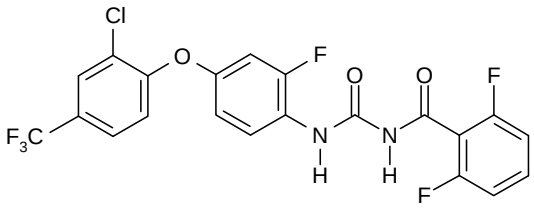
Section A1 **1. Applicant**

BPD Annex Point IIA1

1.1	**Applicant**	BASF Agro B.V Wädenswil Branch Steinacherstrasse 101 CH-8820 Wädenswil (Switzerland) **Representative for BASF in Europe:** BASF Avenue Hamoir, 14 B- 1180 Brussels (Belgium) XXXX **Representative for PT 8 [Wood Preservative] on behalf of BASF** XXX XXXX
1.2	**Manufacturer of Active Substance** (if different)	BASF Agro B.V Wädenswil Branch Steinacherstrasse 101 CH-8820 Wädenswil (Switzerland) **Location** XXX
1.3	**Manufacturer of Product(s) (if different)**	Formulator of the biocidal products: Basiment Holzwurm BV U 1551: Basiment Holzwurm BV Konzentrat XXX

Section A2 **2. Identity of the active substance**

Subsection (Annex Point)			Official use only
2.1	**Common name (IIA2.1)**	Flufenoxuron	
2.2	**Chemical name (IIA2.2)**	1-[4-(2-chloro-alpha, alpha,alpha-trifluoro-para-tolyloxy)-2-fluorophenyl]-3-(2,6-difluorobenzoyl)urea	X
2.3	**Manufacturer´s development code number(s) (IIA2.3)**	BAS 307 I, BASF Reg.No. 243 154, AC 811 678, CL 811 678, WL 115 110	
2.4	**CAS No and EC numbers (IIA2.4)**	None allocated	X
2.4.1	**CAS-No**	101463-69-8	
	Isomer n	Not relevant	
2.4.2	**EC-No**	None allocated	X
2.4.3	**Other**	CIPAC # 470	
2.5	**Molecular and structural formula, molecular mass (IIA2.5)**		
2.5.1	**Molecular formula**	C21 H11 ClF6 N2 O3	
2.5.2	**Structural formula**		
2.5.3	**Molecular mass**	488.8	X
2.6	**Method of manufacture of the active substance (IIA2.1)**	Business Confidential Information given in a separate folder - BCI	

Section A2	**2. Identity of the active substance**				
2.7	**Specification of the purity of the active substance, as appropriate (IIA2.7)**	g/kg >= 950	g/l not applicable	% w/w >=95.0	% v/v not applicable	X
2.8	**Identity of impurities and additives, as appropriate (IIA2.8)**	Business Confidential Information in a separate folder - BCI				
2.8.1	**Isomeric composition**	Not applicable				
2.9	**The origin of the natural active substance or the precursor(s) of the active substance (IIA2.9)**	Not applicable as not of natural origin. See Document IIIA 2.6-BCI				X

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	~~21/12/2004~~02/03/10
Evaluation	**2.2 Chemical name** IUPAC name: *1-[4-(2-chloro-alpha, alpha,alpha-trifluoro-para-tolyloxy)-2-fluorophenyl]-3-(2,6-difluorobenzoyl)urea* CA name: N-[[[4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-fluorophenyl]amino]carbony]-2,6-difluorobenzamide. **2.4 CAS No and EC numbers** Non entry field **2.4.2 EC-No** ~~*None allocated*~~ 417-680-3 **2.5.3 —Molecular mass** *488.8* g/mol **2.7 Specification of the purity of the active substance** ~~*>= 950 g/kg*~~ A new specification document (BASF DocID 2009/1100263) which has been established on basis of a new 5-batches analysis was provided by the applicant. The purity of the active substance is min 960 g/kg (96.0% w/w) **2.9 Origin of the natural active substance——** For the precursor, see *Document IIIA 2.6-BCI*
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Evaluation	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Remarks	

Section A2.10 **BPD Annex Point IIA2.10**	**Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC**

Subsection		**Official use only**
2.10.1 Human exposure towards active substance		
2.10.1.1 Production		
i) Description of process	Description of process : Business Confidential Information in a separate folder BCI Description step process involving potential human exposure during manufacturing: There are only 5 steps where human exposure occurs. 1 **Sampling** the product in solvent 2 **Sampling** the solvent damp solid 3 **Sampling** the dry product 4 **Packing & Sampling** the final product. 5 **Quality Control** testing. The process is operated on 5 shifts. A total of 15 process operators are involved. One operator should be present at each of the above sampling points, and two operators for the packing. Six chemists are involved in testing in the quality control laboratory. All maintenance work is controlled by Permit to Work procedures, which cover the hazards of substances involved, control measures and PPE*. Cleaning is controlled by procedures and relevant PPE is specified to prevent exposure.	
ii) Workplace description	1. The product in solvent is sampled at ~65°C from a closed sampling point. The sample is withdrawn from the bulk liquid by vacuum, and the sample transferred to a glass bottle while wearing PVC suit, PVC gloves and a full face visor. 2. Sampling the damp product. After filtration and before washing/drying the solvent damp product is sampled. The damp product is sampled by a thief type sampling device, through a dedicated sample valve. The filter vessel remains sealed when sampling. When transferring the sample to a bottle, the operator wears a Chemical Disposable Dust Suit, PVC gloves and goggles. 3. This sampling procedure is repeated after washing the	

* PPE = Personal Protective Equipment

Section A2.10 **BPD Annex Point IIA2.10**	**Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC**

	product. 4. Sampling the dry product. The product is sampled, usually once or twice, to ensure it is dry before packing. The technique and PPE are as in 2 above. 5. The pack off and sampling is carried out by two operators in a full air suit, over a period of approx.1 hour. There is LEV to the pack-off head. The polythene liner is sealed to the pack-off head when filling the container. The product is sampled as it is removed from the pack-off head. Analytical Testing. Almost all analytical work with the product in powder form is conducted in fume cupboards. The only exception is the LOD testing, which is carried out in a vacuum oven. The PPE specified for these tasks is protective gloves and safety glasses.
iii) Inhalation exposure	There is no inhalation exposure. The vapour pressure of Flufenoxuron at 20°C is almost zero, and even with lower boiling solvents such as methanol present, the risk from the product is negligible. For sampling the product in solvent, the PPE is specified for splash protection. When packing and sampling the dry powder, there is potential exposure to dust. Typically 15 containers are sampled during the pack-off. There is some dust present during these operations. This is controlled by LEV[1] and the operators also wear a full air suit. There is negligible inhalation exposure in the QC laboratory during analysis of the final product.
iv) Dermal exposure	There is no dermal exposure from sampling the product in solution, sampling the damp or dry product, packing and sampling the final product, or testing the product in the Quality Control (QC) Laboratory.
2.10.1.2Intended use(s)	
1. Professional Users	See Document IIB:
i) Description of application process	“Basiment Holzwurm, BV Konzentrat” “Basiment Holzwurm, BV U 1551” prepared byXXXXX
ii) Workplace description	

[1] LEV = Lowest Exposure Value

Section A2.10 **BPD Annex Point IIA2.10**	**Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC**
iii) Inhalation exposure	
iv) Dermal exposure	
2. Non-professional Users including the general public	See Document IIB: "Basiment Holzwurm, BV Konzentrat" "Basiment Holzwurm, BV U 1551" prepared by XXXXX
(i) via inhalational contact	
(ii) via skin contact	
(iii) via drinking water	
(iv) via food	
(v) indirect via environment	
2.10.2 Environmental exposure towards active substance	
2.10.2.1Production	
(i) Releases into water	There are no aqueous discharges from the process.
(ii) Releases into air	The LEV from dust extractors used for the pack off, are all fitted with HEPA filters, which remove >99.99% of all particulates. The a.i. concentration in the exhaust has not been determined.
(iii) Waste disposal	The total organic waste is typically 4000 l. / mT of product, and total waste for 2003 campaigns was 363mT at typically 2% a.i. All the waste is incinerated.

Section A2.10 **BPD Annex Point IIA2.10**	**Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC**

2.10.2.2Intended use(s)

Affected compartment(s):	See Document IIB: "Basiment Holzwurm, BV Konzentrat" "Basiment Holzwurm, BV U 1551" prepared by XXXXX
water	
sediment	
air	
soil	
Predicted concentration in the affected compartment(s)	
water	
sediment	
air	
soil	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	21/12/2004
Evaluation	Agree with applicant's version
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Evaluation	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Remarks	

+Section A3 **Physical and Chemical Properties of the Active Substance**

Subsection (Annex Point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, temperature, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.1 Melting point, boiling point, relative density (IIA, III.3.1)								
3.1.1 Melting point								
Melting point	EEC A1. 1.4.4.1, (Differential Thermal Analysis) OECD 102	Pure a.i.: purity of 99 % Purity : 99.3%	Melting range: 169-172°C	None	N Y	2 1	Camilleri P. et al., 1986, FX-303-002 XXX Daum A., 2001, XXXX	X
3.1.2 Boiling point								
Boiling point	EEC A2. 1, DTA, OECD 103, OECD 113	Pure a.i.: purity of 99 % Purity : 99.3%		Melting occurs under decomposition, therefore, no boiling point could be observed.	N Y	2 1	Camilleri P. et al., 1986, FX-303-002 (XXXX) Daum A., 2001, XXXX	

Section A3 **Physical and Chemical Properties of the Active Substance**

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.1.3 Bulk density/ relative density								
Bulk/rel. density	EEC A3. 1.4.3 OECD 109 Gas comparison pycnometer	Technical a.i Purity of 99.3 %	The relative density (D_4^{20}) of the technical active ingredient is 1.649 g/cm³.	None	Y	1	Kaestel R., 2001c, XXXX	X
3.2 Vapour pressure (IIA, III.3.2)								
Vapour pressure	EEC A.4	Purity of 97.4%	The vapour pressure of the active substance was found to be 6.52 x 10^{-12} Pa at 20°C and 2.32 x 10^{-11} Pa at 25°C (by extrapolation).	None	Y N	1 1	Langner E.J., 1988, FX-301-002 (XXXX) Rice P., 2000, FX-390-025 (XXXX)	

Section A3 **Physical and Chemical Properties of the Active Substance**

Subsection (Annex Point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, temperature, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.2.1 Henry´s Law Constant (Pt. I-A3.2)	Calculations based on vapour pressure, molecular weight and water solubility		Henry's Law constant at 25 ^{O}C, with a solubility of 3.11 x 10^{-6} mol/m^3 H = 7.46 x 10^{-6} (Pa x m^3/mol).	None	N	1	Rice P., 2000, FX-390-025 (XXXX)	
3.3 Appearance (IIA, III.3.3) **3.3.1 Physical state** **3.3.2 Colour** **3.3.3 Odour**	Visual examination; Organoleptic determination;	Pure a.i. : purity of 99.3% Technical: purity of 99.3 %	Flufenoxuron PAI is a white crystalline solid; the TC is a white, fine powder. Flufenoxuron PAI smells sourish. Flufenoxuron TC has a spicy odour.	None	Y Y	1 1	Kaestel R., 2001, (XXXX Kaestel R., 2001, XXXX	

Section A3 **Physical and Chemical Properties of the Active Substance**

Subsection (Annex Point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, temperature, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.4 Absorption spectra (IIA, III.3.4) **UV/VIS** **IR** **NMR** **MS**	None	None	The structure of flufenoxuron is confirmed by all spectra: UV, IR, NMR and MS The UV spectrum shows a broad transition between 230 and 300 nm with a maximum molar extinction coefficient of 9320 L x mol^{-1} x cm^{-1} at 255 nm.	None	N	2	Fang L.Y., 1996, (XXXX)	
3.4.1 UV/VIS spectra **NMR** **MS**	None	Pure a.i. : purity of 99.2%	The UV spectrum in methanol shows three main peaks at 220, 235 and 254 nm with molar extinction coefficient of 19983 l x mol^{-1} x cm^{-1}, 17880 l x mol^{-1} x cm^{-1}, 19330 l x mol^{-1} x $cm^{-1,}$, respectively. In water , the following results were	None	Y	1	Daum A., 2003, (XXXX)	

Section A3 **Physical and Chemical Properties of the Active Substance**

Subsection (Annex Point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, temperature, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
			obtained: neutral: λ (nm) ε (lx mol $^{-1}$cm^{-1}) 206 31741 252 17403 274 18589 acidic λ (nm) ε(lx mol $^{-1}$cm^{-1}) 220 12073 258 9424 297 10642 basic λ (nm) ε(lx mol $^{-1}$cm^{-1}) 224 19830 237 19571					
3.4.2 IR-Spectra	None	Pure a.i. : purity of 99.2%	IR-spectroscopy: 3248 cm^{-1} and 3108 cm^{-1} amide N-H stretch 1710 cm^{-1} and 1676 cm^{-1} amide C=O stretch 1542 cm^{-1} and 1505 cm^{-1} amide N-H bending	None	Y	1	Daum A., 2003, (XXXX)	

Section A3 **Physical and Chemical Properties of the Active Substance**

Subsection (Annex Point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, temperature, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
			1324 cm^{-1} and 1297 cm^{-1} trifluoromethyl C-F stretch 1135 cm^{-1} :fluoroaromatics C-F stretch 797 cm^{-1}: aromatic C-H "out of plane" → 1,2,3- tri-substitution					
3.4.3 ^{1}H-NMR-Spectra	None	Pure a.i. : purity of 99.2%	^{1}H-NMR (DMSO): reference DMSO-d5: 2.49 ppm, quintet chemical shift ppm / H atom no / multiplicity 11.67 / 22 or 23 / s (broad) 10.38 / 23 or 22 / s (broad) 8.09 / 14 / t* 8.04 / 17 / d 7.71 / 19 / dd 7.63 / 4 / tt* 7.29 / 11 / dd* 7.26 / 3 and 5 / m* 7.20 / 20 / d 6.98 / 13 / ddd* * including coupling to fluorine atoms	None	Y	1	Daum A., 2003, (XXXX)	
3.4.4 MS-Spectra	None	Pure a.i. : purity of 99.2%	Mass spectrum: ESI (negative ion	None	Y	1	Daum A., 2003, (XXXX)	

Section A3 **Physical and Chemical Properties of the Active Substance**

Subsection (Annex Point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, temperature, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
			mode)MS/MS m/z assignment 487 $(M-H)^-$ (quasi molecular íon) 467 $(M-H)^-$ -HF 411 $(M-H)^-$-2HF - HCl 304 C13H7 Cl F4NO$^-$ 289 C14H7F2N2O3$^-$ 156 C7H4F2NO$^-$					

Section A3 **Physical and Chemical Properties of the Active Substance**

Subsection (Annex Point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, temperature, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.5 Solubility in water (IIA, III.3.5)								
Water solubility for Flufenoxuron	EEC A.6.1.3, Column elution method	Purity of 97.4%	Flufenoxuron is nearly insoluble in water. The solubility of flufenoxuron at 25°C in water is nearly independent of the acidity: pH 7: 1.36 µg/l (=1.36ppb) pH 4: 1.86 µg/l (=1.86ppb) pH 9: 3.69 µg/l (=3.69ppb)	None	Y	1	Langner E.J., 1988, XXXX)	
Water solubility - Flufenoxuron degradates	OECD 105 EEC A.6 Column elution	BASF Reg.No.: 4064702; 97%	The solubility in double distilled water at 20°C was determined to be 4.2 mg/L.		Y	1	Bates M., Rice P., 2002, XXXX	
		BASF Reg.No. 102719; 100%	The solubility in double distilled water at 20°C was determined to be 11.1 g/L.					
		BASF Reg.No.: 241208; 99%	The solubility in double distilled water at 20°C was determined to be 3.2 mg/L.					

Section A3 **Physical and Chemical Properties of the Active Substance**

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.6 Dissociation constant	OECD 112	Pure a.i. : purity of 99%	pKa = 10.2	None	N	1	Camilleri P.,Langner E.J., 1986,XXXX)	X
3.7 Solubility in organic solvents, including the effect of temperature on solubility (IIIA, III.1)	OECD 105 Flask method	TC, Purity of 99.2%	The a.i. is rather insoluble in unpolar solvents and of moderate to good solubility in water miscible solvents. The various solubilities at 20 °C (in mg/l solvent) are: n-heptane: < 10 toluene: 3500 dichloromethane: 16000 methanol: 3500 acetone: 83000 ethyl acetate 55000	None	Y	1	Daum A., 2001, XXXX	

Section A3 **Physical and Chemical Properties of the Active Substance**

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.8 Stability in organic solvents used in b.p. and identity of relevant breakdown products (IIIA, III.2)	See: ▪ Document I.2d-1 “Basiment Holzwurm, BV Konzentrat” ▪ Document I.2d-2 “Basiment Holzwurm, BV U 1551” As IIIA 3.8 related to the representative biocidal product(s) for which Janssen Pharamaceutica has prepared BP dossier							

Section A3 **Physical and Chemical Properties of the Active Substance**

Subsection (Annex Point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, temperature, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.9 Partition coefficient n-octanol/water (IIA, III.3.6)								X
Log Pow active substance	OECD 107 Flask method	Pure a.i.: purity of 99 %	The Log P_{OW} of Flufenoxuron PAI is 4.0 and independent of the pH value of the solution. R.M.S comments : an other study according to the HPLC method was submitted. This test is not valid due to surfactant properties of the active substance.	None	Y	1	Langner E.J., 1988, XXXX)	
Log Pow Flufenoxuron degradates	OECD 117 EEC A.8	BASF Reg.No.: 4064702; 97%	Log Pow = 3.12 (Pow = 1318)	For details see chemical glossary	Y	1	Bates M., Ristrocelli D., Rice P., 2002, (XXXX)	
		BASF Reg.No. 102719; 100%	Log Pow = -0.86 (Pow = 0.138)					

Section A3 **Physical and Chemical Properties of the Active Substance**

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
		BASF Reg.No.: 241208; 99%	Log Pow = 3.62 (Pow = 4169)					
3.10 Thermal stability, identity of relevant breakdown products (IIA, III.3.7)	Internal test method CP001 equivalent to OECD 113, but adapted to equipment used	Pure a.i.: purity of 99%	Stable up to 150 °C under N_2 atmosphere and under air	CP001 requests to reduce the weighing to 2-8 mg and the use of a an alumi-nium pan with pierced cover to avoid explosion	Y	1	Daum, A., 2001, (XXXX)	
3.11 Flammability, including auto-flammability and identity of combustion products (IIA, III.3.8)	EEC A.10 EEC A.12(Reasoning)	TC of 97.6 % purity	Flufenoxuron is not highly flammable. Not applicable to solid and because of the chemical structure of the test substance.	None	Y	1	Van Helvoirt J.A.M.W.,1990, FX-330-001 (XXXX)	
	EEC A.16	TC of 97.6 %	No auto-ignition (no exo-	None	Y	1	Van Helvoirt	

Section A3 **Physical and Chemical Properties of the Active Substance**

Subsection (Annex Point)	**Method**	**Purity/ Specification**	**Results** Give also data on test pressure, temperature, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
		purity	thermic or endothermic reaction up to 400 °C).				J.A.M.W.,1990, XXXX)	
3.12 Flash-point (IIA, III.3.9) Flash-point				Not applicable to solids.				

Section A3 **Physical and Chemical Properties of the Active Substance**

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.13 Surface tension (IIA, III.3.10)								
Surface tension	EEC A.5	Pure a.i.: purity of 99.2%	The surface tension of Flufenoxuron is 49.4 mN/m at 1.0 % (w/w). The following values were obtained as a function of time: Surface ten. time 49.47 1 min 54 s 49.40 2 min 07 s 49.35 2 min 20 s 49.33 2 min 33 s 49.30 2 min 46 s Mean value: 49.37 RSD 0.07 %	The influence of time on the surface tension is only described in raw data	Y	1	Kaestel R.,2001, (XXXX)	X
3.14 Viscosity (-)				Not applicable to solids.				
3.15 Explosive properties (IIA, III.3.11)	EEC A.14	TC of 97.6 % purity	Flufenoxuron is not explosive when exposed to thermal or mechanical stress.	None	Y	1	Van Helvoirt J.A.M.W.,Cardinaals J.M., 1990, XXXX)	X

Section A3 **Physical and Chemical Properties of the Active Substance**

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.16 Oxidizing properties (IIA, III.3.12)	EEC A.17	TC of 97.6 % purity	Flufenoxuron has no oxidising properties but the test has not been correctly fulfilled(no preliminary test)	None	Y	3	Van Helvoirt J.A.M.W.,1990, XXXX)	X
3.17 Reactivity towards container material (IIA, III.3.13)	Unlikely to present a concern considering the above physico-chemical properties such as under IIIA 3.15 and IIIA 3.16							

Summary and Conclusions

Flufenoxuron belongs to the chemical family of benzoylureas. The pure (PAI) and the technical grade (TGAI) active ingredient are white, crystalline solids, which melt at 169-172 °C under decomposition. The PAI and TGAI have a sourish to spicy odour. Flufenoxuron is poorly soluble in n-heptane (<0.01 g/l) and in water (1.36 µg/l at pH 7) but of moderate to good solubility in some of the tested organic solvents, *i.e.* acetone (83 g/l) and ethyl acetate (55 g/l). Flufenoxuron has a potential to bioaccumulate with an octanol/water partition coefficient (log Pow) of ~~4.0.~~5.6

Flufenoxuron is neither explosive nor highly flammable, it does not self-ignite and has no oxidizing properties.

Physical and chemical properties of Flufenoxuron degradates

Depending on pH and temperature, flufenoxuron can be degraded to several cleavage products in aqueous media. All three degradation products have no potential to bioaccumulate with an octanol/water partition coefficient ~~of~~ log Pow < 4. Two of them show a low water solubility at 20 °C (3– 4 mg/L), one degradation product (CL 211558) is soluble in water (11.1 g/L).

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	1.1 EVALUATION BY RAPPORTEUR MEMBER STATE
1.2 Date	~~12/07/05~~03/03/2010
Evaluation of applicant's proposition	Applicant's proposition is acceptable with the following amendments: **3.1.1 Melting point** Reference *Daum A., 2001, XXXX*, Result: Melting point around 170°C The melting occurs under decomposition. **3.1.3 Bulk density/relative density** *The relative density (D_4^{20}) of the technical active ingredient is 1.649 ~~g/cm³~~.* **3.6 Dissociation constant** *pKa = 10.2* at T = 25°C **3.9 Partition coefficient** The two studies submitted by the applicant are not acceptable. A new report for the calculation of the Kow with ACD/Labs 6.00 software was provided by the applicant. The result is Log Kow = 5.6. **3.13 Surface tension** *The surface tension of Flufenoxuron is 49.4 mN/m at 1.0 % (w/w)* and T = 20°C **3.16 Explosive properties** *Flufenoxuron is not explosive when exposed to thermal or mechanical stress* (shock and friction). **3.17 Oxidising properties**

	The substance is considered as not oxidising
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(specify)*
Date	**2 GIVE DATE OF COMMENTS SUBMITTED**
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	**2.1.1.1 Discuss if deviating from view of rapporteur member state**
Remarks	

Section A4 1	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.1	IIIA 4.1.1 Flufenoxuron, active substance

			Official use only
		1 REFERENCE	
1.1	**Reference**	**1) Fang L.Y. 1996** Validation of the high pressure liquid chromatographic method M-2636 for the determination of CL 811,678 in technical grade Flufenoxuron (CL 811,678) XXXX unpublished XXXX)	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	FIFRA 40CFR part 160	
2.2	**GLP**	Yes, laboratory was inspected by United States Environmental Protection Agency (US EPA) Office of Enforcement and Compliance Assurance	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	No	
3.1.2	Cleanup	No	
3.2	**Detection**		
3.2.1	Separation method	Reversed phase HPLC	
3.2.2	Detector	UV detection at 254 nm and external calibration.	
3.2.3	Standard(s)	Flufenoxuron, authentic reference substance - PAI	
3.2.4	Interfering substance(s)	None	

Section A4 1	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.1	IIIA 4.1.1 Flufenoxuron, active substance

3.3	**Linearity**	
3.3.1	Calibration range	48.11 mg/100 ml - 151.66 mg/100 ml (corresponding to ca. 48 % -152 % of the test concentration usually applied in the method).
3.3.2	Number of measurements	5
3.3.3	Linearity	The test substance shows a linear analyte response in the investigated concentration range. A coefficient of correlation (r^2) of >0.9999 was observed, and typical results for the linear regression graph were a slope of 52819.7 and an intercept of 89212.3 in a Flufenoxuron PAI concentration range between 48.11 mg/100 ml and 151.66 mg/100 ml, i.e. between 48 % and 152 % of the test concentration usually applied in the method. The % relative change in the specific response was below the limit of 2% relative standard deviation over the range of 50 % to 150 % of the nominal concentration.
3.4	**Specifity: interfering substances**	The identification of the active ingredient is based on comparison of the HPLC retention time of the active ingredient Flufenoxuron to the retention time of the authentic reference substance Flufenoxuron No analytical signal interferences with potential impurities present in Flufenoxuron TGAI are observed. The identity and peak purity of the active substance HPLC peak was proven by UV diode array detection (DAD).
3.5	**Recovery rates at different levels**	According to Guidance Document SANCO/3030/99, the determination of accuracy for the a.s. in the technical material, in terms of recovery data, is not required. However, the specificity, repeatability and linearity proven for method M-2636 strongly support the accuracy of the method for the determination of the active substance in technical Flufenoxuron.
3.5.1	Relative standard deviation	Not applicable
3.6	**Limit of determination**	Not applicable
3.7	**Precision**	
3.7.1	Repeatability	The "within-assay" precision (repeatability, i.e. analysis of 5 TGAI samples on one day by one analyst) yielded a relative standard deviation (% RSD) of 0.35 %. The “between-assay” precision was determined based on six

Section A4 1	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.1	IIIA 4.1.1 Flufenoxuron, active substance

		independent assays of the test substance by two analysts on two different HPLC instruments with two different columns. The overall precision of these assays was calculated to be 0.171 %. The injection precision was tested to be 0.130 % RSD for six consecutive injections. All these precision values met the requirement of not more than 2 % RSD. The mean active ingredient was determined to be 99.34%.
3.7.2	Independent laboratory validation	Not required
		4 APPLICANT'S SUMMARY AND CONCLUSION
4.1	**Materials and methods**	The content of the active substance Flufenoxuron in the technical active ingredient (TC) is determined by dissolving the TC in THF and subsequent reversed phase HPLC using UV detection at 254 nm and external calibration. <u>Test parameters:</u> The specificity of the method was proven by testing of analytical interference of the active substance with impurities potentially present in the TGAI and by investigation of the homogeneity of the active substance HPLC/UV peak. The accuracy was performed by linearity studies. The method was found to be linear over the range of 50 % to 150 % of the nominal concentration based on a linear regression analyses. The precision of the method was proven by the determination of the "within-assay" precision (repeatability), the “between-assay” precision (reproducibility) and the injection precision.
4.2	**Conclusion**	Data regarding specificity, linearity, accuracy and repeatability indicate that Method M-2636 is suitable for determination of the active substance Flufenoxuron in TC.
4.2.1	Reliability	1
4.2.2	Deficiencies	No

Section A4 1	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.1	IIIA 4.1.1 Flufenoxuron, active substance

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	14/01/05
Materials and methods	Acceptable
Conclusion	The applicant's version can be adopted
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A4 1	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.1	IIIA 4.1.2 Relevant breakdown products, isomers, impurities and additives

There are no components of toxicological, ecotoxicological or environmental concern contained in Flufenoxuron TC. Therefore, a respective analytical method is not required.

The Business Confidential Information regarding impurities present in quantities $\geq$ 1 g/kg is provided in document IIIA 4.1-BCI.doc (See separate folder BCI)

Section A4.2 (a)	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.2	4.2 (a) Soil

			Official use only
		1 REFERENCE	
1.1	**Reference**	**1) Kennedy E.M. 1994** Flufenoxuron (WL115110: Cascade): Determination of residues in soil - Development and validation of a liquid chromatographic method XXXX unpublished XXXX)	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	EPA 171-4(b), EPA 40 CFR 158	
2.2	**GLP**	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Flufenoxuron was extracted from soil with a hexane/acetone solvent	
3.1.2	Cleanup	By solid/liquid chomatography using a "Florisil" silica column and s solid phase extraction cartridge	
3.2	**Detection**		
3.2.1	Separation method	HPLC	
3.2.2	Detector	UV absorbance	
3.2.3	Standard(s)	Flufenoxuron	
3.2.4	Interfering	Presence of interferent peak (not identify but lead to a LOQ	

Section A4.2 (a)	**Analytical Methods for Detection and Identification**		
BPD Annex Point IIA, IV.4.2	4.2 (a) Soil		
	substance(s)	proposed at 0.05 mg/kg)	
3.3	**Linearity**		
3.3.1	Calibration range	~~0.01, 0.1 and 1.0 mg/kg~~ 0.05 µg/ml to 0.5 µg/ml	X
3.3.2	Number of measurements	~~3 per fortification level~~ N=6	
3.3.3	Linearity	~~See Table 4.2/1.~~ R2 = 0.9997	X
3.4	**Specifity: interfering substances**	Interfering peak such LOQ set at 0.05 mg/kg	
3.5	**Recovery rates at different levels**	92-106%	
3.5.1	Relative standard deviation	Within the acceptable limit	X
3.6	**Limit of determination**	0.05 mg/kg	
3.7	**Precision**		
3.7.1	Repeatability	~~Not reported~~Relative standard deviation RSD 1.9 – 7.6%	X
3.7.2	Independent laboratory validation	Not reported	
		4 APPLICANT'S SUMMARY AND CONCLUSION	
4.1	**Materials and methods**	A method of analysis to determine residues of Flufenoxuron in soil was derived from an existing method supplied by Shell Research Ltd by substituting a "Florisil" column clean-up for the original HPLC clean-up procedure. Flufenoxuron was extracted from soil with a hexane/acetone solvent mix. An aliquot of the extract was then purified by solid/liquid chomatography using a "Florisil" silica column and s solid phase extraction cartridge. Flufenoxuron was separated by HPLC and detected by UV absorbance. The validation was carried out at recovery levels of 0.01 mg/kg, 0.1 mg/kg and 1.0 mg/kg Flufenoxuron in each of two soil types.	
4.2	**Conclusion**	The validation results are presented in Table 4.2/1. An interferent peak was present in the control soil samples which could not be separated from Flufenoxuron. The recovery values were therefore	

Section A4.2 (a)	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.2	4.2 (a) Soil

		corrected for the mean residue found in the appropriate soil type. After correction, the mean recovery values ranged from 92% to 106%. The presence of this interfering peak means that this method can only achieve a limit of quantification of 0.05 mg/kg.
4.2.1	Reliability	1
4.2.2	Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	~~14/01/05~~03/03/10
Materials and methods	3.3.1 calibration range is 0.05 µg/ml to **10** µg/ml 3.3.3:. R^2 >0.995**0** 3.5.1. Give values. The values are in 3.7.1. 3.7.1. The values are 1,9 – 7,**8** instead of 1,9 – 7,6
Conclusion	The applicant's version ~~can be adopted with the corrections.~~is not acceptable. The validation of the method is not acceptable: there is no confirmatory method, the contribution of interfering peak is not stated, the replication for recovery, repeatability is insufficient.
Reliability	~~1~~4
Acceptability	~~acceptable~~Not acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4.2/1 Validation data for the analytical for the determination of Flufenoxuron in soil

Reference	**Sample matrix**	**Test substance**	**Fortific. level [mg/kg]**	**Average recovery [%]**	**RSD [%]**	**No. of analyses**
Kennedy E.M. 1994	Orchard soil (clay loam)	Flufenoxuron	0.01	106	4.3	3
			0.1	99	3.5	3
			1.0	103	3.4	3
	Coates soil (silty clay)	Flufenoxuron	0.01	103	7.8	3
			0.1	92	1.9	3
			1.0	101	2.6	3

Section A4.2 (a)	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.2	4.2 (a) Soil

			Official use only
		1 REFERENCE	
1.1	**Reference**	**2) Anonymous 1986** Determination of residues of WL115110 in soil - Liquid chromatographic method XXXX unpublished XXXX **3) Anonymous 1989** Determination of residues of WL 129183 in soil - liquid chromatographic method unpublished XXXX)	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No, as description of the method	
2.2	**GLP**	No	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	See 4.2	
3.1.2	Cleanup	See 4.2	
3.2	**Detection**		
3.2.1	Separation method	See 4.2	
3.2.2	Detector	See 4.2	
3.2.3	Standard(s)	See 4.2	

Section A4.2 (a)	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.2	4.2 (a) Soil

3.2.4	Interfering substance(s)	See 4.2
3.3	**Linearity**	
3.3.1	Calibration range	See 4.2
3.3.2	Number of measurements	See 4.2
3.3.3	Linearity	See 4.2
3.4	**Specifity: interfering substances**	See 4.2
3.5	**Recovery rates at different levels**	See 4.2
3.5.1	Relative standard deviation	See 4.2
3.6	**Limit of determination**	See 4.2
3.7	**Precision**	
3.7.1	Repeatability	See 4.2
3.7.2	Independent laboratory validation	See 4.2

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	See 4.2
4.2	**Conclusion**	This report contains a description of the technical procedure for determination of the "urea" metabolite (CL 932338, Reg.no. 4064702) in soil via HPLC/UV. Validation data are not provided. The limit of quantification was calculated to be 0.005 mg/kg. Since a completely new method was developed and validated in 2002 according to the actual guidelines and technical standard, this method is not described further in this chapter
4.2.1	Reliability	2
4.2.2	Deficiencies	No

Section A4.2 (a)	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.2	4.2 (a) Soil

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	14/01/05
Materials and methods	Too many data are missing. That's impossible to state if the applicant's version is acceptable. .
Conclusion	See remark above
Reliability	*4*
Acceptability	Not determined - See remark above .
Remarks	It would be better to with draw this study, since it doesn't provide any information.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A4.2 (a)	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.2	4.2 (a) Soil

A soil method for the analytes Flufenoxuron (BAS 307 I, Reg.No.

Section A4.2 (a)	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.2	4.2 (a) Soil

		243154, CL 811678) and its "urea" metabolite (CL 932338, Reg.No. 4064702) was developed according to current state of the art. First, the method RLA 12637 was validated with a limit of quantification of 0.01 mg/kg. However, since the application rate of Flufenoxuron in vines will be only 40 g/ha, the method was further validated down to a limit of quantification of 0.001 mg/kg	**Official use only**
		1 REFERENCE	
1.1	**Reference**	**4) Jones S. 2002** Method validation fo RLA12637 "HPLC/MS Method for the Determination of BAS 307 I (CL 811678, flufenoxuron) and CL 932338 Residues in Soil" XXXX **5) Smalley R. 2002** Validation of method RLA 12637 for the analysis of BAS 307 I and CL 932338 in soil down to an LOQ of 0.001mg/kg XXXX unpublished XXXX	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s./b.p. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, SANCO/825/00 rev. 6 of 20 June 2000	
2.2	**GLP**	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Preliminary**		

Section A4.2 (a)	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.2	4.2 (a) Soil

	treatment		
3.1.1	Enrichment	With hexane:acetone (80:20, v:v)	
3.1.2	Cleanup	Using solid phase extraction	
3.2	**Detection**		
3.2.1	Separation method	HPLC	
3.2.2	Detector	MS	X
3.2.3	Standard(s)	Flufenoxuron, metabolite CL 932338	
3.2.4	Interfering substance(s)	Not relevant	
3.3	**Linearity**		
3.3.1	Calibration range	~~Table 4.2/2.~~Jones: for both analytes 0.025 to 0.5µg/ml 0.025 µg/ml correspond to 0.005 µg/g 0.5 µg/ml correspond to 0.1 µg/g Smalley: for both analytes 0.001 – 0.25 µg/ml 0.001 µg/ml correspond to 0.0002 µg/g 0.25 mg/ml correspond to 0.05 µg/g	
3.3.2	Number of measurements	~~Table 4.2/2.~~Jones: n=5; Smalley n=7	
3.3.3	Linearity	~~Table 4.2/2.~~Jones: BAS 307I r^2= 0.9999, CL 932338 r^2= 0.9999 Smalley: BAS 307I r^2= 0.9999, CL 932338 r^2= 1.000	
3.4	**Specifity: interfering substances**	~~Table 4.2/2.~~ No interfering substances	
3.5	**Recovery rates at different levels**	Table 4.2/2.	
3.5.1	Relative standard deviation	Table 4.2/2.	
3.6	**Limit of determination**	0.001 mg/kg	
3.7	**Precision**	.	
3.7.1	Repeatability	Table 4.2/2. (see relative standard deviation RSD)	

Section A4.2 (a)	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.2	4.2 (a) Soil

3.7.2	Independent laboratory validation	~~Table 4.2/2.~~No independent lab validation performed

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	**Principle of method** The analytes Flufenoxuron (BAS 307 I) and CL 932338 ("urea" metabolite) in soil are extracted with hexane:acetone (80:20, v:v). The extract is cleaned up using solid phase extraction. Measurement for both compounds is accomplished using HPLC/MS analysis. **Findings** Flufenoxuron (BAS 307 I) and the "urea" metabolite (CL 932338, Reg.No. 4064702) can be confirmed down to the limit of determination of 0.001 mg/kg. Recoveries and repeatability are shown in Table 4.2/2. This method was used for analysis of soil samples from the most recent European terrestrial field soil dissipation study (Jones S., XXXX).
4.2	**Conclusion**	The relevant residue of Flufenoxuron in soil consists of parent only and is covered by BASF method No. RLA 12637. This method fulfills the EU requirements with regard to specificity (no/low interferences at the LOQ), repeatability (recoveries comparably high and consistent), limit of quantification and recovery (average values within 70-110% with an RSD <20%). The method includes also the only significant metabolite found in soil metabolism studies ("urea", CL 932338) although it is not defined as relevant metabolite according to the EU guidance documents on Relevant Metabolites. Confirmatory techniques are not necessary because of the high specificity of the MS-technique used for final measurements. Overall it is concluded, that the described methodology is well suitable to correctly describe the residue situation of Flufenoxuron in soil.
4.2.1	Reliability	1
4.2.2	Deficiencies	No

Section A4.2 (a)	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.2	4.2 (a) Soil

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	~~14/01/05~~03/03/2010
Materials and methods	The applicant's version is acceptable with the following amendments **3.2.2 Detector** Mass transition Flufenoxuron: 489.4 CL 932338: 347.2
Conclusion	The applicant's version can be adopted
Reliability	1
Acceptability	acceptable.
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4.2/2 Validation data for the analytical method RLA 12637 for the determination of Flufenoxuron and CL 932338 in soil

Reference	Sample matrix	Test substance	Fortific. level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses

Table 4.2/2 Validation data for the analytical method RLA 12637 for the determination of Flufenoxuron and CL 932338 in soil

Reference	Sample matrix	Test substance	Fortific. level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Jones S. 2002	Soil (clay)	Flufenoxuron	0.1	77	4.4	5
			0.01	98	12.1	5
	Soil (silty loam)	Flufenoxuron	0.1	93	8.6	5
			0.01	85	5.1	5
Smalley R. 2002	Soil	Flufenoxuron	0.001	90	8.9	5
						5
Jones S. 2002	Soil (clay)	CL 932338	0.1	70	1.6	5
			0.01	90	7.5	5
	Soil (silty loam)	CL 932338	0.1	79	5.7	5
			0.01	78	9.6	5
Smalley R. 2002	Soil	CL 932338	0.001	90	12.0	5

Section A4.2 (b)	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.2	4.2 (b) Water

			Official use only
		1 REFERENCE	
1.1	**Reference**	**6) Anonymous 1986** Determination of residues of WL115110 in water - Liquid chromatographic method XXXX unpublished XXXX	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	Janssen Pharmaceutica	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	See 4.1	
3.1.2	Cleanup	See 4.1	
3.2	**Detection**		
3.2.1	Separation method	See 4.1	
3.2.2	Detector	See 4.1	
3.2.3	Standard(s)	Flufenoxuron	
3.2.4	Interfering substance(s)	See 4.1	
3.3	**Linearity**		
3.3.1	Calibration range	See 4.1	

Section A4.2 (b)	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.2	4.2 (b) Water

3.3.2	Number of measurements	See 4.1
3.3.3	Linearity	See 4.1
3.4	**Specifity: interfering substances**	See 4.1
3.5	**Recovery rates at different levels**	See 4.1
3.5.1	Relative standard deviation	See 4.1
3.6	**Limit of determination**	See 4.1
3.7	**Precision**	
3.7.1	Repeatability	See 4.1
3.7.2	Independent laboratory validation	See 4.1

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	This report contains a description of the technical procedure for determination of Flufenoxuron in water via HPLC/UV after concentration using a C18-cartridge. Validation data are not provided. The limit of quantification was assumed to be approximately 0.01 ug/l after a further cleanup step using a silica Bond Elut cartridge and reversed phase HPLC. Since a completely new method was developed and validated in 2002 according to the actual guidelines and technical standard, this method is not described further in this chapter.
4.2	**Conclusion**	See 4.1
4.2.1	Reliability	2
4.2.2	Deficiencies	No

Section A4.2 (b)	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.2	4.2 (b) Water

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	14/01/05
Materials and methods	The method is only described, but LOQ is not demonstrated, only assumed. Recovery @ LOQ hasn't been verified. Too many raw data are missing, making it impossible to state if the applicant's version is acceptable.
Conclusion	See above
Reliability	4
Acceptability	See above
Remarks	Not described because of superseded by another method : give refernce of this preferred method.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A4.2 (b)	**Analytical Methods for Detection and Identification**
BPD Annex Point IV.4.2	4.2 (b) Water

			Official use only
		1 REFERENCE	
1.1	**Reference**	**7) Smalley R. 2003** Validation of method RLA 12680 for the analysis of BAS 307 I and metabolite CL 932338 in water at an LOQ of 0.01 µg/litre XXXX unpublished XXXX	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s./b.p. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	SANCO/3029/99 rev. 4 (11 July 2000)	
2.2	**GLP**	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Not applicable	
3.1.2	Cleanup	Isolute C18 SPE cartridge	
3.2	**Detection**		
3.2.1	Separation method	~~None~~HPLC	
3.2.2	Detector	MS/MS	
3.2.3	Standard(s)	~~HPLC~~Flufenoxuron, metabolite CL 932338	
3.2.4	Interfering substance(s)	None	
3.3	**Linearity**		

Section A4.2 (b)	**Analytical Methods for Detection and Identification**
BPD Annex Point IV.4.2	4.2 (b) Water

3.3.1	Calibration range	~~0.1 to 0.01 µg/l~~ 0.25 to 1.5 ng/ml
3.3.2	Number of measurements	~~6~~ 5
3.3.3	Linearity	~~See Table 4.2/3.~~ Flufenoxuron r^2= 0.9958 CL 932338 r^2= 0.9961
3.4	**Specifity: interfering substances**	Not relevant because of MS determination
3.5	**Recovery rates at different levels**	See Table 4.2/3.
3.5.1	Relative standard deviation	See Table 4.2/3.
3.6	**Limit of determination**	0.01 µg/l in tap, ground and river water.
3.7	**Precision**	
3.7.1	Repeatability	See Table 4.2/3 (relative standard deviation RSD)
3.7.2	Independent laboratory validation	~~See Table 4.2/3~~ No independent lab validation performed

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1 Materials and methods

Principle of method

Analytical method RLA 12680 has been validated for Flufenoxuron (BAS 307 I) and the "urea" metabolite (CL 932338, Reg.No. 4064702) down to a limit of quantification of 0.01 ug/l in tap, ground and river water.

