14C-Reg.-No. 231 700 - Study of the biokinetics and metabolism in rats
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 2000/1018485

GLP: Yes (Laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

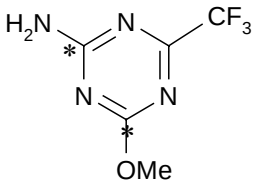
Guideline: EPA/OPPTS 870.7485

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: 14C-Reg.-No. 231 700; (635M04; AMTT); batch: 687-1008, chemical purity > 98 %, radiochemical purity: > 95 %.
Test animals: Wistar rats Chbb:THOM (SPF)

14C-Labelled AMTT (2-amino-4-methoxy-6-(trifluoromethyl)-1,3,5-triazine-2,4-14C) was fed to female Wistar rats at 0.25 mg/kg bw as one single oral dose. The test substance was solubilised in 0.5 % aqueous Tylose for administration. In the excretion experiment the dose solution was administered to five animals and urine and faeces were collected throughout seven days. In the bile experiment four female rats were dosed and only bile was collected up to 48 hours.

Figure B.6.8-1 Structure and position of the ^{14}C-label of AMTT (Reg.-No.: 231 700)



* [14C-Triazine label]

Excretion balances were determined and metabolite patterns were investigated by radio-HPLC from urine, faeces extracts and bile. The relevant transformation products of AMTT were identified.

Findings:
Absorption, Distribution and Excretion of Radioactivity
The amounts of radioactivity excreted within seven days via urine and faeces accounted for 91.9 % and 7.3 % of the dose, respectively. Via bile, 7.3 % of the dose were excreted within 48 hours. Overall, the test substance was rapidly and completely absorbed from the gastrointestinal tract. The excretion of radioactivity occurred predominantly via urine. Within the first 24 hours 53 % of the dose were excreted. Remaining radioactivity in organs and tissues was significantly below 0.5 % of the dose.

Metabolite Patterns and Metabolite Identities

For isolation of metabolites, all matrices were analysed by HPLC. The resulting metabolite pattern of urine mainly showed the demethylated metabolite AHTT (2-amino-4-hydroxy-6-(trifluoromethyl)-1,3,5-triazine) which was identified by mass spectrometry. Including the tautomeric forms of AHTT, the relative quantities accounted for 73.6 % of the dose within the first 72 hours. Remaining AMTT in urine accounted for 7.9 % of the dose. Faeces collected from 0-72 hours contained 4.3 % of the dose as AMTT and 2.3 % of the dose as AHTT and its tautomers. Bile almost exclusively showed AMTT excreted at 6.9 % of the dose within 48 hours.

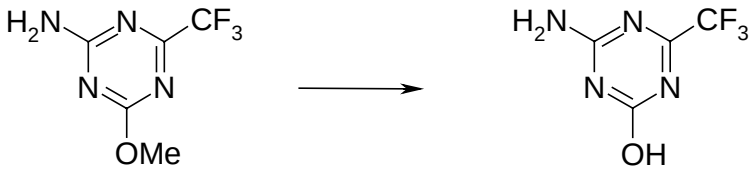
Table B.6.8.2-1: Summary of identified metabolites in urine, faeces and bile after administration of a single oral dose of [triazine-^{14}C]-AMTT at 0.25 mg/kg bw. Excretion in % of dose within specified time period.

Metabolite identity	Urine (0-72 h)	Faeces (0-72 h)	Bile (0-48 h)
	female	female	female
AMTT	7.9	4.3	6.9
AHTT	73.6	2.3	-

Metabolic Pathway

The metabolism of AMTT resulted in the predominant formation of the demethylated compound AHTT and its tautomeric structures [see Figure B.6.8-].

Figure B.6.8-2 Metabolic pathway of ^{14}C-AMTT (635M04) in rats:



AMTT AHTT

Conclusion:

AMTT does not accumulate in rats, but is effectively excreted. The major metabolite AHTT (635M11) is generated by demethylation of the parent compound and is detected as different tautomeric structures.

B.6.8.2.2 Study on the acute oral toxicity

Report: Poelloth C., Hellwig J., 1996
Study on the acute oral toxicity of AMTT, tech. CAS-Nr. [5311-05-7] in rats
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 1996/1000678
and
Poelloth C., 1997
Amendment No. 1 to the report: Study on the acute oral toxicity of AMTT, techn. in rats
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 1997/1000919

GLP: Yes (Laboratory certified by Ministerium fuer Arbeit, Soziales und Gesundheit, Postfach 3180, 55021 Mainz)

Guideline: EEC 92/69

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: AMTT (635M04), techn., CAS-Nr. 5311-05-07; batch no. 27 939/16, purity: 92.3 % - 94.2 %.
Test animals: Wistar rats Chbb:THOM (SPF)

Single administration of the test substance preparation in 0.5 % aqueous Tylose CB 30.000 by gavage to three male and three female fasted Wistar rats at dose level of 200 and to three female fasted Wistar rats at dose level of 2000 mg/kg bw. The application volume was 10 ml/kg bw. The observation period lasted for up to 14 days.

Findings:
The stability of the test substance over the study period was guaranteed. The stability of the test substance in water for 96 hours was confirmed by analysis. The correctness of the concentration of the test substance in the vehicle and its homogeneity were analytically confirmed.
All animals of the 2000 mg/kg bw died within one day after application. There was no mortality in the 200 mg/kg bw dose group. Signs of toxicity in both dose groups comprised impaired or poor general state, dyspnoea, apathy, abdominal or lateral position, staggering, ataxia, atonia, paresis, narcotic-like state, pain reflex absent, twitching, erythema, anemic pallor, exsiccosis, salivation, lacrimation, red clammy snout and eyelid and compulsory gnawing. Body weight development appeared to be normal. During necropsy the animals that died showed erosion/ulcer in the glandular stomach, agonal congestion, discoloration of contents of the urinary bladder, and discoloration of the small intestines and the caecum. There were no macroscopic pathological findings in animals sacrificed at the end of the observation period.

Conclusion:
The oral LD_{50} was found to be > 200 < 2000 mg/kg bw for male and female rats.