Flufenoxuron and CL 932338 are extracted from the water using an Isolute C18 SPE cartridge. Determination of the residues is carried out by HPLC-MS/MS detection. Because of the high specificity of the MS method no further confirmatory method is considered necessary.

Findings

The results of the validation is shown in Table 4.2/3. Recoveries ≥ 85% were obtained for Flufenoxuron and CL 932338 in ground, river and tap water at all fortification levels.

4.2 Conclusion

The relevant residue of Flufenoxuron in water consists of parent only and is covered by BASF method No. RLA 12680. This

Section A4.2 (b)	**Analytical Methods for Detection and Identification**
BPD Annex Point IV.4.2	4.2 (b) Water

		method fulfils the EU requirements with regard to specificity (no/low interferences at the LOQ), repeatability (recoveries comparably high and consistent), limit of quantification and recovery (average values within 70-110% with an RSD <20%). The method includes also the only significant metabolite ("urea", CL 932338) found in aquatic metabolism studies (water/sediment studies) although it is not defined as relevant metabolite according to the EU guidance documents on Relevant Metabolites. Confirmatory techniques are not necessary because of the high specificity of the MS-technique used for final measurements. Overall it is concluded, that the described methodology is well suitable to correctly describe the residue situation of Flufenoxuron in water.
4.2.1	Reliability	1
4.2.2	Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	14/01/05
Materials and methods	The applicant's version is acceptable
Conclusion	The applicant's version can be adopted
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4.2/3 Validation data for the analytical method RLA 12680 for the determination of Flufenoxuron and CL 932338 in water

Reference	Sample matrix	Test substance	Fortific. level [ug/l]	Average recovery [%]	RSD [%]	No. of analyses
Smalley R. 2003	Ground water	Flufenoxuron	0.01	92	3.9	~~6~~5
			0.1	85	5.3	~~6~~5
	River water	Flufenoxuron	0.01	110*	5.8	~~6~~5
			0.1	96	8.4	~~6~~5
	Tap water	Flufenoxuron	0.01	87	3.4	~~6~~5
			0.1	93	6.9	~~6~~5
	Ground water	CL 932338	0.01	98	5.4	~~6~~5
			0.1	94	5.3	~~6~~5
	River water	CL 932338	0.01	94	6.1	~~6~~5
			0.1	106*	6.0	~~6~~5
	Tap water	CL 932338	0.01	95	5.4	~~6~~5
			0.1	94	13.2	~~6~~5

* Two values outside the range of 70-110% recovery

Section A4.2 (b)	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.2	4.2 (c) Air

			Official use only
		1 REFERENCE	
1.1	**Reference**	**8) Zangmeister W. 2003** Validation of analytical method 533: Determination of BAS 307 I (Flufenoxuron) in air by LC/MS-MS XXXX. unpublished XXXX	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s./b.p. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	SANCO/825/00 rev. 6 of 20 June 2000; SANCO/3029/99 rev. 4 (11 July 2000); EEC 91/414	
2.2	**GLP**	Yes (laboratory certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Extracted with a mixture of n-hexane/acetone.	X
3.1.2	Cleanup	Solvent is evaporated to dryness and the residue is dissolved in methanol/water	
3.2	**Detection**		
3.2.1	Separation method	~~None~~LC	
3.2.2	Detector	MS/MS	
3.2.3	Standard(s)	~~LC~~Flufenoxuron	
3.2.4	Interfering	None	

Section A4.2 (b)	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.2	4.2 (c) Air

	substance(s)		
3.3	**Linearity**		
3.3.1	Calibration range	~~0.063 and 0.0001~~ 0.001 to 0.02 µg/l	
3.3.2	Number of measurements	~~6~~5	
3.3.3	Linearity	~~See Table 4.2/4~~ r^2 = 0.9973 – 0.9998	
3.4	**Specifity: interfering substances**	Not relevant because of MS determination	
3.5	**Recovery rates at different levels**	See Table 4.2/4	
3.5.1	Relative standard deviation	See Table 4.2/4	
3.6	**Limit of determination**	0.0001 µg/l.	
3.7	**Precision**		
3.7.1	Repeatability	~~See Table 4.2/4~~Overall repeatability: RSD = 7.9% (n=12)	
3.7.2	Independent laboratory validation	Not required	
	4 APPLICANT'S SUMMARY AND CONCLUSION		
4.1	**Materials and methods**	**Principle of method** After sampling of approximately 540 l air by sucking air (90 l/h) for appr. 6 hours through a Tenax absorber tube (no major break through observed), the absorber tube is closed with 2 plastic caps and transported to the laboratory for analysis or stored at +4°C (storage stability for 7 days proven). For analysis, the Tenax adsorbent is extracted with a mixture of n-hexane/acetone (8:2, v:v). The solvent is evaporated to dryness and the residue is dissolved in methanol/water (7:3, v:v) for gradient LC-MS/MS determination. **Findings**	X

Section A4.2 (b)	**Analytical Methods for Detection and Identification**
BPD Annex Point IIA, IV.4.2	4.2 (c) Air

		Recoveries and repeatability for Flufenoxuron in air are shown in Table 4.2/4.
4.2	**Conclusion**	For air analyses, a HPLC-MS/MS parent method using the common Tenax sampling technique was developed and validated. The method meets all EC requirements regarding specificity (no severe interferences), repeatability (comparable high and consistent results), limit of quantification of 0.0001 µg/l (based on toxicological data) and recovery. It can be concluded that the described method correctly determines the residue level of Flufenxuron in air.
4.2.1	Reliability	1
4.2.2	Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	~~14/01/05~~03/03/10
Materials and methods	3.1.1. This section should also deal with the adsorption of the compound on a TENAX adosrber before the extraction with hexane/acetone. **4.1 Materials and method** The study was performed at 35°C with a humidity of 80%.
Conclusion	The applicant's version can be adopted with ~~the complement in 3.1.1.~~above amendments
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 4.2/4 Validation data for the analytical method for the determination of Flufenoxuron in air

Reference	**Sample matrix**	**Test substance**	**Fortific. level [ug/l]**	**Average recovery [%]**	**RSD [%]**	**No. of analyses**
Zangmeister W. (2003)	Air	Flufenoxuron	0.0001 0.063	88.2 91.8	1.6 11.1	6

Section A4.2 (b) **Analytical Methods for Detection and Identification**

BPD Annex Point IV.4.2 4.2 (d) Animal tissue and human body fluids and tissues

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Since Flufenoxuron is not considered toxic or highly toxic, no residue method is required for animal and human body fluids and tissues according to Commission Directive 96/46/EC.	
Undertaking of intended data submission [ ]	Not applicable	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*16/02/2007*
Materials and methods	*applican'ts version acceptable.*
Conclusion	*Adopt applicant's version*
Reliability	*N a*
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A4.3	**Analytical Methods for Detection and Identification**
BPD Annex Point IIIA, IV.1	4.3 Residue in/on food or feedstuffs

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Not required because of the use recommendations as a wood preservative [PT 8]	
Undertaking of intended data submission [ ]	Not applicable	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	21/02/2005
Evaluation of applicant's justification	Aceptable
Conclusion	Adopt applicant's version
Remarks	1

Section A4.3	**Analytical Methods for Detection and Identification**
BPD Annex Point IIIA, IV.1	4.3 Residue in/on food or feedstuffs

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A5	**Effectiveness against target organisms and intended uses**	
BPD Annex point IIA, V.		
Subsection (Annex Point)	Documents III-B, prepared by XXXX , provide details on this annex point for the 2 representative formulations: ▪ "Basiment Holzwurm, BV Konzentrat" ▪ "Basiment Holzwurm, BV U 1551" to support Annex I listing for Flufenoxuron as a PT8. Therefore only brief input is given.	**Official use only**
5.1 Function (IIA, V.5.1)	PT 8 – Wood Preservative	
5.2 Organism(s) to be controlled and products, organisms or objects to be protected (IIA, V.5.2)	Wood borers	
5.2.1 Organism(s) to be controlled (IIA, V.5.2)	*Hylotrupus bajulus* (longhorn beetle), *Anobium punctun* (furniture beetle), *Lyteus Brunneus* (Powderpost beetle)	**X1**
5.2.2 Products, organisms or objects to be protected (IIA, V.5.2)	Wood	
5.3 Effects on target organisms, and likely concentration at which the active substance will be used (IIA, V.5.3)		
5.3.1 Effects on target organisms (IIA, V.5.3)	Stage: larva (see Document IIIB)	
5.3.2 Likely concentrations at which the A.S. will be used (IIA, V.5.3)	See Table 5/1. The range of variation is intended to cover variables such as exposure conditions, nature, level of infestation, wood species and application methodology	**X2**
PT8	See Table 5/1	

Section A5 **BPD Annex point IIA, V.**		**Effectiveness against target organisms and intended uses**	
5.4	**Mode of action (including time delay) (IIA, V.5.4)**		
5.4.1	**Mode of action**	It is a growth regulator that interferes in chitin production during cuticule development.	
5.4.2	**Time delay**	Related to the moult larva period	
5.5	**Field of use envisaged (IIA, V.5.5)**		
	MG02: Preservatives	Product type PT8 – Wood Preservative Hazard Class: 1, 2 and 3	**X3**
5.6	**User (IIA, V.5.6)**		
	Industrial	Double vacuum or pressure impregnation	**X4**
	Professional	Spray; brush and injection	
	General public	Brush	
5.7	**Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies (IIA, V.5.7)**		
5.7.1	**Development of resistance**	Very low due to the long generation time and the limited number of treatment on subsequent generations	
5.7.2	**Management strategies**	See 5.7.1	
5.8	**Likely tonnage to be placed on the market per year (IIA, V.5.8)**	*confidential*	

Section A5 **Effectiveness against target organisms and intended uses**
BPD Annex point IIA, V.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*April, 7th, 2005*
Materials and methods	X1) Data only provided on *Hylotrupes bajulus.* No existing efficacy data provided on *Anobium punctatum* nor on *Lyctus brunneus*. X2) No efficacy data accepted to support a preventive superficial application method. Data provided to support a preventive impregnation application treatment with 0.75 g flufenoxuron /m^3. X3) No efficacy data accepted to support a preventive superficial application method. X4) No efficacy data accepted to support a preventive superficial application method
Conclusion	According data provided by the applicant, the claim should be: Product type 8, curative treatment against *Hylotrupes bajulus*, and preventive impregnation treatment against wood borers.
Reliability	Trials have been done under quality assurances procedures 1a
Acceptability	Acceptable for curative treatment on *Hylotrupes bajulus* and for preventive impregnation treatment on wood borers.
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 5/1 – Supported uses for Flufenoxuron as a wood preservative (PT 8)

Object and/or situation	**MS/C**[1]	**Product Name**	**Organisms Controlled**	**Formulation**		**Application**			**Applied amount per treatment**			**Remarks:**
(a)		Basiment Holzwurm	(c)	Type (d-f)	Conc. of as (i)	method kind (f-h)	number min max (k)	interval between applications (min)	g as/L min max	water L/m^2 min max	g as/m^2 min max	(m)
Preventive impregnation	EU	Wocosen 100 SL/FL	Wood borers	SL	Flufenoxuron 0.75g/l propiconazole 100	Impregnation (double vacuum, pressure impregnation, injection)	1	N/A	N/A	N/A	0.5-3.0 g/m^3	
		BV U 1551		SL	0.2 g/l							
Curative Superficial	EU	BV Konzentrat	Wood borers	EC	1.0 g/l	Superficial treatment (Spray or brush)	1	N/A	N/A	N/A	~~0.02~~ 0.064 g/m^2	
		BV U 1551		SL	0.2 g/l							

a) *e.g.* biting and suckling insects, fungi, molds; (b) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(c) GCPF Codes - GIFAP Technical Monograph No 2, 1989 ISBN 3-8263-3152-4); (d) All abbreviations used must be explained
(e) g/kg or g/l;(f) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench;
(g) Kind, *e.g.* overall, broadcast, aerial spraying, row, bait, crack and crevice equipment used must be indicated;
(h) Indicate the minimum and maximum number of application possible under practical conditions of use;
(i) Remarks may include: Extent of use/economic importance/restrictions

[1] MS/C=Member state/country

Section A6.1.1	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.1	6.1.1 Acute Oral (limit test)

Official use only

		1 REFERENCE 1
1.1	**Reference**	**1) XXXX** WL115110 (CASCADE): Acute oral toxicity XXXX unpublished XXXX
1.2	**Data protection**	No
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	XXXX
1.2.3	Criteria for data protection	No data protection claimed
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, in compliance with OECD 401
2.2	**GLP**	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	
3.1.1	Lot/Batch number	Batch: XXXX
3.1.2	Specification	See Business Confidential Information folder
3.1.2.1	Description	Powder as described in section 2
3.1.2.2	Purity	97.6%
3.1.2.3	Stability	Not relevant

Section A6.1.1	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.1	6.1.1 Acute Oral (limit test)

3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fischer 344	
3.2.3	Source	XXXX.	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Mean bw (Day 1), males: 200 g, females: 131 g	X
3.2.6	Number of animals per group	5/sex	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Postexposure period	14 days	
3.3.2	Type	Gavage	
3.3.3	Dose	5,000 mg/kg bw	
3.3.4	Vehicle	Carboxymethylcellulose	
3.3.5	Concentration in vehicle	25 % w/w	
3.3.6	Total volume applied	18.6 ml/kg bw	
3.3.7	Controls	None	
3.4	**Examinations**	Clinical observations, body weight determinations, mortality, gross pathology	
3.5	**Method of determination of LD_{50}**	Not applicable	
3.6	**Further remarks**	Not applicable	

Section A6.1.1	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.1	6.1.1 Acute Oral (limit test)

		4 RESULTS AND DISCUSSION.
4.1	**Clinical signs**	No mortality, no overt signs of reaction to treatment
4.2	**Pathology**	No abnormalities detected upon necroscopy examinations.
4.3	**Other**	Not applicable
4.4	**LD_{50}**	Oral LD_{50} was greater than 5,000 mg/kg bw (highest dose tested) for males and females.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	5 males and 5 female Fischer 344 rats (source: XXXX.; mean bw at study initiation, males: 200 g, females: 131 g) received a single oral dose of Flufenoxuron administered by gavage as a 25% w/w suspension in 0.5% (w/v) aqueous carboxymethylcellulose (Specific weight of the suspension: 1.073 g/ml). The test material was administered at the limit dose level of 5,000 mg/kg bw and at a dose volume of 18.6 ml/kg bw. A careful clinical examination was made three times daily for the first and second days and once daily thereafter for the remainder of the 14-day observation period. The initial (day 1), day 7 and day 14 bodyweights were recorded, and changes in bodyweight calculated. All animals survived treatment and were subject to necropsy on day 14. Animals were killed by an intraperitoneal injection of sodium pentobarbitone. The cranial, thoracic and abdominal cavities and viscera were examined and any gross pathological changes recorded, see Table 6.1.1/1 .
5.2	**Results and discussion**	No mortality was observed throughout the 14-day post-treatment period. There were no overt signs of reaction to treatment. All rats had gained weight relative to their Day 1 bodyweights by the end of the 14-day observation period. No abnormalities were detected upon necroscopy examinations.
5.3	**Conclusion**	The acute oral LD_{50} of Flufenoxuron was greater than 5,000 mg/kg bw.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A6.1.1	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.1	6.1.1 Acute Oral (limit test)

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	21/09/2004
Materials and Methods	3.2.5. Age/weight at study initiation : *8-9 weeks old*
Results and discussion	Applicant's version acceptable
Conclusion	Applicant's version acceptable
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.1.1/1 Acute Oral toxicity

Test facility / Reference	**Test substance** **Study type** **Species**	**Results**	**Comment**
XXXX	Flufenoxuron (in CMC) LD_{50} oral gavage Fischer 344 rat	> 5,000 mg/kg bw	No systemic toxicity

Section A6.1.1	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.1	A 6.1.1 Acute Oral

Official use only

		1 REFERENCE 2
1.1	**Reference**	**2) XXXX** Toxicology of insecticides (acyl ureas): The acute oral and percutaneous toxicity, skin and eye irritancy and skin sensitizing potential of WL115110 XXXX unpublished XXXX
1.2	**Data protection**	No
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	XXXX
1.2.3	Criteria for data protection	No data protection claimed
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Not reported, but in general compliance with OECD 401
2.2	**GLP**	No, at the time the study was conducted GLP was not compulsory. However the study was conducted according to the principle of Good Laboratory Practices.
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	
3.1.1	Lot/Batch number	Batch: XXXX
3.1.2	Specification	As described in section 2
3.1.2.1	Description	Powder as described in section 2
3.1.2.2	Purity	99%
3.1.2.3	Stability	Not relevant

Section A6.1.1	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.1	A 6.1.1 Acute Oral

3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Fischer 344
3.2.3	Source	XXXX
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Age: 9-11 weeks; Mean bw (Day 1), males: 221 g, females: 131 g
3.2.6	Number of animals per group	5/sex
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Oral
3.3.1	Postexposure period	14 days
3.3.2	Type	Gavage
3.3.3	Dose	3,000 mg/kg bw
3.3.4	Vehicle	Dimethyl sulphoxide (DMSO)
3.3.5	Concentration in vehicle	30% w/v
3.3.6	Total volume applied	10 ml/kg bw
3.3.7	Controls	None
3.4	**Examinations**	Clinical observations, body weight determinations, mortality, gross pathology
3.5	**Method of determination of LD_{50}**	Not applicable
3.6	**Further remarks**	Not relevant

Section A6.1.1	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.1	A 6.1.1 Acute Oral

4 RESULTS AND DISCUSSION.

4.1	**Clinical signs**	One female rat died on day 2 of the study; there were no other mortalities observed. Clinical signs (6 hours following dosing): chromodacryorrhea (both sexes), abnormal gait (males only), lethargy (females only), increased lacrimation (females only) and blood-tinged urine (females only). These signs resolved in all survivors by study day 2. Body weight gains were unaffected by administration of the test material.
4.2	**Pathology**	No gross pathological changes. In dead female, necropsy showed: compacted powder in the stomach and hemorrhagic petechiae of the stomach mucosa.
4.3	**Other**	Not applicable
4.4	**LD_{50}**	Oral LD_{50} was greater than 3,000 mg/kg bw (highest dose tested) for males and females.

Section A6.1.1	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.1	A 6.1.1 Acute Oral

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	5 male and 5 female Fischer 344 rats (source: XXXX; mean bw at study initiation, males: 221 g, females: 131 g) received a single oral dose of Flufenoxuron administered by gavage as a 30% w/v solution in dimethyl sulphoxide (DMSO). The formulation was prepared on the day of administration. The test material was administered at a dose level of 3,000 mg/kg bw at a dose volume of 10 ml/kg bw. After dosing animals were observed for clinical signs for 14 days. The initial (day 1), day 7, and day 14 body weights were recorded. All animals were subjected to a gross pathological examination.
5.2	**Results and discussion**	One female rat died on day 2 of the study; there were no other mortalities observed. Clinical signs were noted 6 hours following dosing and consisted of chromodacryorrhea (both sexes), abnormal gait (males only), lethargy (females only), increased lacrimation (females only) and blood-tinged urine (females only). These signs resolved in all survivors by study day 2. Body weight gains were unaffected by administration of the test material. In addition, no gross pathological changes were observed during necropsies in the animals which survived to study termination. In the one female that died during the study, compacted powder in the stomach and hemorrhagic petechiae of the stomach mucosa were found at necropsy. Based on the mortality results, the oral LD_{50} was greater than 3,000 mg/kg bw (highest dose tested) for males and females.See Table 6.1.1/2.
5.3	**Conclusion**	The acute oral LD_{50} of Flufenoxuron was greater than 3,000 mg/kg bw.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A6.1.1	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.1	A 6.1.1 Acute Oral

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	21/09/2004
Materials and Methods	Applicant's version acceptable
Results and discussion	Applicant's version acceptable
Conclusion	Applicant's version acceptable
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.1.1/2 Acute Toxicity

Test facility / Reference	**Test substance Study type Species**	**Results**	**Comment**
XXXX	Flufenoxuron (in DMSO) LD_{50} oral gavage Fischer 344 rat	> 3,000 mg/kg bw	1/10 rats administered 3,000 mg/kg bw died; unspecific clinical signs reversible within 2 days

Section A6.1.2	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.2	6.1.2 Acute Dermal

Official use only

1 REFERENCE

1.1	**Reference**	**1) XXXX** Toxicology of insecticides (acyl ureas): The acute oral and percutaneous toxicity, skin and eye irritancy and skin sensitizing potential of WL115110 XXXX unpublished XXXX **2) XXXX** Corrigendum to SBGR.86.042: Toxicology of insecticides (acyl ureas): The acute oral and percutaneous toxicity, skin and eye irritancy and skin sensitizing potential of WL115110 XXXX unpublished XXXX **Note:** The Corrigendum to the original report consists of 1 page, stating that the study was conducted also in accordance to GLP requirements of the JMAFF (Japanese authority)
1.2	**Data protection**	No
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	XXXX
1.2.3	Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Guideline not mentioned in study, but in general compliance with OECD 402; performed according to method of Noakes D.N. & Sanderson D.M.: Brit. J. Ind. Med. 26, 59-64, (1969)
2.2	**GLP**	No, at the time the study was conducted GLP was not compulsory. However the study was conducted according to the principle of Good Laboratory Practices.
2.3	**Deviations**	The surface area of exposed skin was not specifically mentioned in the study report. According to the method applied (XXXX), the exposure area was about 6 cm x 8 cm.

Section A6.1.2	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.2	6.1.2 Acute Dermal

		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch: XXXX	
3.1.2	Specification	See Business Confidential Information folder	
3.1.2.1	Description	Powder as described in section 2	
3.1.2.2	Purity	99%	
3.1.2.3	Stability	Not applicable, as undiluted test substance	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fischer 344	
3.2.3	Source	XXXX.	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Mean bw (Day 1), males: 236 g, females: 158 g	X
3.2.6	Number of animals per group	5/sex	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**		
3.3.1	Postexposure period	14 days	

Section A6.1.2	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.2	6.1.2 Acute Dermal

3.3.2	Area covered	According to method used, exposed area was about 6 cm x 8 cm	X
3.3.3	Occlusion	Occlusive	
3.3.4	Vehicle	Water (drop to moisten test substance)	
3.3.5	Concentration in vehicle	Not applicable	
3.3.6	Total volume applied	Not applicable	
3.3.7	Duration of exposure	24 h	
3.3.8	Removal of test substance	With warm detergent solution	
3.3.9	Controls	None	
3.4	**Examinations**	Clinical observations, body weight determinations, mortality, gross pathology.	
3.5	**Method of determination of LD_{50}**	Not applicable	
3.6	**Further remarks**	Not applicable	
		4 RESULTS AND DISCUSSION.	
4.1	**Clinical signs**	None of the rats died during the study period. There were no overt clinical signs and all rats had gained weight relative to their day 1 bodyweights by the end of the 14-day observation period.	
4.2	**Pathology**	At necropsy there were no macroscopic abnormalities detected.	
4.3	**Other**	Not applicable	
4.4	**LD_{50}**	The acute percutaneous LD_{50} of Flufenoxuron, in rats was greater than 2,000 mg/kg bw (limit dose) under the study conditions.	

Section A6.1.2	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.2	6.1.2 Acute Dermal

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Five male and five female Fischer 344 rats (source: XXXX; mean bw at study initiation, males: 236 g, females: 158 g) were treated with the undiluted test substance (white powder) at the limit dose of 2,000 mg/kg bw, which was applied to the closely shorn dorsal skin under occlusive conditions for 24 hours [exposure area was about 6 cm x 8 cm according to the method cited in the study report]. Prior to application, the test substance was moistened with a few drops of water. After 24 hours of exposure, the treated skin was cleaned with warm detergent solution. The animals were examined for clinical signs and mortality. The initial (day 1), day 7 and day 14 bodyweights were recorded, and changes in body weight calculated. All rats were examined for gross pathological changes at the end of the 14-day observation period.	
5.2	**Results and discussion**	None of the rats died during the study period. There were no overt clinical sign and all rats had gained weight relative to their day 1 bodyweights by the end of the 14-day observation period. At necropsy there were no macroscopic abnormalities detected. The acute percutaneous LD_{50} of Flufenoxuron, in rats was greater than 2,000 mg/kg bw (limit dose) under the study conditions (see Table 6.1.2/1).	
5.3	**Conclusion**	The acute percutaneous LD_{50} of Flufenoxuron, in rats was greater than 2,000 mg/kg bw.	
5.3.1	Reliability	2	X
5.3.2	Deficiencies	No	

Section A6.1.2	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.2	6.1.2 Acute Dermal

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 3.2.5. Age/weight at study initiation: 9-11 weeks old 3.3.2. Area covered: Express surface area of exposed skin in % (OECD 402 : 10% of the whole body surface recommended)
Results and discussion	Applicant's version acceptable
Conclusion	Applicant's version acceptable
Reliability	1
Acceptability	Acceptable
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.1.2/ 3 Acute Dermal Toxicity

Test facility / Reference	**Test substance Study type Species**	**Results**	**Comment**
XXXX	Flufenoxuron LD_{50} dermal Fischer 344 rat	> 2,000 mg/kg bw	No systemic toxicity, no local irritation

Section 6.1.3	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.3	6.1.3 Acute inhalation

			Official use only
		1 REFERENCE	
1.1	**Reference**	**1) XXXX** WL115110: Acute inhalation toxicity study in rats XXXX upublished XXXX **2) XXXX** Addendum to IRI 3689: WL115110: Acute inhalation toxicity study in rats XXXX, unpublished XXXX **Note:** The Addendum to the original report consists of 3 pages, stating that the study was conducted in accordance to GLP requirements of the JMAFF (Japanese authority)	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Guideline not reported, but in general compliance with OECD 403	
2.2	**GLP**	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch: XXXX	
3.1.2	Specification	See Business Confidential Information folder	
3.1.2.1	Description	Powder as described in section 2	
3.1.2.2	Purity	98%	

Section 6.1.3	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.3	6.1.3 Acute inhalation

3.1.2.3	Stability	Not applicable		
3.2	**Test Animals**			
3.2.1	Species	Rat		
3.2.2	Strain	Sprague-Dawley		
3.2.3	Source	XXXX		
3.2.4	Sex	Male and female		
3.2.5	Age/weight at study initiation	Males: 187 g, females: 163 g		X
3.2.6	Number of animals per group	5/sex group and /dose		
3.2.7	Control animals	No		X
3.3	**Administration/ Exposure**	inhalation		
3.3.1	Postexposure period	14 days		
3.3.2	Concentrations	Nominal concentration	8.9 mg/l	
		Analytical concentration	5.1 mg/l	
3.3.3	Particle size	MMAD (mass median aerodynamic diameter) 3.6 µm ± GSD (geometric standard deviation) of 2 µm The amount of the respirable aerosol fraction (< 4.7 µm) that might reach the alveolar region was calculated to be 63.8% (w/w).		
3.3.4	Type or preparation of particles	Dust (Flufenoxuron is a solid)		
3.3.5	Type of exposure	Nose only		
3.3.6	Vehicle	Air		
3.3.7	Concentration in vehicle	See 3.3.8		X

Section 6.1.3	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.3	6.1.3 Acute inhalation

3.3.8	Duration of exposure	4 h
3.3.9	Controls	Vehicle (air)
3.4	**Examinations**	Clinical observations, body weight determinations, mortality, gross pathology, methemoglobin analysis
3.5	**Method of determination of LD_{50}**	Not applicable
3.6	**Further remarks**	None
		4 RESULTS AND DISCUSSION.
4.1	**Clinical signs**	No mortalities were observed in the control and treatment groups during the 14-day observation period following exposure. All control and treatment group animals displayed a subdued behavior immediately following cessation of exposure. No adverse reactions of the rats were observed during or after exposure that could be related to Flufenoxuron treatment. No test substance related effects on body weight development were evident over the study period. Methemoglobin values, measured on blood samples obtained 1 h after cessation of exposure, were similar for the control and Flufenoxuron treated group. Gross pathological examination on completion of the 14-day post exposure observation period revealed no abnormalities attributable to exposure to Flufenoxuron.
4.2	**Pathology**	Methemoglobin values, measured on blood samples obtained 1 h after cessation of exposure, were similar for the control and Flufenoxuron treated group. Gross pathological examination on completion of the 14-day post exposure observation period revealed no abnormalities attributable to exposure to Flufenoxuron.
4.3	**Other**	Not applicable
4.4	**LD_{50}**	The acute inhalation LC_{50} by nose-only exposure to Flufenoxuron dust aerosol was calculated to be greater than 5.1 mg/l.

Section 6.1.3	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.3	6.1.3 Acute inhalation

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Five male and five female Sprague-Dawley rats [source: XXXX.; mean bw (day 1), males: 187 g, females: 163 g] were exposed to a dust aerosol with nose-only exposure at a nominal concentration of 8.9 mg/l Flufenoxuron in air (limit test). A control group consisting of 5 male and 5 female rats was exposed to air only. The concentration was verified analytically. The MMAD (mass median aerodynamic diameter 50%) and the fraction of respirable particles were determined. All the rats were observed for clinical signs at hourly intervals throughout the 4 h exposure period, for the first 1 h post dosing and at least once daily during the subsequent 14 day observation period. Body weights were determined immediately before dosing and on days 2, 3, 4, 7, 10 and 14 post exposure. For analysis of methemoglobin levels, whole blood samples were taken from the tail vein of every rat approx. 1 hour after the 4-h exposure period. All rats were subjected to gross pathology 14 days after exposure.
5.2	**Results and discussion**	The MMAD was determined to be 3.6 µm with a geometric standard deviation of 2.0. The amount of the respirable aerosol fraction (< 4.7 µm) that might reach the alveolar region was calculated to be 63.8% (w/w) (see Table 6.1.3/4). No mortalities were observed in the control and treatment groups during the 14-day observation period following exposure. All control and treatment group animals displayed a subdued demeanour immediately following cessation of exposure. No adverse reactions of the rats were observed during or after exposure that could be related to Flufenoxuron treatment. No test substance related effects on body weight development were evident over the study period. Methemoglobin values, measured on blood samples obtained 1 h after cessation of exposure, were similar for the control and Flufenoxuron treated group. Gross pathological examination on completion of the 14-day post exposure observation period revealed no abnormalities attributable to exposure to Flufenoxuron (see Table A6.1.3/5).
5.3	**Conclusion**	The 4-hour LC_{50} by nose-only exposure to Flufenoxuron dust aerosol was calculated to be greater than 5.1 mg/l.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section 6.1.3	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.3	6.1.3 Acute inhalation

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 3.2.5. Age/weight at study initiation: Age not mentioned 3.2.7. Control animals Yes 3.3.7. Concentration in vehicle: The section 3.3.8. does not give information on the concentration in vehicle. The concentration measured in the vehicle should be provided for all groups.
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version
Reliability	1
Acceptability	Acceptable
Remarks	In Doc IVA, nominal concentration and particle size distribution are only indicated for the dose group n°2.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.1.3/4 Gravimetric concentration and mortalities in the LC_{50} nose-only determination in rats with Flufenoxuron (dust aerosol)

Test Group	**Concentration (mg/l)**		**Mortality (dead animals/animals exposed**	
	Analytical	**Nominal**	**Males**	**Females**
Flufenoxuron	5.1	8.9	0/5	0/5

Table A6.1.3/5 Acute Dermal Toxicity

Test facility / Reference	**Test substance Study type Species**	**Results**	**Comment**
XXXX	Flufenoxuron LC_{50} 4-hour nose-only inhalation Albino (Sprague-Dawley) rat	> 5.1 mg/l (dust aerosol; MMAD 3.6 µm)	No systemic toxicity, no local irritation

Section A6.1.4	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.4	6.1.4-s Acute Dermal Irritation

			Official use only
		1 REFERENCE	
1.1	**Reference**	**1) XXXX** Toxicology of insecticides (acyl ureas): The acute oral and percutaneous toxicity, skin and eye irritancy and skin sensitizing potential of WL115110 XXXX unpublished XXXX **2) XXXXX** Corrigendum to XXXX: Toxicology of insecticides (acyl ureas): the acute oral and percutaneous toxicity, skin and eye irritancy and skin sensitizing potential of WL115110 XXXX unpublished XXXX **Note:** The Corrigendum to the original report consists of 1 page, stating that the study was conducted also in accordance to GLP requirements of the JMAFF (Japanese authority)	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not reported, but in general compliance with OECD 404	
2.2	**GLP**	No, at the time the study was conducted GLP was not compulsory. However the study was conducted according to the principle of Good Laboratory Practices	
2.3	**Deviations**	No	X

Section A6.1.4	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.4	6.1.4-s Acute Dermal Irritation

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	Batch: XXXX
3.1.2	Specification	See Business Confidential Information folder
3.1.2.1	Description	Powder as described in section 2
3.1.2.2	Purity	99%
3.1.2.3	Stability	Not applicable as undulated test substance
3.2	**Test Animals**	
3.2.1	Species	Rabbit
3.2.2	Strain	New Zealand White
3.2.3	Source	XXXX
3.2.4	Sex	Male/female
3.2.5	Age/weight at study initiation	Age: 4–9 months
3.2.6	Number of animals per group	3/sex
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Dermal
3.3.1	Application	
3.3.1.1	Preparation of test substance	Test substance was used as moisted just after application on the skin.
3.3.1.2	Test site and Preparation of Test Site	Intact skin [on a 2 cm x 2 cm test patch]
3.3.2	Occlusion	Semi occlusive
3.3.3	Vehicle	Not applicable as used as undiluted
3.3.4	Concentration in vehicle	Not applicable
3.3.5	Total amount applied	0.5 g
3.3.6	Removal of test substance	Water

Section A6.1.4	**Acute Toxicity**	
BPD Annex Point IIA, VI.6.1.4	6.1.4-s Acute Dermal Irritation	
3.3.7 Duration of exposure	4 h	
3.3.8 Postexposure period	14 days	
3.3.9 Controls	None	X
3.4 Examinations		
3.4.1 Clinical signs	No	
3.4.2 Dermal examination	There were no skin reactions following the application of Flufenoxuron powder to rabbit skin for 4 hours (score grade 0 for erythema and edema at all time points investigated).	
3.4.2.1 Scoring system	The scoring system was identical to that in OECD 404	
3.4.2.2 Examination time points	At 30 minutes after removal of the patch and 1, 24, 48, 72 hours and 7 days after application.	
3.4.3 Other examinations	Not applicable	
3.5 Further remarks	Not applicable	
	4 RESULTS AND DISCUSSION.	
4.1 Average score		
4.1.1 Erythema	0	
4.1.2 Edema	0	
4.2 Reversibility	Not applicable as no skin irritation observed	
4.3 Other examinations	There were no skin reactions following the application of Flufenoxuron powder to rabbit skin for 4 hours (score grade 0 for erythema and edema at all time points investigated).	
4.4 Overall result	Flufenoxuron is not skin irritant under the test conditions	

Section A6.1.4-s	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.4	6.1.4-s Acute Dermal Irritation

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The test substance (0.5 g) was moistened and applied to the intact skin of three male and three female New Zealand White rabbits [Source: XXXX; age: 4–9 months] for 4 hours on a 2 cm x 2 cm test patch under a semi-occlusive dressing. After the patches were removed the treated area was washed with water and gently dried. The animals were observed for skin irritation for 7 days after test material application. Skin readings were performed at 30 minutes after removal of the patch and 1, 24, 48, 72 hours and 7 days after application.	
5.2	**Results and discussion**	There were no skin reactions following the application of Flufenoxuron powder to rabbit skin for 4 hours (score grade 0 for erythema and edema at all time points investigated). Flufenoxuron is not a skin irritant in rabbits.	
5.3	**Conclusion**	Flufenoxuron is not a skin irritant in rabbits.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	X

Section A6.1.4-s	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.4	6.1.4-s Acute Dermal Irritation

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 2.3. Deviations Yes: application area of 4cm² (instead of the 6cm² required in OECD 404) 3.3.9. Controls Yes
Results and discussion	Agree with the applicant's version
Conclusion	5.3.2. Deficiencies Yes
Reliability	1
Acceptability	Acceptable: the smaller area of substance application is not expected to affect the potential irritation effects
Remarks	Weight at the end of the study not provided in Doc IVA
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 6.1.4	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.4	6.1.4-e Acute Eye Irritation

			Official use only
		1 REFERENCE	
1.1	**Reference**	**1) XXXX** Toxicology of insecticides (acyl ureas): The acute oral and percutaneous toxicity, skin and eye irritancy and skin sensitizing potential of WL115110 XXXX unpublished XXXX **2) XXXX** Corrigendum to XXXX: Toxicology of insecticides (acyl ureas): the acute oral and percutaneous toxicity, skin and eye irritancy and skin sensitizing potential of WL115110 XXXX unpublished XXXX **Note:** The Corrigendum to the original report consists of 1 page, stating that the study was conducted also in accordance to GLP requirements of the JMAFF (Japanese authority)	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Guideline not reported, but in compliance with OECD 405	
2.2	**GLP**	No, at the time the study was conducted GLP was not compulsory. However the study was conducted according to the principle of Good Laboratory Practices	
2.3	**Deviations**	No	

Section 6.1.4	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.4	6.1.4-e Acute Eye Irritation

		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch: XXXX	
3.1.2	Specification	See below	
3.1.2.1	Description	Powder as described in section 2	
3.1.2.2	Purity	99%	
3.1.2.3	Stability	Not applicable as undulated test substance	
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	New Zealand White	
3.2.3	Source	XXXX	
3.2.4	Sex	Male/female	
3.2.5	Age/weight at study initiation	Age: 4–9 months	
3.2.6	Number of animals per group	3/sex	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**		
3.3.1	Preparation of test substance	Test substance was used as delivered	
3.3.2	Amount of active substance instilled	0.1 ml (about 25 mg test substance)	
3.3.3	Exposure period	The eyes were not washed.	X
3.3.4	Postexposure period	7 days	

Section 6.1.4	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.4	6.1.4-e Acute Eye Irritation

3.4	**Examinations**	
3.4.1	Ophthalmoscopic examination	Yes
3.4.1.1	Scoring system	Identical to that given in OECD 405
3.4.1.2	Examination time points	The ocular reactions were assessed approximately 1, 24, 48 and 72 hours after application and on day 7
3.4.2	Other investigations	In the event of any corneal damage visualization was aided by the instillation of one drop of 2% fluorescein solution
3.5	**Further remarks**	Not relevant
	4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	No clinical signs
4.2	**Average score**	
4.2.1	Cornea	No effects
4.2.2	Iris	No effects
4.2.3	Conjunctiva	
4.2.3.1	Redness	Slight effects
4.2.3.2	Chemosis	Minimal
4.3	**Reversibility**	Yes
4.4	**Other**	No other observation
4.5	**Overall result**	Flufenoxuron is not eye irritant

Section 6.1.4	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.4	6.1.4-e Acute Eye Irritation

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The potential of Flufenoxuron to cause damage to the conjunctiva, iris or cornea was assessed by a single ocular application of 0.1 ml bulk volume of the test substance (about 25 mg of the test substance) to one eye of three male and three female New Zealand White (NZW) rabbits [Source: XXXX breeding colony; age: 4–9 months]. The eye was not washed. The reactions of the animals were observed immediately after instillation and the initial pain response recorded. The ocular reactions were assessed approximately 1, 24, 48 and 72 hours after application and on day 7. In the event of any corneal damage visualization was aided by the instillation of one drop of 2% fluorescein solution.
5.2	**Results and discussion**	The instillation of Flufenoxuron into the conjunctival sac of one eye of each of six rabbits resulted in no initial pain. No effects on the cornea or iris were noted in any animal at any time point of the study.The only ocular effects observed one 1 hour after instillation were a slight conjunctival redness (grade 1) in all rabbits accompanied by minimal chemosis (grade 1) in two animals. By 24 hours post-dosing only one rabbit had slight conjunctival redness, which had subsided by the next time point of scoring at 48 h post instillation (see Table 6.1.4-e/6).
5.3	**Conclusion**	Flufenoxuron is not an eye irritant according to EU classification criteria.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section 6.1.4	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.4	6.1.4-e Acute Eye Irritation

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 3.3.3. Exposure period 24h
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version
Reliability	1
Acceptability	Acceptable
Remarks	Weight at the end of the study not provided in Doc IVA
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 6.1.4	**Acute Toxicity**
BPD Annex Point IIA, VI.6.1.4	6.1.4-e Acute Eye Irritation

Table 6.1.4-e/6 Flufenoxuron: Eye irritation scores for conjunctival redness in NZW rabbits

	Time point of scoring					Score	Overall mean score
Rabbit	1 h	24 h	48 h	72 h	7 d	24–48–72 h	
840 M	1	0	0	0	0	0	0.06
845 M	1	0	0	0	0	0	
848 M	1	0	0	0	0	0	
816 F	1	0	0	0	0	0	
873 F	1	0	0	0	0	0	
874 F	1	1	0	0	0	0.33	

Section A6.1.5	**Skin sensitisation**
BPD Annex Point IIA, VI.6.1.5	6.1.5 Guinea pig maximisation test (GPMT)

			Official use only
		1 REFERENCE 1	
1.1	**Reference**	**1) XXXX** Toxicology of insecticides (acyl ureas): The acute oral and percutaneous toxicity, skin and eye irritancy and skin sensitizing potential of WL115110 XXXX unpublished XXXX **2) XXXX** Corrigendum to XXXX: Toxicology of insecticides (acyl ureas): the acute oral and percutaneous toxicity, skin and eye irritancy and skin sensitizing potential of WL115110 XXXX unpublished XXXX **Note:** The Corrigendum to the original report consists of 1 page, stating that the study was conducted also in accordance to GLP requirements of the JMAFF (Japanese authority)	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not reported; comparable to OECD 406 (adopted 1981)	
2.2	**GLP**	No, at the time the study was conducted GLP was not compulsory. However the study was conducted according to the principle of Good Laboratory Practices	
2.3	**Deviations**	Yes: no positive control substance was included in the assay	X
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch: XXXX	

Section A6.1.5		**Skin sensitisation**	
BPD Annex Point IIA, VI.6.1.5		6.1.5 Guinea pig maximisation test (GPMT)	
3.1.2	3.1.2 Specification	See below	
3.1.2.1	Description	Powder as described in section 2	
3.1.2.2	Purity	99%	
3.1.2.3	Stability	Not applicable as undiluted test substance	
3.1.2.4	Preparation of test substance for application	a) For <u>intradermal induction:</u> 0.1 ml of 1% test substance solution in corn oil b) <u>for induction:</u> 0.3 ml of 50% (w/w) test substance in petroleum jelly (highest concentration achievable) c) <u>for challenge:</u> 0.3 ml of 50% (w/w) test substance in petroleum jelly (highest concentration achievable)	
3.1.2.5	Pretest performed on irritant effects	Yes	
3.2	**Test Animals**		
3.2.1	Species	Guinea pigs	
3.2.2	Strain	Not indicated	
3.2.3	Source	XXXX	
3.2.4	Sex	Males and female	
3.2.5	Age/weight at study initiation	Mean bw (day 1), males: 556 g; females: 515 g	X
3.2.6	Number of animals per group	Main test: 10/sex in treated groups; 5/sex in control group	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	State study type: Maximization Test based on the Magnusson and Kligman method.	
3.3.1	Induction schedule	Intradermal induction (3 pairs of 0.1 ml injections) followed by a dermal induction one week later	
3.3.2	Way of Induction	Intradermal Epidermal; (semi-) occlusive	
3.3.3	Concentrations used for induction	Intradermal: 1% w/w in corn oil and 1% w/w in a 50:50 mixture of FCA (Freunds Complete Adjuvant) and corn oil Epidermal: 0.3 ml of a 50% w/w mixture with petroleum jelly	

Section A6.1.5		**Skin sensitisation**
BPD Annex Point IIA, VI.6.1.5		6.1.5 Guinea pig maximisation test (GPMT)
3.3.4	Concentration Freunds Complete Adjuvant (FCA)	*Undiluted*
3.3.5	Challenge schedule	Two weeks after epidermal induction
3.3.6	Concentrations used for challenge	0.3 ml of a 50% w/w mixture with petroleum jelly
3.3.7	Rechallenge	No
3.3.8	Scoring schedule	24h, 48h after challenge
3.3.9	Removal of the test substance	Method not indicated
3.3.10	Positive control substance	No
3.4	**Examinations**	
3.4.1	Pilot study	Yes, range finding study
3.5	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Results of pilot studies**	In range-finding investigations, the maximum non-irritant concentration used for 24-h topical occlusive application was found to be a 50% test substance preparation in petroleum jelly (highest concentration achievable). It was possible to inject a 1% test substance preparation in corn oil with a syringe. The concentration was well tolerated locally and systemically
4.2	**Results of test**	
4.2.1	24h after challenge	No positive response
4.2.2	48h after challenge	No positive response
4.2.3	Other findings	Range finding study allows identification of the test substance concentration in the main test.
4.3	**Overall result**	See 5.3

Section A6.1.5	**Skin sensitisation**
BPD Annex Point IIA, VI.6.1.5	6.1.5 Guinea pig maximisation test (GPMT)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Flufenoxuron was tested for its skin sensitizing effect in Guinea pigs [Source: XXXX; mean bw (day 1), males: 556 g; females: 515 g] using the Maximization Test based on the Magnusson and Kligman method.