B.6.8.2.3 Subchronic toxicity study

Report: Mellert W. et al., 2001(j)
Report: AMTT - Subchronic toxicity study with estrus cycle determination and hormone analysis in female Wistar rats. Administration in the diet up to 32 weeks
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 2001/1006078

GLP: Yes (Laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: No test guidelines exist for this type of study.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: AMTT (635M04); batch no. 01185-097, purity: 99.9 %
Test animals: Wistar rats [Chbb:THOM (SPF)]

In order to detect a possible influence of AMTT (2-amino-4-methoxy-6-(trifluoromethyl)-1,3,5-triazine) on estrus cycle, hormonal status and/or cell proliferation in the mammary gland, AMTT was administered to groups of 30 female Wistar rats at dietary concentrations of 0, 40 and 120 ppm up to 32 weeks. The animals were observed for abnormal clinical signs at least once a day. Food consumption, water consumption and body weight were determined. Vaginal smears for estrus cycle determination were prepared and evaluated from day 70 to day 100. After 16 weeks of treatment, blood was taken from each 10 animals per group being in diestrus, and from each 10 animals per group being in proestrus. Luteinising hormone, progesterone, prolactin and estradiol were determined. Thereafter, these animals were subjected to gross-pathological assessment, followed by histopathological examinations. Proliferating cell nuclear antigen (PCNA) was evaluated in the mammary gland.

The remaining 10 animals per group were treated up to 32 weeks. Vaginal smears for estrus cycle determination were prepared and evaluated in these animals from day 147 to day 164 and day 202 to day 224. Towards the end of treatment period, osmotic minipumps were implanted three days prior to necropsy for evaluation of cell proliferation (S-phase response) and apoptosis in the mammary gland. All animals were subjected to gross-pathological assessment without further examinations.

Findings:
The stability of the test substance was proven by reanalysis during the in-life phase of the study. The stability of the test substance in the diet over a period of up to 34 days at room temperature was verified. As the mixtures were stored no longer than this time period, the stability was guaranteed. The homogeneity of the mixtures was verified; the correctness of the concentrations was analytically confirmed.

Table B.6.8.2-2: AMTT - Subchronic toxicity study with estrus cycle determination and hormone analysis in female Wistar rats - Test substance intake

Dietary dose level (ppm)	Test substance intake (mg/kg bw/d)
40	3.6
120	11.1

Clincial signs of toxicity were piloerection and increased water consumption in the high dose group, as well as reduced food consumption and body weight in both treatment groups.

In the estrus cycle determination from day 202 to day 224, the mean days from estrus to estrus were statistically significantly reduced in both dose groups. This was, however, most probably due to the high value (with a high standard deviation) in the control group rather than being a substance-related effect. Moreover, as no effect was seen regarding the single estrus stages, a substance-related effect was not assumed.
Hormone analyses revealed no test substance-related changes in the treated animals either on diestrus or on proestrus.
After both treatment periods, the terminal body weights of dose 1 (40 ppm) and dose 2 (120 ppm) groups showed a significant, dose–dependent decrease which is regarded to be treatment–related. The slightly increased number of erosions/ulcers in the glandular stomach (0/20 in control, 3/20 in group 1, 4/20 in group 2) noted at necropsy (16–week groups only) , is most probably due to the general toxicity ("stress phenomenon"), indicated by the terminal body weight decrease.
Neither macroscopy nor histopathology of the mammary gland, the uterus and the vagina revealed a treatment – related change of structure.
PCNA or BrdU – labeling of the mammary gland did not detect any significant increase of labeling indices after 16 as well as 32 weeks of treatment. Thus, no induction of cell proliferation was detected. The slight significant decrease of the PCNA–labeling index in top dose females is interpreted as being incidental and of no biological significance. A compound–related influence on apoptosis was not found (TUNEL–stain) when the labeling indices of control and top dose female mammary glands of the 32–week treatment group were compared.
Estrogen (Eα) – and progesterone receptors could be recognized in uterus and vagina of control and top dose animals of the 16–week treatment groups. However, the density of receptors did not change, when control and top dose group animals were compared semiquantitatively, indicating no influence on these parameters under the conditions of this study.

In conclusion, the following substance-related effects were obtained (Table B.6.8.2-3):

Table B.6.8.2-3: AMTT - Subchronic toxicity study with estrus cycle determination and hormone analysis in female Wistar rats - substance related effects

Dietary dose level (ppm)	Effects
40 ppm	Reduced food consumption, predominantly in the early phase of the study, reduced body weight (up to 10.5 %), reduced body weight change (24.2 %), slight increase of erosions/ulcers in the glandular stomach (3/20)
120 ppm	Piloerection in 6 animals, reduced food consumption in the early phase of the study, increased water consumption, reduced body weight (13.9 %), reduced body weight change (39.5 %), slight increase of erosions/ulcers in the glandular stomach (4/20).

Conclusion:
Thus, only general signs of toxicity were observed in both treatment groups. The LOAEL was 40 ppm (equal to 3.6 mg/kg bw/d). Estrus cycle determination, hormone analysis as well as PCNA resp. BrdU and TUNEL–stain analysis of mammary glands and a density calculation of estrogen (Eα)– and progesterone receptors in uterus and vagina revealed no treatment-related changes.

B.6.8.2.4 First mutagenicity study

Report: Engelhardt G., Hoffmann H. D., 1996
Report on the study of AMTT, techn. CAS-Nr. [5311-05-7] in the Ames Salmonella/mammalian-microsome mutagenicity test and Escherichia coli / mammalian microsome reverse mutation assay (standard plate test and preincubation test)
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 1996/1000679
and
Engelhardt G., 1997
Amendment No. 1 to the report on the study of AMTT, techn. CAS-Nr. [5311-05-7] in the Salmonella typhimurium/Escherichia coli reverse mutation assay (standard plate test and preincubation test)
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 1997/1000920

GLP: Yes (Laboratory certified by Ministerium fuer Arbeit, Soziales und Gesundheit, Postfach 3180, 55021 Mainz)

Guideline: OECD 471, OECD 472, EEC 92/69 B 13, EEC 92/69 B 14

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: AMTT (635M04), techn., CAS-Nr. 5311-05-07; batch no. 27 939/16, purity: 92.3 % - 94.2 %.
Test system: Salmonella typhimurium strains TA 100, TA 1535, TA 1537 and TA 98 and *Escherichia coli* strain WP2 uvrA
AMTT, techn. CAS-Nr. [5311-05-7] was tested for its mutagenic potential based on the ability to induce back mutations in selected loci of several bacterial strains in the Ames reverse mutation assay. The Salmonella typhimurium strains TA 100, TA 1535, TA 1537 and TA 98 and Escherichia coli strain WP2 uvrA were exposed to the test substance dissolved in DMSO. The study consisted of a standard plate test (with doses ranging from 20 to 5000 µg/plate) and preincubation test (with doses ranging from 20 to 5000 µg/plate) both with and without metabolic activation (Aroclor induced Sprague-Dawley rat liver S-9 mix). Three plates were used per dose for each strain and test condition.
For control purposes and to demonstrate the sensitivity of the test system, negative controls (with and without S9 mix) and positive controls (2-aminoanthracene - with S9 mix; N-

methyl-N'-nitro-N-nitrosoguanidine, 4-nitro-o-phenylendiamine, 9-aminoacridine and N-ethyl-N'-nitrosoguanidin - all without S9 mix) were tested.

A substance is considered positive in this test if the following criteria are met:
- there is at least a doubling of the negative control mutation rate with or without S-9 mix,
- there is a dose-response relationship, and
- the results are reproducible.

Findings:
The stability of the test substance throughout the study period was verified by reanalysis. The stability of AMTT techn. CAS-Nr. [5311-05-7] in the vehicle DMSO and in water has been determined analytically. No test substance precipitation was observed. No bacteriotoxic effect was noticed. The mean number of revertant colonies was not increased in any strain either with or without S-9 activation. Expected increases in revertant colonies were obtained with the positive controls.

Conclusion:
According to the results of the study, AMTT techn. CAS-Nr. [5311-05-7] is not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay.

B.6.8.2.5 Second mutagenicity study

Report: Wollny H.-E. et al., 1999
Gene mutation assay in Chinese hamster V79 cells *in vitro* (V79/HPRT) with AMTT
RCC Cytotest Cell Research GmbH, Roßdorf, Germany Fed. Rep.
unpublished
BASF RegDoc# 1999/10870

GLP: Yes (Laboratory certified by Hessisches Ministerium fuer Umwelt, Energie, Jugend, Familie und Gesundheit, Wiesbaden)

Guideline: EEC 87/302, OECD 476

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: AMTT (635M04); batch no. 01185-097, purity: 99.9 %.
Test system: Chinese hamster ovary (CHO) cells
AMTT, techn. CAS-Nr. [5311-05-7] was tested for its ability to induce gene mutations at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in Chinese hamster ovary (CHO) cells *in vitro*. Two independent experiments were carried out, using two parallel cultures each either with or without the addition of Aroclor 1254-induced Sprague-Dawley rat liver S-9 mix (exogenous metabolic activation).
In an initial range-finding test, precipitation of the test substance occurred at doses > 1000 µg/ml. No changes in osmolarity and pH-values were observed. Thus, the test substance was evaluated at the following doses in the 1st experiment (4 hours exposure): 125.0; 250.0; 500.0; 1000.0; and 2000.0 µg/ml (without and with S-9 mix). A 2nd experiment

for confirmation was performed using the following doses (4 hours exposure): 125.0; 250.0; 500.0; 1000.0; and 2000.0 µg/ml (without and with S-9 mix). After an attachment period of 20 - 24 hours, a treatment period of 4 hours both with and without metabolic activation, an expression phase of about 7 - 9 days and a selection period of about 1 week, the colonies of each test group were stained with 10 % methylene blue in 0.01 % KOH solution and counted. For control purposes and to demonstrate the sensitivity of the test system, negative controls and two appropriate positive control chemicals 7,12-dimethylbenz(a)anthracene (with S9 mix) and Ethylmethane sulfonate (without S9 mix) were tested.
The criteria for a positive response are:

- Increases of the mutation frequencies, that are three times above the spontaneous mutation frequency at least at one of the concentrations in the experiment.
- Evidence of reproducibility of any increase in mutant frequencies.
- Evidence of a dose-response relationship, even if a threefold increase of the mutant frequency is not observed.

Findings:
The stability of the test substance throughout the study period was guaranteed. The stability of AMTT, techn. CAS-Nr. [5311-05-7] in the vehicle DMSO and in water has been confirmed by analysis. No cytotoxicity was observed up to the maximal concentration of 2000 µg/ml. In both main experiments precipitation of the test substance occurred at the highest test concentration of 2000 µg/ml. The negative controls (untreated and vehicle controls) gave mutant frequencies within the range expected for the CHO cell line. Appropriate positive control chemicals led to the expected increase in the frequencies of forward mutations.
The test substance did not cause a relevant increase in the mutant frequencies either without S-9 mix or after adding a metabolising system in both main experiments performed independently of each other. An isolated increase exceeding the threshold of three times of the corresponding solvent control was observed at the maximal concentration of experiment I, culture I (with S-9 mix). This increase was judged as biologically irrelevant since it is based upon statistical fluctuations at such low absolute numbers. The corresponding solvent control is close to the lower border of historical solvent controls. Compared to the corresponding negative control the threshold is not reached. Furthermore the effect was not reproduced in the parallel culture and the absolute value remained well within the range of the historical negative controls.
Thus it can be stated that in this mutagenity assay and under the experimental conditions reported the test substance did not induce gene mutations at the HPRT locus in V 79 cells.

Conclusion:
Under the experimental conditions of this assay, AMTT, techn. CAS-Nr. [5311-05-7] is considered to be non-mutagenic *in vitro* in the CHO/HPRT mutation assay.