In range-finding investigations, the maximum non-irritant concentration used for 24-h topical occlusive application was found to be a 50% test substance preparation in petroleum jelly (highest concentration achievable). It was possible to inject a 1% test substance preparation in corn oil with a syringe. The concentration was well tolerated locally and systemically. Thus, the following concentrations for induction and the challenge were selected for the main test:

Table 6.1.5/7 Test concentrations based on results of range-finding (RF) test

Intradermal induction	0.1 ml of 1% test substance solution in corn oil (RF-test result: slight redness, edges not defined in all Guinea pigs)
Topical induction	0.3 ml of 50% (w/w) test substance in petroleum jelly (highest concentration achievable) (RF-test result: no difference from surrounding skin)
Topical challenge	0.3 ml of 50% (w/w) test substance in petroleum jelly (highest concentration achievable) (RF-test result: no difference from surrounding skin)

RF = Range-finding experiment

The main test was conducted using a group of ten male and ten female Guinea pigs together with a control group of five males and five females.

Section A6.1.5	**Skin sensitisation**
BPD Annex Point IIA, VI.6.1.5	6.1.5 Guinea pig maximisation test (GPMT)

Intradermal induction

The animals were closely shaved in the shoulder region using electric clippers followed by an electric razor; two rows of three injections were made, one on each side of the midline, as follows:

Table 6.1.5/8 Application scheme at intradermal induction

Test animals	**Control group animals**
Two injections (0.1 ml) of Freund's complete adjuvant (FCA)	Two injections (0.1 ml) of Freund's complete adjuvant (FCA)
Two injections (0.1 ml) of 1% Flufenoxuron in corn oil	Two injections (0.1 ml) of corn oil
Two injections (0.1 ml) of 1% Flufenoxuron in 50:50 FCA / corn oil	Two injections (0.1 ml) of 50:50 FCA / corn oil

Topical induction

One week after induction by the intradermal injections, the same area of skin was shaved using electric clippers only. A 4 cm x 4 cm patch of Whatman Number 3 filter paper was moistened with 0.3 ml of the test material, placed over the site of injection and covered with a "Sleek" dressing. The dressing was then securely covered with an 8 cm "Poroplast" elastic adhesive bandage for 48 hours. Similar patches of filter paper (but coated with petroleum jelly only) were applied to the controls.

Topical challenge

Topical challenge was carried out two weeks after the topical induction. Hair was removed from a 3 cm x 3 cm area of one flank by clipping then shaving. A 2 cm x 2 cm patch of Whatman Number 3 filter paper, moistened with 0.1 ml of the appropriately diluted test material, was placed over the shaved area and covered with a 3 cm square of adhesive tape ("Blenderm"), held in place by an 8 cm "Poroplast" elastic adhesive bandage. Controls were also treated with the diluted test material. After 24 hours the patch was removed and the site examined for a response immediately, 24 and 48 hours after its removal.

The response was scored using the following standard four-point

Section A6.1.5	**Skin sensitisation**
BPD Annex Point IIA, VI.6.1.5	6.1.5 Guinea pig maximisation test (GPMT)

scale:

0 No difference from surrounding skin

1 Slight redness, edges not defined

2 Pink/red square with defined edges

3 Beet red square with well defined edges

The result of the test is expressed as the numbers of positive responses (i.e. 1, 2 and 3) shown by the test animals at both 24 and 48 hours after the removal of the challenge patches.

5.2 Results and discussion

The stability of Flufenoxuron suspension in corn oil for at least the duration of administration was demonstrated via HPLC analysis. The stabilities of formulations of the test substance in pure petroleum jelly and corn oil : Freund's Complete Adjuvant 1:1 (v/v) were assessed by a panel of qualified chemists. All these formulations were considered stable for at least 7.5 hours.

The erythema resulting from the topical challenge was scored immediately on removal of the challenge patches and 24 and 48 hours later. None of the twenty test animals showed any positive response at either 24 or 48 hours after removal of the challenge patches. It may be concluded, therefore, that the test material is not a skin sensitizer in Guinea pigs.

5.3 Conclusion

Flufenoxuron was not a skin sensitizer in Guinea pigs according to the Magnusson and Kligman Maximisation Test method.

5.3.1 Reliability

2

5.3.2 Deficiencies

No positive control, no signs of dermal irritation after epidermal induction

Section A6.1.5	**Skin sensitisation**
BPD Annex Point IIA, VI.6.1.5	6.1.5 Guinea pig maximisation test (GPMT)

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 2.3. Deviations Yes: Age of animals not reported 21h after removing the patch, the study area should be shaved off again before the observation at 48h Weight at the end of the study not provided 3.2.5. Age/weight at study initiation: Age of animals not reported
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version
Reliability	2
Acceptability	Not acceptable: deviations reported may affect the maximisation test results
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.1.5	**Skin sensitisation**
BPD Annex Point IIA, VI.6.1.5	6.1.5 Guinea pig maximisation test (GPMT)

			Official use only
		1 REFERENCE 2	
1.1	**Reference**	**3) XXXX** BAS 307 I (Flufenoxuron) – Maximization Test in Guinea pigs. XXXX unpublished XXXX.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes 96/54/EC B.6, OECD 406, EPA OPPTS 870.2600, Japan / MAFF	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	BAS 307 I (Flufenoxuron**)**	
3.1.1	Lot/Batch number	XXXX	
3.1.2	Specification	See below	
3.1.2.1	Description	Solid / white	
3.1.2.2	Purity	99.1 % (for details see Certificate of Analysis No. 211387_1)	
3.1.2.3	Stability	The stability under storage conditions over the study period was guaranteed (See Certificate of Analysis).	
3.1.2.4	Preparation of test substance for application	For induction and for challenge: homogenised in 1% cleaned sodium carboxymethylcellulose (CMC) solution in bi-distilled water	
3.1.2.5	Pretest performed	Yes	

Section A6.1.5		**Skin sensitisation**	
BPD Annex Point IIA, VI.6.1.5		6.1.5 Guinea pig maximisation test (GPMT)	
	on irritant effects		
3.2	**Test Animals**		
3.2.1	Species	Guinea pigs	
3.2.2	Strain	HsdPoc: DH	
3.2.3	Source	XXXX	
3.2.4	Sex	female	
3.2.5	Age/weight at study initiation	Age: 8–9 weeks Body weight at Day 0: 496–549g, 520.1 ± 18.8 g	
3.2.6	Number of animals per group	10 females in treated group, 5 females in control group	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	State study type: Maximization Test based on the Magnusson and Kligman method.	
3.3.1	Induction schedule	Intradermal induction (3 pairs of 0.1 ml injections) followed by a dermal induction one week later	
3.3.2	Way of Induction	Intradermal followed by epidermal induction for 48 h under occlusive conditions	
3.3.3	Concentrations used for induction	Intradermal: 5% w/w in corn oil and 5% w/w in a 50:50 mixture of FCA (Freunds Complete Adjuvant) and corn oil Epidermal: 50% w/w in 1% aqueous carboxymethylcellulose (CMC)	X
3.3.4	Concentration Freunds Complete Adjuvant (FCA)	Freund's Complete Adjuvant (FCA) emulsified with 0.9% aqueous NaCl solution in a ratio of 1:1	
3.3.5	Challenge schedule	Two weeks after epidermal induction (Day 21 of study)	
3.3.6	Concentrations used for challenge	25% w/w in 1% aqueous CMC	
3.3.7	Rechallenge	No; since no borderline results were observed, a 2nd challenge was not performed	
3.3.8	Scoring schedule	24h, 48h after challenge	
3.3.9	Removal of the test substance	Epidermal induction and challenge sites were washed with water after removal of the patch.	

Section A6.1.5		**Skin sensitisation**
BPD Annex Point IIA, VI.6.1.5		6.1.5 Guinea pig maximisation test (GPMT)
3.3.10	Positive control substance	No concurrent positive control tested in this study. However, a separate study with Alpha-Hexylcinnamaldehyde is performed twice a year in the laboratory and is included as an appendix to the report. The last control study was started 5 months before the Flufenoxuron study
3.4	**Examinations**	
3.4.1	Pilot study	Yes, range finding study
3.5	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Results of pilot studies**	After the intradermal induction intense erythema and swelling were observed at the injection sites at which only Freund's complete adjuvant / 0.9% NaCl solution (1:1) was applied. Intradermal injections of a 5% test substance preparation in 1% CMC-solution in bi-distilled water caused moderate and confluent erythema and swelling. At the injection sites of a 5% test substance preparation in Freund's complete adjuvant / 0.9% aqueous NaCl-solution (1:1) intense erythema and swelling were seen. In the epidermal pre-test no skin findings were observed in the animals treated with a 25% test substance preparations 24 and 48 hours after removal of the patch. Discrete or patchy erythema was noted in 2 out of 3 animals treated with a 50% test substance preparation 24 hours after removal of the patch.
4.2	**Results of test**	
4.2.1	24h after challenge	No positive response
4.2.2	48h after challenge	No positive response

Section A6.1.5	**Skin sensitisation**
BPD Annex Point IIA, VI.6.1.5	6.1.5 Guinea pig maximisation test (GPMT)

4.2.3	Other findings	After the intradermal induction intense erythema and swelling were observed at the injection sites at which only Freund's complete adjuvant / 0.9% NaCl solution (1:1) was applied. Intradermal injections of a 5% test substance preparation in 1% CMC-solution in bi-distilled water caused moderate and confluent erythema and swelling. At the injection sites of a 5% test substance preparation in Freund's complete adjuvant / 0.9% aqueous NaCl-solution (1:1) intense erythema and swelling were seen in all test group animals. The control group animals, injected with 1% CMC-solution in bi-distilled water did not show any skin reactions. A 50% formulation of 1% CMC-solution in bi-distilled water with Freund's adjuvant/0.9% aqueous NaCl-solution (1 : 1) caused intense erythema and swelling in all control group animals. The epicutaneous induction with a 50% test substance preparation in 1% CMC-solution in bi-distilled water led to incrustation, partially open (caused by the intradermal induction) and moderate and confluent erythema in addition to swelling in all test group animals.
4.3	**Overall result**	None of the 10 test animals showed positive responses at 24 or 48 hours after removal of the challenge patches. Thus, the test material was considered not to have a sensitizing effect on the skin of Guinea pigs in the Maximization test.

Section A6.1.5	**Skin sensitisation**
BPD Annex Point IIA, VI.6.1.5	6.1.5 Guinea pig maximisation test (GPMT)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The purpose of this study was to assess the potential sensitizing properties of Flufenoxuron in a Magnusson and Kligman Maximization test according to OECD 406 and 96/54/EC B.6. Details of the technical conduct are given above.

5.2 Results and discussion

Preliminary test:

Intradermal injection of

- Freund's adjuvant/0.9% aqueous NaCl-solution (1 : 1),
- a 5% test substance preparation in 1% CMC-solution, and
- a 5% test substance preparation in Freund's adjuvant/0.9% aqueous NaCl-solution (1 : 1)

caused moderate to intense erythema and swelling at the injection site [see Table 6.1.5/9].

Occlusive epidermal application of a 25% Flufenoxuron suspension in 1% aqueous CMC for 48 hours did not result in any dermal irritation [see Table 6.1.5/10]. In contrast, after occlusive application of a 50% Flufenoxuron suspension for 48 hours, discrete to moderate erythema were observed at the application site 1 hours after removal of the dressings. Irritation resolved within 48 hours.

The control animals were not treated since the 1% CMC-solution in distilled water used as formulating agent was not expected to influence the result of the study.

Based on the results of the preliminary tests concentrations of 5% Flufenoxuron in the respective vehicles were used for intradermal induction. Epidermal induction was performed using a 50% suspension of Flufenoxuron, whereas the challenge was performed with a 25% suspension of Flufenoxuron in 1% CMC.

Main study:

Induction: Like in the preliminary test the injection of Freund's adjuvant / 0.9% aqueous NaCl-solution (1 : 1) and of 5% Flufenoxuron in the mixture of Freund's adjuvant / 0.9% aqueous NaCl resulted in intense erythema and swelling (Grade 3) at the injection site. At the injection site of the 5% Flufenoxuron suspension moderate and confluent erythema with swelling was observed (Grade 2 E). All 10 animals reacted homogenously to the intradermal injections.

All control animals displayed intense erythema and swelling (Grade 3) after injection of Freund's adjuvant containing suspensions, whereas the injection of the vehicle (1% aqueous CMC) caused not skin reactions.

Section A6.1.5		**Skin sensitisation**
BPD Annex Point IIA, VI.6.1.5		6.1.5 Guinea pig maximisation test (GPMT)
		After epidermal induction with a 50% Flufenoxuron preparation in 1% aqueous CMC moderate and confluent erythema with swelling and partially open incrustations were observed in all treatment group animals. Challenge: The challenge with a 25% test substance preparation in 1% CMC-solution in doubly distilled water did not cause any skin reactions in any animal of the control group and the test group 24 and 48 hours after removal of the patches. Positive control: The results of the positive control study are summarized in Table 6.1.5/11. The positive control with Alpha-hexylcinnamaldehyde techn. 85% demonstrated that the study design was able to detect sensitizing compounds in Guinea pigs under the laboratory conditions chosen.
5.3	**Conclusion**	Based on the results of this study and applying the EU evaluation criteria it is concluded that Flufenoxuron has no sensitizing properties under the test conditions chosen.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A6.1.5	**Skin sensitisation**
BPD Annex Point IIA, VI.6.1.5	6.1.5 Guinea pig maximisation test (GPMT)

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	21/09/2006
Materials and Methods	**Revisions/Amendments:** 2.3 Deviations Control animals were not treated by epidermal application during induction since 1% aqueous CMC solution was not expected to cause irritation. 3.3.3 Concentrations used for induction *Intradermal: 5% w/w in ~~corn~~* 1% aqueous carboxymethylcellulose *and 5% w/w in a 50:50 mixture of FCA (Freunds Complete Adjuvant) and ~~corn oil~~* 1% aqueous carboxymethylcellulose.
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version
Reliability	1
Acceptability	Acceptable
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.1.5/9 Skin irritation scores 24 hours after interadermal injection - Preliminary test

Animal #	Application site	A) Freund's adjuvant / 0.9% aqueous NaCl-solution (1 : 1)	B) 5% Flufenoxuron preparation in 1% CMC-solution	C) 5% Flufenoxuron in A)
1	left	3	2 E	3
	right	3	2 E	3
2	left	3	2 E	3
	right	3	2 E	3

Grading: 2: moderate and confluent erythema (E = swelling)
3: intense erythema and swelling

Table 6.1.5/10 Skin irritation scores after epidermal application - Preliminary test

Animal #	Readings 1 hour after removal of the patch		Readings 24 hours after removal of the patch		Readings 48 hours after removal of the patch	
Application site	Right flank middle	Left flank middle	Right flank middle	Left flank middle	Right flank middle	Left flank middle
Flufenoxuron concentration in 1% CMC	50%	25%	50%	25%	50%	25%
79	2	0	1	0	0	0
80	2	0	1	0	0	0
81	1	0	0	0	0	0

Grading: 1: discrete or patchy erythema
2: moderate and confluent erythema

Table 6.1.5/11 Results of pos. control substance test (Alpha-hexylcinnamaldehyde techn. 85%)

	Challenge					
	Test substance 5% in Lutrol® E 400			**Vehicle control: Lutrol® E 400**		
	24 h	**48 h**	**Total**	**24 h**	**48 h**	**Total**
Control group	0/5	0/5	0/5	0/10	0/10	0/10
Test group	10/10	9/10	10/10	0/20	0/20	0/20

Induction (intradermal): test substance 5% in paraffin oil
Induction (epicutaneous): test substance 10% in Lutrol® E 400
Challenge (epicutaneous): test substance 5% in Lutrol® E 400

x/y: number of positive reactions/number of animals tested (reading at 24 h and/or 48 h after removal of the patch)

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

Introduction

Studies on absorption, distribution, excretion and metabolism of BAS 307 I in mammals were investigated using the [fluroaniline-U-^{14}C]- and [benzoyl-U-^{14}C]-labelled compound. Flufenoxuron was several times renamed/renumbered due to company acquisitions. Therefore, several numbering systems exist for this compound in the various reports. All designations for the parent compound are listed below.

Flufenoxuron (trade name CASCADE): BAS 307 I; CL811678; WL115110; RegNo. 243154

Figure 6.2/ 1 Flufenoxuron; [fluoroaniline-U-^{14}C]-labelled (synonyme aniline-label)

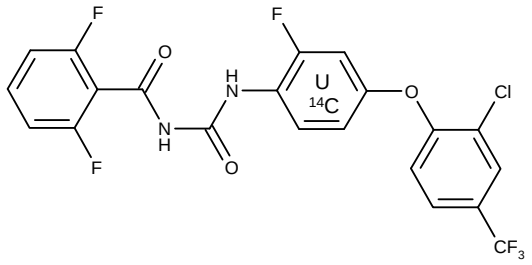
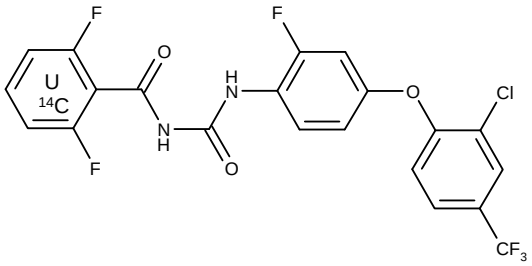


Figure 6.2/ 2 Flufenoxuron; [benzoyl-U-^{14}C]-labelled (synonym benzamide or amide-label)



Official use only

1 REFERENCE 1

1.1 Reference

1) XXXX
The fate of (^{14}C-aniline)-WL115110 in the fischer 344 rat following a single low oral dose of 3.5 mg per kg bodyweight
XXXX
unpublished
XXXX

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
		2) XXXX Corrigendum to XXXX: The fate of (^{14}C-aniline)-WL115110 in the fischer 344 rat following a single low oral dose of 3.5 mg per kg bodyweight XXXX unpublished XXXX **3) XXXX** Addendum to XXXX: The fate of (^{14}C-aniline)-WL115110 in the fischer 344 rat following a single low oral dose of 3.5 mg per kg bodyweight XXXX unpublished XXXX	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Company with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, EPA 85-1	X
2.2	**GLP**	No, at the time the study was conducted GLP was not compulsory. However the study was conducted according to the principle of Good Laboratory Practices.	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Flufenoxuron Batch/purity: XXXX([^{14}C]-Flufenoxuron): 99.4%, specific activity 35.38 µCi/mg	
3.1.2	Specification		

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
3.1.2.1	Description	Radiolabelled test substance	
3.1.2.2	Purity	Give purity in % of active substance	X
3.1.2.3	Stability	Stable	
3.1.2.4	Radiolabelling	^{14}C-aniline Flufenoxuron	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fisher 344	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	The male rats weighed 209 to 240 grams. The female rats weighed 130 to 150 grams; age not reported	X
3.2.6	Number of animals per group	5/sex/group (main test)	
3.2.7	Control animals	Yes, 1 male and 1 female	
3.3	**Administration/ Exposure**	Single oral	
3.3.1	Preparation of test substance	The radiolabeled test substance was formulated in a mixture containing 10% w/w of dimethyl sulfoxide (DMSO) in Mulgofen EL719 surfactant (polyethoxylated castor oil) and 80% isotonic saline solution immediately prior to dosing. The dose was administered orally by gavage at a constant volume of 2.5 mL/kg with a disposable syringe and a steel ball-tipped needle. The actual dose amount was based on individual animal body weight. The animals were fasted overnight with free access to water prior to dosing. The mean dose of ^{14}C-BAS 307 I administered to individual test animals was 3.29 mg/kg b.w (males 3.31-3.37 mg/kg, females 3.08-3.49 mg/kg) for the test animals in the main study and 4.54 mg/kg b.w.(males, 4.36-4.52 mg/kg, females, 4.55-4.74 mg/kg) for the test animals used in the CO_2 study.	X
3.3.2	Concentration of test substance	3.5 mg/kg bw	
3.3.3	Specific activity of test substance	35.38 µCi/mg	
3.3.4	Volume applied	2.5 mL/kg	
3.3.5	Size of test site	Oral gavage	
3.3.6	Exposure period	Single dose	

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
3.3.7	Sampling time	Daily for 7 days post dosing for urine and feces	
3.3.8	Samples	At sacrifice: whole blood, liver, spleen, kidneys, peri-renal fat, gastrointestinal tract (including contents), gonads, lungs, hearts, muscle, bone, bone marrow, skin (including feet and tail) and brain.	
		4 RESULTS AND DISCUSSION	
4.1	**Toxic effects, clinical signs**	No adverse effects in either the control or ^{14}C-Flufenoxuron treated animals were observed.	
4.2	**Dermal irritation**	Not relevant	
4.3	**Recovery of labelled compound**	Total recovery: 96.43% for male and 92.65% for female	
4.4	**Percutaneous absorption**	Not relevant	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	<u>Test animals</u> Fischer 344 strain male and female rats were obtained from XXXX. The male rats weighed 209 to 240 grams. The female rats weighed 130 to 150 grams. No age was given. <u>Method of dosing</u> The radiolabeled test substance was formulated in a mixture containing 10% w/w of dimethyl sulfoxide (DMSO) in Mulgofen EL719 surfactant (polyethoxylated castor oil) and 80% isotonic saline solution immediately prior to dosing. The dose was administered orally by gavage at a constant volume of 2.5 mL/kg with a disposable syringe and a steel ball-tipped needle. The actual dose amount was based on individual animal body weight. The animals were fasted overnight with free access to water prior to dosing. The mean dose of ^{14}C- Flufenoxuron administered to individual test animals was 3.29 mg/kg b.w (males 3.31-3.37 mg/kg, females 3.08-3.49 mg/kg) for the test animals in the main study and 4.54 mg/kg b.w.(males, 4.36-4.52 mg/kg, females, 4.55-4.74 mg/kg) for the test animals used in the CO_2 study. <u>Sampling:</u> <u>Pilot CO_2 Study</u>	X

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

After dosing, 2 males and 2 females were placed into individual Nalgene metabolism cages designed for the separation and collection of expired carbon dioxide (CO_2) and organic volatiles, urine and feces. Expired organic volatiles were collected in a trap maintained in a freezing mixture of solid CO_2/acetone. Expired CO_2 was trapped in 5M sodium hydroxide mixture. Organic volatiles and expired CO_2 were collected for 24 hours post-treatment. Urine and feces were collected 24 hours post-treatment. After the final collection at 24 hours post-treatment, two animals (one male and one female) were subjected to qualitative radioanalysis by whole body autoradiography (WBA). Urine and feces from this pilot experiment were radioanalysed, but not further characterized. Quantitative analysis of the tissue samples from this group was not performed.

Main Study

After dosing, 5 males and 5 females (treated group) and 1 male and 1 female (control group) were individually maintained in Nalgene metabolism cages enabling the separate collection of urine and feces. At the end of the animal holding interval, cages were rinsed with a small volume of deionized water:methanol (1:1, v/v), this sample was designated the cage wash The excreta samples were collected on ice. Urine and feces were collected at daily for 7 days post-dosing.

Earlier (4,8 and 12 h) and intermediate (36 h) sampling intervals were not collected, since information obtained from the preliminary CO_2 study indicated urine to be a minor route of elimination. The following tissues were collected at sacrifice: whole blood, liver, spleen, kidneys, peri-renal fat, gastrointestinal tract (including contents), gonads, lungs, hearts, muscle, bone, bone marrow, skin (including feet and tail) and brain. All organs and tissue samples (except blood) were individually weighed and stored frozen (-18°C), blood was stored at 4°C. The remaining carcass was mixed with an equal amount (w/v) of water and homogenized.

Analytical Methods

Specific radioactivity was determined by high performance liquid chromatography (HPLC) of the analyte and radiochemical purity by HPLC and TLC. Radiopurity of formulated ^{14}C-Flufenoxuron was >99%.

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

Total Radioactive Residues (TRR) of daily urine, cage wash, and CO_2 and volatile traps were assayed by liquid scintillation counting of aliquots. The TRR in feces, tissues, and organs were determined by combustion of aliquots to ^{14}C-CO_2. Skin and carcass samples were solubilized using a mixture of sodium hydroxide, water, methanol and Triton-X. Aliquots of the solubilized skin were taken for LSC.

Any tissue that contained greater than 2% of the administered dose was extracted with organic solvent and was radioassayed (quantitavely and qualitatively) for the amount of extractable radioactivity and the nature of the metabolites. Tissue samples collected were liver, fat, GI tract, skin and carcass from male and female animals.

Radiopurity: TLC on Merck silica gel plate.

Radioactivity was detected by autoradiography and radiochromatogram scanning.

Extraction of feces

For the qualitative and quantitative examination of the eliminated radioactivity in the feces, homogenate samples (10% w/w) from days 1 to 7 were combined (time pool samples) from each treated animals prior to analysis. Each animal was examined individually. Fecal samples (3.5-4.5 g) were extracted with organic solvent. Feces samples were mixed with 3-4 time the sample weight of ethyl acetate:methanol (3:1, v/v) and extracted on a roller mixer for 20 minutes at room temperature The mixtures were centrifuged (2500 G) for 10 minutes and the supernatant removed. The solid residue was mixed a further amount of solvent mixture (3-4 times w/w) and extracted as described. Extraction of the post-extracted residue with twice (v/v) the amount of solvent was performed. Following three extractions with ethyl acetone:methanol solution, the particulate reside was mixed twice with acetonitrile (15 mL) and extracted as described above. The radioactivity in the air-dried residue, characterized as unextractable residue, was combusted followed by LSC. The respective supernatant fractions from ethyl acetate:methanol and acetontirle extractions were pooled and made up to 100 mL. Triplicate aliquot samples (0.5 mL) were taken for radioanalysis by LSC. The organo-soluble radioactivity was concentrated and analysed by TLC.

The air-dried post-extracted PES were further analyzed by Soxhlet extraction with acetontirle, and acid-hydrolysis (2N hydrochloric acid) followed by extraction with organic solvent.

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

TLC of the radioactivity released by the acid hydrolysis was attempted.

Preparative TLC of fecal extracts on Whatman PLK5F Linear K silica gel plates was used to isolate the exclusive radio component in the day1/2 group pool fecal extracts from male and female test animals. The area of the silica gel containing the exclusive component was removed and the component recovered by ethyl acetate extraction. The extract was concentrated and examined by TLC, HPLC and MS.

Whole Body Autoradiography

Carcasses from two animals (one male and one female) from the CO_2 study, sacrificed 24 h post-dose were preserved for sectioning by freezing in hexane/solid carbon dioxide for 30 minutes prior storage at –20°C. Longitudinal whole-body sections, approximately 30 µm in thickness, were cut on a cryomicrotome. At least four sections were taken at various levels of each animal on a strip of adhesive tape. Each strip was freeze dried and two sections from each level were placed in contact with X-ray film, typically for 30 days, prior to development of the X-ray film.

Extraction of Tissues (Liver, Fat, Intestines, Skin and Carcass)

Respective male and female group pool homogenates were prepared. Samples (2.5 g) were extracted twice with 10 ml of ethyl acetate: methanol (3:1/v/v) followed by two extractions with 10 mL of acetonitrile. The extracts were pooled, and assayed by LSC and TLC. The radioactivity in the air-dried post-extracted liver residue (PES) was determined by combustion. The post-extracted residue was extracted twice with 10 - 15 ml of methylene chloride. The radioactivity in the extract was determined by LSC. An aliquot of the residual air-dried PES-1 was subjected to Soxhlet extraction with acetonitrile and acid hydrolysis. The aqueous-acid extracted radioactivity was extracted with diethyl ether and the residual PES-2 from the acid hydrolysis was extracted with acetonitrile. The extracts were combined, concentrated and analysed by TLC.

Fat, skin and carcass were extracted with methylene chloride. The sample was filtered, and the filtrate concentrated to dryness. The residue was reconstituted in hexane and partitioned with acetonitrile (3x). The hexane and acetonitrile fraction were separated.

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

		The acetonitrile fraction was concentrated to a small volume and analysed by LSC, HPLC and TLC. The hexane fraction was analysed for radioactivity by LSC. Intestine was extracted with ethyl acetate: methanol followed by acetonitrile, similar to that described for feces. Control liver, fat, intestine, skin and carcass and controls fortified with [^{14}C-aniline]-Flufenoxuron were also extracted to assess recovery and stability of compound. Lyophilized urine fractions were subjected to hydrolytic enzymes (ß-glucuronidase and arylsulphatase) at pH 5.0 in 0.1 M sodium acetate buffer at 37°C overnight. The chemical structure was compared with authentic standard by co-TLC or confirmed by mass spectrometry. Non-radioactive standards: UV detector on a HPLC system.
5.2	**Results and discussion**	The stability, homogeneity and correctness of the test substance preparation was analytically verified. No adverse effects in either the control or ^{14}C-Flufenoxuron treated animals were observed. <u>Pilot CO_2 Study</u> A pilot showed there was negligible radioactivity (<0.01%) in the expired air as carbon dioxide or volatile metabolites. The results showed that elimination of [^{14}C-aniline]-Flufenoxuron-related radiocarbons by respiration is nil. Twenty-four hours post-dose, approximately 10% of the dose was detected in the feces of male and female animals respectively, and 1.5 and 1.9% respectively in the male and female urine. The rates of elimination of the radioactivity via the urine and feces in this preliminary group were similar to those observed in the main study. <u>Main study</u> Seven days post-dose, approximately 23% of the dose was detected in the feces of male and female animals respectively, and <5% in the male and 5% in female urine. Overall, 93-96% of the administered dose was recovered from male and female test animals. There were no major sex-related differences in elimination of radioactivity. Results of the elimination of radioactivity are summarised in Table 6.2/ 1. <u>Tissue distribution</u> Blood and tissue concentrations in female rats and male rats were comparable. Mean tissue recoveries (% of the administered dose)

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

as well as the mean concentrations of radioactive residues in tissues (TRR[µg/g]) are summarised in Table 6.2/ 2.

Whole-body autoradiography

Results from the whole-body autoradiography indicated that the radioactivity was extensively distributed throughout the carcass at 24 h post dosing. There were substantial concentrations of radioactivity, as dark area in the autoradiograph, associated with the fat (subcutaneous and abdominal), gastrointestinal tract, bone marrow and skin. The autoradiographs from male and female test animals were similar.

Metabolism

Unchanged parent was the major component in the extracts of feces, fat, skin, intestinal contents and carcass. The results are summarised as follows.

Urine (4.75% of the administered dose male, 5.13% of the administered dose female):

Extraction of the urine at pH 2 with ethyl acetate partitioned a mean of 21.8% of the recovered radioactivity (males, 15.9% TRR; females, 27.7% TRR) into the organic phase. The overall recovery was >97% of the TRR. TLC of the organo-soluble radioactivity showed at least ten metabolites, each accounting for <0.5% of the administered dose. There was very little (<0.1%) unchanged Flufenoxuron detected in the urine. Two of the minor components were identified by co-chromatography (TLC); WL129183 (Reg. No. 4064702; 0.02-0.06% of the administered dose) and WL115096 (Reg. No. 241208; 0.02-0.07% of the administered dose). From the water-soluble radioactivity (78% TRR), enzyme hydrolysis released less than 1% of TRR as organo-soluble radioactivity. This has not been used for further investigations.

Feces (23.9% of dose male, 21.1% of dose female):

Extraction of the radioactive residue in feces samples Day-1 to Day-7, showed that 14.2-15.8% of the administered dose of time pooled samples was extractable, and 6.9-8.0% was unextractable. TLC analysis of the ethyl acetate: methanol fraction, containing the majority of the extracted radioactivity, showed the presence of qualitatively similar metabolic profiles at day 1, day 3 and day 7. These were similar to the time pooled samples from individual animals. The major radioactive product in the feces extract was unchanged Flufenoxuron, accounted for 6%, 0.4% and 0.3% of

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

the administered dose respectively in male and female day 1, day 3 and day 7 samples, respectively. Products of less than 1% each of the administered dose were detected in the extracts of the feces. These were identified by co-chromatography (TLC) as WL132612 (Reg. No. 4110959), WL129183 (Reg. No. 4064702) and WL115096 (Reg. No. 241208).

Following sequential extraction of the excreta (group pool, time pool and group time pool samples) with organic solvents of differing polarities, unextractable residues accounted for up to 9% of the administered dose. Following Soxhlet extraction a portion of the radioactive dose was extracted with acetonitrile (4.3% of the administered dose), and released by acid hydrolysis of these unextracted residues in feces. However, no further attempt was made to identify the nature of these extracted residues due to the low concentration of radioactivity or difficulty in chromato-graphy due to co-extractive matrices.

Liver (1.80% of dose male, 1.61% of dose female):

Following extraction of the group pool liver by ethyl acetate: methanol followed by acetonitrile, approximately 74% (males, 76%, females 72.8%) of the TRR was in the combined organic phases. By comparison, all of the recovered radioactivity of the [^{14}C-aniline]- Flufenoxuron-fortified control was found in the organic phase. Quantitative analysis by TLC showed one major radioactivity product comprising 1% of the administered dose (males, 1.1%; females, 1.0%). This component was coincident with Flufenoxuron. The remaining radioactivity present in the extract was composed of at least three minor components. These were not identified. Metabolite profiles in male and female extracts appeared to be qualitatively similar. Attempts were made to study the nature of the unextracted liver residues. Further extractions with methylene chloride and Soxhlet extraction with acetonitrile were ineffective in releasing any additional radioactivity. Acid hydrolysis (2N HCl for 2 h) followed by extractions of both the acid supernatant and residual PES with organic solvents (diethyl ether and acetonitrile, respectively) released about 25% of the residual radioactivity (<0.1% of the administered dose). TLC of the released radioactivity indicated that it was polar, and no attempt was made to characterize this product

Fat (7.03% of dose male, 7.87% of dose female):

Substantial concentrations of [^{14}C-aniline]-Flufenoxuron-derived residues were detected in the fat tissue of male (11.39 µg/g) and female (11.01 µg/g) test animals. This accounted for 7.03% and

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

7.87% of the administered dose, respectively. Qualitative and quantitative analysis of the chemical nature and chromatographic distribution of the radioactive residue was carried out. The acetonitrile fraction containing the majority of the radioactivity from fat samples (>91% TRR in male and female test animals, >89% TRR in fortified control) was analysed by TLC. A single radioactive peak, coincident with unchanged [^{14}C-aniline]-Flufenoxuron was detected in the male and female fat. Unchanged Flufenoxuron accounted for 10 µg/g (males 9.8 µg/g, females 10 µg/g) of the administered dose. The combined total of all other unknown minor non-discrete radioactive products was less than 0.9% of the administered dose (males, 1.4%; females, 0.5%) of the administered dose.

Intestines (7.62% of dose male, 6.99% of dose female):

Concentrations of [^{14}C-aniline]-Flufenoxuron-derived residues in intestines accounted for 7.62% (male, 2.69 µg/g) and 6.99% (female, 2.32 µg/g) of the administered dose. Qualitative and quantitative analysis of the chemical nature and chromatographic distribution of the radioactive residue was carried out. Following sequential extraction with ethyl acetate:methanol and acetonitrile, 94.5% of the recovered radioactivity [male: 94.7% (2.55 µg/g); female: 94.2% (2.19 µg/g)] were in the organic phase. TLC analysis showed one major radioactive component coincident with Flufenoxuron. This comprised 6.09% (male, 6.41%, female 5.78%) of the administered dose. The combined total of the other minor radioactive products was approximately 0.8% for both sexes.

Skin (12.23% of dose male, 19.37% of dose female):

Since the level of radioactivity associated with the residual skin accounted for approximately 12.23% (male, 1.65 µg/g) and 19.37% (female, 2.53 µg/g) of the administered dose, the qualitative and quantitative analysis of the chemical nature of the TRR was carried out.

Following disruption (by grinding with liquid nitrogen and extraction of the combined male/female skin samples with methylene chloride, 96.9% of the recovered radioactivity (equivalent to 15.3% of the administered dose) was found in the organic phase. The unextracted residue was not analysed further. After partition of the extracted radioactivity between hexane and acetonitrile, all the entire (99.9%) of the recovered radioactivity was found in the acetonitrile fraction. TLC showed a major radioactive component coincident with Flufenoxuron which was detected in the combined male/female/skin sample. This comprised

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

12.9% of the administered dose (mean of male and female). The combined total of all other minor radioactive products was less than 0.9% of the administered dose; these were not characterised.

Carcass (38.12% of dose male, 29.5% of dose female):

The level of radioactivity associated with the residual carcass accounted for approximately 38.12% (male, (1.03 µg/g) and 29.5% (female, 0.85 µg/g) of the administered dose. Therefore, the qualitative and quantitative analysis of the chemical nature of the TRR was carried out. Following methylene chloride extraction, 87.3-89.1% of the TRR was extracted. Solvent partitioning of the extractable residue between acetonitrile and hexane showed that essentially the entire radioactivity partitioned into acetonitrile. A single major radioactive component coincident with unchanged Flufenoxuron and comprising 27.8% (male, 31%; female, 24.7%) of the administered dose was detected. The combined total of all other minor radioactive products was less than 0.69% (male, 1.3%; female, 0.08%).

Muscle:

Low concentrations of [^{14}C-aniline]-Flufenoxuron-derived residues were detected in the muscle tissue male (0.26 µg/g) and female (0.26 µg/g) test animals. At the end of the experiment, this amounted to 3.69% (male) and 3.59% (female) of administered dose.

Kidney:

Moderate concentrations of [^{14}C-aniline]-Flufenoxuron-derived residues were detected in the kidney tissue male (1.16 µg/g) and female (0.87 µg/g) test animals. At the end of the experiment, this amounted to 0.28% (male) and 0.22% (female) of administered dose.

Blood:

Low concentrations of [^{14}C-aniline]- Flufenoxuron equivalent residues were detected in the blood of male (0.21 µg/g) and female (0.21 µg/g) test animals. At the end of the study this was estimated to account for 0.43% and 0.44% percent of the administered dose in male and female test animals.

Other tissues:

Residues in other tissues were <2% of the dose and were not extracted.

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
5.3 Conclusion	Fischer 344 strain male and female rats received a single oral dose of [^{14}C-aniline]- Flufenoxuron in DMSO/Mulgofen at 3.50 mg/kg b w. During the seven-day post-dose, total recoveries for the male and female dose groups ranged from 93-96%. Of the dose recovered, 5% in urine (males, 4.75%; females, 5.13%), 24.9% in feces (males, 28.77%; females 21.09%), 33.8% in carcass (males, 38.12%; females, 29.50%), 15.8% in skin (males, 12.23%; females, 19.37%), 7.3% in the intestine (males, 7.62%; females, 6.99%), 7.4% in fat (males, 7%; females 7.8%), 3.6% in muscle (males, 3.69%; females, 3.59%), 1.7% in liver (males, 1.80%; females, 1.61%), 0.25% in kidney (males, 0.28%; females, 0.22%) and <1% in the other samples. Negligible amounts of radioactivity (< 0.1%) were found in volatile organic compound trap and in the carbon dioxide trap. Fecal excretion was the primary elimination route, and the majority of the radioactivity was eliminated within 48 hours after dosing. There were no major sex differences in absorption or elimination of radioactivity for rats dosed with [^{14}C-aniline]- Flufenoxuron. Fat contained up to 11µg/g Flufenoxuron-derived residues. Flufenoxuron-derived residues (1-4 µg/g) were also found in bone marrow, heart, lung, brain, ovary and gonads. The unchanged parent was the single component of the residue in the fat, feces, skin and carcass. There were negligible amounts (<0.2%) of unchanged Flufenoxuron in the urine, however; trace amounts (0.2%) of two metabolites (WL115096, Reg. No. 241208, and WL129183, Reg. No. 4110959) identified in the urine indicated that the absorbed Flufenoxuron was metabolized by cleavage of the bond adjacent to the 2, 6-difluorobenzoyl moiety.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 2.1. Guideline study EPA OPPTS 870.7485 3.1.2.2. Purity 99.4% 3.2.5. Age/weight at study initiation: Age not reported
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version Based on the radioactivity in the urine, cage wash, carcass and organs, the minimal absorbed fraction in 168 hours was 72.52 % in males and 71.56% in females.
Reliability	1
Acceptability	Acceptable
Remarks	In 3.3.1. and 5.1: *10% w/w DMSO* + 10% w/w Mulgofen + *80% w/w isotonic saline solution*
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.2/ 1 Excretion balance at 48 h and 168 h post dosing (expressed as % of the administered dose)

	MALE	FEMALE
Dose [mg/kg b w]	3.5	3.5
Label	Aniline	Aniline
Application site	oral	oral
Application mode	single	single
48 h		
Urine 0-48	2.04	2.46
Feces 0-48	13.18	12.28
Subtotal	15.22	14.74
168 h		
Urine 0-168	4.75	5.13
Cage wash	0.15	0.08
Feces 0-168	23.86	21.09
Carcass + organs	67.67	66.35
Total	96.43	92.65

Table 6.2/ 2 Recovery in tissues (% of the administered dose) and tissue concentrations (TRR [µg/g]) at 168 h post dosing

	MALE		FEMALE	
Dose [mg/kg b w]	3.5		3.5	
Label	Aniline		Aniline	
Application site	oral		oral	
Application mode	single		single	
	% of dose	TRR [µg/g]	% of dose	TRR [µg/g]
Liver	1.80	1.38	1.61	1.39
Kidney	0.28	1.16	0.22	0.87
Intestine	7.62	2.69	6.99	2.32
Skin	12.23	1.65	19.37	2.53
Carcass	38.12	1.03	29.50	0.85
Fat[1)]	7.03	11.39	7.87	11.01
Blood	n.d.	0.21	n.d.	0.21
Others[2)]	0.60	n.d.	0.79	n.d.
Subtotal	67.68	n.d.	66.35	n.d.

1) Fat samples were sub-samples for analysis, the remainder is in the carcass.

2) Includes heart, brain, lung spleen, gonads and sub-samples of tissues removed at necropsy like muscle, blood

n.d. not determined

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

Official use only

The absorption, distribution, and elimination of Flufenoxuron in 3 male and 3 female Fischer 344 rats with cannulated bile ducts were investigated following a single oral dose of 3.5 mg/kg b w. The exposure time after dosing was 48 hours. The experiments were performed with ^{14}C- Flufenoxuron labelled in the aniline-ring.

1 REFERENCE 2

1.1	**Reference**	**4) XXXX** Excretion of an oral dose of (Aniline ^{14}C) WL 115110 in bile duct-cannulated rats XXXX unpublished XXXX)
1.2	**Data protection**	No
1.2.1	Data owner	BASF
1.2.2	Company with letter of access	XXXX
1.2.3	Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Not reported, however in general principles with US EPA 85-1
2.2	**GLP**	Yes, (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	Batch: XXXX
3.1.2	Specification	As given in section 2

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
3.1.2.1	Description	Radiolabelled test substance: see 6.2-1 introduction	
3.1.2.2	Purity	98%, specific activity 31.5 µCi/mg	
3.1.2.3	Stability	Stable	
3.1.2.4	Radiolabelling	^{14}C-aniline Flufenoxuron	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fisher 344	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	The rats weighed 150-170 grams and aged 9 weeks (males) or 13 weeks (females).	
3.2.6	Number of animals per group	3/sex/group (main test)	
3.2.7	Control animals	Yes, receiving unlabelled test substance	
3.3	**Administration/ Exposure**	Single oral	
3.3.1	Preparation of test substance	The radiolabeled test substance was formulated in a mixture containing 10% w/w of dimethyl sulfoxide (DMSO) in Mulgofen EL719 surfactant (polyethoxylated castor oil) and 80% isotonic saline solution immediately prior to dosing.	X
3.3.2	Concentration of test substance	3.5 mg/kg bw	
3.3.3	Specific activity of test substance	Specific activity 31.5 µCi/mg	
3.3.4	Volume applied	2.5 mL/kg	
3.3.5	Size of test site	Oral gavage	
3.3.6	Exposure period	Single dose	
3.3.7	Sampling time	1) 0-24 and 24-48 h periods after dosing for measurement of radioactivity; 2) at sacrifice	
3.3.8	Samples	1) Urine, feces and bile 2) Gastro-intestinal tract and carcass	

Section A6.2	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**
BPD Annex Point IIA, VI.6.2	

		4 RESULTS AND DISCUSSION	
4.1	**Toxic effects, clinical signs**	No adverse effects in either the control or ^{14}C- Flufenoxuron treated animals were observed.	
4.2	**Dermal irritation**	Not relevant	
4.3	**Recovery of labelled compound**	About 90% for male and female	
4.4	**Percutaneous absorption**	Not relevant	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	<u>Animals</u> Fischer 344 male and female rats were obtained from XXXX. The rats weighed 150-170 grams and aged 9 weeks (males) or 13 weeks (females). <u>Method of dosing</u> The radiolabeled test substance was formulated in a mixture containing 10% w/w of dimethyl sulfoxide (DMSO) in Mulgofen EL719 surfactant (polyethoxylated castor oil) and 80% isotonic saline solution immediately prior to dosing. The dose was administered orally by stomach cannula at a constant volume of 0.4 mL (2.5 mL/kg). The actual dose amount was based on individual animal body weight. Males (160-164 g) were dosed on June 30, 1992 and females (160-164 g) were dosed on July 3, 1992. Males received 0.575 mg (3.51-3.59 mg/kg) and females received 0.554 mg (3.38-3.46 mg/kg). <u>Sampling</u> After dosing, urine, feces and bile were collected during 0-24 and 24-48 h periods after dosing for measurement of radioactivity. Gastro-intestinal tract and carcass were taken at sacrifice. <u>Analytical Methods</u> Prior to each dose, the radiochemical purity of the dose material in the dose vehicle was measured by TLC on Merck silica gel plate (5 x 20 and 20 x 20 cm).	X

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

Total Radioactive Residues (TRR):

Liquid samples were assayed by liquid scintillation counting. Solid samples (feces homogenates and GI tract homogenates) were assayed by combustion/LSC. Carcasses were solubilized overnight in aqueous sodium hydroxide (3.3 M): methanol:Triton X-405 (6:3:1, v/v/v). After cooling, the total volume of solution was measured and a sub-sample taken. Duplicate aliquots were taken, acidified and assayed for radioactivity by LSC.

Radioactivity was detected by autoradiography and radiochromatogram scanning.

HPLC coupled with UV detector (254 nm) and radio detector:

Spherisorb S50DS2, 25 cm x 4.6 mm

Mobile Phase: Pump A: 0.0025 M sodium acetate pH 6.5), Pump B: acetonitrile

Gradient: Time 0: 50% A, 50% B, Time 30, 100% B

Flow rate 2 mL/min

One-minute fractions of column eluate were collected for 30 minutes using a Frac 100 fraction collector and radioassayed. These measurements were used to determine the recovery of radioactivity and the proportion of sample radioactivity associated with unchanged Flufenoxuron and its metabolites.

Samples of unhydrolyzed and acid-hydrolyzed bile were analyzed by TLC.

For establishing chromatographic correspondence of non-labeled reference standards and the radioactive metabolites of Flufenoxuron, the reference standards were co-chromatographed with the radioactive samples. Co-chromatographic correspondence was assessed by visual inspection of the TLC plate and its autoradiography.

5.2 Results and discussion

The stability, homogeneity and correctness of the test substance preparation was analytically verified. No adverse effects in either the control or ^{14}C- Flufenoxuron treated animals were observed**.**

Excretion balance

After single oral doses of ^{14}C- Flufenoxuron at a nominal level of 3.5 mg/kg to 3 male and 3 female rats with cannulated bile ducts, means of 19.7% (males) and 6.7% (females) of the administered dose were excreted in bile over the 48 hour collection period. Means of 2.6% (male) and 1.6% (female) were excreted in the urine (including cage wash) during 48 hours after dosing. In this time period, means of 4.0% (male) and 30.2% (female) of the

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

dose were excreted in faeces. After sacrifice at 48 hours, means of 5.0% (male) and 4.4% (female) of the dose were in the GI tract, radioactivity remaining in the carcass accounted for means of 59.1% (male) and 47.3% (female).

Total amount of absorption (urine + cage-wash + bile + carcass) in 48 hours was 81.4% of the low dose rate of 3.5 mg/kg b.w. for male rats and 55.6% for the female rats

Metabolism: characterisation of radioactive bile metabolites:

HPLC of the [^{14}C-aniline]-Flufenoxuron-derived bile residues showed a high proportion of polar unresolved metabolites, which accounted for the majority of the radioactivity in each sample, 79.1% TRR (males) and 73.7% TRR (females). The remaining major component co-chromatographed with Flufenoxuron and accounted for 16.3% TRR (males) and 20.9% TRR (females), equivalent to 3.2% and 1.4% of the administered dose, respectively.

A very minor region with a similar retention time to WL115096 (Reg. No. 241208) accounted for 0.9% TRR (males) and 0.6% TRR (females), equivalent to 0.2% and 0.04% of the administered dose.

After acid-hydrolysis of bile samples, co-chromatography (HPLC) with Flufenoxuron and WL115096 (Reg. No. 241208) showed that Flufenoxuron accounted for 13.4% (male) and 18.2% (female) of the sample radioactivity, equivalent to 2.6% and 1.2% of the administered dose, respectively. These proportions were similar to unhydrolyzed bile. Radioactivity co-eluting with WL115096 (Reg. No. 241208) was identified, accounting for 5.9% (male) and 6.5% (female) of the sample radioactivity, equivalent to 1.2% and 0.4% of the administered radioactivity. This was an increase over the proportions measured prior to hydrolysis. The major component in each sample remained as highly polar material, but 'others' increased when compared to unhydrolyzed samples and a new peak with a retention time of 6 minutes was detected. This indicated that some polar material had been hydrolyzed. The conditions were sufficient to release WL115096 (Reg. No. 241208) from any polar aniline based conjugates. In a control experiment, Flufenoxuron was stable under the conditions of hydrolysis used.

5.3 Conclusion

Fischer 344 strain male and female rats received an intra-gastric oral dose of [^{14}C-anline]- Flufenoxuron in DMSO/Mulgofen at nominal level of 3.50 mg/kg b.w. There were sex differences in the disposition of radioactivity. In males, absorption appeared to

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
		be almost quantitative with only 4% of the dose excreted in the feces, representing unabsorbed material. In females, 30.2% of the dose was excreted in the feces. In both sexes, urinary excretion of radioactivity was low and a substantial proportion of the dose remained unexcreted after 48 hours. Biliary excretion of radioactivity accounted for (mean values) 19.7% and 6.7% of the dose, respectively in male and female animals after 48 hours. Unchanged parent was excreted in bile in small amounts, representing 3.2% of the dose in male animals and 1.4% of the dose in female animals. The identification of Flufenoxuron was confirmed by co-chromatography (TLC and HPLC). There were very low levels of WL115096 (Reg. No. 241208) (<0.2%). Trace amounts (1.2%, males; 0.4%, female) of WL115096 (Reg. No. 241208) were released following acid hydrolysis of the polar bile radioactivity. In a previous study (D. R. Hawkins, et. al., 1992, XXXX), cited in .III A 6.2/4) with [^{14}C-benzoyl]-Flufenoxuron, only abut 4.5% of the dose was excreted in bile by cannulated rats of both sexes, but urinary excretion was higher than in the present study. These differences arise as a consequence of in vivo cleavage of the benzoyl urea linkage. This results in different metabolites being formed from the different labeled forms of Flufenoxuron which can be excreted by different routes. Higher molecular weight metabolites from aniline labeled Flufenoxuron would be more likely excreted in the bile. The sex difference in biliary excretion observed in the present study may be due to differences in the capacities in male and females animals to perform certain biotransformation reaction. Total amount of absorption (urine + cagewash + bile + carcass) in 48 hours was 81.4% of the low dose rate of 3.5 mg/kg b.w. for male rats and 55.6% for the female rats. The unchanged parent compound was the single component of the residue in the fat, feces, skin and carcass. There was negligible amounts (<0.2%) of unchanged Flufenoxuron in the urine, however; trace amounts (0.2%) of two metabolites (WL115096 (Reg. No. 241208) and WL129183 (Reg. No. 4064702)) identified in the urine indicated that the absorbed Flufenoxuron was metabolised by cleavage of the bond adjacent to the 2, 6-difluorobenzoyl moiety.	
5.3.1	Reliability	2	X
5.3.2	Deficiencies	No	

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 3.3.1. Preparation of test substance […] 10% w/w Mulgofen […] No indication about photoperiod, temperature or humidity
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version 5.1. Materials and methods 10% w/w Mulgofen 5.3.1. Reliability 1
Reliability	1
Acceptability	Acceptable
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.2/ 3 Excretion and retention of radioactivity by rats with cannulated bile ducts at 48 h post dosing expressed as % of the administered dose

	MALE	FEMALE

Dose [mg/kg b w]	3.5	3.5
Label	Aniline	Aniline
Application site	oral	oral
Application mode	single	single
Bile (0-24)	11.9	3.9
Bile (0-48)	7.8	2.7
Total Bile	19.7	6.6
Urine (0-24)	1.2	0.6
Urine (0-48)	1.3	0.9
Cage-wash	0.1	0.1
Total Urine and Cage-wash	2.6	1.6
Faeces (0-24)	0.6	11.4
Faeces (0-48)	3.4	18.8
Total Faeces	4.0	30.2
G.I.T.	5.0	4.4
Residual carcass	59.1	47.3
Total Carcass	64.1	51.8
Total Recovery	90.3	90.2
% Absorbed in 48 hours[1)]	81.4	55.6

1) Total urine, cage-wash, bile and residual carcass.

Table 6.2/ 4 Quantification of Flufenoxuron-derived residues in bile samples

Component	HPLC retention time (min)	Male		Female	
		% TRR	% of dose	% TRR	% of dose
Polars	1-4	79.1	15.6	73.7	4.9
Reg. No. 241208	11	0.9	0.2	0.6	0.04
Flufenoxuron	15	16.3	3.2	20.9	1.4
Others [a)]	-	3.8	0.7	4.7	0.3

a) Radioactivity distributed through regions of the chromatogram other than those stated. Did not contain discrete peaks

Table 6.2/ 5 Quantification of Flufenoxuron-derived residues in acid-hydrolysed bile samples

Component	HPLC retention time (min)	Male		Female	
		% TRR	% of dose	% TRR	% of dose
Polars	1-5	65.7	12.9	61.7	4.1
B1 [a)]	6	7.8	1.5	8.3	0.6

Reg. No. 241208	11	5.9	1.2	6.5	0.4
Flufenoxuron	15	13.4	2.6	18.2	1.2
Others [b)]	-	7.1	1.4	5.4	0.4

a) Radioactivity distributed through regions of the chromatogram other than those stated. Did not contain discrete peaks

b) Unknown components. Probably the same in both sexes

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

The absorption, distribution, elimination and metabolism of Flufenoxuron in male and female Fischer 344 rats were investigated following a single high oral dose of 350 mg/kg b w. The experiments were performed with ^{14}C- Flufenoxuron labelled in the aniline-ring. Five male and five female rats were used for the excretion balance studies as well as tissue distribution and metabolism.

Official use only

1 REFERENCE 3

1.1 Reference

5) XXXX
The fate of (^{14}C-aniline)-WL115110 in the fischer 344 rat following a single high oral dose of 350 mg per kg
XXXX
unpublished
XXXX

6) XXXX
Corrigendum/addendum to XXXX: The fate of (^{14}C-aniline)-WL115110 in the fischer 344 rat following a single high oral dose of 350 mg per kg
XXXX
unpublished
XXXX)

7) XXXX
Addendum to XXXX: The fate of (^{14}C-aniline)-WL115110 in the fischer 344 rat following a single high oral dose of 350 mg per kg
XXXX
unpublished
XXXX

1.2 Data protection No

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
1.2.1	Data owner	BASF	
1.2.2	Company with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not report, similar to US EPA 85-1	
2.2	**GLP**	No, at the time the study was conducted GLP was not compulsory. However the study was conducted according to the principle of Good Laboratory Practices.	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	Batch: XXXX	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	Radiolabelled test substance: see 6.2-1 introduction	
3.1.2.2	Purity	> 99.2%, specific activity 0.35 µCi/mg	
3.1.2.3	Stability	Stable	
3.1.2.4	Radiolabelling	^{14}C-aniline Flufenoxuron	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fisher 344	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Male: 207 to 230 grams, female: 139 to 156 grams	X
3.2.6	Number of animals per group	5/sex/group (main test)	
3.2.7	Control animals	Yes, 1 male and 1 female (receiving unlabelled test substance)	

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**
3.3	**Administration/ Exposure**	Single oral
3.3.1	Preparation of test substance	The radiolabeled test substance and unlabeled test substance were each formulated in a mixture containing 30% w/w of dimethyl sulfoxide (DMSO) in Mulgofen EL719 surfactant (polyethoxylated castor oil) immediately prior to dosing.
3.3.2	Concentration of test substance	350 mg/kg bw
3.3.3	Specific activity of test substance	0.35 µCi/mg
3.3.4	Volume applied	6 ml/kg
3.3.5	Size of test site	Not applicable, oral gavage
3.3.6	Exposure period	Single dose
3.3.7	Sampling time	1) 24h, 48h and 72 h post dosing 2) At sacrifice
3.3.8	Samples	1) urine and feces 2) whole blood, liver, spleen, kidneys, peri-renal fat, gastrointestinal tract (including contents), gonads, lungs, hearts, muscle, bone, bone marrow, skin (including feet and tail) and brain.
		4 RESULTS AND DISCUSSION
4.1	**Toxic effects, clinical signs**	No adverse effects in either the control or ^{14}C-Flufenoxuron treated animals were observed.
4.2	**Dermal irritation**	Not relevant
4.3	**Recovery of labelled compound**	Total recovery: 89.5-96.2% in all experiments
4.4	**Percutaneous absorption**	Not relevant
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Animals Fischer 344 strain male and female rats were obtained from XXXX. The male rats weighed 207 to 230 grams. The female rats weighed 139 to 156 grams.

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

Dosing

The treatment of Fischer rats included one dose group and one control group. One group received a dose of [^{14}C-aniline]-Flufenoxuron at 350 mg/kg. The animals in the control group received unlabeled Flufenoxuron at 350 mg/kg.

The radiolabeled test substance and unlabeled test substance were each formulated in a mixture containing 30% w/w of dimethyl sulfoxide (DMSO) in Mulgofen EL719 surfactant (polyethoxylated castor oil) immediately prior to dosing. The dose was administered orally by gavage at a constant volume of 6 mL/kg with a disposable syringe and a steel ball-tipped needle. The actual dose amount was based on individual animal body weight. Animals were fasted 18 h with free access to water prior to dosing.

Since test animals were exposed to a high dose level of Flufenoxuron, the control animals designated for the study were treated with non-radioactive active ingredient formulated in DMSO/Mulgofen to ensure than any potentially adverse effects on the normal renal clearance or fecal constituency of the animals under study was addressed in the control group as well.

After dosing, the animals were placed into individual Nalgene metabolism cages designed for the separation and collection of expired carbon dioxide (CO_2) and organic volatiles, urine and feces.

Sampling

In a pilot study with 2 males and 2 females, expired organic volatiles were collected in a trap maintained in a freezing mixture of solid CO_2/acetone. Expired CO_2 was trapped in 5M sodium hydroxide mixture. Organic volatiles and expired CO_2 were collected for 24 hours post-treatment. Urine and feces were collected 24 hours post-treatment. After the final collection at 24 hours post-treatment, the animals were sacrificed. Urine and feces from this pilot experiment were radioanalysed, but not further characterised.

In the main study, five males and five females (treated group) were given C-14 dose and 1 male and 1 female (control group) received non-radioactive dose. The excreta samples were collected on ice. Urine and feces were collected at 24, 48, and 72 h post-dosing. At the end of the animal holding interval, cages were rinsed with a small volume of deionized

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

water:methanol(1:1, v/v), this sample was designated as the cage wash. Earlier (4, 8 and 12 h) and intermediate (36 h) sampling intervals were not included, since information obtained from the pilot study indicated urine to be a very minor route of elimination of radioactivity. The following tissues were collected at sacrifice: whole blood (7 mL), liver, spleen, kidneys, peri-renal fat, gastrointestinal tract (including contents), gonads, lungs, hearts, muscle, bone, bone marrow, skin (including feet and tail) and brain. All organs and tissue samples (except blood) were individually weighed and stored frozen, blood was stored at 4°C. The remaining carcass was mixed with an equal amount (w/v) of water and homogenised.

Analytical Methods

Specific radioactivity was determined by high performance liquid chromatography (HPLC) of the analyte and standard [^{14}C-aniline]-Flufenoxuron.

Radiopurity: TLC on Merck silica gel plate. Radioactivity was detected by autoradiography and radiochromatogram scanning.

Total Radioactive Residues (TRR) of daily urine, cage wash, CO_2 and volatile traps were assayed by liquid scintillation counting of aliquots. The TRR residual carcass, tissues, and organs were determined by combustion of aliquots to ^{14}C-CO_2. Skin tissue samples were solubilised using a mixture of sodium hydroxide, water, methanol and Triton-X. Aliquots of the solubilised skin were taken for LSC. Feces were extracted with organic solvents (methanol:water) and the extracts analysed by LSC, the residual post-extracted solid was combusted followed by LSC.

Extraction of feces

The samples (1-2 gm) of feces were extracted with organic solvent. Feces samples were mixed with 3-4 time the sample weight of ethylacetate : methanol (3:1, v/v) and extracted on a roller mixer for 20 minutes at room temperature. The mixtures were centrifuged (2500 g) for 10 minutes and the supernatant removed. The solid residue was mixed a further amount of solvent mixture (3-4 times v/w) and extracted as described. A final extraction of the post-extracted residue with twice (v/w) the amount of solvent provided the final extraction. The respective supernatant fractions were combined and each pooled sample was made up to 25 mL. Triplicate aliquot samples (0.5 mL) were

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

taken for radioanalysis by LSC. For the qualitative and quantitative examination of the radioactivity recovered in the fecal extracts, aliquots of each Day-1, Day-2 and Day-3. The fecal extracts were combined from each test animal (time pool samples). The [^{14}C-aniline]-Flufenoxuron-derived radioactivity present in the time pool samples from the individual test animals and in the group time pool samples were analysed by HPLC.

Preparative TLC of fecal extracts on Whatman PLK5F Linear K silica gel plates was used to isolate the exclusive radio component in the day1/2 group pool fecal extracts from male and female test animals. The area of the silica gel containing the exclusive component was removed and the component recovered by ethyl acetate extraction. The extract was concentrated and examined by TLC, HPLC and MS.

Extraction of fat

A 2-3 g composite fat sample was extracted with methylene chloride. The sample was filtered, and the filtrate concentrated to dryness. The residue was reconstituted in hexane and partitioned with acetonitrile (3X). The hexane and acetonitrile fraction were separated. The acetonitrile fraction was concentrated to a small volume and analysed by LSC, HPLC and TLC. The hexane fraction was analysed for radioactivity by LSC.

Extraction of carcass

A 10 g composite homogenised carcass sample was extracted with methylene chloride. The sample was filtered, and the filtrate concentrated to dryness. The residue was reconstituted in hexane and partitioned with acetonitrile (3X). The hexane and acetonitrile fractions were separated. The acetonitrile fraction was concentrated to a small volume and analysed by LSC, HPLC and TLC. The hexane fraction was analysed for radioactivity by LSC.

Chemical structure identification

Non-radioactive standards: UV detector on a HPLC system.

Metabolites were compared with authentic standard by co-TLC or confirmed by mass spectrometry.

5.2 Results and discussion

The stability, homogeneity and correctness of the test substance preparation was analytically verified. No adverse effects in either the control or ^{14}C-Flufenoxuron treated animals were observed.

Pilot study

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

A pilot showed there was negligible radioactivity (<0.01%) in the expired air as carbon dioxide or volatile metabolites. The results showed that elimination of ^{14}C-Flufenoxuron-related radiocarbons by respiration is nil. Twenty-four hours post-dose, approximately 57 and 47% of the dose was detected in the feces of male and female animals respectively, and 0.18 and 0.33% respectively in the male and female urine.

Main study: Excretion balance

Following a single oral dose of 350 mg/kg b w, the administered Flufenoxuron was rapidly eliminated from the rat. In 48 hours after dosing, the majority of dose was recovered in the feces. Seventy-two hours post-dose, approximately 85% of the dose was detected in the feces of male and female animals respectively, and <1% in the male and female urine. There was no major sex - related differences in elimination of radioactivity.

The overall recovery of radioactivity was in the range of 89.5 – 96.2% in all experiments (see table 6.2-3/1).

Tissue distribution

The absorbed Flufenoxuron-related residue was distributed throughout the body as radiocarbons were detected at 3.2 to 203 µg/g in all tissue and organ matrices of males and female test animals (table 6.2-3/2). The mean residual percent of administered radioactivity in blood, carcasses, and tissues at 3-day post dose ranged from <0.1% to 4.44%.The mean concentration of radioactive residue was detected at 3.2-3.7 µg/g in blood, 192-203 µg/g in fat, 4.5-4.5 µg/g in muscle, 13.8-14.1µg/g in kidneys, and 24.3-24.8 µg/g in liver (0.31-0.36% of the administered dose). For other tissues that were examined, the mean concentration of radioactive residues were 6.5-7.7 µg/g in spleen, 12.3 -13.6 µg/g in lungs, 9.9-11.5 µg/g in heart, 1.0-2.1 µg/g in bone, 21.6-52.6 µg/g in bone marrow, 18.1-24.6 µg/g in skin (1.28-1.71% of the administered dose), 7.3-8.2 µg/g in brain, 7.9-52.0 µg/g in gonads. Fat showed the highest concentration of residues (192-203 µg/g) and muscle the lowest (4.5-4.6 µg/g). The animal carcasses showed 12.6-13.7 µg/g of radioactive residues (4.08-4.44% of the administered dose). Blood and tissue concentrations in male and female rats were comparable.

Rate of absorption

Total amount of absorption of the high dose rate of 350 mg/kg b w (urine + liver + skin + carcass) in 72 hours was 6.1% for male rats and 7.1% for the female rats.

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

Metabolism

The metabolic fate of [^{14}C-aniline]-Flufenoxuron in the rat was defined by analyzing metabolic products in the feces, fat and carcass. Unchanged parent was the major component in the extracts of feces, fat and carcass. Since the urine, liver, kidney and muscle from male and from female test animals each accounted for substantially less than 5% of the administered radioactive dose, no attempts were made to investigate the profile of [^{14}C-aniline]-Flufenoxuron degradation products present.

Feces (85.4% of dose male, 84.2% of dose female):

The radioactive residue in feces samples Day-1, Day-2 and day-3 were extractable with organic solvents based on the TRR in the Day 1 and 2 pooled sample from male and female animals, 74.7-79.7% of the administered dose were extracted. 1.13-1.19% of the administered dose were unextractable. For the Day 3 feces sample 2.2-4.7% of the administered dose were extracted and 0.22-0.25% unextractable.

Fecal radiocarbons were composed mainly of unchanged parent, no other discrete radioactive products of greater than 1% of the administered dose were detected in the extracts of the feces. Unchanged Flufenoxuron accounted for 78% (males 79%, females 77%) of the administered dose in the 0-72 h sample. Approximately 3% (males 3.2%, females 2.2%) accounted for Flufenoxuron in 48-72 h fecal extracts.

Fat (3.9% of dose in both male and female):

Extraction of the fat homogenate (192-203 µg/g, 3.9% of the administered dose in males and females) showed 86.4-87.9% (166 - 181 µg/g) of fat TRR was extractable into methylene chloride and 12.1-13.6% (25-26 µg/g) was unextractable. The acetonitrile extracts, following solvent partitioning of the methylene chloride residue between hexane and acetonitrile, contained the majority (97%, 161- 174 µg/g) of the adipose radioactivity (males 96.7% and females 95.9%). Less than 4% of the residual radioactivity was hexane-soluble, this was not examined further. Following HPLC analysis, 97.2-97.3% of the activity in the acetonitrile extract was identified as unchanged Flufenoxuron (equivalent to 156-169 µg/g), the remainder of the radioactivity (~3%) was not characterized.

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

Carcass (4.08% of dose male, 4.44% of dose female):

[^{14}C-aniline]-Flufenoxuron-derived residues were detected in the residual carcass of male (12.6 µg/g) and female (13.7 µg/g) test animals 72 h post-dose. Since the level of radioactivity associated with the residual carcass accounted for approximately 4% (males 4.08%, females 4.44%) of the administered dose, qualitative and quantitative analysis of the chemical nature of the TRR was carried out. Following methylene chloride extraction and solvent partitioning of the extractable residue between acetonitrile and hexane, the majority of the radioactivity of the administered dose (3.8%; males 3.31%, females 4.21%) was contained in the acetonitrile extract. Unchanged Flufenoxuron accounted for the single radiocomponent in the carcass extract. This accounted for 3.6% of the administered dose (males 3.18%, females 4.04%). The combined total of all other minor (non-discrete) radioactive products was less than 0.2% (males 0.13%, females 0.17%) of the administered dose. These were not characterized.

5.3	**Conclusion**	Fischer 344 strain male and female rats received a single high oral dose of [^{14}C-aniline]-Flufenoxuron at 350 mg/kg b w. During the three-day post-dose, total recoveries for the male and female dose groups ranged from 92-93%. Less than 1% of the dose was recovered in the urine (male, 0.33-0.45%; female, 0.49-0.75%). Total radioactivity of 84.8- 85.4% was recovered in feces and 4.08- 4.44% in carcass. Negligible amounts of radioactivity (< 0.1%) were found in volatile organic compound trap and in the carbon dioxide trap. Fecal excretion was the primary elimination route, and the majority of the radioactivity was eliminated within 48 hours after dosing. There were no major sex differences in absorption or elimination of radioactivity for rats dosed with [^{14}C-aniline]- Flufenoxuron. Fat contained the highest residues (192-203 µg/g, 3.9% of the dose). Residues in the other tissues and organs ranged from 3- 25 µg/g (<0.1-0.36% of the dose).
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 3.2.5. Age?
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version Based on the radioactivity in urine, cage wash, carcass and organs, the minimal absorbed fraction was 7.42 % in males and 9% in females
Reliability	1
Acceptability	Acceptable
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.2/ 6 Excretion balance (% of the administered dose) at 24 h, 48 h and 72 h post dosing

	MALE	**FEMALE**
Dose [mg/kg b w] Label	350 Aniline	350 Aniline

Application site Application mode	oral single	oral single
24 h		
Urine 0-24 Faeces 0-24 Subtotal	0.2 55.5 55.7	0.2 34.7 35.0
48 h		
Urine 24-48 Faeces 24-48 Subtotal	0.1 27.3 27.4	0.2 44.2 44.4
72 h		
Urine 0-72 Cage wash Feces 0-72 Subtotal Carcass + organs Total	0.4 0.02 85.4 85.7 7.0 92.7	0.6 0.1 84.2 84.9 8.3 93.2

Table 6.2/ 7 Recovery in tissues (µg/g) at 72 h post dosing

	MALE	**FEMALE**
Dose [mg/kg b w] Label Application site Application mode	350 Aniline oral single	350 Aniline oral single
72 h		
Blood Liver Kidney Muscle Fat Gut wall Gut contents Gonads Lungs Bone marrow	3.2 24.3 14.1 4.5 192.0 76.5 21.9 7.9 12.3 21.6	3.7 24.8 13.8 4.6 202.5 88.8 43.8 52.0 13.6 52.6

Skin	18.1	24.6
Brain	7.3	8.2
Carcass	12.6	13.7

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

		39 Fischer 344 strain female rats received 28 daily single oral doses of [^{14}C-aniline]-Flufenoxuron at 3.5 mg/kg b w followed by a depuration period of 177 days to measure the rates of accumulation, rates of elimination, and half-life values for Flufenoxuron-derived residues in selected tissues.	**Official use only**
		1 REFERENCE 4	
1.1	**Reference**	**8) XXXX** (^{14}C-aniline)-WL115110: Accumulation and depletion from tissues following 28 successive, daily oral low doses (3.5 mg per kg) to female fischer 344 rats XXXX unpublished XXXX **9) XXXX** Corrigendum to XXXX: (^{14}C-aniline)-WL115110: Accumulation and depletion from tissues following 28 successive, daily oral low doses (3.5 mg per kg) to female fischer 344 rats XXXX unpublished XXXX **10) XXXX** (^{14}C-aniline)-WL115110: Accumulation and depletion from tissues following 28 successive, daily oral low doses (3.5 mg per kg) to female fischer 344 rats. II. Nature of the residue in fat XXXX unpublished XXXX **11) XXXX** Corrigendum to XXXX: (^{14}C-aniline)-WL115110: Accumulation and depletion from tissues following 28 successive, daily oral low doses (3.5 mg per kg) to female fischer 344 rats II. Nature of the residue in fat XXXX unpublished XXXX	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Company with letter of access	XXXX	

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, EPA 85-1	
2.2	**GLP**	Yes, (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch: XXXX	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	Radiolabelled test substance: see 6.2-1 introduction	
3.1.2.2	Purity	>99%	X
3.1.2.3	Stability	Stable	
3.1.2.4	Radiolabelling	14C-aniline Flufenoxuron	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fisher 344	
3.2.3	Source	XXXX.	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	142 to 155 grams at the start of the experiment. No age given.	
3.2.6	Number of animals per group	39 females	
3.2.7	Control animals	None	
3.3	**Administration/ Exposure**	Oral gavage	
3.3.1	Preparation of test substance	The radiolabeled test substance was formulated in a mixture containing 10% w/w of dimethyl sulfoxide (DMSO) in Mulgofen EL719 surfactant (polyethoxylated castor oil) and 80% isotonic saline solution. Dose solutions were prepared four times for each week; on days 1, 8, 15 and 22 of the accumulation phase.	X

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**
3.3.2	Concentration of test substance	3,5 mg/kg bw
3.3.3	Specific activity of test substance	3.789 µCi/mg
3.3.4	Volume applied	2.5 mL/kg
3.3.5	Size of test site	Not applicable
3.3.6	Exposure period	28 days
3.3.7	Sampling time	Groups of 3 animals were sacrificed on days 2, 8, 15, 22 of the accumulation phase and days 29, 30, 32, 35, 42, 56, 70, 95 and 205 of the depuration phase.
3.3.8	Samples	The following tissues were collected at sacrifice: whole blood, liver, kidney, fat (perirenal) gastro-intestinal (GI) tract (cardiac sphincter to anus) plus contents, ovaries, bone (both femurs, for bone marrow analysis), skin and remaining carcass. Any excess fat adhering to the surface of the excised organs or tissue (particularly skin and GI tract) was removed and added to the remaining carcass.
		4 RESULTS AND DISCUSSION
4.1	**Toxic effects, clinical signs**	No obvious signs of toxicity were observed in the animals during the course of the study.
4.2	**Dermal irritation**	Not relevant
4.3	**Recovery of labelled compound**	See 5.2 below
4.4	**Percutaneous absorption**	Not relevant
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	<u>Animals</u> Fischer 344 strain female rats were obtained from XXXX. The female rats weighed 142 to 155 grams at the start of the experiment. No age given. <u>Dosing</u> The radiolabeled test substance was formulated in a mixture containing 10% w/w of dimethyl sulfoxide (DMSO) in Mulgofen EL719 surfactant (polyethoxylated castor oil) and 80% isotonic

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
	saline solution. Dose solutions were prepared four times for each week; on days 1, 8, 15 and 22 of the accumulation phase. The animals received a daily dose of approximately 3.5 mg per kg of ^{14}C-Flufenoxuron by oral intubation at a constant dose volume of 2.5 ml/kg. Each animal received the same volume of dose for a 7 day period (4 periods, Days 1-7, 8-14, 15-21, 22-28), which was determined by the mean body weight of all animals. The animals were weighed every 7 days and the dose volume adjusted accordingly. <u>Sampling</u> After dosing, the animals were housed in 13 groups of 3 in a suspended cage system and were given free access to food and water. Groups of 3 animals were sacrificed on days 2, 8, 15, 22 of the accumulation phase and days 29, 30, 32, 35, 42, 56, 70, 95 and 205 of the depuration phase. The following tissues were collected at sacrifice: whole blood, liver, kidney, fat (perirenal) gastro-intestinal (GI) tract (cardiac sphincter to anus) plus contents, ovaries, bone (both femurs, for bone marrow analysis), skin and remaining carcass. Any excess fat adhering to the surface of the excised organs or tissue (particularly skin and GI tract) was removed and added to the remaining carcass. Samples taken at 29 days (maximum residue), 56, 70 and 95 days were selected for analysis as being representative of the fat depletion profile. Pooled samples were prepared by taking 0.3-0.4 g subsamples from individual fat samples at each selected time point to give a total weight of about 1.0 g. All organs and tissue samples were stored frozen (except blood, stored at 4°C). The remaining carcass was homogenized with an equal amount of water (w/v), the GI tract plus contents was homogenized. Whole body autoradiography: One additional female rat received 28 daily oral doses of ^{14}C-Flufenoxuron (3.5 mg/kg) and was housed individually. Twenty-four hours after the final dose, it was sacrificed and prepared for whole-body autoradiography. Longitudinal whole-body sections were prepared, at least four sections were taken from the various levels of the animal on a strip of 3M Scotch tape and freeze dried. Two sections from each level were placed in contact with X-ray film and stored at –20°C. One X-ray film from each level was developed after 4 weeks exposure, and the other after 12 weeks exposure. <u>Analytical Methods</u> Specific radioactivity was determined by high performance liquid	X

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

chromatography (HPLC) of the analyte and radiochemical purity by HPLC and TLC.

Radiopurity of formulated ^{14}C-Flufenoxuron (Batch 1) was determined by TLC on the first and on the last day of use.

Total Radioactive Residues (TRR) in tissues and organs were determined by combustion of aliquots to ^{14}C-carbon dioxide. Skin and carcass samples were solubilised using a mixture of sodium hydroxide, water, methanol and Triton-X. Aliquots of the solubilised skin were taken for LSC.

Total Radioactive Residues (TRR) in extracts of fat tissue was determined be liquid scintillation counting. TRR in the post-extracted solids was determined by combustion to carbon-14 carbon dioxide followed by liquid scintillation counting.

One g aliquot sub-samples of abdominal fat were extracted with methylene chloride. The extracts were filtered and the unextracted residue collected on filter paper. The filtrate was concentrated to dryness and the residue reconstituted in hexane. The hexane was back extracted with acetonitrile. The hexane and acetonitrile fractions were assayed for radioactivity by LSC. The unextracted residue/filter paper was combusted followed by liquid scintillation counting. The acetonitrile extract was concentrated and subjected to TLC under the following conditions:

TLC: Merck silica gel plates.

Single dimensional TLC using diethyl ether:hexane (2:1, v/v).

Radioactivity was detected by radiochromatogram scanning and quantified by scraping the zones of silica gel followed by mixing with methanol and counting.

Non-radioactive standards: UV detection of authentic unlabeled reference compounds.

5.2 Results and discussion

TLC of ^{14}C-Flufenoxuron, on the first and on the last day of use indicated that the formulated test material was stable.

No obvious signs of toxicity were observed in the animals during the course of the study.

<u>Tissue distribution</u>

Residue concentrations increased with the number of administered doses throughout the treatment period (28 day). The increase was apparently linear with respect to time for most tissues; in skin an apparent exponential type relationship was observed, suggesting that equilibrium concentration (plateau) was close to being achieved. However, equilibrium concentrations

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

were not observed during the 28 day dosing schedule.

Peak concentrations of radioactivity were determined in tissue samples obtained on days 29 and 30 (24 and 48 hours after the final dose, respectively). The highest residue concentration, expressed as µg/g equivalents of Flufenoxuron, was located in the fat (144 µg/g), the other tissue residues decreased in the rank order bone marrow (33 µg/g) > ovary (23 µg/g) > G. I. tract (18 µg/g) > skin (18 µg/g) > liver (16 µg/g) > carcass (15 µg/g) > kidney (11 µg/g) > blood (3 µg/g). These show a similar rank order to those seen after single oral dose of ^{14}C- Flufenoxuron at a both 3.5 and 350 mg/kg to Fischer 344 rats (Huckle, 1986, 1987).

After termination of dosing, the tissue residues decreased with time. The mean half-life was 34 days; values ranged from 28 days (fat) to 48 days (liver). The rank order was as follows: liver (48) > kidney (38) > marrow (34) > 34 = ovary (34) > skin (33) > blood (32) > GI tract (29) > carcass (28) = fat (28).

Whole body autoradiography

Results from the whole-body autoradiography indicated that the radioactivity was extensively distributed throughout the entire carcass. The darkest regions were associated with the fat (subcutaneous and abdominal). The qualitative distribution of radioactivity as shown by whole body autoradiography in the present study is similar after single oral and multiple oral treatments.

Extraction

Following extraction of the abdominal fat samples from days 29, 56, 70 and 95, the acetonitrile fraction contained 95.7- 97.3% of the recovered radioactivity (equivalent to 140 µg/g at day 29, 62.5 µg/g at day 56, 44 µg/g at day 70 and 24 µg/g at day 90). The hexane fraction contained 0.2-1.4% of the fat sample TRR's (equivalent to 0.26 - 0.35 µg/g). 1.7 -3.6% of the TRR were in the post-extracted residues which accounted for 0.4 to 3.6 µg/g.

Identification

TLC of the acetonitrile fraction showed a single radioactive product with chromatographic properties indistinguishable from those of authentic Flufenoxuron. Following quantification of plates, ^{14}C- Flufenoxuron accounted for 97.8% (97.2-98.4%) of the radioactivity applied to the TLC plates; and 2.2% (1.6-2.3%) was in the remaining zones of radioactivity. Thus, Flufenoxuron accounted for 93.3-95.7% (23.7 - 137.8 µg/g) of the TRR in

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
	abdominal fat.	
5.3 Conclusion	Fischer 344 strain female rats received single daily oral doses of ^{14}C-Flufenoxuron in DMSO/Mulgofen at 3.50 mg/kg b.w for 28 days followed by a depuration period of 177 days to measure the rates of accumulation, rates of elimination, and half-life values for ^{14}C-Flufenoxuron-derived residues in selected tissues. Peak concentrations of radioactivity were detected at 24 and 48 hour after the final dose. Although an equilibrium concentration (plateau level) was close to being achieved in skin, for the majority of tissues, equilibrium concentrations were not observed during the 28 day dosing schedule. Whole body autoradiography of one treated rat indicated that the radioactivity was well distributed throughout the carcass, with fat showing the highest concentrations of radioactivity (144 µg/g), and the lowest tissue residues were detected in the kidney (11 µg/g). Blood residues were 3 µg/g. The fat residue was characterized as being unchanged ^{14}C-Flufenoxuron in samples obtained at the time points showing peak residue levels (29 days), and at later time points representative of the residue depletion profile, days 56, 70 and 95. After termination of the dosing, the tissue residues decreased with time. The mean elimination half-life was 34 days, with liver having the highest half-life (48 days) and the carcass and fat the lowest (28 days). The rank order was as follows: liver (48) > kidney (38) > marrow (34) > 34 = ovary (34) > skin (33) > blood (32) > GI tract (29) > carcass (28) = fat (28).	
5.3.1 Reliability	1	X
5.3.2 Deficiencies	No	X

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 3.1.2.2. Purity: Add specific activity
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version
	Revisions/Amendments: 5.3.2. Deficiencies Yes (only females tested)
Reliability	2, only females tested
Acceptability	Acceptable
Remarks	3.3.1 and 5.1 10% w/w Mulgofen
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

Official use only

The biokinetics and metabolism of [^{14}C-benzoyl]-Flufenoxuron have been studied after single oral low level doses (3.5 mg/kg) and high level doses (350 mg/kg) to rats. The biliary excretion after low level doses and tissue distribution of radioactivity after both single low and high level doses has also been studied. The proportions and nature of metabolites have been investigated as well as the tissue distribution (qualitatively) by whole-body autoradiography at 4 hours after single oral low level doses.