B.6.8.2.6 Third mutagenicity study

Report: Engelhardt G., Leibold E., 2004
Report: *In vitro* chromosome aberration assay with Reg. No. 231700 (metabolite of BAS 635 H) in V79 cells
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep., unpublished,
BASF RegDoc# 2004/1014204

GLP: Yes

(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guideline: OECD 473, EEC 92/69 B 10

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Reg. No. 231 700 (BH 635-5 = AMTT); batch No. 00831-237, purity: 99.9 %.
Test system: V79 cells, with and without metabolising system.
The substance Reg. No. 231 700 (= AMTT) was assessed for its potential to induce structural chromosomal aberrations (clastogenic activity) and/or changes in the number of chromosomes (aneugenic activity) in V79 cells *in vitro* both in the presence and in the absence of a metabolising system.
According to an initial range-finding cytotoxicity test for the determination of the experimental doses and taking into account the cytotoxicity actually found in the main experiments, the following doses were evaluated in two independent experiments:

Table B. 6.8.2-4: Experimental design

Experiment	Exposure period	Sampling time	S-9 mix	Dose levels [µg/mL]]
1st assay	4 hours	18 hours	With & without	0 - 500 - 1000 – 2000
2nd assay	18 hours	18 hours	Without	0 - 125 - 250 – 500
	18 hours	28 hours	Without	0 – 1000
	4 hours	28 hours	With	0 - 500 - 1000 – 2000

About 2 - 3 hours prior to harvesting the cells, Colcemid was added to arrest cells at a metaphase-like stage of mitosis (c-metaphases). After preparation of the chromosomes and staining with Giemsa, 100 metaphases for each culture in the case of the test substance and vehicle controls, or 50 cells for each culture in the case of the concurrent positive controls, were analysed for chromosomal aberrations.

Findings:
The negative controls (vehicle controls) gave frequencies of aberrations within the range expected for the V79 cell line. Both of the positive control chemicals, i.e. EMS and cyclophosphamide, led to the expected increase in the number of cells containing structural chromosomal aberrations.

Chromosome analysis – 1st experiment

Clastogenic mode of action
After a treatment time of 4 hours no relevant increase in the number of chromosomally damaged cells was observed either without S-9 mix or after the addition of a metabolising system. The types and frequencies of aberrations are given in the following tables.

Aneugenic mode of action
No increase in the number of cells with changes in the number of chromosomes was demonstrated either without S-9 mix or after the addition of a metabolising system.

Table B.6.8.2-5: Chromosome analysis-1st experiment

	Vehicle control	500 µg/mL	1000 µg/mL	2000 µg/mL	pos. control
Assay without S-9 mix; 4-hour exposure, 18-hour sampling time, positive control: 350 µg EMS/mL					
Metaphases incl. gaps	5 (2.5 %)	13 (6.5 %)	6 (3.0 %)	12 (6.0 %)	21 %**
Metaphases excl. gaps	1 (0.5 %)	9 (4.5 %*)	2 (1.0 %)	3 (1.5 %)	16 %**
Assay with S-9 mix; 4-hour exposure, 18-hour sampling time, positive control: 0.5 µg CPP/mL					
Metaphases incl. gaps	10 (5.0 %)	8 (4.0 %)	14 (7.0 %)	12 (6.0 %)	18 %**
Metaphases excl. gaps	4 (2.0 %)	1 (0.5 %)	6 (3.0 %)	7 (3.5 %)	17 %**

* p ≤ 0.05, ** p ≤ 0.01; Fisher's exact test with Bonferroni-Holm correction

Chromosome analysis – 2nd experiment

Clastogenic mode of action
Both with and without S-9 mix, no increase in the number of structurally damaged metaphases was observed either after a treatment time of 4 hours or after a continuous treatment of 18 hours at both sampling times, i.e. 18 and 28 hours. The types and frequencies of aberrations are given in the following table.

Aneugenic mode of action
No increase in the number of cells with changes in the number of chromosomes was demonstrated either with and without S-9 mix.

Table B.6.8.2-6: Chromosome analysis-2nd experiment

	Vehicle control	125 µg/mL	250 µg/mL	500 µg/mL	pos. control
Assay without S-9 mix; 18-hour exposure, 18-hour sampling time, positive control: 350 µg EMS/mL					
Metaphases incl. gaps	13 (6.5 %)	8 (4.0 %)	13 (6.5 %)	6 (3.0 %)	19 %**
Metaphases excl. gaps	3 (1.5 %)	3 (1.5 %)	4 (2.0 %)	4 (2.0 %)	17 %**

* p ≤ 0.05, ** p ≤ 0.01; Fisher's exact test with Bonferroni-Holm correction

	Vehicle control	1000 µg/mL	pos. control	-	-
Assay without S-9 mix; 18-hour exposure, 28-hour sampling time, positive control: 0.5 µg CPP/mL					
Metaphases incl. gaps	11 (5.5 %)	8 (4.0 %)	25 %**	-	-
Metaphases excl. gaps	7 (3.5 %)	5 (2.5 %)	25 %**	-	-

* p ≤ 0.05, ** p ≤ 0.01; Fisher's exact test with Bonferroni-Holm correction

	Vehicle control	500 µg/mL	1000 µg/mL	2000 µg/mL	pos. control
Assay with S-9 mix; 4-hour exposure, 28-hour sampling time, positive control: 0.5 µg CPP/mL					
Metaphases incl. gaps	7 (3.5 %)	12 (6.0 %)	13 (6.5 %)	7 (3.5 %)	21 %**
Metaphases excl. gaps	6 (3.0 %)	4 (2.0 %)	3 (1.5 %)	3 (1.5 %)	18 %**

* p ≤ 0.05, ** p ≤ 0.01; Fisher's exact test with Bonferroni-Holm correction

Mitotic Index
A dose-dependent suppression of the mitotic activity was observed after continuous treatment of 18 hours at both sampling times, i.e. 18 and 28 hours without metabolic system. Likewise, cell count was reduced and growth inhibition was observed under all experimental conditions.