1 REFERENCE 5

1.1	**Reference**	**12) XXXX** The metabolism of ^{14}C -WL115110 in rats XXXX unpublished XXXX **13) XXXX** Report amendment no. 1: The metabolism of ^{14}C -WL115110 in rats XXXX unpublished XXXX
1.2	**Data protection**	No
1.2.1	Data owner	BASF
1.2.2	Company with letter of access	XXXX
1.2.3	Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Not reported but similar to US EPA 85-1
2.2	**GLP**	Yes, (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	Batch no. XXXX

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	Radiolabelled test substance: see introduction 6.2-1	
3.1.2.2	Purity	98%	X
3.1.2.3	Stability	Stable	
3.1.2.4	Radiolabelling	^{14}C-aniline Flufenoxuron	X
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fisher 344	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	The male rats, aged 7-9 weeks, weighed 209 to 240 grams. The female rats, aged 9-11 weeks, weighed 150 to 170 grams.	
3.2.6	Number of animals per group	maximum 9/sex/group (main test)	
3.2.7	Control animals	None	
3.3	**Administration/ Exposure**	Single oral	
3.3.1	Preparation of test substance	The radiolabeled test substance was formulated in a mixture containing 10% w/w of dimethyl sulfoxide (DMSO) in Mulgofen EL719 surfactant (polyethoxylated castor oil) and 80% isotonic saline solution immediately prior to dosing. The dose was administered orally by gavage with a disposable syringe and a steel ball-tipped needle. The actual dose amount was based on individual animal body weight. The nominal dose of [^{14}C-benzoyl]-Flufenoxuron administered to individual test animals was 3.5 mg/kg b.w for the low dose treatments, and 350 mg/kg for the high dose treatments.	X
3.3.2	Concentration of test substance	3.5 and 350 mg/kg bw	
3.3.3	Specific activity of test substance	35 µCi/mg	
3.3.4	Volume applied	Not reported	
3.3.5	Size of test site	Not applicable	
3.3.6	Exposure period	Single dose	

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
3.3.7	Sampling time	1) <u>Excretion study</u> : urine was collected during 0-6 h, 6-24 h and then at 24-hour intervals up to 168 hours Expired air was collected at 0-24 and 24-48 hours after dosing. Feces were collected during 24-hour intervals up to 168 h.	X X
		2) <u>Blood and plasma studies:</u> at pre dose and 0.25, 0.5, 1, 2, 3, 4, 6, 9 and 24 hours after dosing followed by further samples at either daily intervals or at 2-3 day intervals at later times until 336 hours after dosing.	X
		3) B<u>iliary excretion study:</u> urine and feces at 0-24 h and 24-48 h for the measurement of radioactivity. Bile was collected at 3-hourly intervals for 0-48 hours. After 48 hours, animals were sacrificed and the gastrointestinal tracts removed and taken for measurement as well as the remaining carcass.	X
		4) <u>Quantitative tissue distribution studies</u> Groups of six rats (3M, 3F) were sacrificed at 4, 20 and 168 hours post-dose . The following tissues and organs were removed from the carcass: liver, kidneys, heart, lungs, brain, eyes, gonads (testes or ovaries) spleen, pancreas, adrenals, thyroid, thymus, gastro-intestinal tract, uterus and samples of muscle (gluteus superficial), bone (femur), bone marrow and fat (subcutaneous and perirenal). The remaining carcass was retained for measurement of radioactivity. Three animals of the same sex were housed in one cage <u>5) whole-body autoradiography</u> sacrificed 4 hours after treatment for sections of tissue slices	X
3.3.8	Samples	See 3.3.7	
		4 RESULTS AND DISCUSSION	
4.1	**Toxic effects, clinical signs**	Not reported	X
4.2	**Dermal irritation**	Not relevant	

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
4.3	**Recovery of labelled compound**	Total recovery> 95% for male and for female	
4.4	**Percutaneous absorption**	Not relevant	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	<u>Animals</u> Fischer 344 strain male and female rats were obtained from XXXX. The male rats, aged 7-9 weeks, weighed 209 to 240 grams. The female rats, aged 9-11 weeks, weighed 150 to 170 grams. Animals were not fasted during the dosing period. <u>Dosing and housing of animals</u> The radiolabeled test substance was formulated in a mixture containing 10% w/w of dimethyl sulfoxide (DMSO) in Mulgofen EL719 surfactant (polyethoxylated castor oil) and 80% isotonic saline solution immediately prior to dosing. The dose was administered orally by gavage with a disposable syringe and a steel ball-tipped needle. The actual dose amount was based on individual animal body weight. The nominal dose of [^{14}C-benzoyl]-Flufenoxuron administered to individual test animals was 3.5 mg/kg b.w for the low dose treatments, and 350 mg/kg for the high dose treatments. After dosing, test animals used in the pharmacokinetics experiments (whole blood and plasma studies), and quantitative tissue distribution studies were housed in stainless steel cages with suspended mesh floors. For excretion-balance experiments, the animals were housed in individual glass metabolism cages designed for separate collection of urine and feces. For biliary excretion studies, the rats were housed in restraining cages designed for separate collection of urine and feces. <u>Sampling</u> In the excretion study (3 males and 3 females for each low and high dose), urine was collected during 0-6 h, 6-24 h and then at 24-hour intervals up to 168 hours. Expired air from each metabowl was pulled through two traps containing ethanolamine:2-ethoxyethanol. Radioactivity in expired air traps was collected at 0-24 and 24-48 hours after dosing. Feces were collected during 24-hour intervals up to 168 h. The cage interiors	X

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

were washed with water after sacrifice. The gastrointestinal tract, cardiac sphincter to anus (including contents) was removed from each animal and the remaining carcass retained for measurement of radioactivity.

In the blood and plasma studies (6 males and 6 females for each low and high dose), blood samples (2 x 0.1 mL) were withdrawn from the tail vein into heparinized tubes at pre dose and 0.25, 0.5, 1, 2, 3, 4, 6, 9 and 24 hours after dosing followed by further samples at either daily intervals or at 2-3 day intervals at later times until 336 hours after dosing. The two (0.1 mL) samples of whole-blood from six rats (3M and 3F) were used for direct measurement. Samples of whole blood from the other six rats (3M, 3F) were used for the measurement of radioactive content in samples of plasma (2 x 0.05 mL) after removal of the cells by centrifugation. Three animals of the same sex were housed in one cage.

Urine and feces of the biliary excretion study (3 males and 3 females, low dose) were collected at 0-24 h and 24-48 h for the measurement of radioactivity. Bile was collected at 3-hourly intervals for 0-48 hours. After 48 hours, animals were sacrificed and the gastrointestinal tracts removed and taken for measurement as well as the remaining carcass.

Quantitative tissue distribution studies (9 males and 9 females for each low and high dose):

Groups of six rats (3M, 3F) were sacrificed at 4, 20 and 168 hours post-dose. These times were selected (from plasma level data) to nominally correspond to Cmax, ½ Cmax and Cterm, respectively. At these times, a blood sample (8-10 mL) was taken and a sub-sample was used for separation of plasma by centrifugation. The following tissues and organs were removed from the carcass: liver, kidneys, heart, lungs, brain, eyes, gonads (testes or ovaries) spleen, pancreas, adrenals, thyroid, thymus, gastro-intestinal tract, uterus and samples of muscle (gluteus superficial), bone (femur), bone marrow and fat (subcutaneous and perirenal). The remaining carcass was retained for measurement of radioactivity. Three animals of the same sex were housed in one cage.

<u>Whole-body autoradiography (low dose: 1 male and 1 female):</u>

Both animals were sacrificed 4 hours after treatment. The animals were prepared for whole-body autoradiography. Sections of tissue slices obtained at several levels through the carcass between the level of the kidney and the level of the spinal cord were, prepared. The sections were mounted on Cellux tape and freeze

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

dried before placing them in contact with X-ray film , exposure times for film were typically 30 days. The relative concentrations of radioactivity in the various tissues were estimated by visual inspection.

Analytical Methods

- Total Radioactive Residues (TRR):

Feces were extracted by homogenization with methanol, and after centrifugations, radioactivity measured in both extracts and post-extracted solids. Daily urine, cage washings, CO_2 traps, plasma, solvent extracts were assayed by liquid scintillation counting of aliquots. Carcasses were digested for 48 h at 55° C in sodium hydroxide (2M) in 30% methanol (v/v) containing Triton X-405. Tissues suitable for solubilization were incubated at 50°C for 24 hours with solubilizer. Radioactivity in post-extracted fecal samples, whole-blood, gastrointestinal tract, liver and spleen was determined after combustion.

Radioactive components on thin-layer plates were detected by autoradiography or were located and measured directly using a Berthold Linear Analyzed.

- Identification of metabolites/degradation products:

Samples of 0-24 and 24-48 hour urine from the low dose excretion study, 0-24 and 24-48 hour methanol extracts of feces from the low and high dose extraction studies and the 20 h fat extracts from the low dose tissue distribution study were pooled by combining representative proportions from each animal to give samples for male and female rats. These were analyzed directly by TLC. The Flufenoxuron-derived metabolites/degradation products formed in the rat were characterized by co-chromatographic comparison using different systems with the following reference compounds: 2, 6-difluorobenzoic acid, Reg. No. 206955; 2, 6-difluoro-benzamide, Reg. No. 102719; 1-(2-fluoro-4-hydroxyphenyl)-3-(2, 6-difluorobenzoyl)urea.

- TLC systems: Merck Silica gel plates:

System 1: dichloromethane

System 2: diethyl ether:n-hexane (2:1, v/v)

System 3: toluene:ethyl acetate (1:1, v/v)

System 5: chloroform:methanol:acetic acid (64:25:4, v/v/v)

TLC systems Whatman KC18 F reverse phase

System 6: methanol:water (80:20, v/v)

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**
		Fat, skin and carcass were extracted with methylene chloride. The samples were filtered, and the filtrates concentrated to dryness. The residues were reconstituted in hexane and partitioned with acetonitrile (3X). The hexane and acetonitrile fractions were separated. The acetonitrile fractions were concentrated to a small volume and analyzed by LSC, HPLC and TLC. The hexane fractions were analyzed for radioactivity by LSC. Intestine was extracted with ethyl acetate:methanol followed by acetonitrile, similar to that described for feces. Control liver, fat, intestine, skin and carcass and controls fortified with [^{14}C-benzoyl]-Flufenoxuron were also extracted to assess recovery and stability of the test compound. Lyophilized urine fractions were subjected to hydrolytic enzymes (ß-glucuronidase and arylsulphatase) at pH 5.0 in 0.1 M sodium acetate buffer at 37°C overnight.
5.2	**Results and discussion**	<u>Excretion studies, low oral dose</u> After **single low oral doses** of [^{14}C-benzoyl]-Flufenoxuron at 3.5 mg/kg to 2 male and 3 female rats: - Means of 30 % of the administered dose (male) and 24 % of the administered dose (female) were excreted in the urine during 7 days (including cagewash); - Means of 19 % of the administered dose (male) and 12 % of the administered dose (female) were excreted via feces during 7 days after dosing. Most of the radioactivity was excreted in 0 – 48 hours (15 % male, 9 % female). Radioactivity in the 0-24 and 24 – 48 hour expired air traps was below the limit of quantification (LOQ =0.03 % of the administered dose). At sacrifice after 168 hours, radioactivity in the remaining carcasses accounted for 46 % of the administered dose (male) and 59 % of the administered dose (female) with 1 % of the administered dose (male) and 2 % of the administered dose (female) in the gastro-intestinal tracts (including contents). Thus, means of 95 % of the administered dose (male) and 96 % of the administered dose (female) were recovered (Table 6.2/9). <u>Excretion studies, high oral dose</u> After **single high oral doses** of [^{14}C-benzoyl]-Flufenoxuron at 350 mg/kg to 3 male and 3 female rats, means of 0.7 % of the administered dose (male) and 0.5 % of the administered dose

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

(female) were excreted in the urine during 7 days (including cagewash).

Means of 93 % of the administered dose (male) and 102 % of the administered dose (female) were excreted via feces during 7 days after dosing.

Most of the radioactivity was excreted in 0 – 48 hours (91 % of the administered dose for both male and female).

Radioactivity in the 0-24 and 24 – 48 hour expired air traps was below the limit of quantification (LOQ =0.03 % of the administered dose).

At sacrifice after 168 hours, radioactivity in the remaining carcasses and the gastro-intestinal tracts (including contents) accounted for < 1 % of the administered dose (male and female). Thus, means of 94 % of the administered dose (male) and 103 % of the administered dose (female) were recovered (Table 6.2/ 9).

Low dose biliary excretion

After **single low oral doses** of [^{14}C-benzoyl]-Flufenoxuron to 3 male and 3 female rats with cannulated bile ducts:

- Means of 14 % of the administered dose (male) and 10 % of the administered dose (female) were excreted in the urine (including cagewash) during 48 hours;
- Means of 5 % of the administered dose (male) and 5 % of the administered dose (female) were excreted in the bile during 48 hours after dosing.
- At 48 hours after dosing, means of 11 % of the administered dose (male) and 4 % (female) were excreted in the faeces.
- After the sacrifice at 48 hours after dosing, radioactivity in the remaining carcasses accounted for 61 % of the administered dose (male) and 78 % of the administered dose (female). Overall means of 4 % of the administered dose (male) and 3 % of the administered dose (female) were measured in the gastro-intestinal tract (including contents).

Thus, means of 94 % of the administered dose (male) and 100 % of the administered dose (female) administered were recovered (Table 6.2-5/3)

Total amount of absorption (urine + cagewash + bile + carcass) in 48 hours was 79.76% of the low dose rate of 3.5 mg/kg b.w. for male rats and 92.15% for the female rats. The mean value was 85.96 %.

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

Pharmacokinetics

The pharmacokinetic parameters are summarised in the following table. Concentrations C_{max} of radioactivity in plasma and whole-blood were higher after high level doses (0.8 – 1.1 µg equivalents/ml) compared to after low level doses (0.3 – 0.4 µg equivalents/ml). However, there was no substantial increase in the AUC in plasma and whole-blood after high level doses (21.3 – 42.1 µg x hr ml^{-1}) compared to after low-level doses (25.4 – 62.5 µg x hr ml^{-1}).

Low dose plasma

After single low oral doses of [^{14}C-benzoyl]-Flufenoxuron to 3 male and 2 female rats, peak mean concentrations of radioactivity in the plasma of 0.27 µg equivalents/ml (male) and 0.39 µg equivalents/ml (female) were measured at 6 hours after dosing. Mean concentrations of radioactivity in the plasma then declined bi-exponentially with an initial half-life of approximately 6 hours and a terminal half-life of 155 to 428 hours. Mean concentrations in the female rat plasma were higher than in the male rat plasma.

At 336 hours after dosing, concentrations had declined to 0.02 µg equivalents/ml (male) and 0.06 µg equivalents/ml (female). Means of the areas under the plasma concentration-time-curves (AUC) until 48 hours were 25.4 µg x hr ml^{-1} (male) and 62.5 µg x hr ml^{-1} (female).

Low dose whole blood

After single low oral doses of [^{14}C-benzoyl]-Flufenoxuron to 3 male and 3 female rats, peak mean concentrations of radioactivity in whole blood of 0.42 µg equivalents/ml (male) and 0.60 µg equivalents/ml (female) were measured at 3 – 4 hours and 3 hours after dosing respectively. Mean concentrations of radioactivity in whole blood then declined bi-exponentially with an initial half-life of approximately 5-8 hours and a terminal half-life of 178 to 327 hours. Mean concentrations in the female rat blood were generally slightly higher than in the male blood at the later times.

At 336 hours after dosing, concentrations had declined to 0.04 µg equivalents/ml (male) and 0.06 µg equivalents/ml (female). Means of the areas under the blood concentration-time-curves (AUC) until 72 hours were 38.6 µg x hr ml^{-1} (male) and 62.3 µg x hr ml^{-1} (female).

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

High dose plasma

After single high oral doses of [^{14}C-benzoyl]-Flufenoxuron to 3 male and 3 female rats, peak mean concentrations of radioactivity in the plasma of 0.8 µg equivalents/ml (male) and 1.1 µg equivalents/ml (female) were measured at 4 - 6 hours after dosing. Mean concentrations of radioactivity in the plasma then declined with a terminal elimination half-life of approximately 13 - 22 hours measured between 6 and 48 hours.

At 48 hours after dosing, concentrations had declined to below the limit of reliable measurement (<0.27 µg equivalents/ml). Therefore, results from measurements after 72 hour were not processed. Means of the areas under the plasma concentration-time-curves (AUC) until 48 hours were 24.1 µg x hr ml^{-1} (male) and 21.3 µg x hr ml^{-1} (female).

High dose whole blood

After single high oral doses of [^{14}C-benzoyl]-Flufenoxuron to 3 male and 3 female rats, peak mean concentrations of radioactivity in whole blood of 0.75 µg equivalents/ml (male) and 1.0 µg equivalents/ml (female) were measured at 3 hours after dosing. Mean concentrations of radioactivity in whole blood then declined with a terminal elimination half-life of 37 hours measured between 3 and 48 hours in female rats. The concentrations in male rats could not be resolved into an exponential component.

At 72 hours after dosing, concentrations had declined to 0.33 µg equivalents/ml (male) and <0.21 µg equivalents/ml (female). Means of the areas under the blood concentration-time-curves (AUC) until 72 hours were 42.1 µg x hr ml^{-1} (male) and 26.1 µg x hr ml^{-1} (female).

Absorption rate constants

The absorption rate constants of radioactivity from plasma concentration data after low oral doses were 1.0431 and 1.0311 per hour in males and females, respectively. They accounted for 1.0330 and 1.7349 in males and females respectively following high oral doses. These rate constants corresponded to mean absorption half-lives in plasma at 0.66 and 0.67 hours in male and female rats respectively at the low dose and 0.67 and 0.40 hours respectively at the high dose.

The absorption rate constants of radioactivity from whole-blood

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
	concentration data after low level oral doses were 0.9953 and 0.6128 per hour in males and females, respectively, and 1.1060 and 1.7349 in males and females respectively following high level oral doses. These rate constants corresponded to mean absorption half-lives in plasma at 0.70 and 1.13 hours in male and female rats respectively at the low dose and 0.65 hours in female rats at the high dose. The concentration-time profile in male rats after high level doses could not be resolved into its exponential components. Tissue distribution **Single oral low dose**: After single low level oral doses of [^{14}C-benzoyl]-Flufenoxuron, 43-49% of the dose was found in the carcass at 4 hours increasing to 71-78% at 20 hours before decreasing to 39-47% at 168 hours after dosing. At 4 hours after dosing, the tissues with the largest amounts of radioactivity were the GI-tract (29-36% of the administered dose) and the muscle (26-30% of the administered dose). These amounts decreased at later times with 4% of the dose present in the GI tract at 20 hours and 1-2% of the dose at 168 hours. Levels decreased more slowly in the muscle, to 11-19% and 5-6% of the dose, respectively, at 20 and 168 hours after dosing. Lower levels were observed in the liver with 9-10% of the dose observed at 4 hours decreasing to around 1% at 168 hours. Radioactivity in the fat at 4 hours after dosing accounted for 7% (subcutaneous) and 3% (perirenal) of the dose and this increased to 23% (subcutaneous) and 9-10% (perirenal) at 20 hours before decreasing slightly, accounting for 15-17 % subcutaneous) and 7% (perirenal) at 168 hours after dosing. Apart from bone marrow, blood and kidney had 1-2% of the dose at 4 hours after dosing, the levels observed in all other tissues were <1% of the dose (Table 6.2/ 12). At 4 hours after dosing, the highest concentrations of radioactivity were in the adrenals, GI-tract, liver, ovaries, thyroid and bone marrow (8-28 µg/g). Tissues with intermediate concentrations of radioactivity were the kidney, lungs, pancreas, perirenal fat and subcutaneous fat (4-7 µg/g). Concentrations in other tissues were < 4 µg/g. At 20 hours after dosing, the highest concentrations of radioactivity were in the perirenal fat and subcutaneous fat (15-17 µg/g). Levels in other tissues had generally decreased with intermediate levels observed in the adrenals, bone marrow, ovaries, pancreas and thyroid (4-8 µg/g). Concentrations in other tissues were < 2 µg/g.	X

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

At 168 hours after dosing, the concentrations of radioactivity were generally lower than at 20 hours. Highest concentrations were still in the perirenal fat and subcutaneous fat (9-11 µg/g). Intermediate levels were observed in the adrenals, bone marrow, pancreas and thyroid (2-3 µg/g). Concentrations in other tissues were in the range of 0.05 -1 µg/g (Table 6.2/ 13).

Single high oral dose: After single high oral doses of [^{14}C-benzoyl]-Flufenoxuron, 1% of the dose was found in the carcass at 4 hours increasing to 1-4% at 20 hours before decreasing to 0.4% at 168 hours after dosing. At 4 hours after dosing, most of the radioactivity was associated with the GI tract (109-118% of the dose) and this decreased through the study, accounting for 12-19% and 0.1% at 20 hours and 168 hours, respectively. Radioactivity in the muscle accounted for 0.22-0.25% at 4 hours after dosing and decreased to below the limit of detections at 168 hours after dosing. Radioactivity in the fat at 4 hours after dosing accounted for 0.05-0.06% (subcutaneous) and 0.02% (perirenal) of the dose. This increased to 0.12-0.27% (subcutaneous) and 0.05-0.12% (perirenal) of the dose at 20 hours after dosing before decreasing slightly, accounting for 0.17-0.21% (subcutaneous) and 0.07-0.09% (perirenal) of the dose at 168 hours. Apart from radioactivity in the liver, 0.07-0.11% of the dose at 4 hours after dosing, decreasing to 0.01-0.03% of the dose at 168 hours. Levels in all other tissues were generally 0.01% of the dose or less (Table 6.2/ 14).

At 4 hours after dosing, the highest concentration of radioactivity was in the GI-tract, 4000-5000 µg/g. Intermediate levels were observed in the adrenals, bone marrow, kidney, liver, ovaries and pancreas (4-8 µg/g). Levels were lower in the perirenal fat and subcutaneous fat (2-3 µg/g). In all other tissues, the concentrations were 1-3 µg/g.

At 20 hours after dosing, concentrations had increased in the perirenal fat and the subcutaneous fat (6-14 µg/g). Levels in the other tissues were generally lower than at 4 hours with the highest concentration was still observed in the GI tract (270, 670 µg/g). Intermediate levels were observed in the adrenals, bone marrow and pancreas (2-7 µg/g). Levels in the other tissues were still either below the limit of detection or in the range of 1-3 µg/g).

At 168 hours after dosing, the levels of radioactivity in most tissues were below the limit of reliable measurement. Concentrations were highest in the perirenal and subcutaneous fat (9 µg/g). The only other tissues with any detectable radioactivity

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

were the GI tract, liver, adrenals (female only) and pancreas (1-3 µg/g), see Table 6.2/ 15.

Whole Body Autoradiography

The highest concentrations of radioactivity were in stomach contents, brown fat, small intestine contents and the preputial and clitoral glands. Intermediate concentrations were found in kidney, fat, exorbital lachrymal gland, spleen, liver, muscle, infra-orbital lachrymal gland, lung, pancreas, bone marrow, adrenals, Harderian gland, salivary gland, myocardium, brain, thyroid, thymus and spinal cord. In the female rat, intermediate levels were also observed in the uterus wall and the ovary.

Metabolism

The metabolic fate of [^{14}C-benzoyl]-Flufenoxuron was defined by analyzing the metabolic products in urine, feces and fat. Metabolites found were 2, 6-difluorobenzoic acid, Reg. No. 206955 and 2, 6-difluorobenzamide, Reg. No. 102719. Unchanged parent was the single predominant component in fat and feces.

Urine: In urine of rats after a low level dose of [^{14}C-benzoyl]-Flufenoxuron, the major metabolite co-chromatographed with 2, 6-difluorobenzoic acid, accounting for 8-9% of the dose in 0-24 hours and 2-4% of the dose in 24-48 hours from male and female rats. A second metabolite was found to co-chromatograph with 2, 6-difluorobenzamide and accounted for 0.2% and 0.1% of the dose during 0-24 and 24-48 hours. Three further polar metabolites (A, B and C) were separated in a more polar TLC system, these accounted for 0.4-0.8, 0.2-0.4% and 0.1-0.3%. There was no unchanged parent detected in the urine (Table 6.2/ 16).

Feces: In both high and low level dose studies, the extracts of the feces showed only one radioactive component which co-chromatographed with unchanged parent. In the low level study, unchanged Flufenoxuron accounted for 12-16% of the dose during 0-24 hours and 2-3% of the dose during 24-48 hours in male and female rats, respectively. After high level doses of [^{14}C-benzoyl]-Flufenoxuron radioactivity corresponding to parent compound in the feces extract accounted for 78% and 68% of the dose during the 0-24 hours and 13% and 22% of the dose during

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

24-48 hours in male and female rats, respectively (

Table 6.2/ 17).

Fat: Only one component was identified in extracts of subcutaneous fat taken 20 hours after low level dosing. This was shown to co-chromatograph with Flufenoxuron. It accounted for 15-16 µg/g at 20 hours after dosing.

X

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

5.3 Conclusion

The biokinetics, metabolism, biliary excretion and tissue distribution of [^{14}C-benzoyl]-Flufenoxuron was studied in Fischer 344 strain male and female rats following treatment with [^{14}C-benzoyl]-Flufenoxuron in DMSO/Mulgofen. Biliary excretion studies were conducted at nominal single oral low doses (3.50 mg/kg b.w). Tissue distribution, metabolism, and biokinetics studies were conducted at nominal single oral low doses (3.50 mg/kg b.w) and nominal single oral high doses (350 mg/kg b.w).

After the single oral low doses, 24 % (female) to 30% (male) of the dose was excreted in the urine and 12 %(male) to 19% (female) of the dose was in the feces 168 hours post-dose. At 168 hours, 46 % (male) to 59% (female) of the dose was in the remaining carcass with 1-2% in the GI tract (including contents). Overall, 94% (male) and 100% (female) of the administered dose was recovered.

After the single oral high doses, only 0.5-0.7% of the dose was excreted in the urine and 93 % (male) to 102% (female) of the dose was in the feces during 168 hours post-dose. At 168 hours, <1% of the administered dose was in the remaining carcass and GI tract plus contents. Overall, 94% (male) and 103% (female) of the administered dose was recovered.

Following the low and high dose, most of the radioactivity was excreted in the 0-48 h urine and feces, although appreciable amounts were also excreted at later times, particularly in the urine after the low level dose. There was no appreciable difference in the excretion of radioactivity by male and female rats. It was no detectable radioactivity in the expired air.

In the biliary excretion study of Flufenoxuron, 10% (female) to 14% (male) of the dose was in the urine, about 5% in the bile and 4 % (female) to 11% (male) in the feces 48 hours after dosing. At 48 hours post-dose, 61% (male) and 78% (female) of the administered dosed remained in the carcass. Overall, 94% (male) and 100% (female) of the administered dose was recovered in the

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

biliary excretion study.

Total amount of absorption (urine + cagewash + bile + carcass) in 48 hours was 79.76% of the low dose rate of 3.5 mg/kg b.w. for male rats and 92.15% for the female rats. The mean value was 85.96 %.

In plasma, peak of mean concentrations of radioactivity occurred at 6 hours (0.3- 0.4 µg/mL, low dose) and 4-6 hours (0.8-1.1 µg/mL, high dose). The absorption half-lives of concentrations in plasma were 0.66-0.67 hours (1.03 -1.04 h^{-1}) after low doses and 0.40-0.67 hours (1.03 - 1.73 h^{-1}) after high doses. After low doses, concentrations of radioactivity in plasma declined bi-exponentially with an initial half-life of 6 h and a terminal half-life of 200-400 h. After high doses, concentrations declined with an approximate terminal elimination half-life of 13-22 h. Peak of mean concentrations of radioactivity in whole-blood occurred at 3-4 hours (0.4- 0.6 µg/mL, low dose; 0.8-1.0 µg/mL, high dose). After low doses, concentrations of radioactivity in whole-blood declined bi-exponentially with an initial half-life of approximately 5-8 h and a terminal half-life of 200-300 h. After high doses, concentrations declined with an approximate terminal elimination half-life of 37 h in female rats.

Tissues with highest concentrations of 6- 28 µg/g at 4 hours after both low and high doses were adrenals, GI tract (including contents), liver and bone marrow apart from the very high concentration of 4000-5000 µg/g in the GI tract after high doses. In addition, at 4 hours post-dose, there were also higher concentrations in thyroid and ovaries after low doses (9-12 µg/g). At 20 hours after both low and high doses, concentrations decreased apart from fat in which concentrations increased 2 -4 fold to 6-17 µg/g. At 168 hours after both low and high doses, the highest concentrations of 9-11 µg/g were in fat. At 168 hours, highest concentrations of 2-3 µg/g in tissues other than fat were in the adrenals (female only), GI tract and pancreas after high level doses. Concentrations of radioactivity in plasma were typically 10-fold lower than in tissues at 20 hours after both low and high doses and at 168 hours after low doses. The qualitative distribution of radioactivity was assessed by whole-body autoradiography at 4 hours after a single low doses of [^{14}C-benzoyl]-Flufenoxuron. Highest concentrations were in the stomach contents, brown fat, small intestine contents and the preputial and clitoral glands.

The unchanged Flufenoxuron was the single component of the residue in the fat and feces. There was no unchanged parent detected in the urine. The major metabolite (8-9% of the

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

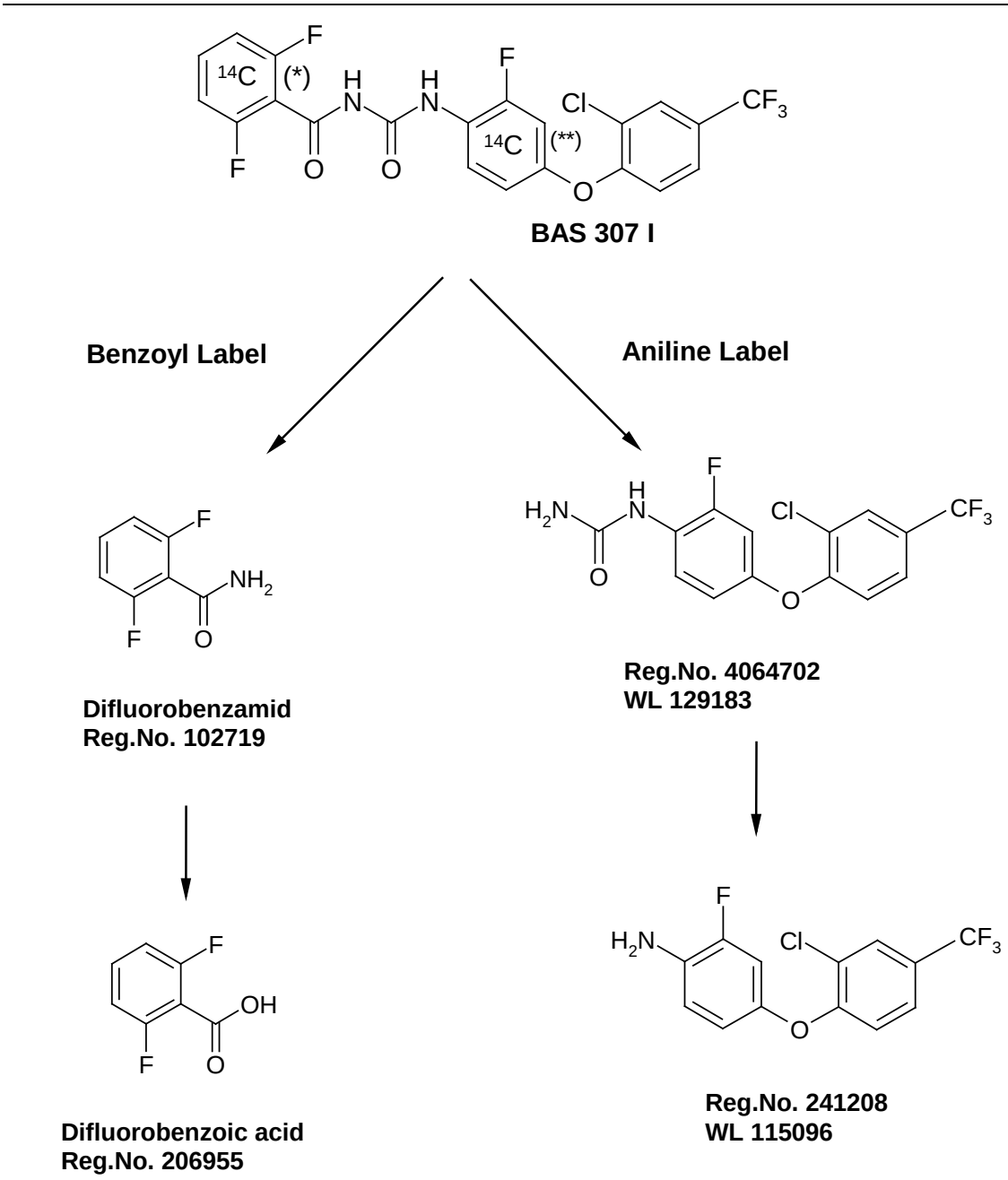
administered dose at 0-24 hours, 2-4% of the dose at 24-48 hours) was identified as 2, 6-difluorobenzoic acid, Reg. No. 206955. A second minor metabolite 0.2% and 0.1% at 0-24 and 24-48 hours was identified as 2, 6-difluorobenzamide, Reg. No. 102719. There were three polar unidentified metabolites, accounting for 0.1% to 0.8% of the dose. The identified metabolites in the urine indicated that the absorbed Flufenoxuron was metabolised by cleavage of the bond adjacent to the 2, 6-difluorobenzoyl moiety.

On the basis of these finding, the proposed metabolic pathway for Flufenoxuron in the rat is shown as follows:

Section A6.2

BPD Annex Point IIA, VI.6.2

Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study



(*) position of ^{14}C-label (difluoroamide)
(**) position of ^{14}C-label (fluoroaniline)

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 3.1.2.2. Purity 99%, specific activity 35µCi/mg 3.1.2.4. Radiolabelling 14C-benzamide flufenoxuron 3.3.1. Preparation of the substance 10% w/w Mulgofen 3.3.7. Add precisions about the number of animals for each study excretion study 3M, 3F blood and plasma study 6M, 6F biliary excretion 3M, 3F tissue distribution 9M, 9F whole body autoradiography 1M, 1F
Results and discussion	Agree with the applicant's version **Revisions/Amendments:** 4.1. Toxic effects, clinical signs One female rat died, 15 min after dosing, in the low dose plasma study (cause unknown). One male had laboured breathing and so was killed 24h after dosing, in the low dose excretion study. Moreover, in some cases, rats were lethargic shortly after dosing.
Conclusion	Agree with the applicant's version **Revisions/Amendments:** 5.1. 10% Mulgofen 5.2. *Metabolism, feces-low level study: Flufenoxuron accounted for* 12-6% *of the dose* Change this percentage in the report study to IVD.2. Absorption rate constants The absorption rate constants of radioactivity from plasma concentration data after low oral doses were 1.0431 and 1.0311 per hour in males and females, respectively: The corresponding unit for these values is not specified. The absorption rates need to be expressed, at least, in %.
Reliability	1

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

Acceptability	Acceptable: required modifications are only precisions to be added
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.2/ 8 Mean excretion by rats after single low oral doses of [^{14}C-benzoyl]- Flufenoxuron at 3.5 mg/kg b w

Sample	Time (hours)	Mean male (n = 2) [1)]	Mean female (n = 3)	Overall mean (n = 5)
Urine	0 – 6	1.70	1.92	1.83
	6 – 24	8.44	8.39	8.41
	24 – 48	4.80	3.05	3.75
	48 – 72	3.23	2.04	2.52
	72 – 96	2.64	1.97	2.24
	96 – 120	2.06	1.69	1.84
	120 – 144	2.27	1.69	1.92
	144 – 168	2.04	1.58	1.77
Total urine	0 – 168	27.16	22.34	24.26
Cagewash	168	2.55	1.63	2.00
Total urine and Cagewash	0 – 168	29.71	23.97	26.26
Faeces	6 – 24	13.05	6.03	8.84
	24 – 48	2.03	3.46	2.89

	48 – 72	0.87	0.64	0.73
	72 – 96	0.70	0.50	0.58
	96 – 120	0.64	0.42	0.50
	120 – 144	0.62	0.48	0.54
	144 – 168	0.60	0.39	0.47
Total faeces	0 – 168	18.50	11.92	14.55
G-I-tract (including contents)	168	1.49	1.88	1.72
Carcass	168	45.53	58.72	53.44
Total		95.22	96.49	95.98
% Absorbed [2)] in 168 hours		75.24	82.69	79.70

[1)] One rat had laboured breathing and was therefore killed about 24 hours after dosing. The rat's lung were conjested.