Table B.6.8.2-7: Mitotic Index

Time (hours)		Dose level	% Rel.
Exposure	Sampling	Vehicle control DMSO	100
18	18	125 µg/mL	73.8
18	18	250 µg/mL	58.4
18	18	500 µg/mL	43.9
18	18	1000 µg/mL	*
18	18	1500 µg/mL	**
18	18	350 µg EMS /mL	42.1
Exposure	Sampling	Vehicle control DMSO	100
18	28	250 µg/mL	87.5
18	28	500 µg/mL	81.7
18	28	1000 µg/mL	54.9
18	28	1500 µg/mL	*
18	28	350 µg EMS /mL	68.9

* no or only few metaphases for evaluation
** no or only few metaphases for evaluation and of poor quality

Discussion:
In the present study a suppression of the mitotic activity was observed. The cause for that suppression remained unclear. It might be an indication for cytotoxicity already occurring in the lowest dose of 125 µg/mL. In this case the cell line V79 is proved to be too sensitive for this test substance. On the other hand a lower mitotic activity might be caused by spindle cell poisons, for example carbendazim. Since the micronucleus test in NMRI mice revealed negative results it might be concluded that AMTT does not exert clastogenic properties.
Conclusion:
On the basis of the results of the present study, AMTT did not cause any relevant increase in the number of structurally aberrant metaphases incl. and excl. gaps at both sampling times either without S-9 mix or after adding a metabolising system in two experiments performed independently of each other. No increase in the frequency of cells containing numerical aberrations was demonstrated either.
AMTT is not considered to be a clastogenic or an aneugenic agent under in-vitro conditions in V79 cells.

B.6.8.2.7 Fourth mutagenicity study

Report: Voelkner W. et al., 1998
Micronucleus assay in bone marrow cells of the mouse after a single intraperitoneal administration of AMTT
RCC Cytotest Cell Research GmbH, Roßdorf, Germany Fed. Rep.
unpublished
BASF RegDoc# 1998/11043

GLP: Yes (Laboratory certified by Hessisches Ministerium fuer Umwelt, Energie, Jugend, Familie und Gesundheit, Wiesbaden)

Guideline: OECD 474, EEC 92/69, B 12

Deviations: In deviation to the protocol for a short period the relative humidity was higher than 70 % (highest value 88 %). In deviation to the protocol for the test groups vehicle and test article at preparation interval 48 hours 4000 instead of 2000 PCEs were scored for micronuclei. These deviations had no influence on the integrity and validity of the study.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: AMTT (635M04); batch no. 27939-141 CP031929, purity: 99.8 %.
Test animals: NMRI mice
AMTT, techn. CAS-Nr. [5311-05-7] was tested for its potential to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of male mice. For this purpose the test substance was formulated in DMSO (dimethylsulfoxide) and was administered once intraperitoneally to male animals in a volume of 4 ml/kg bw. After 24 hours and 48 hours post application the bone marrow cells were collected for micronuclei analysis.
Five animals were evaluated per test group for the occurrence of micronuclei. 2000 or 4000 (test group at preparation interval 48 hours; two independent evaluations of 2000 PCEs in order to verify the results) polychromatic erythrocytes (PCEs) per animal were scored for micronuclei. To describe a cytotoxic effect due to the treatment with the test article the ration between polychromatic and normochromatic erythrocytes (NCEs) was determined in the same sample. As a negative control the vehicle, DMSO was applicated to male mice by the same route. As a positive control, 30 mg cyclophosphamide (CPP)/kg body weight dissolved in deionised water, were administered to male animals once intraperitoneally in a volume of 10 ml/kg body weight.
The following dose levels of the test substance were investigated:
- 24 hours preparation interval: 6.25; 12.5; and 25.0 mg/kg bw.
- 48 hours preparation interval: 25.5 mg/kg bw.

The highest dose was estimated by pre-experiments to be close to the maximum tolerated dose. The animals expressed toxic reactions. The test chemical is to be considered positive in this assay if the following criteria are met:
- A dose-related increase in the number of micronucleated polychromatic erythrocytes or a statistically significant positive response for at least one of the test points.

Findings:
The stability of the test substance was determined by reanalysis. The stability of AMTT, techn. CAS-Nr. [5311-05-7] in DMSO for a period of 4 hours was determined by analysis. The concentrations of the test substance in the vehicle were analytically determined. The administration of the test substance led to evident signs of toxicity. Application of the positive control chemical cyclophosphamide led to a statistically significant increase of induced micronucleus frequency. The mean number of normochromatic erythrocytes was not substantially increased after treatment with the test article as compared to the mean value of NCEs of corresponding vehicle controls indicating that AMTT had no cytotoxic properties in the bone marrow.
In comparison to the corresponding vehicle controls there was no significant or biologically relevant enhancement in the frequency of the detected micronuclei at preparation interval 24 hours after administration of the test article. A statistically enhancement of the micronucleus frequency at preparation interval 48 hours is considered not to be of biological relevance,

since the frequency was within the historical negative control range. Additionally, the micronucleus frequency was lower than the vehicle control value at preparation interval 24 hours, indicating that the statistical significance was rather caused by the low vehicle control value than by a test article induced increase of the micronucleus frequency.

Conclusion:
The test substance AMTT, techn. CAS-Nr. [5311-05-7] did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the mouse and therefore is considered to be non-mutagenic in this micronucleus assay.

B.6.8.2.8 Pre-/postnatal screening toxicity study in Wistar rats

Report: Schilling K. et al., 2001
AMTT and BisSH - Pre-/postnatal screening toxicity study in Wistar rats - Oral administration (gavage)
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 2001/1003834

GLP: No

Guideline: No test guidelines exist for this type of study.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: AMTT (635M04); batch no. CP031929, purity: 99.8 %, BisSH (impurity Reg.-No. 302571); batch no. CP 031930, purity: 99.7 %.
Test animals: Wistar rats [Chbb:THOM (SPF)]
The study was conducted in order to prove that the effects observed in the 2-generation study with tritosulfuron containing 2.45 % AMTT derive from AMTT and not from tritosulfuron nor BisSH (the other major impurity). AMTT and BisSH (also an impurity of tritosulfuron) were tested for their effects on pregnant Wistar rats during gestation and lactation and consequences on pre-/postnatal development of the progeny. The test substances were administered to 8 mated female Wistar rats/group at doses of 20 and 50 mg/kg bw/d for AMTT and 5 and 30 mg/kg bw/d for BisSH from day 6 post coitum up to day 9 post partum at latest (depending on dam/pup survival). A standard dose volume of 5 ml/kg bw was used; the control group (8 females) was dosed with the vehicle only (0.5 % Tylose CB 30.000 in doubly distilled water). The dams were allowed to deliver. All AMTT treated pups from both dose groups died within 3 days after birth; therefore, the study was terminated and the surviving animals and pups were sacrificed without further examinations with the following exceptions: all surviving dams of AMTT treated dose groups and seven dams of the control group (6 while nursing, 1 after five days of withdrawal) were sacrificed by decapitation. The blood was collected and stored at – 80 °C. The exsanguinated animals were necropsied and assessed by gross pathology. Parts of the female mammary gland were fixed, processed and assessed histopathologically.

Findings:
The following test substance-related adverse findings were obtained:
AMTT; 50 mg/kg bw/d

Maternal toxicity: 4/5 dams showing distocia had to be sacrificed, the other was found dead. One dam delivered fetuses (8 in total; 6 liveborn, 2 stillborn). Severely affected impaired health status (piloerection, lateral position, unsteady gait, apathy, partial eye closure chromodacryorrhea, urine smeared fur in all or single dams). No nesting activity in 3 dams. The dam that delivered showed insufficient care of the pups and nursed insufficiently. Reduced food consumption during the whole treatment period. Impaired body weight gains during gestation days 6-20 (70 % below control). 2 dams with gastroenteritis, stomach with ulcerations and large intestine bloody feces. Moderate (grade 3) involution of the mammary gland tissue with milky fluid in dilated mid-sized milk ducts in the examined dam.
Developmental toxicity: All pups died until 1 day post partum (viability index 0 %). All liveborn pups showed clinically no or little milk in the stomach, hypothermia; necropsy revealed 3 pups with an empty stomach.
AMTT; 20 mg/kg bw/d
Maternal toxicity: Piloerection in 3/8 dams during the second third of gestation and in all dams within lactation. All dams showed insufficient care of the pups and nursed their pups insufficiently. Reduced food consumption during measured lactation days 0-7 post partum (57 % lower than control). Increased water consumption during gestation days 13-14 (53 % above control) but reduced values during measured lactation days 4-5 and 7-8 post partum (up to 57 % below control). Lower mean body weight gains during gestation days 6-20 (26 % below control) and mean body weights during lactation days 6-9 (up to 15 % below control). Morphologically a resting (inactive) mammary gland tissue was noted in all dams. Three animals showed a slight focal milk production. All pups died until day 3 post partum (viability index 0 %). Lower pup body weights on day 1 post partum.
Developmental toxicity: All pups showed clinically no or little milk in the stomach, hypothermia; necropsy revealed an empty stomach in the vast majority of pups.
BisSH treatment: No treatment-related adverse effects were observed in the both BisSH treatment groups.

Conclusion:
The oral application of AMTT induced severe maternal and developmental toxicity at 20 mg/kg bw/d and at 50 mg/kg bw/d.

B.6.8.2.9 Study of a possible bond of AMTT and Tritosulfuron to the estrogen receptor

Report: Vollmer G., 2000
Study of a possible bond of Reg.-No. 231 700 and Reg.-No. 271 272 to the estrogen receptor in the cytosol from the endometrial RUCA-I adenocarcinoma cell line
Molecular Medicine Luebeck Medical University, Luebeck, Germany Fed. Rep.
unpublished
BASF RegDoc# 2000/1019272

GLP: No

Guideline: No test guidelines exist for this type of study.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: 635M04 (Reg.-No. 231 700; AMTT) and Reg.-No. 271 272 (tritosulfuron)
Test system: Endometrial RUCA-I-adenocarcinoma cell line of rats
The bonding affinity of the substances tritosulfuron (Reg.-No. 271272) and AMTT (Reg.-No. 231700) to the estrogen receptor was determined in the endometrial RUCA-I-adenocarcinoma cell line of the rat.
The relative bonding affinity of the substances was measured in a competition assay as compared with a certain amount of unlabeled estradiol (50 pmol/l) as an internal control. Nonylphenol was used as the positive control in a concentration of 1 µmol/l. First, the relative bonding affinity of the two test substances was roughly estimated as compared with the internal control, estradiol; then, the relative bonding affinity was determined exactly in a concentration of 50 µM in a further four experiments with the test substances. For the determination of the total bond, (2,4,6,7-3H)- estradiol was added in different concentrations to 100 µl of cytosolic extract (control or treated with the test substances). Unlabeled estradiol was used to expel the radioactive hormone from the specific bonding site (estrogen receptor) and therefore determine the nonspecific bond.

Findings:
All four experiments carried out produced data which could be evaluated for the calculation of the bonding affinities of the test substances to the estrogen receptor. The relative bonding affinities of AMTT (Reg.-No. 231700) were about 0.000045 % with reference to estradiol and those of tritosulfuron (Reg.-No. 271272) were 0.000025 %. As compared with nonylphenol, the bonding affinities of the test substances were 2000 x weaker for AMTT and more than 4000 x weaker for tritosulfuron.

Conclusion:
In the presence of endogenous estrogens, the bonding capacity of tritosulfuron and AMTT to the estrogen receptor is regarded as extremely low. A biological effect of the substances, the activation of the receptor-mediated gene expression, is extremely unlikely.