2) Total urine, cage wash and carcass

Table 6.2/ 9 Mean excretion by rats after single high oral doses of [^{14}C-benzoyl]- Flufenoxuron at 350 mg/kg b w

Sample	Time (hours)	Mean male (n = 3)	Mean female (n = 3)	Overall mean (n = 5)
Urine	0 – 6	0.16	0.13	0.15
	6 – 24	0.17	0.17	0.17
	24 – 48	0.08	0.05	0.07
	48 – 72	0.06	0.03	0.05
	72 – 96	0.05	0.03	0.04
	96 – 120	0.04	0.03	0.03
	120 – 144	0.04	0.03	0.03
	144 – 168	0.04	0.02	0.03
Total urine	0 – 168	0.65	0.49	0.57
Cage wash	168	0.02	0.01	0.02
Total urine and Cage wash	0 – 168	0.67	0.50	0.59
Faeces	6 – 24	77.71	68.64	73.18
	24 – 48	13.02	22.24	17.63
	48 – 72	2.01	10.00	6.01
	72 – 96	0.11	0.58	0.35

	96 – 120	<0.10	0.10	0.10
	120 – 144	<0.10	<0.10	<0.10
	144 – 168	<0.10	<0.10	<0.10
Total faeces	0 – 168	92.82	101.50	97.16
G-I-tract (including contents)	168	0.01	0.01	0.01
Carcass	168	0.54	0.87	0.71
Total		94.04	102.87	98.45
% Absorbed [1)] in 168 hours		1.21	1.37	1.30

1) Total urine, cage wash and carcass

Table 6.2/ 1 Mean excretion (retention) of radioactivity by rats after with cannulated bile ducts single low oral doses of ^{14}C-Flufenoxuron at 3.5 mg/kg b w

Sample	Time (hours)	Mean male (n = 3)	Mean female (n = 3)	Overall mean (n = 5)
Urine	0 – 24	7.01	5.88	6.45
	24 – 48	6.91	3.57	5.24
Cage wash	48	0.51	0.19	0.35
Total urine and Cage wash	0 – 48	14.43	9.64	12.04
Bile	0 – 6	0.40	0.76	0.57
	6 – 24	1.80	2.25	2.03
	24 – 48	2.45	1.50	1.99
Total bile	0 – 48	4.65	4.51	4.58
Faeces	0 – 24	3.68	0.75	2.21
	24 – 48	7.36	3.28	5.32
Total faeces	0 – 48	11.03	4.03	7.53
G-I- tract (including content)	48	3.59	3.45	3.52
Carcass	48	60.68	78.00	69.34
Total		94.39	99.63	97.01
% Absorbed [1)] in 48 hours		79.76	92.15	85.96

1) Total urine, cage wash, bile and carcass

Table 6.2/ 10 Biokinetic parameters derived from plasma levels vs. time curves after single oral administration of [^{14}C-benzoyl]-Flufenoxuron to male and female rats

Dose level	3.5 mg/kg oral		350 mg/kg oral	
Sex	Male	Female	Male	Female
C_{max} [µg/g]	0.27	0.39	0.77	1.10
T_{max} [h]	6	6	4	6
T ½ [h] absorption	0.66	0.67	0.67	0.40
T ½ [h] elimination	155	428	22 [1)]	13 [1)]
AUC [µg x h/ml]	25.4	62.5	24.1	21.3

[1)] High-dose half-lifes were only approximations due to data being close to the limit of reliable measurement

Table 6.2/ 11 Biokinetic parameters derived from blood levels vs. time curves after single oral administration of [^{14}C-benzoyl]-Flufenoxuron to male and female rats

Dose level	3.5 mg/kg oral		350 mg/kg oral	
Sex	Male	Female	Male	Female
C_{max} [µg/g]	0.42	0.60	0.75	1.02
T_{max} [h]	3	3	3	3
T ½ [h] absorption	0.70	1.13	-- [1)]	0.64
T ½ [h] elimination	178	327	-- [1)]	37 [2)]
AUC [µg x h/ml]	38.6	62.3	42.1	26.1

[1)] Concentration-time-profile could not be resolved into its exponential components

[2)] High-dose half-lifes were only approximations due to data being close to the limit of reliable measurement

Table 6.2/ 12 Mean concentrations of radioactivity in tissues of rats at different times after single low oral doses of [^{14}C-benzoyl]-Flufenoxuron, expressed as % of the administered dose

Time at sacrifice	4 hours		20 hours		168 hours	
Tissue	Male	Female	Male	Female	Male	Female
Adrenal glands	0.05	0.09	0.02	0.03	0.01	0.01
Bone marrow	0.79	1.74	0.43	0.84	0.20	0.32
Carcass	42.89	48.88	77.77	71.23	39.00	46.65
G.I. Tract	35.80	28.96	4.20	3.86	1.69	1.27
Kidneys	0.99	1.00	0.36	0.31	0.11	0.10
Liver	9.56	8.52	3.14	2.52	1.08	0.89
Lungs	0.54	0.63	0.26	0.23	0.08	0.09
Muscle	26.43	29.72	18.75	10.75	4.86	6.24

Ovaries	-	0.06	-	0.03	-	0.01
Pancreas	0.57	0.70	0.58	0.45	0.22	0.20
Perirenal fat	3.07	2.56	10.41	8.86	7.15	7.03
Subcutaneous fat	6.60	6.77	23.44	22.55	16.88	14.80
Thyroid gland	0.01	0.01	0.01	<0.01	<0.01	<0.01
Whole-blood	1.10	1.45	0.34	0.38	0.11	0.12

Table 6.2/ 13 Mean concentrations of radioactivity in tissues of rats at different times after single low oral doses of [^{14}C-benzoyl]-Flufenoxuron, expressed as µg/g

Time at sacrifice	4 hours		20 hours		168 hours	
Tissue	Male	Female	Male	Female	Male	Female
Adrenal glands	18.96	28.28	7.70	7.93	2.93	2.67
Bone marrow	7.75	17.27	4.14	8.40	1.66	2.94
Carcass	1.99	2.24	3.57	3.31	1.55	1.97
G.I. Tract	16.87	14.73	1.49	1.74	0.72	0.66
Kidneys	4.87	5.22	1.68	1.63	0.52	0.55
Liver	8.60	8.74	2.34	2.39	0.77	0.82
Lungs	4.37	5.18	1.93	1.82	0.65	0.71
Muscle	2.01	2.27	1.37	0.83	0.32	0.45
Ovaries	-	8.91	-	5.18	-	0.93
Pancreas	5.75	6.81	5.26	4.20	2.18	1.76
Perirenal fat	5.23	4.38	17.11	15.29	10.45	11.27
Subcutaneous fat	4.50	4.65	15.41	15.56	9.87	9.47
Thyroid gland	9.14	12.47	4.83	4.60	2.03	1.75
Whole-blood	0.54	0.72	0.16	0.19	0.05	0.05

Table 6.2/ 14 Mean concentrations of radioactivity in tissues of rats at different times after single high oral doses of [^{14}C-benzoyl]-Flufenoxuron, expressed as % of the administered dose

Time at sacrifice	4 hours		20 hours		168 hours	
Tissue	Male	Female	Male	Female	Male	Female
Adrenal glands	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Bone marrow	0.01	0.02	0.01	0.01	<0.01	<0.01
Carcass	1.17	0.78	1.40	3.91	0.37	0.43
G.I. Tract	118.40	109.40	11.72	19.07	0.12	0.05
Kidneys	0.01	0.01	0.01	<0.01	<0.01	<0.01
Liver	0.11	0.07	0.05	0.03	0.03	0.01
Lungs	0.01	0.01	<0.01	<0.01	<0.01	<0.01
Muscle	0.25	0.22	0.14	0.12	<0.11	<0.10
Ovaries	-	<0.01	-	<0.01	-	<0.01
Pancreas	0.01	0.01	0.01	0.01	<0.01	<0.01
Perirenal fat	0.02	0.02	0.12	0.05	0.09	0.07
Subcutaneous fat	0.06	0.05	0.27	0.12	0.21	0.17
Thyroid gland	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Whole-blood	0.02	0.02	<0.02	<0.02	<0.02	<0.02

Table 6.2/ 15 Mean concentrations of radioactivity in tissues of rats at different times after single high oral doses of [^{14}C-benzoyl]-Flufenoxuron, expressed as µg/g

Time at sacrifice	4 hours		20 hours		168 hours	
Tissue	Male	Female	Male	Female	Male	Female
Adrenal glands	13.89	13.28	7.13	4.86	<4.50	3.10
Bone marrow	7.46	12.50	3.99	6.78	<2.03	<5.47
Carcass	4.07	2.72	4.78	13.91	1.09	<1.48
G.I. Tract	4141.40	4684.70	271.02	673.72	3.25	2.05
Kidneys	4.67	3.86	1.77	1.10	<0.55	0.53
Liver	7.54	6.17	2.55	2.02	1.29	1.02
Lungs	3.45	3.25	1.84	1.28	<0.61	0.64
Muscle	1.42	1.30	0.74	0.70	<0.54	<0.55
Ovaries	-	4.02	-	2.12	-	<2.12
Pancreas	3.86	3.98	3.83	2.49	1.66	2.42
Perirenal fat	3.48	2.00	14.32	6.16	9.30	9.35
Subcutaneous fat	3.08	2.70	13.03	6.58	8.89	8.67
Thyroid gland	<20.0	<13.60	<18.32	<16.96	<11.07	<15.48
Whole-blood	0.62	0.77	<0.58	<0.58	<0.57	<0.58

Table 6.2/ 16 Proportions of radioactive components in urine of rats after single low doses of Flufenoxuron, expressed as % of the administered dose

Components	Male		Female	
	0 – 24 h	24 – 48 h	0 – 24 h	24 – 48 h
A	0.5	0.6	0.8	0.4
B	0.3	0.3	0.4	0.2
C	0.2	0.1	0.3	0.2
2,6-difluorbenzoic acid	8.6	3.5	8.0	2.1
2,6-difluorbenzamid	0.2	<0.1	0.2	0.1
Other	0.1	0.2	0.2	<0.1
Polar fraction	0.1	<0.1	0.2	0.1

Table 6.2/ 17 Proportions of radioactive components in faecal extracts of rats after single low and high doses of Flufenoxuron, expressed as % of the administered dose

Dose group Components	Male		Female	
	0 – 24 h	24 – 48 h	0 – 24 h	24 – 48 h
Low dose: Flufenoxuron	12	2	6	3
High dose: Flufenoxuron	78	13	68	22

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

Fischer 344 strain male rats were fed diets containing zero, 0.005, 0.05, 0.5, 5, 50, or 500 mg/kg feed of unlabelled Flufenoxuron for 100 days. Rats were sacrificed and omental samples from each dose group were analyzed for Flufenoxuron. Groups of five animals were dosed at different dose levels.

The study was undertaken to examine the relationship between dietary intake and fat storage of Flufenoxuron over a wide range of dietary concentrations. The exposure period of 100 days was chosen as being in excess of three elimination half-lives for Flufenoxuron in rats to achieve a steady state or plateau value.

Official use only

		1 REFERENCE 6
1.1	**Reference**	**14) XXXX** WL115110 (Cascade): Residues in the body fat of rats following ingestion in diet for 100 days XXXX unpublished XXXX
1.2	**Data protection**	No
1.2.1	Data owner	BASF
1.2.2	Company with letter of access	XXXX
1.2.3	Criteria for data protection	No data protection claimed
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Not report, description given hereafter
2.2	**GLP**	Yes, (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)
2.3	**Deviations**	Not applicable
		3 MATERIALS AND METHODS
3.1	**Test material**	
3.1.1	Lot/Batch number	Batch no. XXXX
3.1.2	Specification	As given in section 2

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
3.1.2.1	Description	Radiolabelled test substance: not applicable as test substance for this study unlabelled	X
3.1.2.2	Purity	97.4%	
3.1.2.3	Stability	Stable	
3.1.2.4	Radiolabelling	Not applicable	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fisher 344	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	X
3.2.5	Age/weight at study initiation	7-8 weeks	
3.2.6	Number of animals per group	5/sex/group	X
3.2.7	Control animals	Yes, basal diet	X
3.3	**Administration/ Exposure**	In food	
3.3.1	Preparation of test substance	For all except the 500 mg/kg diet, the unlabeled test substance was incorporated into diet by adding a solution in acetone, tumbling the mixture in a steel drum. For the control diet, acetone alone was added to diet. For the 500 mg/kg diet, fine Flufenoxuron powder was mixed with diet, and the mixture was tumbled in a steel drum. Once mixed, diets were sealed in plastics bags and delivered to the animal unit.	
3.3.2	Concentration of test substance	0, 0.005, 0.05, 0.5, 5, 50, or 500 mg/kg feed of unlabelled Flufenoxuron	
3.3.3	Specific activity of test substance	Not applicable	
3.3.4	Volume applied	Not applicable	
3.3.5	Size of test site	Not applicable	
3.3.6	Exposure period	For 100 days	
3.3.7	Sampling time	At necropsy	
3.3.8	Samples	Liver, a sample of omental fat, testicular fat, and peri-renal fat	

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

		4 RESULTS AND DISCUSSION	
4.1	**Toxic effects, clinical signs**	Not reported	
4.2	**Dermal irritation**	Not relevant	
4.3	**Recovery of labelled compound**	Total recovery > 93%	X
4.4	**Percutaneous absorption**	Not relevant	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	<u>Animals</u> Fischer 344 strain male rats were obtained as weanlings XXX. The rats were 7 to 8 weeks old at the start of the exposure. No age given.	X
		<u>Dosing</u> For all except the 500 mg/kg diet, the unlabeled test substance was incorporated into diet by adding a solution in acetone, tumbling the mixture in a steel drum. For the control diet, acetone alone was added to diet. For the 500 mg/kg diet, fine Flufenoxuron powder was mixed with diet, and the mixture was tumbled in a steel drum. Once mixed, diets were sealed in plastics bags and delivered to the animal unit.	
		<u>Sampling</u> At necropsy, the liver, a sample of omental fat supplemented where necessary with testicular fat, and a sample of peri-renal fat were removed. The liver and peri-renal fat was taken as reserve samples. They were not analysed. The samples were stored at –18°C until they were analysed. In addition to the main study animals, stock rats were necropsied to provide material for analysis recovery.	
		<u>Analytical Methods</u> Diet:	

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

Diets were analysed, where possible, to demonstrate that they contained the desired concentration of Flufenoxuron. This was conducted for all diets containing 500 to 0.5 mg/kg. None of the diets contained 0.05 or 0.005 mg/kg of Flufenoxuron were analyzed, because the method employed for diet analysis lacked the sensitivity and precision necessary for those two dose concentration.

For the 500 mg/kg and 50 mg/kg diets, Flufenoxuron was extracted from the diet with acetone using a Soxhlet extractor. The extract was evaporated to dryness and the residue dissolved in acetonitrile. The resulting solution was analyzed by HPLC with ultraviolet detection.

For the 5 mg/kg, 0.5 mg/kg and control diets, the Flufenoxuron is extracted from the diet by shaking with acetonitrile, and the extract is purified by partitioning with hexane. The acetonitrile layer is rendered aqueous, and extracted with hexane. After evaporation of the hexane layer, the residue is dissolved in the HPLC mobile phase and analyzed by HPLC using ultraviolet absorption detection.

Tissue:

A portion of the fat sample, about 2 g in weight, was mixed with anhydrous sodium sulphate in a beaker and extracted, by boiling and decantation, with a mixture of acetone and hexane. The solvent was evaporated off, and the residue dissolved in hexane. Lipid was removed by extracting the hexane with acetonitrile. The lipid stayed preferentially with the hexane phase, while Flufenoxuron partitioned into the acetonitrile. The acetonitrile phase was rendered aqueous, and extracted with hexane. The hexane extract was evaporated to dryness, and the residue dissolved in a mixture of hexane, ethanol, and glacial acetic acid and analyzed by HPLC using ultraviolet detections. The amount of Flufenoxuron in the sample was calculated after comparison of the responses obtained from sample extracts with those of known strength solutions of Flufenoxuron.

Samples of body fat chosen randomly from five animals of each dose group were analysed. In addition, control and control fat to which known amounts of Flufenoxuron had been added were analysed to show that the analysis method was working well.

5.2 Results and discussion

Diet Concentrations Distribution and Stability

Three batches of diets were made for the study, and they were all

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

checked by analysis to show that the desired concentrations of Flufenoxuron had been achieved. The limit of determination to the required degree of accuracy was about 0.1 mg/kg. No Flufenoxuron was found in control diet at the limit of detection of 0.1 mg/kg. The results obtained for the other dose groups were all satisfactory. The quantity of Flufenoxuron found in the diet, expressed as percentage of the desired value was 96-103% for the 500 mg/kg diet, 91-97% for the 50 mg/kg diet, 93-94% for the 5 mg/kg diet, and 94-105% for the 0.5 mg/kg diet.

The Flufenoxuron was uniformly distributed in the diet based on analysis of samples taken from the top, middle and bottom of each mix of diet. The results expressed as percentage of the nominal value were: for 500 mg/kg diet, 101-102% for the top, 89-100% for the middle and 91-95% for the bottom; for 50 mg/kg, 92% for the top, 91% for the middle and 90-91% for the bottom; for the 5 mg/kg diet, 97-98% for the top, 91-97% for the middle and 95-97% for the bottom and for the 0.5 mg/kg diet, 94-99% for the top, 91-97% for the middle and 95-97% for the bottom.

The stability of Flufenoxuron in the diet was carried out with the 0.5 mg/kg diet. The concentrations were found 104% and 109% of the nominal value, demonstrating the stability of that diet during storage for 42 days. Since the stability of Flufenoxuron in diets at concentrations of 1 to 50000 mg/kg had been demonstrated for other studies, on the basis of that work, the diets for this experiment were assumed to be stable for their period of use (100 days).

Recovery Determinations for fat and diet

Results for recovery determination in which known amounts of Flufenoxuron were added at 0.5, 5 and 50 mg/kg to diet and at 0.5, 1, 2, 10 and 500 mg/kg to fat samples are shown as follows. For the diet, the average recoveries were 102-112% at 0.5 mg/kg, 91-93% at 5 mg/kg and 96-102% at 50 mg/kg. For the fat, the average recoveries were 102% at 0.5 mg/kg, 94-100% at 1 mg/kg, 96-104% at 2 mg/kg, 96-97% at 10 mg/kg and 93.5-95% at 500 mg/kg.

Fat Residue

For the zero and 0.005 mg/kg dose group, no residues of Flufenoxuron were found at a limit of detection of 0.1 mg/kg (µg/g). For all other dose groups, the concentrations of Flufenoxuron were readily measurable. For the 0.05 mg/kg, the 0.5 mg/kg, the 5 mg/kg, and the 50 mg/kg treatment groups, the average concentrations found in the fat were 0.198, 1.67, 18.6 and

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
		188 mg/kg, respectively. These were 3.96, 3.34, 3.72 and 3.76 times, respectively, and as high as those in the diets. For the 500 mg/kg dose group, the average concentration in the fat (230 mg/kg) was only 0.46 times as high as that in the diet. Indeed, the average concentration of Flufenoxuron in the fat of those animals was only 22% higher than the average (188 mg/kg) for animals fed 50 mg/kg of Flufenoxuron.	
5.3	**Conclusion**	Fischer 344 strain male rats were fed diets containing zero, 0.005, 0.05, 0.5, 5, 50, or 500 mg/kg of Flufenoxuron for 100 days. Rats were sacrificed, and omental samples from each dose group were analyzed for Flufenoxuron. For the diets containing 0.05 to 50 mg/kg of Flufenoxuron, the concentration in the fat averaged 0.198 to 188 µg/g. For the animals fed 0.005 mg/kg of Flufenoxuron, no measurable amounts of Flufenoxuron were found at a limit of detection of 0.1 mg/kg. For the 500 mg/kg dose group, the average concentration of Flufenoxuron in fat was 230 µg/g. This was only 22% higher than the average (188 mg/kg) for animals fed 50 mg/kg of Flufenoxuron.	
5.3.1	Reliability	1	X
5.3.2	Deficiencies	No	X

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 2.1. Guideline study: Guideline not reported 3.1.2.1 Description WL115110 (CASCADE®) 3.2.4. Only male rats mentioned in the study report 3.2.6 Number of animals per group 10/group (one sex) 3.2.7 Number of control animals 20 No precisions about weight, temperature, humidity and photoperiod during the experiment
Results and discussion	Agree with the applicant's version **Revisions/Amendments:** 4.3. Recovery of the labelled compound: As the substance is unlabelled, this parameter is not relevant.
Conclusion	Agree with the applicant's version **Revisions/Amendments:** 5.1. Animals Delete the last sentence "No age given" Fischer 344 strain male rats were obtained as weanlings from XXXX. The rats were 7 to 8 weeks old at the start of the exposure 5.2. A table with the real measured concentrations of the substance in each mix of diet should be provided instead of the results expressed as a "percentage of the desired value" 5.3.1. Reliability 2 5.3.2. Deficiencies Yes (no females)
Reliability	2: no females tested, no precision about weight evolution
Acceptability	Acceptable
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

The absorption and disposition of Flufenoxuron in two male and two female beagle dogs were investigated following a single oral dose of 3.5 mg/kg b w. The experiments were performed with ^{14}C-Flufenoxuron labelled in the aniline-ring.

Official use only

1 REFERENCE 7

1.1	**Reference**	**15) XXXX** The absorption and disposition of ^{14}C-WL 115110 in the dog after a single oral administration XXXX unpublished XXXX **16) XXXX** Amendment no. 1: The absorption and disposition of ^{14}C-WL115110 in the dog after a single oral administration XXXX unpublished XXXX **17) XXXX** Amendment no. 2: The absorption and disposition of ^{14}C-WL115110 in the dog after a single oral administration XXXX unpublished XXXX
1.2	**Data protection**	No
1.2.1	Data owner	BASF
1.2.2	Company with letter of access	XXXX
1.2.3	Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Not report, description given hereafter
2.2	**GLP**	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch no. XXXX	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	Radiolabelled test substance: see introduction 6.2-1	
3.1.2.2	Purity	99%	
3.1.2.3	Stability	Stable	
3.1.2.4	Radiolabelling	Not applicable	X
3.2	**Test Animals**		
3.2.1	Species	Dog	
3.2.2	Strain	Beagle	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	11.6-13.7 kg, about 6 months old	
3.2.6	Number of animals per group	2/sex/group	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Single oral dose	
3.3.1	Preparation of test substance	The test substance was applied in an aqueous formulation containing mulgofen EL-719, DMSO (dimethylsulphoxide) and saline. The radiochemical purity of the dose in solution, determined via thin-layer-chromatography, was >98 %.	
3.3.2	Concentration of test substance	3.5 mg/kg bw	
3.3.3	Specific activity of test substance	Specific activity 3.48 µCi/mg	
3.3.4	Volume applied	Not applicable	X
3.3.5	Size of test site	Not applicable	
3.3.6	Exposure period	For 100 days	X

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
3.3.7	Sampling time	Urine: 0 - 6, 6 - 24, 24 - 48, 48 – 72, 72 – 96, 96 – 120, 120 – 144, and 144 – 168 hours Feces: at 24 hour intervals up to 168 hours Blood samples: at pre-dose, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144 and 168 hours after administration of the dose At necropsy: The liver, kidney, thyroid, spleen, adrenals, brain, lungs, pancreas, ovaries/testes, uteruses (females only) and samples of muscle, fat (perirenal and subcutaneous), bone marrow and blood	
3.3.8	Samples	See 3.3.7	
		4 RESULTS AND DISCUSSION	
4.1	**Toxic effects, clinical signs**	Not reported	X
4.2	**Dermal irritation**	Not relevant	
4.3	**Recovery of labelled compound**	Total recovery > 93%	
4.4	**Percutaneous absorption**	Not relevant	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Animals Four adult beagle dogs (2 male, 2 female, bodyweight 11.6 to 13.7 kg, aged about 6 months) were obtained from XXXX. Dosing The test substance was applied in an aqueous formulation containing mulgofen EL-719, DMSO (dimethylsulphoxide) and saline. The radiochemical purity of the dose in solution, determined via thin-layer-chromatography, was >98 %. Animals were allowed free access to food and water except for 16 hours prior and 4 hours after dosing during which time food was withheld. Each dose was weighed in a syringe and administered by gavage. The dosing catheter was washed down with water into the dog's stomachs.	

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

Excretion

Three of the animals suffered severe diarrhoea during the first 30 minutes to 1 hour after dosing. This was collected as a separate sample for each animal. Urine was collected in solid CO_2-cooled containers during 0 - 6, 6 - 24, 24 - 48, 48 – 72, 72 – 96, 96 – 120, 120 – 144, and 144 – 168 hours. Faeces were collected at 24 hour intervals up to 168 hours. Aqueous cage washings were made at 24-hour intervals up to 168 hours. All samples were stored at –20°C prior to analysis.

Plasma kinetics

Blood samples (6 ml) were taken from the jugular vein at pre-dose, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144 and 168 hours after administration of the dose. Whole blood (about 1 ml) was retained for measurement of haematocrit and radioactivity in samples taken at pre-dose and 1, 4, 8, 24, 48, 72, 96, 120, 144 and 168 hours after dosing. Remaining blood samples were centrifuged to separate the cells which were discarded and the plasma retained for analysis. Plasma was stored at –20°C except for the case of undergoing analysis. Whole-blood was stored at 4°C prior to haematocrit analysis after which blood was stored at –20°C except for the case of undergoing radiochemical analysis.

Tissue residues

The animals were killed by pentobarbitone overdose at 168 hours after dosing. The liver, kidney, thyroid, spleen, adrenals, brain, lungs, pancreas, ovaries/testes, uteruses (females only) and samples of muscle, fat (perirenal and subcutaneous), bone marrow and blood (ca. 200 ml) were taken for analysis.

Blood was centrifuged to separate the cells which where dispatched to Sittingbourne Research Centre for analysis.

Preparation of samples and measurement of radioactivity

Faeces were diluted with water (ca. 2:1 by weight) and mixed to a fine paste prior to analysis by combustion. Diarrhoea samples which contained urine and cage washings taken at the time of sampling, were centrifuged and the separated extract was collected and the pellet allowed to dry. Radioactivity was analysed in the extract as well as the dried pellet. Large tissue samples were finely minced and homogenised prior to analysis. Bone marrow samples were combusted whole.

Samples of urine, cage washing, plasma and diarrhoea extract

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

were mixed with scintillator cocktail MI31 (Canberra Packard). Samples of faecal homogenate, whole blood, and tissues were combusted in oxygen using automatic Sample Oxidiser, products of combustion were absorbed into Optisorb1 (LKB) and mixed with OptisorbS. Recoveries of radioactivity from C-14 standards for sample oxidisers combusted in the oxidiser were >95 %. Measurements of radioactivity were corrected for oxidiser efficiency.

Radioactivity was measured by liquid scintillation counting with automatic standard quench correction.

Extraction of excreta samples for TLC

Pro rata pools of faecal homogenates were made for male faeces of days 1 (0 – 24 h), 2 (24 – 48 h), 1+2 (0 - 48 h) as well of female faeces. Samples of pooled faeces were extracted three times with ethyl acetate: methanol (3:1, v/v). Diarrhoea extracts and pellets were pooled and extracted in the same way as faecal samples. Urine (from one male animal, 0 – 6 hours) was adjusted to pH 1 with 1 N HCl and extracted four times with 2 volumes of ethyl acetate.

For TLC, extracts were concentrated under vacuum by rotary film evaporation and taken up in a suitable volume of methanol.

5.2 Results and discussion

Excretion and tissue distribution of radioactivity

Following single oral doses of Flufenoxuron, most of the radioactivity was excreted in the faeces and diarrhoea (58 % of the administered dose, males and 64 % of the administered dose, females). Diarrhoea samples were produced by both male dogs (14.0 % and 50.8 % of the administered dose) and by one female dog (13.4 % of the administered dose) within one hour of dosing. Most of the radioactivity in the faeces was excreted during the first 24 hours after dosing (18.5 % males, 49.3 % females) with a further 6.9 % and 8.1 % respectively being gradually excreted during 24 to 168 hours.

Small amounts of radioactivity were found in the urine of 3 animals (1.7 – 4.0.% of the administered dose), the 0 – 6 hours urine of one male dog contained 13.2 % of the administered dose. Due to the dark discoloration of this particular sample it appeared that most of the urinary radioactivity was a contamination either by faeces or diarrhoea. Means of 1.2 % of the administered dose (males) and 0.76 % of the administered dose (females) were detected in the cage washings.

Residual radioactivity in tissues was calculated as 17.5 % of the

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

administered dose and 17.6 % of the administered dose respectively in males and females, most of which resided in the fat (13.7 % male and 13.4.% female) and in the muscle (2.2 % of the administered dose male, 2.3 % of the administered dose female).

Concentrations of radioactivity in plasma and whole blood

In plasma, mean concentrations of radioactivity reached a peak of 0.389 µg/ml (male dogs) and 0.424 µg/ml (female dogs) at 4 hours after dosing. They declined to means of 0.212 µg/ml (male) and 0.228 µg/ml (female) respectively at 24 hours. Concentrations declined slowly (0.167 µg/ml male dogs and 0.191 µg/ml female dogs at 168 hours after dose administration) with an estimated half-life of 28 days.

In the whole-blood, concentrations of radioactivity rose to a peak of 0.246 µg/g (males) and 0.282 µg/g (females) at 4 hours and declined to 0.215 µg/g (males) and 0.250 µg/g (females) at 8 hours. Concentrations of radioactivity then rose to 0.261 µg/g (males) and 0.332 µg/g (females) at 168 hours. The initial peak and decline in radioactivity in the whole-blood was related mainly to concentrations of radioactivity in the plasma. The later rise during 8 to 168 hours was presumably due to gradual uptake of radioactivity by the cells. There was an increase in the cell/plasma ratio of radioactivity from 0.21 and 0.32 (males and females respectively) at 4 hours to 2.5 and 2.8 at 168 hours.

Mean concentrations of radioactivity in the blood cells rose from 0.082 µg/g (male dogs) and 0.129 µg/g (female dogs) at 4 hours to 0.235 µg/g and 0.284 µg/g respectively at 24 hours and then rose more gradually to final concentrations of 0.410 µg/g and 0.535 µg/g respectively at 168 hours.

Concentrations of radioactivity in tissues

Mean concentrations of radioactivity were similar in the corresponding tissues of male and female dogs. The greatest concentrations were detected in the perirenal fat (3.03 µg/g, males, 2.80 µg/g, females) and the subcutaneous fat (3.20 µg/g, males, 3.16 µg/g, females) followed by bone marrow (1.43 µg/g, males, 1.08 µg/g, females) and liver (0.70 µg/g, males, 0.85 µg/g, females). The lowest amounts were found in muscle (0.18 µg/g, males, 0.20 µg/g, females), plasma and brain (0.14 µg/g, both males and females). Most tissues apart from the brain contained concentrations of radioactivity equal to or greater than in the

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
	plasma. Proportions of radioactive components in extracts of urine, faeces and diarrhoea. Between 95 to 100 % of radioactivity in urine, faeces and diarrhoea collected during 24 hours after dosing, was extractable in suitable solvents. Between 80 and 82 % of radioactivity in faeces was extractable from 24 – 48 hours faecal samples. Almost all extractable radioactivity in urine and diarrhoea (ca. 38 % of the administered dose in males and 6 % of the administered dose in females) was identified by TLC. Means of 16.5 % and 46.8 % of the administered dose in males and females respectively corresponded to the unchanged Flufenoxuron in 0 – 24 hour faecal samples and means of 0.4 % and 0.5 % of the administered dose respectively corresponded to the unchanged parent in 24 - 48 hour samples. One minor metabolite, co-eluting with WL 115096, Reg. No 241208, was only present in significant amounts in 24 - 48 hour faecal samples, see Table 6.2/ 22. <u>Estimation of rates of absorption</u> Assuming that the absorption of Flufenoxuron can be described by a first-order process, the absorption rate constants of Flufenoxuron following oral doses to dogs were 0.83 per hour in males and 0.74 per hour in females. These rate constants corresponded to mean absorption half lives of 0.84 hours and 1.04 hours in male and female dogs respectively (Table 6.2/ 23). It is important to note that the terminal phase of the plasma C-14 concentration-time curves were not well characterised and, therefore, values of the exponents and coefficients describing the curve should be regarded as approximations.	
5.3 Conclusion	The absorption, disposition and metabolism of Flufenoxuron in two male and two female beagle dogs were investigated following a single oral dose of [^{14}C-aniline]-Flufenoxuron at a dose level of 3.5 mg/kg bodyweight. During 7 days after dosing, means of 58 % of the administered dose, males, and 64 % of the	X

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**
		administered dose, females, were excreted via feces and diarrhoea. Means of 8.5 % dose and 2.9 % dose were detected in the urine of male and female dogs, respectively. A mean of 17.6 % of the administered dose was retained in the tissues at 168 hours, most of which resided in the subcutaneous fat (13.4 % dose) and muscle (2.2 % dose). A mean estimated total of 85 % of the administered dose was recovered from excreta and tissues. Means of 16.9 % and 46.8 % of the administered dose in faeces of males and females respectively as well as 6.4 % of dose in male urine corresponded to the unchanged Flufenoxuron. One minor metabolite, co-eluting with WL 115096, Reg. No 241208, was only present in 0.1 % of the administered dose in 24 - 48 hour faecal samples.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 3.1.2.4. Radiolabelling: 14C-aniline flufenoxuron 3.3.4. Volume applied: 2 ml solution/kg of bodyweight 3.3.6. Exposure period not relevant because it is a single oral dose, 100 days correspond to the post exposure period
Results and discussion	Agree with the applicant's version **Revisions/Amendments:** 4.1. Toxic effects, clinical signs Two males and one female had diarrhoea between 30 min and 1 hour after dosing 5.2. Results and discussion / Estimation of rates of absorption An answer to the following question would have been useful: Why are expressed the absorption rates per hour? Based on the radioactivity found in urine, cage wash and tissues, the minimal absorbed fraction was 27.29 % and 21.23 % in males and females, respectively. This value is probably largely underestimated because of the diarrhea.
Conclusion	Agree with the applicant's version
Reliability	1
Acceptability	Acceptable, with absorption rates expressed in %
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.2/ 18 Excretion balance (percent of administered dose) after single oral dose of [^{14}C-aniline]- Flufenoxuron at 3.5 mg/kg b w to male and female beagle dogs

Animal number		**1 M**	**2 M**	**Mean (M)**	**3 F**	**4 F**	**Mean (F)**
Urine	0 - 6 h	13.16	0.21	6.69	0.04	0.06	0.05
	6 - 24 h	0.30	0.28	0.29	0.26	0.69	0.48
	24 - 48 h	0.24	0.47	0.36	0.33	0.69	0.51
	48 - 72 h	0.20	0.31	0.26	0.24	0.66	0.45
	72 - 96 h	0.23	0.34	0.29	0.24	0.54	0.39
	96 - 120 h	0.16	0.28	0.22	0.20	0.50	0.35
	120 - 144 h	0.19	0.26	0.23	0.19	0.43	0.31
	144 - 168 h	0.17	0.25	0.21	0.17	0.46	0.32
Subtotal urine		**14.65**	**2.40**	**8.52**	**1.67**	**4.03**	**2.85**
Faeces	0 - 24 h	25.67	11.38	18.53	66.21	32.29	49.25
	24 - 48 h	1.37	2.08	1.73	1.23	3.24	2.23
	48 - 72 h	0.97	1.38	1.17	1.05	1.84	1.45
	72 - 96 h	1.40	1.07	1.24	0.93	1.80	1.37
	96 - 120 h	0.70	1.13	0.91	0.80	1.58	1.19
	120 - 144 h	1.05	0.97	1.01	0.84	1.21	1.03
	144 - 168 h	0.74	1.00	0.87	0.53	1.04	0.79
Subtotal faeces		**31.90**	**19.01**	**25.46**	**71.59**	**43.00**	**57.30**
Diarrhoea		**13.98**	**50.84**	**32.41**	**--**	**13.39**	**64.00**
Cage wash		1.42	1.03	1.23	0.73	0.78	0.76
Tissues		20.34	14.73	17.54	12.39	22.84	17.62
Total recovery		**82.29**	**88.01**	**85.15**	**86.38**	**84.04**	**85.21**

Table 6.2/ 19 Concentrations of radioactivity in the plasma (µg/ml) and the whole blood (µg/g) after single oral dose of [^{14}C-aniline]-Flufenoxuron at 3.5 mg/kg b w to two male and two female beagle dogs

Sampling	**1 M**		**2 M**		**3 F**		**4 F**	
time (hours)	**Plasma**	**Blood**	**Plasma**	**Blood**	**Plasma**	**Blood**	**Plasma**	**Blood**
Pre-dose	< 0.009	< 0.013	< 0.009	< 0.013	< 0.009	< 0.013	< 0.009	< 0.013
0.5	0.087		0.133		0.093		0.118	
1	0.220	0.054	0.226	0.077	0.137	0.057	0.247	0.064
1.5	0.369		0.260		0.174		0.362	
2	0.459		0.271		0.210		0.488	
4	0.514	0.320	0.264	0.172	0.224	0.156	0.624	0.408
6	0.443		0.228		0.202		0.585	
8	0.378	0.283	0.188	0.146	0.193	0.146	0.476	0.353
12	0.284		0.157		0.155		0.351	
24	0.226	0.242	0.198	0.202	0.138	0.150	0.317	0.358
48	0.191	0.243	0.170	0.215	0.116	0.164	0.256	0.370
72	0.210	0.267	0.159	0.217	0.131	0.191	0.247	0.395
96	0.189	0.280	0.161	0.227	0.111	0.179	0.269	0.452
120	0.196	0.286	0.157	0.244	0.108	0.191	0.243	0.448
144	0.194	0.296	0.158	0.251	0.105	0.190	0.246	0.442
168	0.197	0.297	0.137	0.224	0.121	0.194	0.261	0.470

Table 6.2/ 20 Concentrations (µg/g) and tissue/plasma ratios of radioactivity in the tissues of male dogs sacrificed at 168 hours after single oral doses of [^{14}C-aniline]- Flufenoxuron at 3.5 mg/kg b w

	1 M			2 M		
Tissue	**µg/g**	**% of dose**	**Tissue/plasma ratio**	**µg/g**	**% of dose**	**Tissue/plasma ratio**
MUSCLE	0.218	2.57	1.1	0.148	1.75	1.1
Liver	0.793	0.57	4.0	0.610	0.53	4.5
Fat (periren.)	3.690	0.18	19	2.360	0.10	17
Fat (subcut.)	3.700	15.7	19	2.700	11.4	20
Kidney	0.490	0.06	2.5	0.356	0.05	2.6
Bone marrow	1.830	0.19	9.3	1.020	0.11	7.5
Whole-blood	0.297	0.81	1.5	0.224	0.61	1.6
Plasma	0.197	0.30	1.0	0.137	0.21	1.0

Table 6.2/ 21 Concentrations (µg/g) and tissue/plasma ratios of radioactivity in the tissues of female dogs sacrificed at 168 hours after single oral doses of [^{14}C-aniline]- Flufenoxuron at 3.5 mg/kg b w

	3 F			4 F		
Tissue	**µg/g**	**% of dose**	**Tissue/plasma ratio**	**µg/g**	**% of dose**	**Tissue/plasma ratio**
Muscle	0.126	1.47	1.0	0.272	3.17	1.0
Liver	0.508	0.43	4.2	0.854	0.93	4.6
Fat (periren.)	1.870	0.19	15	3.730	0.23	14
Fat (subcut.)	2.290	9.56	19	4.020	16.8	15
Kidney	0.265	0.04	2.2	0.613	0.09	2.3
Bone marrow	0.627	0.07	5.2	1.540	0.16	5.9
Whole-blood	0.194	0.52	1.6	0.470	1.26	1.8
Plasma	0.121	0.18	1.0	0.261	0.39	1.0

Table 6.2/ 22 Proportions of radioactive components in extracts of faeces from dogs, separated by TLC, expressed as % of the administered dose

Substance	Male				Female		
	0 – 24 h	24 – 48 h	0 – 48 h	Urine 0–6h	0 – 24 h	24 – 48 h	0 – 48 h
Origin (TLC)	0.8	0.9	1.4	-	0.9	1.0	2.0
Flufenoxuron	16.5	0.4	16.9	6.4	46.4	0.5	46.9
Reg.No. 241208	-	0.1	0.2	-	-	0.1	0.4
Others(not ident.)	0.5	<0.1	0.8	0.2	0.6	0.1	0.7

Table 6.2/ 23 Kinetic parameters describing the plasma radioactivity concentration – time profiles after single oral doses of [^{14}C-aniline]-Flufenoxuron to dogs

	1 M	**2 M**	**3 F**	**4 F**	**Mean**
T_{max} (h)	4.0	2.0	4.0	4.0	3.5
C_{max} (mg/kg)	0.514	0.217	0.224	0.624	0.408
K_{el} (h^{-1})	0.00067	0.00188	0.00117	0.00101	0.00118
$T_{1/2}$ (h)	1035	368	592	686	670

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

			Official use only
		1 REFERENCE 8	
1.1	**Reference**	**18) XXXX** WL 115110 Kinetic accumulation/ elimination study in dogs XXXX unpublished XXXX	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Company with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No – Study Plan can be summarised as follow: Seven female Beagle dogs were administered Flufenoxuron via the diet for 19 consecutive weeks at a constant concentration of nominally 500 mg/kg feed by a daily food ration of 400 g. An additional female dog received untreated diet for control purposes. The food consumption was recorded daily for all animals. The analysis of the test diets was performed with 7 samples of the weekly mixing batches. On completion of the dosing period, 24 h after the last offering of treated diet, (i.e. Day 134 of the study) animals 2, 3, 4 and control animal 1 were sacrificed to provide tissues for analysis of Flufenoxuron. Animals 5 and 6 were sacrificed 4 weeks later (Day 162 of the study). Animals 7 and 8 were sacrificed a further 4 weeks later (Day 190 of the study). Animal 5,6,7, and 8 received an untreated diet from Day 134 on.	
2.2	**GLP**	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)	
2.3	**Deviations**	No	X
		3 MATERIALS AND METHODS	

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	Batch no. XXXX	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	Radiolabelled test substance: not applicable	X
3.1.2.2	Purity	97.9%	
3.1.2.3	Stability	Stable	
3.1.2.4	Radiolabelling	Not applicable	
3.2	**Test Animals**		
3.2.1	Species	Dog	
3.2.2	Strain	Beagle	
3.2.3	Source	XXXX	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	16.2-23.4 kg, about 4 to 7 years old	
3.2.6	Number of animals per group	7	
3.2.7	Control animals	No	X
3.3	**Administration/ Exposure**	In food	
3.3.1	Preparation of test substance	Not reported	
3.3.2	Concentration of test substance	500 mg/kg fee	
3.3.3	Specific activity of test substance	Not applicable	
3.3.4	Volume applied	Not applicable	X
3.3.5	Size of test site	Not applicable	
3.3.6	Exposure period	For 19 weeks	
3.3.7	Sampling time	1) Blood samples and subcutaneous fat: Days –1, 2, 3, 4, 7 and weekly thereafter until Day 56; further samples were taken on Days 77, 98, 119, 133 and fortnightly thereafter until Day 189. 2) Tissue samples (liver, kidney, muscle, fat and bone marrow) at sacrifice	
3.3.8	Samples	See 3.3.7	

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

4 RESULTS AND DISCUSSION

4.1	**Toxic effects, clinical signs**	There were no adverse clinical signs or reactions to treatment with Flufenoxuron during the dosing or off-test phase of the study
4.2	**Dermal irritation**	Not relevant
4.3	**Recovery of labelled compound**	Not applicable
4.4	**Percutaneous absorption**	Not relevant

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Animals

Eight obese, purpose bred, female Beagle dogs were obtained from XXXX. The animals were 4 to 7 years old and weighed 16.2 to 23.4 kg.

Sampling

Whole blood samples (ca. 5 ml) from all animals were collected from the jugular vein on Days –1, 2, 3, 4, 7 and weekly thereafter until Day 56; further samples were taken on Days 77, 98, 119, 133 and fortnightly thereafter until Day 189.

Subcutaneous fat samples (ca. 3 g) from all animals were removed by biopsy from the flanks under local anaesthesia at the same time as blood sampling.

Animals were sacrificed by intravenous injection of pentobarbitone sodium followed by exsanguination. Tissue samples (liver, kidney, muscle, fat and bone marrow) were taken for analysis.

Analysis of Flufenoxuron in blood, fat, bone marrow, and tissue samples using analytical method SAMS 457-2.

Blood and fat samples taken during the study were analysed at the labs of XXXX).

Bone marrow and tissue samples (muscle, kidney, liver, and fat) taken from the sacrificed animals were analysed at Shell XXXX.

Blood samples (IRI) were extracted twice with hexane at pH 3 (HCl) by means of mechanical shaking followed by

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

centrifugation. The combined extracts were then evaporated to dryness, re-dissolved in acetonitrile and analysed by HPLC-UV (column: Hypersil ODS, mobile phase: acetonitrile/water 70/30, detection wavelength: 254 nm).

Fat samples (IRI) were mixed with anhydrous sodium sulphate, macerated with scissors and homogenised with acetone/hexane 90/10. After centrifugation, the supernatant liquid was passed through a short column containing anhydrous sodium sulphate. The pellet was resuspended in the extraction solvent and treated as before. The combined eluates were evaporated to dryness, redissolved in hexane and partitioned into acetonitrile. The acetonitrile phase was washed with hexane and evaporated to dryness, the resulting residue was reconstituted in the HPLC mobile phase and transferred to HPLC (as described above).

Bones were split with bone clippers, bone marrow was then scraped using appropriate spatulas. Bone marrow and tissue samples (SRC) (except for liver) were mixed with anhydrous sodium sulphate, macerated with scissors and homogenised with hexane. Acetonitrile previously saturated with hexane (acetonitrile/ hexane: ca. 8/2) was added and the mixture was homogenised again. After centrifugation, the supernatant liquid was passed through a short column containing anhydrous sodium sulphate. Liver samples were extracted twice with hexane saturated acetonitrile.

The blending vessel was rinsed with further acetonitrile/ hexane solution. The combined acetonitrile phases were transferred to a separating funnel (liver-extracts: hexane was added for separation), shaken well and allowed to stand until phase separation was observed. The acetonitrile layer was transferred to a second separating funnel, washed with hexane and evaporated to dryness. The solid residuum was dissolved in the mobile phase (hexane + ethanol + acetic acid, 97+3+0.2) and injected onto a normal phase HPLC clean-up system (column: Spherisorb-5 NH_2, 25 cm x 9 mm I.D., mobile phase: see above, flow rate: 4.0 ml/min, detection wavelength: 254 nm, Flufenoxuron retention time: ca. 15 min).

The fraction corresponding to Flufenoxuron was evaporated to dryness, reconstituted in the RP-HPLC mobile phase (see below), and cleaned-up again by HPLC (column: Spherisorb-5 ODS 2, 25 cm x 9 mm I.D., mobile phase: acetonitrile + water + triethylamine, 80+20+0.2, flow rate: 4.0 ml/min, detection wavelength: 254 nm, Flufenoxuron retention time: ca. 4 min).

For analysis, the collected fraction was evaporated to dryness, reconstituted in the normal phase HPLC solvent and injected into

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

HPLC (column: Spherisorb-5 NH_2, 25 cm x 4.6 mm I.D., mobile phase: hexane + ethanol + acetic acid, 97+3+0.2, flow rate: 2.0 ml/min, detection wavelength: 254 nm, Flufenoxuron retention time: ca. 7 min).

Blood samples (SRC) were mixed with silica gel and extracted three times with acetone. The extracts were passed through a short column containing anhydrous sodium sulphate, combined and evaporated to dryness, reconstituted in acetonitrile, and partitioned twice with hexane (previously saturated with acetonitrile). To the acetonitrile phase, water and sodium chloride were added and extracted three times with hexane, previously saturated with acetonitrile. The combined hexane phases were evaporated to dryness, reconstituted in the normal phase HPLC mobile phase and cleaned-up as described above.