B.6.15 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner
AIIA-5.8.1	Czich, A.	1999	In vitro chromosome aberration assay in Chinese hamster V79 cells with reg.-no. 335 182 (BH 635-3). 633600 ! 32M0576/969428 ! #BASF 99/11504 GLP, unpublished TOX2001-944	Y	BAS
AIIA-5.8.1	Engelhardt, G.	2001	Amendment no. 3 to the report Salmonella typhimurium/Escherichia coli reverse mutation assay (standard plate test and preincubation test) with PS 335 182. 40M0576/964420 ! 2001/1007726 GLP, unpublished TOX2001-942	Y	BAS
AIIA-5.8.1	Engelhardt, G.	2000	Amendment no. 1 to the report Salmonella typhimurium/Escherichia coli reverse mutation assay (standard plate test and preincubation test) with PS 335 182. 40M0576/964420 ! 2000/1019291 GLP, unpublished TOX2001-941	Y	BAS
AIIA-5.8.1	Engelhardt, G.	1999	Amendment no. 1 to the report Salmonella typhimurium/Escherichia coli reverse mutation assay (standard plate test and preincubation test) with PS 335 182. 40M0576/964420 ! 1999/11629 GLP, unpublished TOX2001-940	Y	BAS
AIIA-5.8.1	Engelhardt, G. and Hoffmann, H.D.	1999	In vitro chromosome aberration assay with reg.-no. 292 564; BH 635-2 in V79 cells. 32M0599/964427 ! 1999/11684 GLP, unpublished TOX2001-935	Y	BAS
AIIA-5.8.1	Engelhardt, G. and Hoffmann, H.D.	1999	In vitro chromosome aberration assay with reg.-no. 335 184 (BH 635-4) in V79 cells. 32M0577/964426 ! 1999/11685 GLP, unpublished TOX2001-950	Y	BAS
AIIA-5.8.1	Engelhardt, G. and Hoffmann, H.D.	2000	Salmonella typhimurium / escherichia coli reverse mutation assay (standard plate test and preincubation test) with reg.-no. 373 906. 40M0298/004076 ! 2001/1006087 GLP, unpublished TOX2001-952	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner
AIIA-5.8.1	Engelhardt, G. and Hoffmann, H.D.	2000	In vitro gene mutation test with reg.-no. 373 906 in CHO cells (HPRT locus assay). 50M0298/004079 ! 2001/1006073 GLP, unpublished TOX2001-953	Y	BAS
AIIA-5.8.1	Engelhardt, G. and Hoffmann, H.D.	2000	Cytogenetic study in vivo with reg.-no. 373 906 in the mouse micronucleus test after two intraperitoneal administrations. 26M0298/004077 ! 2000/1018736 GLP, unpublished TOX2001-954	Y	BAS
AIIA-5.8.1	Engelhardt, G. and Hoffmann, H.D.	1998	Salmonella typhimurium/escherichia coli reverse mutation assay (standard plate test and preincubation test) with reg.-no. 335 184 (BH 635-4). 40M0577/964422 ! 1998/11635 GLP, unpublished TOX2001-948	Y	BAS
AIIA-5.8.1	Engelhardt, G. and Hoffmann, H.D.	1998	Salmonella typhimurium/escherichia coli reverse mutation assay (standard plate test and preincubation test) with PS 335 182. 40M0576/964420 ! #BASF 98/10810 GLP, unpublished TOX2001-939	Y	BAS
AIIA-5.8.1	Engelhardt, G. and Hoffmann, H.D.	1999	Salmonella typhimurium / escherichia coli reverse mutation assay (standard plate test and preincubation test) with reg.-no. 292 564; BH 635-2. 40M0599/964425 ! 1999/11412 GLP, unpublished TOX2001-933	Y	BAS
AIIA-5.8.1	FitzGerald, R.E.	2007	TBSA: Assessment of Reproduction Toxicity Potential (2007/1005045) TOX2007-605	Y	BAS
AIIA-5.8.1	Gamer, A.O. and Hoffmann, H.D.	2000	Reg. no. 373906 - Acute oral toxicity study in Wistar rats. 10A0298/001071 ! 2001/1006074 GLP, unpublished TOX2001-955	Y	BAS
AIIA-5.8.1	Gamer, A.O. and Leibold, E.	2003	TBSA - Acute oral toxicity study in rats BASF DocID 2003/1021650 GLP, unpublished TOX2004-1605	Y	BAS
AIIA-5.8.1	Kaspers, U. et al.	2003	TBSA - Subacute toxicity study in Wistar rats administration in the diet for 4 weeks BASF DocID 2003/1004048 GLP, unpublished TOX2003-1461	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner
AIIA-5.8.1	Kaspers, U. et al.	2003	TBSA - Repeated dose oral toxicity study in Wistar rats administration in the diet for 4 weeks BASF DocID 2003/1004049 GLP, unpublished TOX2003-1462	Y	BAS
AIIA-5.8.1	Kaspers, U. et al.	2003	Reg. No. 335 184 (metabolite of BAS 635 H) - Subacute toxicity study in Wistar rats administration in the diet for 4 weeks BASF DocID 2003/1022011 GLP, unpublished TOX2003-1606	Y	BAS
AIIA-5.8.1	Kirsch, P. and Hildebrand, B.	1995	Study on the acute oral toxicity of TBSA in rats. 10A0148/941038 ! #BASF 95/11408 GLP, unpublished TOX2001-936	Y	BAS
AIIA-5.8.1	Mellert, W., Deckardt, K., Gembardt, Ch. and Van Ravenzwaay, B.	2001	Reg.-no. 335182 (BH 635-3) - Subchronic toxicity study in Wistar rats administration in the diet for 3 months. 50C0576/96195 ! 2001/1006072 GLP, unpublished TOX2001-947	Y	BAS
AIIA-5.8.1	Honarvar, N.	2006	Micronucleus assay in bone marrow cells of the rat with Reg.-No. 292564 (RCC-Study no. 1055401 ! BASF no. 06M0599/969032 ! 2006/1040668) TOX2007-604	Y	BAS
AIIA5.8.1	Honarvar, N.	2006	In vivo Unscheduled DNA Synthesis in Rat Hepatocytes with Reg.-No. 292564 (RCC-Study no. 1055402 ! BASF no. 80M0599/969031 ! 2006/1040669) TOX2007-603	Y	BAS
AIIA-5.8.1	Schneider, S. et al.	2004	TBSA - One-Generation Reproduction Toxicity Study in Wistar Rats Range finding study (with 4 weeks premating) continuous dietary administration BASF DocID 2004/1017198 GLP, unpublished TOX2004-1424	Y	BAS
AIIA-5.8.1	Schneider, S. et al.	2004	TBSA - One-generation reproduction toxicity study in Wistar rats range finding study (with 10 weeks premating) continuous dietary administration BASF DocID 2004/1017197 GLP, unpublished TOX2004-1425	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner
AIIA-5.8.1	Wiemann, C.	1998	Amendment no. 1 - Reg.-no. 335 182 (BH 635-3): Acute oral toxicity in rats. 10A0576/961216 ! 1999/11221 GLP, unpublished TOX2001-946	Y	BAS
AIIA-5.8.1	Wiemann, C.	1999	Amendment no. 1 - Reg.-no. 292564; BH 635-2: Acute oral toxicity in rats. 10A0599/961217 ! 1999/10381 GLP, unpublished TOX2001-938	Y	BAS
AIIA-5.8.1	Wiemann, C. and Hellwig, J.	1998	Reg.-no. 335 182 (BH 635-3): Acute oral toxicity in rats. 10A0576/961216 ! #BASF 98/10843 GLP, unpublished TOX2001-945	Y	BAS
AIIA-5.8.1	Wiemann, C. and Hellwig, J.	1999	Reg.-no. 335 184 (BH 635-4): Acute oral toxicity in rats. 10A0577/961218 ! #BASF 99/10213 GLP, unpublished TOX2001-951	Y	BAS
AIIA-5.8.1	Wiemann, C. and Hellwig, J.	1999	Reg.-no. 292564; BH 635-2: Acute oral toxicity in rats. 10A0599/961217 ! #BASF 99/10099 GLP, unpublished TOX2001-937	Y	BAS
AIIA-5.8.1	Wollny, H.-E.	1999	Gene mutation assay in Chinese hamster V79 cells in vitro (V79 /HPRT) with reg.-no. 335 182; BH 635-3. 640402 ! 50M0576/969430 ! 1999/12026 GLP, unpublished TOX2001-943	Y	BAS
AIIA-5.8.1	Wollny, H.-E.	1999	Gene mutation assay in Chinese hamster V79 cells in vitro (V79 /HPRT) with reg.-no. 335 184; BH 635-4. 640403 ! 50M0577/969431 ! 1999/12016 GLP, unpublished TOX2001-949	Y	BAS
AIIA-5.8.1	Wollny, H.-E.	1999	Gene mutation assay in Chinese hamster V79 cells in vitro (V79 /HPRT) with reg.-no. 292 564; BH 635-2. 640401 ! 50M0599/969429 ! 1999/11691 GLP, unpublished TOX2001-934	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner
AIIA-5.8.2	Engelhardt, G.	1997	Amendment no. 1 to the report on the study of AMTT, techn. cas-nr. [5311-05-7] (ZHT test substance no.: 96/38) in the salmonella typhimurium/escherichia coli reverse mutation assay (standard plate test and preincubation test). 40M0038/964034 ! 1997/1000920 GLP, unpublished TOX2001-961	Y	BAS
AIIA-5.8.2	Engelhardt, G. and Hoffmann, H.D.	1996	Report on the study of AMTT, techn. cas-nr. [5311-05-7] (ZHT test substance no.: 96/38) in the Ames salmonella/mammalian.microsome mutagenicity test and escherichia coli / mammalian-microsome reverse mutation assay (standard plate test and preincubation test). 40M0038/964034 ! 1996/1000679 GLP, unpublished TOX2001-960	Y	BAS
AIIA-5.8.2	Engelhardt, G. and Leibold, E.	2004	*In vitro* chromosome aberration assay with Reg. No. 231700 (metabolite of BAS 635 H) in V79 cells BASF DocID 2004/1014204 GLP, unpublished TOX2004-1426	Y	BAS
AIIA-5.8.2	Leibold, E., Hafemann, C. and Hoffmann, H.D.	2001	14C-eg. no. 231 700 - Study of the biokinetics and metabolism in rats. 02B0491/986012 ! 2000/1018485 GLP, unpublished TOX2001-956	Y	BAS
AIIA-5.8.2	Mellert, W., Deckardt, K., Kaufmann, W. and Van Ravenzwaay, B.	2001	AMTT - Subchronic toxicity study with estrus cycle determination and hormone analysis in female Wistar rats administration in the diet up to 32 weeks. 99S0100/98131 ! 2001/1006078 GLP, unpublished TOX2001-959	Y	BAS
AIIA-5.8.2	Poelloth, C.	1997	Amendment no. 1 to the report study on the acute oral toxicity of AMTT, techn. in rats. 10A0038/961019 ! 1997/1000919 GLP, unpublished TOX2001-958	Y	BAS
AIIA-5.8.2	Poelloth, C. and Hellwig , J.	1996	Study on the acute oral toxicity of AMTT, techn. cas-nr. [5311-05-7] in rats. 10A0038/961019 ! 1996/1000678 GLP, unpublished TOX2001-957	Y	BAS