The method produces a reliable limit of determination of 0.03 mg/kg.

5.2 Results and discussion

Clinical signs

There were no adverse clinical signs or reactions to treatment with Flufenoxuron during the dosing or off-test phase of the study. In week 6, animal 4 had a large abscess, probably as a result of an infection introduced during biopsy. This was drained and the animal treated with Streptophen® (Glaxovet Ltd., Uxbridge, UK) and Clamoxyl® (Beecham Animal Health, Brentford, UK). The animal showed complete recovery after 12 days.

All animals showed a gradual weight loss over the 19 weeks of treatment, being 3.5 kg in the control animal and 0.6 to 2.9 kg in the treated animals. It is considered that these results were due to the daily rationing of food to 400 g.

There was no effect on food consumption as a result of treatment with Flufenoxuron. Control animal 1 showed a certain degree of inappetance for the first few weeks of the study. Food consumption for this animal gradually improved to a level comparable to other animals on the study by week 12.

Actual dose level

Analysis of the test diet gave the following results: deviation from the nominal 500 mg/kg varied, in absolute terms, from –10.0 % to +6.1 %, i.e. 450.2 mg/kg to 530.7 mg/kg. Homogeneity of test diet as indicated by triplicate sampling at each time point showed a good degree of homogeneity. Variation with samples at each time point was an average of 1.7 % of actual concentration.

From the food consumption data (calculated at weekly intervals),

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

the average daily dose of Flufenoxuron received by each animal was calculated (based on a nominal 500 mg/kg in the diet). The data are shown in Table 6.2-8/ 24.

Blood sample analysis

Individual and mean values for treated animals are presented in Table 6.2-8/ 25. The mean results show an approximately parabolic rise in blood concentration of Flufenoxuron until the end of treatment at Day 133. In the off-test recovery phase (Days 134-189) there was an approximately exponential decline in Flufenoxuron concentration in blood. There was considerable individual variation in blood concentrations at each time point. This was attributed to the variations and fluctuations in ingested amounts of test material via feed intake. Semi-log regression analysis of the off-test recovery phase data gave a straight line plot from which the calculated half-life of Flufenoxuron in whole blood was 33 days with 95 % confidence limits of 25 and 41 days.

Fat sample analysis

Individual and mean values for treated animals are presented in Table 6.2-8/3. The mean results show an approximately parabolic rise in fat Flufenoxuron residues during the treatment period. In the off-test phase (Days 134-189) an approximately linear decline in Flufenoxuron levels was observed. There was considerable variation in individual animal results at each time point. This was again attributed to the individual animal variations and fluctuations in ingested amounts of test material via feed intake. Linear regression analysis of the off-test recovery phase data gave a straight line plot from which the calculated half-life of Flufenoxuron in fat was 28 days, 95 % confidence limits being 12 and 46 days.

Tissue sample analysis

Results of analysis of tissues of individual dogs are presented in Table 6.2-8/ 28. The results from dogs sacrificed at each time point have been combined to produce mean tissue concentrations. These mean results have been used for the calculation of Flufenoxuron half live for each tissue type based on the assumption that the decay processes are simple first order and, therefore, exponential in form. The results of this regression analysis are summarised in Table 6.2-8/ 29.

5.3 Conclusion

The gradual weight loss seen in all animals during the study was

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
	related to the animals obese condition at the start of the study and the on-study rationing to 400 g per day. There was no evidence of inappetance due to formulation of the diet with Flufenoxuron at 500 mg/kg, nor were there any signs of adverse clinical reactions to treatment. The rise and subsequent decline in blood concentration and fat levels of Flufenoxuron were consistent with bioaccumulation and elimination of the test material. In conclusion, Flufenoxuron administered to dogs at 500 mg/kg in the diet for 19 consecutive weeks resulted in accumulation of the test material in fat and a corresponding rise in blood concentration. The distribution of Flufenoxuron between blood, fat, bone marrow, liver, and kidney was similar to that found for the rat. An exception was the concentration in bone marrow which was relatively higher in the dog. Elimination of Flufenoxuron from the tissues during the off-test recovery period was at a rate corresponding to mean half-lives of 20 to 38 days.	
5.3.1 Reliability	1	X
5.3.2 Deficiencies	No	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 2.3. Deviations Not relevant 3.1.2.1. Description WL115110 3.2.7. Control animal Yes, 1 3.3.4. Volume applied 200 mg/dog/day
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version
Reliability	3
Acceptability	Acceptable, study plan does not follow any guideline but the results give us relevant information on metabolism of flufenoxuron
Remarks	

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.2-8/ 24Average daily intake of Flufenoxuron during 19 weeks dosing period

Animal number	Dose level [mg/dog day]	Body weight at the end of dosing period	Dose level [mg/kg bw]
1	0	12.4	0.00
2	182	15.8	11.52
3	149	16.0	9.31
4	126	14.6	8.63
5	196	16.1	12.17
6	188	19.0	9.89
7	187	15.6	11.99
8	191	18.7	10.21

Table 6.2-8/ 25 Concentration of Flufenoxuron in blood expressed as mg/kg bw

Day of Study	Animal number							Mean
	2	3	4	5	6	7	8	
Mean daily dose mg/kg bw	11.52	9.31	8.63	12.17	9.89	11.99	10.21	10.53
Pretrial -1	0	0	0	0	0	0	0	0
Treatment Period								
2	0.094	0.000	0.000	0.031	0.148	0.076	0.000	0.050
3	0.074	0.000	0.000	0.000	0.000	0.000	0.000	0.011
4	0.000	0.000	0.000	0.000	0.000	0.034	0.031	0.009
7	0.061	0.000	0.042	0.044	0.082	0.082	0.031	0.049
14	0.153	0.039	0.080	0.069	0.072	0.134	0.082	0.090
21	0.250	0.203	0.144	0.157	0.129	0.152	0.089	0.156
28	0.248	0.096	0.154	0.178	0.136	0.253	0.121	0.169
35	0.353	0.226	0.161	0.253	0.218	0.180	0.156	0.221
42	0.285	0.204	0.199	0.296	0.218	0.226	0.207	0.234
49	0.389	0.391	0.336	0.396	0.312	0.342	0.293	0.351
56	0.447	0.259	0.330	0.359	0.327	0.340	0.283	0.335
77	0.457	0.246	0.340	0.360	0.363	0.308	0.350	0.346
98	0.518	0.180	0.400	0.375	0.409	0.299	0.344	0.361
119	0.674	0.258	0.379	0.382	0.456	0.442	0.387	0.425
133	0.534	0.348	0.431	0.389	0.617	0.306	0.441	0.438

Table 6.2-8/ 26 Concentration of Flufenoxuron in blood expressed as mg/kg bw (continued)

Recovery Period								
147				0.262	0.412	0.165	0.317	0.289
161				0.150	0.255	0.122	0.247	0.194
175						0.095	0.160	0.128
189						0.038	0.144	0.076

Table 6.2-8/ 27 Concentration of Flufenoxuron in fat expressed as mg/kg bw

Day of Study	Animal number							Mean
	2	3	4	5	6	7	8	
Mean daily dose mg/kg bw	11.52	9.31	8.63	12.17	9.89	11.99	10.21	10.53
Pretrial -1	0	1.44	0	0	0	0	0	0*
Treatment Period								
14	6.65	7.09	5.82	6.43	6.77	5.94	5.80	6.36
28	14.57	5.13	11.86	12.11	12.69	8.69	12.69	11.11
42	27.79	12.99	18.73	16.75	19.59	17.24	15.84	18.42
56	30.27	20.85	26.95	27.46	30.47	30.41	21.37	26.83
77	37.15	20.60	31.89	29.94	33.75	28.13	30.66	30.30
98	44.21	24.74	33.53	35.21	33.22	22.92	28.91	30.39
119	43.83	22.01	29.35	23.82	29.82	32.87	35.30	31.00
134	37.56	23.05	37.45	31.47	18.04	70.86	35.40	36.26
Recovery Period								
147				23.48	30.65	18.72	26.05	24.73
161				13.68	23.44	13.55	16.28	16.74
175						4.85	12.80	8.83
189						2.11	0.56	1.34

* The mean value was calculated excluding the value determined for animal 3. This was, however, close to the limit of reliable determination for this animal on the particular analytical occasion in question (ca. 1 mg/kg). No explanation can be given for this anomalous result since no peak corresponding to Flufenoxuron was found in the samples chromatographed immediately prior to this sample.

Table 6.2-8/ 28 Concentration of Flufenoxuron in blood, fat, and tissues of individual animals at the day of sacrifice expressed as mg/kg bw

Tissue sample	Study day	133	133	133	133	161	161	189	189
	Animal no	1	2	3	4	5	6	7	8
Fat		0.03	43.20	19.30	40.00	13.60	19.70	3.90	6.80
Blood		0.03	0.39	0.25	0.33	0.15	0.31	0.09	0.14
Muscle		<0.03	7.81	4.21	3.26	1.12	6.14	0.76	1.30
Kidney		<0.03	2.30	1.09	0.61	1.10	1.39	0.20	0.46
Liver		<0.03	4.43	1.88	4.48	1.19	1.33	0.41	0.53
Bone marrow		0.04	22.74	13.20	47.08	12.17	11.16	3.56	5.12

Table 6.2-8/ 29 Results of regression analysis

Tissue	Half-life (days)	95% Confidence Interval (days)
Fat	22	15 - 42
Blood	38	22 - 130
Muscle	25	13 – 777 [1)]
Kidney	31	14 - 163
Liver	20	14 - 32
Bone marrow	23	14 - 54

Large confidence intervals arise from the poor fit of the data to the exponential model in some cases, which in turn arises from variation in the analytical determinations.

[1)] The very high upper confidence limit for muscle is largely a result of the high residue found in dog 6. This may be a result of differing fat contents in the muscle samples, since fat residues were much higher

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

Official use only

1 REFERENCE 9

1.1	**Reference**	**19) XXXX.** WL115110 Percutaneous penetration of the 10 DC formulation in the rat in vivo XXXX unpublished XXXX
1.2	**Data protection**	No
1.2.1	Data owner	BASF
1.2.2	Company with letter of access	XXXX
1.2.3	Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

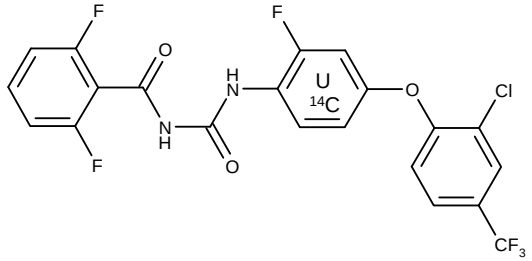
2.1	**Guideline study**	No – This study was performed prior to the release of official dermal penetration study guidelines. The percutaneous penetration of [aniline-^{14}C] WL115110 has been investigated in rats in vivo when applied at two concentrations (formulation concentrate and field strength dilution) of the 10 DC formulation. The exposure time (8 h) was selected to represent a typical working period. After exposure, the dose site was washed using a soap/water routine selected to represent a typical decontamination procedure. The distribution of absorbed material was studied in various tissues (blood, fat and remaining carcass). Additionally, the fate of material remaining at the site of administration after washing was investigated in groups of animals maintained for periods of 24 and 72 h post dose.
2.2	**GLP**	Yes (Laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	Flufenoxuron (labelled and non-labelled)

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

3.1.1	Lot/Batch number	Batch no. for XXXX
3.1.2	Specification	As given in section 2
3.1.2.1	Description	Radiolabelled test substance: not applicable
3.1.2.2	Purity	a) > 97% b) 98% (radiochemical purity); c) blend of a) and b)
3.1.2.3	Stability	Stable
3.1.2.4	Radiolabelling	[U ^{14}C] – aniline Flufenoxuron



3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Fischer 344
3.2.3	Source	XXXX
3.2.4	Sex	Female
3.2.5	Age/weight at study initiation	140.6 to 173.6 g, about 56-70 day old
3.2.6	Number of animals per group	4
3.2.7	Control animals	Yes, 1 per treatment group
3.3	**Administration/ Exposure**	Dermal

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**
3.3.1	Preparation of test substance	For the preparation of the field dilution To the entire quantity of sample a), i.e. 130 µCi = 2.16 mg 20 µ1 of the blank formulation was added. After sonication mains supply water (pH 6.35) was added to a final standard volume of 20 ml. The nominal concentration was 0.11 mg a.i. per ml. For the preparation of the concentrated formulation sample c) (see 3.1.1.; 0.5404 g) was mixed with 5.4 ml blank formulation concentrate. The nominal concentration was 0.1001 g a.i. per ml.
3.3.2	Concentration of test substance	See 3.3.1
3.3.3	Specific activity of test substance	a) not applicable b) 54.06 µCi/mg c) 1.24 µCi/mg
3.3.4	Volume applied	~ 200 µl
3.3.5	Size of test site	10 cm²
3.3.6	Exposure period	8 hours
3.3.7	Sampling time	Either after 0, 8, 24, or 72 hours; Urine and feces were sampled after 8, 24, and 72 hours (where applicable)
3.3.8	Samples	Urine, feces, cage wash, protective devices, blood, abdominal fat, skin application site, carcass and cotton swabs used for washing the application site
		4 RESULTS AND DISCUSSION
4.1	**Toxic effects, clinical signs**	There were no adverse clinical signs or reactions to treatment with Flufenoxuron during the dosing. Body weight loss was observed in all groups including control animals. This was attributed to the provision of food on the floor of the cage as opposed to the normal hopper arrangement.
4.2	**Dermal irritation**	Not observed
4.3	**Recovery of labelled compound**	Formulation concentrate: 89.5 to 93.3% Field dilution: 56.9 to 81.4%
4.4	**Percutaneous absorption**	Very low

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Four groups of 4 female Fischer 344 rats (XXXX., 56 to 70 days old) were administered either ~200 µl of the formulation concentrate or a field use aqueous dilution (1:900) thereof. Additionally, each one control rat was administered the blank formulation (formulation not containing active ingredient) or the diluted blank formulation.

Twenty-four hours prior to dosing typically 20 cm² of the upper dorsal surface was clipped free of hair under light diethyl ether anesthesia. Immediately before administration of the labelled formulation, an 'O'-ring (effective area 10 cm²) was glued to the skin using a cyanoacrylate adhesive. The applied dose (syringe weighed before and after application) was allowed to air dry for about 1.5 minutes. After that a non-occlusive protective device (nylon mesh, mesh size 50 microgrids/cm²) was glued to the upper 'O'-ring surface. No protective device was applied to the zero-hour group (see below). After dosing the animals were placed in metabolism cages for the collection of urine and feces.

One group of animals was killed immediately after drying of the dose and immediate washing of the skin (0-hour group). This group served as a surrogate for a spillage/occupational accident situation where immediate wash off occurs.
The skin application site of the other 3 groups was washed 8 hours after application. One of these groups was killed immediately after skin washing, the other two groups 24 and 72 hours after administration. The latter two groups received an additional skin wash prior to sacrifice. The control rats received skin washings 8 and 24 hours after administration of the blank formulation and were killed after the second skin wash. Accordingly the exposure/sacrifice times in this experiment were 0/0, 8/8, 8/24 and 8/72.

The skin wash was performed under diethyl ether anesthesia with a disposable cotton swab soaked with a 10% w/w aqueous soap dilution. The skin was then rinsed twice with water soaked cotton swabs and subsequently dried twice with dry cotton swabs.

Except of the 0-hour group rats, which were killed by CO_2 inhalation, the animals were killed by i.p. injection of a pentoparbitone overdose. The following samples were taken: whole blood, abdominal fat, dosing site (skin) and the remaining carcass. No blood and fat samples were taken for the 0-hour group.

Radioactivity was determined either by direct LSC (urine, cage wash, cotton swabs (after extraction with cyclohexanone for 30

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

minutes) and dose check samples), oxygen combustion (feces, blood and fat) or after solubilisation (dosing site, protective mesh and remaining carcass (after homogenisation)).

The normal background in the various biological samples was individually determined in order to establish a significantly valid limit of reliable measurement. Tissues obtained from control animals were analysed. The normal background level was measured by at least triplicate separate determinations of each control sample. The limit of detection (LD) of net radioactivity (in dpm) was calculated using the formula LD = CV + 3 SD + 1 where CV is the mean value of n determinations of the control sample and SD is the standard deviation these measurements. The limit of reliable measurement was set at 2 x the net LD (i.e. LD - mean control value) above the control value.

5.2 Results and discussion

Clinical signs

There were no adverse clinical signs or reactions to treatment with Flufenoxuron during the dosing. Body weight loss was observed in all groups including control animals. This was attributed to the provision of food on the floor of the cage as opposed to the normal hopper arrangement.

Actual dose level

The available dose was defined as applied radioactivity minus the radioactivity on the protective mesh. The available doses are given in Table 6.2/30. The average dose over all groups was 1,72 and 0.0019 mg/cm² for the formulation concentrate and the field dilution, respectively.

Dermal penetration

The study provides no summary tables listing and summarizing the radioactivity values obtained in the different sample types. The author of this summary compiled a summary table based on the individual values given in the appendix section of the report. Some of the values calculated during this exercise do not correspond to the values given in the report. In some cases this is simply due to rounding errors in other cases the reason remains unclear. Despite of these differences, the conclusion of the report is not significantly altered.

The total recovery of radioactivity was in the range of 89.5 to 93.3% for the formulation concentrate [see Table 6.2/ 31]. The recovery for the field dilution was considerably lower (56.9 to 81.4%). The latter was considered to be due to the low water solubility of the test article.

Most of applied test article was removable by skin washes (48 to

Section A6.2 **BPD Annex Point IIA, VI.6.2**		**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**	
		78.1% for the formulation concentrate and 33.1 to 65% for the field dilution. The highest amounts of radioactivity were recovered in the washes immediately after administration (0-hour groups). There was no consistent decrease of skin residues with time. Although the skin residues were lower after 72 than 24 hours this decrease was not accompanied by simultaneous increase of absorbed dose. Thus, the skin residues are not considered to result in relevant continued absorption. The numerical changes are most likely an expression of biological and experimental variability rather than an expression of continued dermal uptake. Despite of the presence of substantial amounts of the penetration enhancer N-methyl-2-pylolidone and of cyclohexanone (each about 340 g/l) dermal penetration of Flufenoxuron was remarkably low. For the formulation concentrate a dermal penetration rate of < 0.16% was reported by the study director. Based on the data presented in Table 6.2/ 31, dermal penetration (sum of dose found in urine, feces, blood, fat and remaining carcass) was 0.05, 0.96 and 0.35% after 8, 24, and 72 hours. In absence of a consistent trend a mean dermal absorption of about 0.5% may be assumed for the formulation concentrate. For the field dilution a dermal penetration rate of < 1.9% was reported. Based on the data presented in Table 6.2/ 31, dermal penetration was 0.77% after 8 hours and 3.46 and 3.77% after 24 and 72 hours. Since there is no significant change of the amount of absorbed material between 24 and 72 hours, the dermal absorption of Flufenoxuron in the field dilution is about 3.5%. The validity of the Flufenoxuron absorption rate of the field dilution is somewhat compromised by the low recovery of radioactivity. However, it is know that dermal penetration of very lipophilic and bulky molecules is low. The molecular weight of Flufenoxuron is 488.77, i.e. near to the limit of 500 above that a minimal dermal absorption is to be expected. Flufenoxuron is also very lipophilic as indicated by a $logP_{o/w}$ of 4. Therefore, in absence of fully valid data, the assumption of a default dermal penetration factor of 10% for diluted Flufenoxuron preparations is sufficiently conservative.	
5.3	**Conclusion**	Dermal penetration of formulated Flufenoxuron is low.	
5.3.1	Reliability	2	X
5.3.2	Deficiencies	No summary tables are available; radioactivity of skin and ‘O’-rings were determined together, low recovery at the field dilution	

Section A6.2 **BPD Annex Point IIA, VI.6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	Agree with the applicant's version
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version
Reliability	3
Acceptability	As it is written in the summary, the validity of the flufenoxuron absorption rate of the field dilution is somewhat compromised by the low recovery of radioactivity. Based on its physico-chemical properties, flufenoxuron is a borderline case for considering a dermal absorption of 10 % (according to the TGD on risk assessment). Nevertheless, a default dermal penetration factor of 10 % for diluted flufenoxuron preparations is considered as sufficiently conservative. This value will need to be confirmed by the submission of an adequate study at product authorisation level.
Remarks	**IUCLID not provided**
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.2/ 30: Flufenoxuron DC 10 Dermal penetration study: Applied dose

	Applied dose					
	Formulation concentrate			Field dilution		
Sacrifice time	[mg/animal]	[mg/kg b.w.]	[mg a.i./cm²]	[mg/animal]	[mg/kg b.w.]	[mg a.i./cm²]
0h	17.0985	112.30	1.7098	0.0195	0.12	0.0020
8h	17.5283	111.32	1.7528	0.0191	0.12	0.0019
24h	17.1477	110.42	1.7148	0.0184	0.108	0.0018
72h	17.0259	112.23	1.7026	0.0185	0.1095	0.0018
Overall Mean	17.20	111.57	1.72	0.0189	0.112	0.0019
Standard Deviation	0.22	0.89	0.02	0.001	0.004	0.000

Table 6.2/ 31: Dermal penetration of Flufenoxuron formulated as a 10 DC preparation

Sacrifice time	% of dose							
	0-hours		8-hours		24-hours		72 hours	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Formulation concentrate								
0h wash	78.12	7.82						
8h wash			47.99	2.61	54.62	5.00	54.92	9.94
24h wash					16.86	3.55		
72 h wash							12.91	3.06
Skin wash total	78.12	7.82	47.99	2.61	71.48	5.01	67.83	7.81
Skin residue	15.08	6.97	41.55	3.79	18.67	3.09	24.64	4.98
Cage wash	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.02
unabsorbed	**93.20**	**1.16**	**89.54**	**4.63**	**90.16**	**2.25**	**92.48**	**3.03**
Urine	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.00
Feces	0.00	0.00	0.00	0.00	0.38	0.58	0.17	0.32
Carcass	0.13	0.27	0.04	0.05	0.57	0.65	0.17	0.25
Blood	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fat	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.00
Absorbed	**0.13**	**0.27**	**0.05**	**0.05**	**0.96**	**1.23**	**0.35**	**0.57**
Recovery (total)	93.33	0.94	89.58	4.64	91.12	1.49	92.83	2.78

Table 6.2/ 31: Dermal penetration of Flufenoxuron formulated as a 10 DC preparation

Sacrifice time	% of dose							
	0-hours		8-hours		24-hours		72 hours	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Field Dilution								
0h wash	65.01	6.16						
8h wash			33.08	4.86	26.96	5.70	29.75	1.30
24h wash					9.83	2.78		
72 h wash							8.67	0.64
Skin wash total	65.01	6.16	33.08	4.86	36.79	8.26	38.41	0.70
Skin residue	16.03	2.70	29.36	4.20	16.27	2.36	19.16	1.33
Cage wash	0.00	0.00	0.03	0.04	0.25	0.18	0.12	0.08
Unabsorbed	**81.04**	**7.10**	**62.47**	**2.17**	**53.31**	**9.62**	**57.69**	**0.97**
Urine	0.00	0.00	0.00	0.00	0.12	0.09	0.23	0.10
Feces	0.00	0.00	0.01	0.01	0.60	0.58	0.65	0.39
Carcass	0.32	0.30	0.75	0.65	2.59	1.98	2.70	1.47
Blood	0.00	0.00	0.00	0.00	0.01	0.02	0.01	0.01
Fat	0.00	0.00	0.01	0.01	0.15	0.16	0.18	0.08
Absorbed	**0.32**	**0.30**	**0.77**	**0.67**	**3.46**	**2.81**	**3.77**	**1.97**
Recovery (total)	81.36	7.30	63.24	2.38	56.77	7.29	61.45	1.61

Section A6.3	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.3	6.3.1 Oral administration (28-day study) - Rat

			Official use only
		1 REFERENCE 1	
1.1	**Reference**	**1) XXXX** WL115110: A 28 day feeding study in rats XXXX unpublished- XXXX **2) XXXX** Corrigendum to XXXX: WL115110: A 28 day feeding study in rats XXXX unpublished XXXX Note: the corrigendum consists of 1 page, stating that the study was conducted also in accordance to GLP requirements of JMAFF (Japanese authority)	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Guideline not reported, but in general compliance with OECD 407	
2.2	**GLP**	No, (at the time the study was conducted GLP was not compulsory. However the study was conducted according to the principle of Good Laboratory Practices)	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch: XXXX)	
3.1.2	Specification	As given in section 2	

Section A6.3	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.3	6.3.1 Oral administration (28-day study) - Rat

3.1.2.1	Description	As given in section 2	
3.1.2.2	Purity	99%	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fischer 344	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age at study initiation: 6-8 weeks	X
3.2.6	Number of animals per group	7/sex/group	
3.2.7	Control animals	Yes, 14/sex	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	28 days	
3.3.2	Frequency of exposure	Daily	
3.3.3	Postexposure period	None	
3.3.4	**Oral**		
3.3.4.1	Type	In food	
3.3.4.2	Concentration	50; 500; 5,000; 10,000 and 50,000 ppm, equivalent to an average daily compound intake of 4.8; 49; 475; 997 and 5,147 mg/kg bw in males and 5.3; 51; 505; 1,032 and 5,290 mg/kg bw in females respectively.	
3.3.4.3	Vehicle	None	
3.3.4.4	Concentration in vehicle	Not applicable	
3.3.4.5	Total volume applied	Not applicable	
3.3.4.6	Controls	Basal diet	

Section A6.3	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.3	6.3.1 Oral administration (28-day study) - Rat

3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Twice daily on weekdays, once daily on weekends and holidays
3.4.1.2	Mortality	Twice daily on weekdays, once daily on weekends and holidays
3.4.2	Body weight	At weekly intervals
3.4.3	Food consumption	At weekly intervals
3.4.4	Water consumption	No
3.4.5	Ophthalmoscopic examination	No
3.4.6	Haematology	Yes
3.4.7	Clinical Chemisty	Yes
3.4.8	Urinalysis	No
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes, liver, spleen, kidneys, testes, heart, brain and adrenals
3.5.2	Gross and histopathology	Yes
3.5.3	Other examinations	None
3.5.4	Statistics	A two-way analysis of variance with treatment and block as factors was used for all variates. Following analysis of variance, differences between the control and treated group means were assessed for significance using the Williams' t test (Williams, 1971; Williams, 1975). On occasions where a monotonic dose response could not be assumed, Dunnett's test was used (Dunnett, 1964). Body and organ weights are reported as analysed with initial body weight as a covariate provided a significant covariance relationship was observed. Organ weights were further analysed using terminal body weight as a covariate. Although not a true covariance analysis, because the terminal body weights are dependent upon treatment, the analysis does provide an aid to the interpretation of organ weights when there are differences in terminal body weights. The analysis attempts to estimate what the organ weights would have been if all the animals had had the same terminal body weight.

Section A6.3	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.3	6.3.1 Oral administration (28-day study) - Rat

		Food intakes, clinical chemistry and haematology variates were examined using analysis of variance. When analysis of variance was considered to be inappropriate, Wilcoxon's two-sample rank sum test with ties was used (Hill and Peto, 1971; Lehman, 1961). (For references see study report)	
3.6	**Further remarks**	None	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	Clinical signs were limited to pale feces for males and females fed 50,000 ppm, which was attributed to unabsorbed test material	
4.1.2	Mortality	No mortalities at any concentration level	
4.2	**Body weight gain**	Increased food consumption in males at 50,000 ppm; for details see 5.2	
4.3	**Food consumption and compound intake**	Increased terminal body weight of 50,000 ppm males; for details see 5.2	
4.4	**Ophtalmoscopic examination**	Not applicable	
4.5	**Blood analysis**		
4.5.1	Haematology	No relevant effects; for details see 5.2	
4.5.2	Clinical chemistry	No relevant effects; for details see 5.2	X
4.5.3	Urinalysis	Not applicable	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	No relevant findings; for details see 5.2	X
4.6.2	Gross and histopathology	No effects	
4.7	**Other**	Not applicable	

Section A6.3	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.3	6.3.1 Oral administration (28-day study) - Rat

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Flufenoxuron was fed to five groups of 7 male and 7 female Fischer 344 rats [XXXX; age at study initiation: 6-8 weeks] at dietary concentrations of 50; 500; 5,000; 10,000 and 50,000 ppm for 28 days. A concurrent control group of 14 males and 14 females was fed basal diet.

All animals were subjected to clinical examination regularly during the study. Rats were examined for gross deviations in food and water consumption; body weights and food intake were determined at weekly intervals. At termination of the 28-day study period, rats were killed, terminal body weights and organ weights (liver, spleen, kidneys, testes, heart, brain and adrenals) were recorded. Blood samples were obtained for clinical chemistry and hematological examinations. Furthermore, methemoglobin determinations were performed using the non-specific CO-Oximeter method. Histopathological examinations were performed on a comprehensive set of organs from the control and 50,000 ppm group, and from organs of rats of other treatment groups that showed abnormal gross pathological changes.

5.2 Results and discussion

The test substance was judged to be stable over the study duration. All diets were shown to be within ± 10% of the nominal concentration and to be mixed homogeneously.

The test substance intake calculated on the basis of body weight and food consumption data is summarized in the table below:

Table 6.3.1/12 Test substance intake

Dietary dose level (ppm)	Mean test substance intake (mg/kg bw/d)		
	Males	Females	Combined
50	4.8	5.3	5.0
500	49	53	51
5000	475	534	505
10,000	997	1,067	1,032
50,000	5,147	5,432	5,290

There were no mortalities during the 28-day study period. Clinical signs were limited to pale feces for males and females

Section A6.3	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.3	6.3.1 Oral administration (28-day study) - Rat

fed 50,000 ppm, which was attributed to unabsorbed test material. Food consumption values for all treated females and males at 50, 500, 5,000 and 10,000 ppm were comparable to controls. However, 50,000 ppm males exhibited increased food consumption from weeks 2 to 4 compared to controls. Increased food consumption for males at 50,000 ppm was reflected by an increased mean body weight for males at this concentration, resulting in an increased overall body weight gain (13%) compared to controls. Female rats in all groups exhibited comparable mean body weights and overall body weight gains.

No treatment-related adverse hematological effects were noted in the rat after 28 days of test material administration. An apparent slight increase in methemoglobin, observed at and above 500 ppm in males and at 5,000 ppm and higher doses in females was considered to be a false-positive finding. The concentrations measured were below the level of accuracy ascribed to the IL 282 CO-Oximeter by the manufacturer (less than 2%) for detection of methemoglobin. Furthermore, data from the 2-yr oral feed study at 3-months showed no effect on methemoglobin at all when a specific methemoglobin detection method was used [see II A 5.8.2/1, FX-905-002 Evelyn K.A., Malloy H.T. 1938], whereas the non-specific CO-Oximeter method showed changes qualtitatively similar to the rat 28- and 90-day feeding studies.

X

Table 6.3.1/13 Methemoglobin detection (CO-Oximeter method) after 4-week treatment

Test		**Dose levels (ppm)**					
Parameter		**0**	**50**	**500**	**5,000**	**10,000**	**50,000**
Methemo	M	0.6	0.5	0.9*	1.2**	1.2**	1.1**
globin							
[g/dl]	F	0.6	0.5	0.8	1.3**	1.2**	1.2**

Statistical evaluation: * = p< 0.05; ** = p<0.01 (William's test)

Clinicochemical findings are summarised in Table 6.3.1/14.

A statistically significant increase in cholesterol was observed in females at 5,000 to 50,000 ppm as compared to controls. However, this finding was not considered biologically significant, because there was no dose-response relationship. Furthermore, the magnitude of the increases was slight, and there were no similar findings in males. Other incidental statistically significant changes in clinical chemistry parameters were not considered biologically significant, since they were not noted in the 90-day

Section A6.3	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.3	6.3.1 Oral administration (28-day study) - Rat

		and/or 2-year chronic rat dietary studies and/or showed no dose-response relationship at higher concentrations of test material. A summary of organ weight changes is given in Table 6.3.1/15. Statistically significant increases in absolute spleen, heart, kidney and liver weights were noted for males at 50,000 ppm, which likely reflected the increased body weight gain of males at this treatment level. Spleen and heart weights adjusted for terminal body weights for 50,000 ppm males were slightly but statistically significantly increased. However, in the absence of corresponding histopathological or hematological changes in males, these slight increases were not considered adverse. There were no organ weight effects in females at any concentration of test material [see Table 6.3.1/15]. There were no treatment-related gross pathological or histopathological changes observed.	
5.3	**Conclusion**	Given the lack of adverse treatment-related effects, the NOAEL for this study was 50,000 ppm, the highest concentration tested. The NOAEL is equivalent to an average daily intake of approximately 5,147 mg/kg bw in males and 5,432 mg/kg bw in females (calculated from food consumption data).	X
5.3.1	LO(A)EL	Not relevant	X
5.3.2	NO(A)EL	50 000 ppm, highest tested concentration (equivalent to 5,147 mg/kg bw in males and 5,432 mg/kg bw in females)	X
5.3.3	Other	None	
5.3.4	Reliability	1	
5.3.5	Deficiencies	No	

Section A6.3	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.3	6.3.1 Oral administration (28-day study) - Rat

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	Applicant's version acceptable
Results and discussion	3.2.5 Age/weight at study initiation Which weight? 4.5.2. for males, decreasing triglyceride level at 5000 ppm is relevant and must be mentioned in the report; as well, lower beta globulin levels in high dose females should be mentioned in the report
Conclusion	5.2. Table 6.3.1/1 Values in columns "Females" and "Combined" in contradiction with values reported in 3.3.4.2.: which are correct? - Concerning the level of MetHb, there is a contradiction between the study report ("increase observed is toxicologically significant") and the study summary ("false-positive finding"). Moreover, results are equivocal due to the method used. For male rats, if we take into account the lower triglyceride levels measured, a NOAEL could be determined at 500 ppm; for female rats, the lower beta globulin levels observed lead to a NOAEL of 10000 ppm. **NOAEL 500 ppm for males and 10000 ppm for females**
Reliability	2
Acceptability	Acceptable with the modification of NOAEL and LOAEL according to the previous remarks.
Remarks	-
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*

Section A6.3	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.3	6.3.1 Oral administration (28-day study) - Rat

Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.3.1/14 Clinico-chemical findings

Test parameter		**Dose levels (ppm)**					
		0	**50**	**500**	**5,000**	**10,000**	**50,000**
Cholesterol (mM)	M	1.44	1.38	1.40	1.43	1.33*	1.30**
	F	2.07	2.01	2.07	2.23*	2.23*	2.18*
Triglycerides (mM)	M	1.25	1.17	1.17	1.04*	1.08*	0.97**
	F	0.65	0.65	0.61	0.59	0.58	0.65
Inorganic phosphate (mM)	M	3.08	3.12	3.10	3.18	3.32	3.35*
	F	2.78	2.87	2.80	2.62	2.77	2.60
Albumin [g/l]	M	53.70	53.71	51.87	54.03	54.10	50.80
	F	52.34	52.60	54.28	53.80	54.64	56.10*
Beta-globulin [g/l]	M	19.81	19.39	21.46	19.94	19.81	21.47
	F	22.41	21.98	21.33	21.94	21.17	19.92*

Statistical evaluation: * = p< 0.05; ** = p<0.01 (William's test)

Table 6.3.1/15 Organ weight changes of the heart and spleen

Test parameter		**Dose levels (ppm)**					
		0	**50**	**500**	**5,000**	**10,000**	**50,000**
Terminal bw [g]	M	240.5	245.8	244.5	229.1	247.8	248.4
	F	157.3	153.1	147.2*	151.3	154.8	150.9
Heart wt. (absolute) [g]	M	0.79	0.84	0.8	0.79	0.82	0.86**
	F	0.58	0.57	0.58	0.59	0.58	0.57
Heart wt. (adjusted) [g]	M	0.8	0.83	0.81	0.81	0.82	0.84**
	F	0.57	0.57	0.59	0.6	0.59	0.57
Spleen wt (absolute)	M	0.57	0.6	0.56	0.56	0.59	0.62**
[g]	F	0.42	0.42	0.41	0.41	0.43	0.41
Spleen wt (adjusted)	M	0.57	0.59	0.56	0.58	0.59	0.61*
[g]	F	0.41	0.42	0.41	0.42	0.43	0.42

Statistical evaluation: * = p< 0.05; ** = p<0.01 (William's test)

Section A6.3	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.3	6.3.1 Oral administration (28-day study) - Mice

			Official use only
		1 REFERENCE 2	
1.1	**Reference**	**3) XXXX** Flufenoxuron (WL115110): A 28 day range-finding feeding study in mice XXXX unpublished XXXX	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not reported; in general compliance with OECD 407	
2.2	**GLP**	No - For the scope of a range finding study the study is nevertheless considered to be fully valid since it was conducted in a GLP accredited laboratory and the study procedures are adequately described in the report.	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	Batch: XXXX	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	As given in section 2	
3.1.2.2	Purity	99%	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Mice	
3.2.2	Strain	B6C3F_1 ("C57/C3H F_1 hybrid")	
3.2.3	Source	XXXX	

Section A6.3	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.3	6.3.1 Oral administration (28-day study) - Mice

3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age at study initiation: 5 weeks	
3.2.6	Number of animals per group	7/sex/group	
3.2.7	Control animals	Yes, 14/sex	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	28 days	
3.3.2	Frequency of exposure	Daily	
3.3.3	Postexposure period	None	
3.3.4	**Oral**		
3.3.4.1	Type	In food	
3.3.4.2	Concentration	50; 500; 5,000; 10,000 and 50,000 ppm, equivalent to a mean daily compound intake of 10.5; 110; 1,091; 2,142 and 9,820 mg/kg bw in males and 14.0; 142, 1,353; 2,811 and 12,157 mg/kg bw in females, respectively.	
3.3.4.3	Vehicle	None	
3.3.4.4	Concentration in vehicle	Not applicable	
3.3.4.5	Total volume applied	Not applicable	
3.3.4.6	Controls	Basal diet	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes, daily	X
3.4.1.2	Mortality	Yes, daily	X
3.4.2	Body weight	Yes, weekly intervals	
3.4.3	Food consumption	Yes, weekly intervals	
3.4.4	Water consumption	No	

Section A6.3	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.3	6.3.1 Oral administration (28-day study) - Mice

3.4.5	Ophthalmoscopic examination	No	
3.4.6	Haematology	Yes	
3.4.7	Clinical Chemisty	Yes	
3.4.8	Urinalysis	No	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes, liver, spleen, kidneys, testes, heart, brain and testes	X
3.5.2	Gross and histopathology	Yes	
3.5.3	Other examinations	None	
3.5.4	Statistics	For numeric data the significance levels of the differences between the control and treated means (adjusted if necessary) were determined using the William's t test (Williams, 1971; 1972) whenever justifiable. Dunnett's test (Dunnett, 1964) was used if a monotonic dose response could not be assumed. If for a variate there were serious doubts about the validity of the assumption required for the analysis of variance, the Wilcoxon two-sample rank sum test (Hill and Peto, 1971; Lehman, 1961) was used and the results of this analysis reported. Bodyweights were analysed with initial bodyweight as a covariate. In order to adjust for differences in terminal bodyweight, organ weights were also considered with terminal bodyweight as covariate. The incidences of histopathology lesions were compared with control values by Fisher's exact test (Fisher, 1950). For full references see study report.	
3.6	**Further remarks**	None	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	Clinical signs were limited to pale feces for males and females fed 50,000 ppm, which was attributed to unabsorbed test material	
4.1.2	Mortality	Three moribund animals were removed from the studies. Based on histopathological examination, this condition was not test substance related.	

Section A6.3	**Repeated dose toxicity**	
BPD Annex Point IIA, VI.6.3	6.3.1 Oral administration (28-day study) - Mice	

4.2	**Body weight gain**	Mean body weights and overall body weight gains for males at all treatment levels were comparable to controls. Although females fed 50,000 ppm exhibited a slight (8%) reduction in body weight at week 2 compared to controls, overall body weight gain for 50,000 ppm females at study termination was comparable to controls. This slight reduction in body weight for 50,000 ppm females at week 2 likely reflected reduced food consumption observed at this time.	
4.3	**Food consumption and compound intake**	Females at 50,000 ppm exhibited reduced food consumption at weeks 2 and 4 compared to controls. Food consumption was unaffected at all other treatment levels.	
4.4	**Ophtalmoscopic examination**	Not applicable	
4.5	**Blood analysis**		
4.5.1	Haematology	No effects	
4.5.2	Clinical chemistry	No effects	
4.5.3	Urinalysis	Not applicable	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	Absolute organ weights were comparable among all male and female groups	X
4.6.2	Gross and histopathology	No effects	
4.7	**Other**	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Flufenoxuron technical was fed to five groups of 7 male and 7 female B6C3F_1 ("C57/C3H F_1 hybrid") mice [XXXX, age at study initiation: approx. 5 weeks] at dietary concentrations of 50; 500, 5,000, 10,000 and 50,000 ppm for 28 days. A concurrent control group of 14 males and 14 females was fed basal diet. Mice were checked for clinical signs daily. Bodyweight and food intake were measured weekly. On day 26, orbital blood samples were taken for haematology. At termination, the mice were necropsied and blood samples taken by cardiac puncture for clinical chemistry. All organs were subjected to macroscopic examination and brain, liver, kidney, spleen, heart and testes were weighed. Histopathology was performed on a comprehensive set of organs from the control and top dose groups (50,000 ppm),	

Section A6.3	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.3	6.3.1 Oral administration (28-day study) - Mice

decedents at 10,000 ppm, and thymus, spleen and submaxillary and mesenteric lymph nodes of survivors at 5,000 and 10,000 ppm.

5.2 Results and discussion

Analyses showed that achieved Flufenoxuron concentrations, as well as stability and homogeneity in the diet preparation were acceptable in all cases.

The test substance intake calculated on the basis of body weight and food consumption data is summarized in Table 6.3.1.2/16.

Table 6.3.1.2/16 Test substance intake

Dietary dose level (ppm)	Mean test substance intake (mg/kg bw/d) Males	Females
50	10.5	14.0
500	110	142
5000	1,091	1,353
10000	2,142	2,811
50000	9,820	12,157

Three female mice became moribund and were removed from the study (two at 10,000 ppm on days 26 and 27 and one at 50,000 ppm on day 14). Histological examination of the three moribund mice revealed necrosis of the lymphoid tissue. No treatment related changes were observed in any of the other tissues of these animals. It was concluded that the pathological changes and the moribund condition of the three females was not directly attributable to Flufenoxuron toxicity.

Clinical signs were limited to pale feces for males and females fed 50,000 ppm, which was attributed to unabsorbed test material. Females at 50,000 ppm exhibited reduced food consumption at weeks 2 and 4 compared to controls. Food consumption was unaffected at all other treatment levels. Mean body weights and overall body weight gains for males at all treatment levels were comparable to controls. Although females fed 50,000 ppm exhibited a slight (8%) reduction in body weight at week 2 compared to controls, overall body weight gain for 50,000 ppm females at study termination was comparable to controls. This slight reduction in body weight for 50,000 ppm females at week 2 likely reflected reduced food consumption observed at this time.