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner
AIIA-5.8.2	Schilling, K., Gembardt, Chr. and Van Ravenzwaay, B.	2001	AMTT and BisSH - Pre-/postnatal screening toxicity study in Wistar rats oral administration (gavage). 19R0100/98014 ! 2001/1003834 not GLP, unpublished TOX2001-964	Y	BAS
AIIA-5.8.2	Völkner, W.	1998	Micronucleus assay in bone marrow cells of the mouse after a single intraperitoneal administration of AMTT. 614800 ! 26M0100/989050 ! #BASF 98/11043 GLP, unpublished TOX2001-963	Y	BAS
AIIA-5.8.2	Vollmer, G.	1999	Study of a possible bond of reg.-no. 231700 and reg.-no. 271272 to the estrogen receptor in the cytosol from the endometrial RUCA-I adenocarcinoma cell line. 99P0100/989150 and 99P0042/989151 not GLP, unpublished TOX2001-965	Y	BAS
AIIA-5.8.2	Wollny, H.-E.	1999	Gene mutation assay in Chinese hamster V79 cells in vitro (V79 /HPRT) with AMTT. 631700 ! 50M0100/989153 ! 1999/10870 GLP, unpublished TOX2001-962	Y	BAS

Codes of owner

BAS: BASF Aktiengesellschaft