Section A6.3	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.3	6.3.1 Oral administration (28-day study) - Mice

		There were no treatment-related effects on hematology or clinical chemistry parameters. Absolute organ weights were comparable among all male and female groups. A statistically significant increase in the heart weight adjusted for terminal body weight of 50,000 ppm females was observed compared to controls. However, in the absence of any increase in absolute heart weights or corresponding histopathological effects in this organ, as well as the lack of a similar finding in longer-term (90-day and 2-year) studies, this slight increase was not considered adverse. There were no treatment-related gross pathological or histopathological changes observed.
5.3	**Conclusion**	Given the lack of adverse treatment-related effects, the NOAEL for this study was 50,000 ppm, the highest concentration tested. The NOAEL is equivalent to an average daily intake of approx. 9,820 mg/kg bw in males and 12,157 mg/kg bw in females.
5.3.1	LO(A)EL	Not relevant
5.3.2	NO(A)EL	50 000 ppm, highest tested concentration (equivalent to 9,820 mg/kg bw in males and 12,157 mg/kg bw in females.)
5.3.3	Other	None
5.3.4	Reliability	2
5.3.5	Deficiencies	No

Section A6.3	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.3	6.3.1 Oral administration (28-day study) - Mice

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 3.4.1.1. Clinical signs *Twice daily on weekdays, once daily on weekends and holidays* 3.4.1.2. Mortality *Twice daily on weekdays, once daily on weekends and holidays* 3.5.1. Delete one “testes”
Results and discussion	Agree with the applicant’s version **Revisions/Amendments:** 4.6.1. Organ weights *[…] However, female heart weight presents a slight increase.*
Conclusion	Agree with the applicant’s version LO(A)EL: - NO(A)EL: 50000 ppm
Reliability	2
Acceptability	The study report is incomplete: appendix with description of the tested substance, data and observations for each animal, statistical tests realized on these data must be provided to fully accept this study. The study report should be completed. This study is however not used in the risk assessment.
Remarks	-
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*

Section A6.3	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.3	6.3.1 Oral administration (28-day study) - Mice
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.3	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.3	6.3.2 dermal

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Acute toxicity studies did not indicate any adverse findings when Flufenoxuron was tested by the dermal route at limit dose levels. Therefore, no short-term toxicity studies by the dermal route was conducted.	
Undertaking of intended data submission [ ]	Not applicable	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Evaluation of applicant's justification	Agree with applicant's justification
Conclusion	Waiving accepted
Remarks	

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.3	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.3	6.3.3 inhalation

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Acute toxicity studies did not indicate any adverse findings when Flufenoxuron was tested by inhalation. Furthermore, short-term inhalation toxicity studies are not necessary due to the low volatility of Flufenoxuron. Therefore, no short-term toxicity studies by the inhalation route were conducted.	
Undertaking of intended data submission [ ]	Not applicable	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Evaluation of applicant's justification	Discussions with the notifiant took place during the evaluation phase. As the formulation to be assessed is a liquid, no exposure to the neat Flufenoxuron powder will occur and the waiving for inhalation repeated dose toxicity was hence accepted. In addition, the formulation has a low volatility and spraying aqueous dilution is not expected to generate substantial amounts of droplets < 50 µm. During risk characterisation, exposition levels will be particularly checked and margin of safety will be determined taking into account this lack of data.
Conclusion	Waiving accepted
Remarks	

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*

Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.4	**Repeated dose toxicity (subchronic oral)**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (90-day study) - Rat

Official use only

1 REFERENCE 1

1.1	**Reference**	**1) XXXX** WL115110: A 90 day feeding study in rats XXXX unpublished XXXX **2) XXXX** Corrigenda/Addenda to XXXX: WL115110: A 90 day feeding study in rats XXXX unpublished XXXX **Note:** 5-page addendum with a) discussion of stability/homogeneity of test substance sample which was used for preparation of the treatment group diets; b) correction of minor mistakes in text and some tables; c) updated GLP compliance statement. The addendum/corrigendum does not alter the interpretation of the study. **3) XXXX** Corrigenda/Addenda to SBGR.86.256: WL115110: A 90 day feeding study in rats XXXX unpublished XXXX **Note:** Correction of two footnotes in Summary tables 2 and 3 of the original report. The addendum/corrigendum does not alter the interpretation of the study
1.2	**Data protection**	No
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	XXXX
1.2.3	Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Not reported; in compliance with OECD 408
2.2	**GLP**	No

Section A6.4	**Repeated dose toxicity (subchronic oral)**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (90-day study) - Rat

		(at the time the study was conducted GLP was not compulsory. However the study was conducted according to the principle of Good Laboratory Practices)	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch: XXXX)	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	As given in section 2	
3.1.2.2	Purity	96.6%	X
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fischer 344	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age at study initiation: 6-8 weeks	
3.2.6	Number of animals per group	10/sex/group	
3.2.7	Control animals	20/sex	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	90 days	
3.3.2	Frequency of exposure	Daily	
3.3.3	Postexposure period	None	
3.3.4	Oral		

Section A6.4	**Repeated dose toxicity (subchronic oral)**	
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (90-day study) - Rat	
3.3.4.1 Type	In food	
3.3.4.2 Concentration	50; 500; 5,000; 10,000 and 50,000 ppm equivalent to an average compound intake 3.5; 35; 351; 689; 3,637 mg/kg bw/day in males and 4.1; 41; 399; 820 and 4,151 mg/kg bw/day in females	
3.3.4.3 Vehicle	None	
3.3.4.4 Concentration in vehicle	Not applicable	
3.3.4.5 Total volume applied	Not applicable	
3.3.4.6 Controls	Basal diet	
3.4 Examinations		
3.4.1 Observations		
3.4.1.1 Clinical signs	At least once a day	X
3.4.1.2 Mortality	At least once a day	X
3.4.2 Body weight	Weekly intervals	
3.4.3 Food consumption	Weekly intervals	
3.4.4 Water consumption	No	
3.4.5 Ophthalmoscopic examination	On 5 rat/sex for control and highest dose (50,000 ppm); before and after exposure period	
3.4.6 Haematology	At termination of study	
3.4.7 Clinical Chemisty	At termination of study	
3.4.8 Urinalysis	5 /sex/dose group and 10/sex/control group	X
3.5 Sacrifice and pathology		
3.5.1 Organ Weights	Liver, spleen, kidneys, testes, heart, brain, uterus and adrenal glands	X
3.5.2 Gross and histopathology	Yes	
3.5.3 Other examinations	None	
3.5.4 Statistics	A two-way analysis of variance with treatment and block as factors was used for all variates. Following analysis of variance, differences between the control and treated group means were assessed for significance using the William's t test (Williams,	

Section A6.4	**Repeated dose toxicity (subchronic oral)**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (90-day study) - Rat

		1971; Williams 1975). On occasions where a monotonic dose response could not be assumed, Dunnett's test was used (Dunnett, 1964). Body and organ weights are reported as analysed with initial body weight as a covariate provided a significant covariance relationship was observed. Organ weights were further analysed using terminal body weight as a covariate. Although not a true covariance analysis, the analysis does provide an aid to the interpretation of organ weights when there are differences in terminal body weights. The analysis attempts to estimate what the organ weights would have been if all the animals had had the same terminal body weight. Food intakes, clinical chemistry, urine volume and osmolality, and haematology variates other than counts of banded neutrophils (BANDS), were examined using the analysis of variance. Wilcoxon's two sample rank sum test (Hill and Peto, 1971; Lehman, 1961) was used for BANDS and for urine analysis variates other than volume and osmalality. (Detailed references see study report)	
3.6	**Further remarks**	None	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	Clinical signs were limited to pale feces for males and females fed 50,000 ppm and for a few animals fed 10,000 ppm, which was attributed to unabsorbed test material.	
4.1.2	Mortality	No mortalities at any dose level	
4.2	**Body weight gain**	Comparable mean and overall bodyweight between treated and control groups	
4.3	**Food consumption and compound intake**	Comparable to the control, except for males at 50,000 ppm where increased food consumption was noted from Week 7 to termination.	
4.4	**Ophtalmoscopic examination**	No treatment-related effects	
4.5	**Blood analysis**		
4.5.1	Haematology	Indication for a mild anemia in females at ≥ 500 ppm. No comparable changes in males but examination of bone marrow indicated (like in females) an increased erythropoiesis. For details see 5.2.	X

Section A6.4 **BPD Annex Point IIA, VI.6.4**		**Repeated dose toxicity (subchronic oral)** 6.4.1 Oral administration (90-day study) - Rat	
4.5.2	Clinical chemistry	No adverse treatment-related effects; for details see 5.2.	X
4.5.3	Urinalysis	No treatment-related effects	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	No adverse treatment-related effect, details given under 5.2	X
4.6.2	Gross and histopathology	No treatment-related effect, details given under 5.2	
4.7	**Other**	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Flufenoxuron was fed to five groups of 10 male and 10 female Fischer 344 rats [XXXX; age at study initiation: 6-8 weeks] at dietary concentrations of 50; 500; 5,000; 10,000 and 50,000 ppm for 90 days. A concurrent control group of 20 males and 20 females was fed basal diet. All animals were subjected to careful clinical examination at least once daily. Ophthalmological examination was performed on 5 rats of each sex from the control and the high-dose group before and after the 90-day study period. Body weights and food consumption were determined at weekly intervals for all animals. Blood samples were taken at termination of the study for assessment of clinicochemical and hematological parameters. Generation of methemoglobin was assessed using the nonspecific CO-Oximeter method. Urinalyses were conducted on five animals of each sex from each of the treated groups, and from ten controls of each sex. All animals were subjected to detailed post-mortem examination and all macroscopic abnormalities recorded. Terminal body weights and the weights of the liver, spleen, kidneys, testes, heart, brain, uterus and adrenal glands were recorded. Histopathology was performed on a comprehensive set of organs from rats of the control and 50,000 ppm group, and on lungs, liver and kidney tissues from all test groups.	
5.2	**Results and discussion**	The test substance was judged to be stable over the study duration. All diets were shown to be within ± 10% of the nominal concentration and to be mixed homogeneously. Test substance intakes calculated on the basis of body weight and food consumption data are presented in Table 6.4.1/17.	

Section A6.4 **Repeated dose toxicity (subchronic oral)**

BPD Annex Point IIA, VI.6.4 6.4.1 Oral administration (90-day study) - Rat

Table 6.4.1/17 Test substance intake

Dietary dose level	Mean test substance intake (mg/kg bw/d)		
(ppm)	Males	Females	Combined
50	3.5	4.1	3.8
500	35	41	38
5,000	351	399	375
10,000	689	820	754
50,000	3,637	4,151	3,894

There were no mortalities during the 90-day study period. Clinical signs were limited to pale feces for males and females fed 50,000 ppm and for a few animals fed 10,000 ppm, which was attributed to unabsorbed test material. Food consumption values for all treated females and males at 50; 500; 5,000 and 10,000 ppm were comparable to controls. However, 50,000 ppm males exhibited increased food consumption from week 7 to study termination compared to controls. Male and female rats in all groups exhibited comparable mean body weights and overall body weight gains.

Hematological findings are summarized in Table 6.4.1/18. At 500 ppm and above, females exhibited hematological changes indicative of a mild anemia, including statistically significant decreases in hemoglobin (dose-related) and increases in mean erythrocyte diameter. At 5,000 ppm and above decreases in erythrocyte count and hematocrit were additionally seen in females, accompanied by a statistically significant increase in the percentage of reticulocytes compared to controls. Mild anemia was confirmed in females by evaluation of bone marrow smears: A statistically significant decrease in myeloid/erythroid ratios, indicative of a slight compensatory increase in erythropoiesis, was observed for females at 50,000 ppm, as compared to control and 50 ppm animals, the only other groups examined for myeloid/erythroid ratios.

In males there were no indications of anemia from assessment of blood samples (statistically significant changes in mean corpuscular volume and mean corpuscular hemoglobin concentration in treatment group males were considered to be of dubious toxicological significance).

However, the bone marrow smears from high-dose group males indicated an increase in erythropoiesis (i.e. statistically significant decrease in the myeloid/erythroid ratio) as compared to control

Section A6.4	**Repeated dose toxicity (subchronic oral)**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (90-day study) - Rat

and 50 ppm animals.

Assessment of methemoglobin revealed a small but statistically significant increase in methemoglobin at all dose levels [see Table 6.4.1/18]. The increases were dose related, but plateaued at 5,000 ppm to approximately three times the control value. Such changes could be expected based on literature data showing that acyl urea compounds similar to Flufenoxuron, produced methemoglobin [Maas et al.: Chem. Pflanzenschutz, 6, 423 (1981)]. The levels of methemoglobin detected (less than 2%) however, were below the level of accuracy ascribed to the IL 282 CO-Oximeter by the manufacturer raising the suspicion that they could have been false positive values. As the animals had been necropsied at the time of blood sample, the values could not be confirmed using another method of analysis.

An opportunity for confirmatory analysis, however, did occur at the 13 week interim bleed of a subsequent 2-year rat feeding study with Flufenoxuron [see IIIA 6.5, XXXX]. In the 2-year rat study, methemoglobin was estimated using the specific binding of methemoglobin to cyanide by the method of Evelyn and Malloy [Evelyn K.A.,Malloy H.T., 1938, Microdetermination of oxyhemoglobin, methemoglobin, and sulfhemoglobin in a single sample of blood, Journal of Biological Chemisty, 126, 655-662, XXXX], as well as the (unspecific) CO-Oximeter method. The results show no methemoglobin when the method of Evelyn and Malloy was used, but a dose-related increase in methemoglobin, very similar to that seen in the present 90-day study, when the CO-Oximeter was used [see Table 6.4.1/19].

Selected blood samples from the 13 week interim bleed of the 2-year oral feed study were then stored at ambient temperature for one day and the methaemoglobin reanalyzed using the CO-Oximeter. In view of the high levels of methemoglobin reductase in rat blood, the methemoglobin should have been completely reduced in the time. However, the results showed no difference in the methemoglobin content, before and after storage, indicating that the CU-Oximeter was measuring something other than methemoglobin. One may conclude from these experiments on the 13-week interim blood samples that feeding Flufenoxuron causes interference to the absorbance measurements of the CO-Oximeter, but does not cause methemoglobinemia. There must, therefore, be a strong presumptive case for arguing that methemoglobinemia was not present in this reported 90-day study.

Clinicochemical findings are summarized in [see Table 6.4.1/20]. At 500 ppm and above, females exhibited statistically significant increases in mean cholesterol, similar to that observed in the 28-

Section A6.4	**Repeated dose toxicity (subchronic oral)**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (90-day study) - Rat

		day dietary study. However, this finding was not considered biologically significant, because there was no dose-response relationship; furthermore, the magnitude of the increases was slight and there were no similar findings in males. At 50,000 ppm, males exhibited slight, statistically significant increases in aspartate and alanine aminotransferase levels. However, these increases were not considered biologically significant since there were no corresponding liver histopathological effects. There were no treatment-related effects on urinary or ophthalmology parameters. A summary of organ weight changes is presented in Table 6.4.1/21. At 5,000 ppm and higher dose levels, the increase of female spleen weights (absolute and adjusted values) were statistically significant compared to control levels. These slight increases, although not dose-related, were likely related to the hematological effects noted for females at these dietary concentrations of test material. At 10,000 and 50,000 ppm, male rats exhibited statistically significant increases in heart weights (adjusted for terminal body weight). However, in the absence of histopathological changes, these slight increases were not considered adverse. No other toxicologically significant changes in organ weights were noted. There were no treatment-related gross pathological or histopathological changes observed.	
5.3	**Conclusion**	Based on hematology changes in females at 500 ppm, the NOAEL for this study was 50 ppm. The NOAEL is equivalent to an average daily intake of approx. 3.5 mg/kg bw for males, 4.1 mg/kg bw for females and 3.8 mg/kg bw/d for males and females combined.	
5.3.1	LO(A)EL	5000 ppm, equivalent to about 351mg/kg b.w./day for male and 399 mg/kg b.w/day for female rats	X
5.3.2	NO(A)EL	500 ppm, equivalent to about 3.5 mg/kg b.w. /day for male and 4.1 mg/kg b.w/day for female rats	X
5.3.3	Other	None	X
5.3.4	Reliability	1	
5.3.5	Deficiencies	No	

Section A6.4	**Repeated dose toxicity (subchronic oral)**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (90-day study) - Rat

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	06/10/2004
Materials and Methods	3.1.2.2. Purity *96.6% at TO and then decreasing purity until 91.6%* 3.4.1.1. Clinical signs *Twice daily on weekdays and once daily on weekends and holidays* 3.4.1.2. Mortality *Twice daily on weekdays and once daily on weekends and holidays* 3.4.8. Urinalysis Precise when urinalysis was realised and how were collected samples 3.5.1. Organ weights Epididymides, ovaries and thymus were not weighted as provided by the guideline 408.
Results and discussion	Applicant's version acceptable provided these remarks are taken into account : 4.5.1. Haematology […] *A slight increase of MetHb for female even with the use of the 2nd method (0.3 to 1.6%; Table 6.4.1/3) was observed (after 3-month treatment in the 2-yr rat study)* 4.5.2. *Modifications of the levels of cholesterol (increase for males 500-50000 ppm), triglycerid (decrease for both sex from 5000 ppm), serum enzymes and bilirubin (increase)* 4.6.1. Organ weights *Weight of spleen and liver, for females, and of heart for males increase while weight of brain and testes decrease*
Conclusion	NOAEL and LOAEL reported in the conclusion of the study summary (5.3.1 and 5.3.2) are wrong: they need to be modidied accordingly. Based on hematological changes in females, the LO(A)EL was 500 ppm (~41 mg/kg bw/d) and the NO(A)EL was 50 ppm (~4.1 mg/kg bw/d) for females. Based on clinico-chemical findings in males, the LO(A)EL was 5000 ppm (~351 mg/kg bw/d) and the N(O)AEL was 500 ppm (~35 mg/kg bw/d) for males. 5.3.3. *Anemia is most important for females than for males*
Reliability	1
Acceptability	Acceptable with the corrections above

Section A6.4	**Repeated dose toxicity (subchronic oral)**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (90-day study) - Rat

Remarks	-
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.4.1/18 Hematological findings in 90-day oral rat study

Test parameter		**Dose level (ppm)**					
		0	**50**	**500**	**5,000**	**10,000**	**50,000**
RBC [10^{12}/l]	M	9.37	9.45	9.29	9.46	9.40	9.46
	F	9.15	9.05	9.05	8.68**	8.70**	8.56**
Hb [g/dl]	M	13.7	13.7	13.6	13.6	13.6	13.8
	F	14.2	14.0	13.8*	13.6**	13.3**	13.2**
Ht [ratio]	M	0.450	0.449	0.446	0.446	0.445	0.448
	F	0.464	0.460	0.457	0.442**	0.438**	0.432**
Mean erythrocyte diam. [µm]	M	5.8	5.7	5.8	5.7	5.8	5.8
	F	5.8	5.7	6.0*	6.0*	5.9**	5.9**
MCV [fl]	M	48.0	47.5	48.0	47.1*	47.4*	47.4*
	F	50.7	50.9	50.5	51.0	50.4	50.5
MCHC [g/dl]	M	30.3	30.6	30.4	30.6	30.5	30.9**
	F	30.6	30.4	30.2	30.9	30.4	30.4
Reticulocytes [%]	M	0.8	N.D.	N.D.	N.D.	N.D.	0.7
	F	0.7	0.7	1.0	1.3**	1.3**	1.4**
Myeloid : Erythroid ratio	M	1.9	1.9	N.D.	N.D.	N.D.	0.8**
	F	1.8	1.7	N.D.	N.D.	N.D.	1.0**
Methemoglobin [%]	M	0.8	1.1**	1.1**	1.6**	1.5**	1.6**
	F	0.5	0.7*	0.8**	1.4**	1.4**	1.5**

Statistical evaluation: * = p< 0.05; ** = p<0.01 (William's test); N.D. = not determined

Table 6.4.1/19 Methemoglobin determinations after 3-month treatment in the 2-yr rat study

Dose concentration (ppm)	Amount of methemoglobin found, as a percentage of total hemoglobin			
	Males		Females	
	Method of Evelyn & Malloy	CO-Oximeter method	Method of Evelyn & Malloy	CO-Oximeter method
0	0.58	0.3	0.63	0.3
1	0.57	0.3	0.92	0.4
5	0.42	0.3	0.79	0.3
50	0.63	0.5**	0.67	0.6**
500	0.52	0.6**	0.61	0.6**
5000	0.75	0.9**	0.80	1.1**
50000	0.57	1.3**	0.80	1.6**

Table 6.4.1/20 Clinicochemical findings

Test parameter		Dose level (ppm)					
		0	50	500	5,000	10,000	50,000
Cholesterol (mM)	M	1.97	2.02	1.90	1.98	1.82	1.89
	F	2.15	2.17	2.33*	2.47**	2.42**	2.47**
Triglycerides (mM)	M	1.03	1.08	1.04	0.73*	0.68**	0.69**
	F	0.62	0.52	0.63	0.44*	0.50*	0.49*
ASAT [I.U./l]	M	91.3	93.9	107.7	101.6	100.7	116.0**
	F	104.7	102.1	104.2	101.4	95.6	94.0
ALAT [I.U./l]	M	52.4	56.8	64.1	58.9	59.1	66.4*
	F	53.1	54.1	58.4	51.2	49.5	46.1

Statistical evaluation: * = p< 0.05; ** = p<0.01 (William's test)

Table 6.4.1/21 Organ weight changes of the heart and spleen

Treatment (ppm)		**0**	**50**	**500**	**5,000**	**10,000**	**50,000**
Terminal bw [g]	M	310.9	316.5	306.8	307.5	303.0	308.2
	F	181.9	180.3	182.9	179.4	183.3	176.4
Heart wt. (absolute) [g]	M	0.88	0.92	0.86	0.91	0.93	0.92
	F	0.62	0.62	0.64	0.61	0.62	0.59
Heart wt. (adjusted) [g]	M	0.88	0.91	0.87	0.92	0.94**	0.92*
	F	0.62	0.63	0.63	0.62	0.62	0.60
Spleen wt (absolute) [g]	M	0.64	0.65	0.62	0.64	0.62	0.63
	F	0.42	0.45	0.44	0.47**	0.49**	0.47**
Spleen wt (adjusted) [g]	M	0.64	0.64	0.63	0.64	0.63	0.64
	F	0.42	0.45	0.44	0.47**	0.48**	0.48**

Adjusted = adjusted for terminal body weight.

Statistical evaluation: * = $p < 0.05$; ** = $p < 0.01$ (William's test)

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1-2 Oral administration (90-day study) - Mice

Official use only

1 REFERENCE 2

1.1	**Reference**	**4) XXXX** WL115110: A 90 day feeding study in mice XXXX unpublished XXXX **5) XXXX** Corrigenda/Addenda to XXXX: WL115110: A 90 day feeding study in mice XXXX unpublished XXXX **Note:** 5-page addendum with a) discussion of stability/homogeneity of test substance sample which was used for preparation of the treatment group diets; b) correction of minor mistakes in text and some tables, and c) updated GLP compliance statement. The addendum/corrigendum does not alter the interpretation of the study **6) XXXX** Corrigenda/Addenda to XXXX: WL115110: A 90 day feeding study in mice XXXX unpublished XXXX **Note:** 1-page addendum with information on the justification of dose selection. The addendum does not alter the interpretation of the study
1.2	**Data protection**	No
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	XXXX
1.2.3	Criteria for data protection	No data protection claimed

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1-2 Oral administration (90-day study) - Mice

		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not reported; in compliance with OECD 408	
2.2	**GLP**	No (at the time the study was conducted GLP was not compulsory. However the study was conducted according to the principle of Good Laboratory Practices)	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch: XXXX	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	As given in section 2	
3.1.2.2	Purity	96.6%	X
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Mice	
3.2.2	Strain	B6C3F_1 ("C57/C3H F_1 hybrid")	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age at study initiation: 6-8 weeks	
3.2.6	Number of animals per group	10/sex/group (+10 mice/group/sex for blood chemical chemistry analysis)	
3.2.7	Control animals	Yes, 20/sex (+ 20 mice/sex for blood chemical chemistry analysis)	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	90 days	
3.3.2	Frequency of exposure	Daily	

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1-2 Oral administration (90-day study) - Mice

3.3.3	Postexposure period	None	
3.3.4	Oral		
3.3.4.1	Type	In food	
3.3.4.2	Concentration	50; 500; 5,000; 10,000 and 50,000 ppm equivalent to average daily compound intakes of 10; 103; 1,069; 2,139 and 11,071 mg/kg bw in males and 12; 124; 1,247; 2,482 and 12,619 mgg bw in females, respectively.	
3.3.4.3	Vehicle	None	
3.3.4.4	Concentration in vehicle	Not applicable	
3.3.4.5	Total volume applied	Not applicable	
3.3.4.6	Controls	Basal diet	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Twice daily during weekday, and once daily at week-ends	
3.4.1.2	Mortality	At least once a day	X
3.4.2	Body weight	Weekly intervals	
3.4.3	Food consumption	Weekly intervals	
3.4.4	Water consumption	No	
3.4.5	Ophthalmoscopic examination	5 rat/sex for control and highest dose (50,000 ppm); before and after exposure period	
3.4.6	Haematology	At termination of study	
3.4.7	Clinical Chemisty	At termination of study	
3.4.8	Urinalysis	No	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Liver, spleen, kidneys, testes, heart, brain, uterus and adrenal glands	
3.5.2	Gross and histopathology	Yes	
3.5.3	Other examinations	None	

Section A6.4		**Repeated dose toxicity**	
BPD Annex Point IIA, VI.6.4		6.4.1-2 Oral administration (90-day study) - Mice	
3.5.4	Statistics	A two-way analysis of variance with treatment and block as factors was used for all variates. Following analysis of variance, differences between the control and treated group means were assessed for significance using the William's t test (Williams, 1971; Williams 1975). On occasions where a monotonic dose response could not be assumed, Dunnett's test was used (Dunnett, 1964). Body and organ weights are reported as analysed with initial body weight as a covariate provided a significant covariance relationship was observed. Organ weights were further analysed using terminal body weight as a covariate. Although not a true covariance analysis, the analysis does provide an aid to the interpretation of organ weights when there are differences in terminal body weights. The analysis attempts to estimate what the organ weights would have been if all the animals had had the same terminal body weight. Food intakes, clinical chemistry and haematology variates other than counts of banded neutrophils (BANDS), were examined using the analysis of variance. Wilcoxon's two sample rank sum test (Hill and Peto, 1971; Lehman, 1961) was used for BANDS, atypical lymphocytes, and in females only, gamma glutamyl transpeptidase values. (For exact references see study report)	
3.6	**Further remarks**	None	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	Clinical signs were limited to pale feces for males and females fed at 50,000 ppm, which was attributed to unabsorbed test material	
4.1.2	Mortality	No treatment-related mortality. For details see 5.2.	X
4.2	**Body weight gain**	Decreased body weight gain in males at 50,000 ppm	
4.3	**Food consumption and compound intake**	Comparable to the control	
4.4	**Ophtalmoscopic examination**	No treatment-related effects	

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1-2 Oral administration (90-day study) - Mice

4.5	**Blood analysis**		
4.5.1	Haematology	Indication for a slight anemia in both sexes at 50,000 ppm. For details see study summary under 5.2	
4.5.2	Clinical chemistry	Increase of total bilirubin at ≥ 500 ppm. Decrease of blood urea nitrogen at ≥ 10, 000 ppm. For details see study summary under 5.2	X
4.5.3	Urinalysis	Not applicable	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	Increased of terminal body weight adjusted liver weights at ≥ 500 ppm that however did not display a dose-response relationship and was not accompanied by corroborative clinical chemistry and histopathological findings. For details see study summary under 5.2	
4.6.2	Gross and histopathology	No treatment related effects	
4.7	**Other**	Not applicable	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Flufenoxuron was fed to five groups of 10 male and 10 female B6C3F_1 ("C57/C3H F_1 hybrid") mice [XXXX; age at study initiation: 6-8 weeks] at dietary concentrations of 50; 500; 5,000; 10,000 and 50,000 ppm for 90 days. A concurrent control group of 20 males and 20 females was fed basal diet. Additional 10 mice/sex/group and 20 control group mice/sex were included to provide blood samples for clinical chemistry analysis.

All animals were subjected to careful clinical examination twice daily during weekdays and once daily at weekends. Ophthalmological examination was performed on 5 mice of each sex from the control and the high-dose group before and after the 90-day study period. Body weights and food consumption were determined at weekly intervals for all animals. Blood samples were taken at termination of the study for assessment of clinicochemical and hematological parameters including assessment of methemoglobin (using a specific detection method according to Evelyn and Malloy). All animals were subjected to detailed post-mortem examination and all macroscopic abnormalities recorded.

Terminal body weights and the weights of the liver, spleen, kidneys, testes, heart, brain, uterus and adrenal glands were recorded. Histopathology was performed on a comprehensive set

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1-2 Oral administration (90-day study) - Mice

		of organs from rats of the control and 50,000 ppm group, and on lungs, liver and kidney tissues from all test groups.
5.2	**Results and discussion**	The test substance was judged to be stable for the duration of the study. All diets were shown to be within ±11% of the nominal concentration and to be mixed homogeneously. The test substance intake is summarized in Table 6.4.1/22. Two females from 5,000 ppm group, two males from 10,000 ppm group, and one female from the 50,000 ppm group either died or were sacrificed when moribund during the study There were no findings at necroscopy indicative of the cause of death. In view of the absence of specific pathological findings and the lack of a dose-response relationship, these deaths were not considered to be test substance-related. Clinical signs were limited to pale feces for males and females fed 50,000 ppm, which was attributed to unabsorbed test material. Food consumption was generally comparable for all groups. There was no effect on body weight or body weight gain for females at any concentration of test material. In males, mean body weights for males at 50,000 ppm were slightly but statistically significantly decreased at the end of the study period [see Table 6.4.1/23]. Results of hematological and clinicochemical examinations are summarized in Table 6.4.1/24. Hematology evaluations revealed minimal albeit statistically significant decreases in erythrocyte count, hemoglobin and hematocrit for males at 50,000 ppm compared to controls. In addition, both males and females showed a statistically significant and generally dose-related increase in serum bilirubin from 500 to 50,000 ppm, as compared to controls. These aforementioned hematological and clinicochemical changes were indicative of a slight anemia. There was no evidence of methemoglobinemia. Additional clinical chemistry changes included statistically significant decreases in blood urea nitrogen for males at 50,000 ppm and for females at 10,000 and 50,000 ppm compared to controls. These decreases were dose-related in females. Liver weights adjusted for terminal body weights were marginally increased over control values, attaining statistical significance in both sexes at 500 ppm and higher dose levels [by up to 8%, see Table 6.4.1/25]. However, given the lack of a convincing dose-response relationship and in the absence of any corresponding clinical chemistry or histopathology changes, it must be doubted that these increases were related to treatment. There were no treatment-related gross pathological or histopathological changes observed.

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1-2 Oral administration (90-day study) - Mice

5.3	**Conclusion**	Based on increased serum bilirubin at 500 ppm in males and females, the NOAEL for this study was 50 ppm (equivalent to an average daily intake of approx. 10 mg/kg bw/d for males and 12 mg/kg bw/d for females.
5.3.1	LO(A)EL	500 ppm, equivalent to daily intakes of about 103 mg/kg bw in males and 124 mg/kg bw in females
5.3.2	NO(A)EL	50 ppm; equivalent to about 10 mg/kg b.w/day for male and 12 mg/kg b.w./day for female mice
5.3.3	Other	None
5.3.4	Reliability	1
5.3.5	Deficiencies	No

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1-2 Oral administration (90-day study) - Mice

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 3.1.2.2. Purity *96.6% at TO and then decrease until 94.1%* 3.4.1.2. Mortality *Twice daily on weekdays and once daily on weekends and holidays*
Results and discussion	**Revisions/Amendments:** 4.5.2 Clinical chemistry […] Moreover, we can observe a decrease of glucose for females 5000 ppm (table 3 page 17) and an increase of bilirubin for both sexes. 4.1.2. Mortality 5 mice died or were killed during the study but the mortality cause was unknown
Conclusion	Agree with the applicant's version LO(A)EL: 500 ppm NO(A)EL: 50 ppm
Reliability	1
Acceptability	Acceptable
Remarks	-
	6 COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.4.1/22 Test substance intake

Dietary dose level	**Mean test substance intake (mg/kg bw/d)**	
(ppm)	**Males**	**Females**
50	10	12
500	103	124
5,000	1,069	1,247
10,000	2,139	2,482
50,000	11,071	12,619

Table 6.4.1/23 Body weight changes

Treatment (ppm)		**0**	**50**	**500**	**5,000**	**10,000**	**50,000**
Body weight [g] (week 13)	M	33.4	34.0(102%)	32.4 (98%)	32.7 (98%)	32.2 (97%)	30.7 (93%)
	F	27.5	27.1 (99%)	27.5(100%)	26.1 (95%)	27.9(101%)	26.8 (97%)
Body weight gain [g] (week 1-13)	M	10.4	11.2(108%)	8.9 (86%)	10.3 (99%)	9.2 (88%)	8.1 (78%)
	F	7.4	7.1 (96%)	7.5(101%)	6.2 (84%)	7.8(105%)	7.3 (99%)

Statistical evaluation; ** = p<0.01 (William's test)

Table 6.4.1/24 Hematological and clinicochemical findings

Treatment (ppm)		**0**	**50**	**500**	**5,000**	**10,000**	**50,000**
RBC [10^{12}/l]	M	10.12	10.10	10.13	10.00	10.02	9.68**
	F	10.31	10.28	10.18	9.97	10.38	10.21
Hemoglobin[g/dl]	M	15.7	15.6	15.6	15.5	15.5	15.1**
	F	16.1	15.9	15.9	15.8	16.2	16.0
Hematocrit [ratio]	M	0.453	0.455	0.455	0.448	0.451	0.435**
	F	0.459	0.456	0.459	0.450	0.465	0.454
Reticulocytes [%]	M	0.4	N.D.	N.D.	N.D.	N.D.	0.6
	F	0.4	N.D.	N.D.	N.D.	N.D.	0.4
Myeloid/erythroid ratio	M	2.5	N.D.	N.D.	N.D.	N.D.	2.2
	F	2.2	N.D.	N.D.	N.D.	N.D.	2.1
Methemoglobin [%]	M	0.4	0.3	0.3	0.3	1.2	0.2
	F	0.3	0.3	0.2	0.1	0.6	0.5
Bilirubin	M	2.9	3.3	4.4**	5.5**	6.5**	6.3**
	F	4.2	4.5	5.3**	5.8**	5.9**	6.7**
Triglyceride	M	2.15	1.99	2.12	1.86	1.51**	1.59**
	F	1.64	1.64	1.57	1.53	1.50	1.31
Urea nitrogen		11.7	11.3	10.7	10.3*	10.5	9.9**
		10.9	10.2	10.7	10.5	9.7*	9.1**

Statistical evaluation: * = p< 0.05; ** = p<0.01 (William's test)

Table 6.4.1/25 Organ weight changes

Treatment (ppm)		**0**	**50**	**500**	**5,000**	**10,000**	**50,000**
Terminal body weight [g]	M	33.7	34.5	33.9	32.1	32.8	30.8**
	F	28.1	27.3	27.9	26.9	28.3	26.4
Liver weight, absolute [g]	M	1.59	1.69	1.69	1.66	1.68	1.56
	F	1.44	1.42	1.50	1.48	1.54	1.50
Liver weight, adjusted for bw [g]	M	1.57	1.64	1.68*	1.68*	1.69*	1.64*
	F	1.42	1.43	1.49*	1.50*	1.51**	1.54**
Heart weight, absolute [g]	M	0.17	0.17	0.17	0.17	0.18	0.18*
	F	0.14	0.14	0.14	0.14	0.15*	0.15*
Heart weight, adjusted for bw [g]	M	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	F	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Statistical evaluation: * = p< 0.05; ** = p<0.01 (William's test); N.S. = Covariance relationship is not statistically significant

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (90-day study) - Dog

			Official use only
		1 REFERENCE 3	
1.1	**Reference**	**7) XXXX** WL115110: 13 week oral toxicity study in dogs XXXX unpublished XXXX **8) XXXX** Addendum to XXXX - WL115110: A 13 week oral toxicity study in dogs XXXX unpublished XXXX **Note:** The Corrigendum to the original report consists of 3 pages, stating that the study was conducted also in accordance to GLP requirements of the JMAFF (Japanese authority) **9) XXXX** Supplement to XXXX report no. XXXX (WL115110: 13 week oral toxicity study in dogs). A 13 week no effect level XXXX unpublished XXXX **Note:** The Supplement to the original report consists of 10 pages, comparing study results with findings of the 52-week dog study [see XXXX] in support of establishing a NOAEL for the 3-month dog study. **10) XXXX** Supplement to XXXX report no. XXXX (WL115110 : 13 week oral toxicity study in dogs) XXXX unpublished XXXX **Note:** The supplement to the original report consists of 7 pages, presenting evidence for possible contamination of control dogs; the implication on validity of the study is discussed.	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data	No data protection claimed	

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (90-day study) - Dog

	protection		
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	EPA 82-1; in general compliance with OECD 409	
2.2	**GLP**	Yes (Laboratory certified by the Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)	
2.3	**Deviations**	Yes, (a) Analyses of feed and renal fat suggested that control group dogs might have been exposed to the test substance, although the possibility could not be ruled out that contamination of feed and renal fat occurred during sampling of the probes. However, hematological parameters determined in control group dogs were generally within the historical control range. (b) Because a diet formulation error resulted in underdosing of the 5,000 ppm animals for the first 2 weeks of treatment, the duration of dietary test substance administration was extended to 15 weeks for all groups.	X
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch: XXXX)	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	As given in section 2	
3.1.2.2	Purity	98%	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Dog	
3.2.2	Strain	Beagle dog	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age at study initiation: 5-7 months	
3.2.6	Number of animals per group	4/sex/group	
3.2.7	Control animals	Yes, 4/sex	

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (90-day study) - Dog

3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	90 days	X
3.3.2	Frequency of exposure	Daily	
3.3.3	Postexposure period	None	
3.3.4	Oral		
3.3.4.1	Type	In food	
3.3.4.2	Concentration	500; 5,000 and 50,000 ppm equivalent to mean daily compound intakes of 18; 263 and 1,961 mg/kg bw in males and 21; 173 and 1,988 mg/kg bw in females	X
3.3.4.3	Vehicle	None	
3.3.4.4	Concentration in vehicle	Not applicable	
3.3.4.5	Total volume applied	Not applicable	
3.3.4.6	Controls	Basal diet	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Daily	
3.4.1.2	Mortality	Daily	
3.4.2	Body weight	Weekly intervals	
3.4.3	Food consumption	Daily	
3.4.4	Water consumption	Quantitative measurements of water consumption over a 24-h period were monitored pretrial and prior to the completion of the treatment period	
3.4.5	Ophthalmoscopic examination	Undertaken pretrial and prior to the completion of the treatment period	
3.4.6	Haematology	Yes, all animals at weeks –1, 9, 12 and 15. Additional haematological investigations were also undertaken during Week 2 (all animals), Week 4 (0 and 5,000 ppm) and limited parameters were assayed for all animals in Week 12.	

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (90-day study) - Dog

3.4.7	Clinical Chemisty	At weeks –1, 9 and 15
3.4.8	Urinalysis	No
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	At study termination in all surviving dogs
3.5.2	Gross and histopathology	All animals on study
3.5.3	Other examinations	Electrocardiographic measurements were undertaken pretrial and prior to the completion of the treatment period
3.5.4	Statistics	Haematology, clinical chemistry, organ weight data and body weight gain were analysed for homogeneity of variance using the 'F-max' test. If the group variance appeared homogeneous a parametric ANOVA was used and pairwise comparisons made via Student's t-test. If the variances were inhomogeneous, log or square root transformations were used in an attempt to stabilise the variances. For consistency the means presented in summary tables are the untransformed values although the accompanying indicators of levels of significance may have been derived from log or square transformed means.
3.6	**Further remarks**	None
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Limited to pallor of the gums and/or sclera for several 50,000 ppm male and female animals during weeks 13, 14 and 15; toxicological significance unclear since comparable signs were not observed in 1-year dog study. For details see study summary under 5.2.
4.1.2	Mortality	No mortalities at any concentration level
4.2	**Body weight gain**	Comparable to the control
4.3	**Food consumption and compound intake**	Comparable to the control.
4.4	**Ophtalmoscopic examination**	Comparable to the control.

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (90-day study) - Dog

4.5	**Blood analysis**		
4.5.1	Haematology	Mild anaemia at all dose levels. For details see study summary under 5.2.	X
4.5.2	Clinical chemistry	Increased cholesterol levels in males at ≥ 5,000 ppm. For details see study summary under 5.2.	
4.5.3	Urinalysis	Not applicable	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	Increased absolute and relative liver weights in males at ≥ 5,000 ppm. For details see study summary under 5.2	
4.6.2	Gross and histopathology	No treatment-related macroscopic changes; bone marrow hyperplasia and increased deposition of yellow pigment in bone marrow, see study summary under 5.2	X
4.7	**Other**	No treatment-related findings. For details see study summary under 5.2	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Flufenoxuron was fed to three groups of 4 male and 4 female Beagle dogs [source: XXXX; age at study initiation: 5–7 months] at dietary concentrations of 500; 5,000 and 50,000 ppm. A concurrent control group of 4 males and 4 females was fed basal diet. Because a diet formulation error resulted in underdosing of the 5,000 ppm animals for the first 2 weeks of treatment, the duration of dietary test substance administration was extended to 15 weeks for all groups.

Diets containing Flufenoxuron at the required concentrations were prepared at the beginning of each study week. The test substance was incorporated directly into plain ground diet and mixed in a Winkworth Change Drum Mixer for 20 min. Diet samples were assayed by the Sponsor in respect of the following: Homogeneity of the achieved mix was assessed by taking 100 g samples of diet from 6 positions immediately after the diet had been mixed. This was undertaken for all treatment groups including Controls for Week 1 diet mixes. Stability was also assessed for all treatment groups by taking 2 x 100 g random samples on day 7 from the batches of diet mixed for Week 1. As the diet samples assays performed by the Sponsor gave unexpected results, all archive samples retained for all dose groups during the study period were analysed at the test facility using the method supplied by the Sponsor.

The animals were observed daily for any signs of ill health or

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (90-day study) - Dog

		reaction to treatment. Food consumption was monitored daily and body weight measurements were undertaken at weekly intervals throughout the dosing period. Quantitative measurements of water consumption over a 24-h period were monitored pretrial and prior to the completion of the treatment period. Ocular examinations and electrocardiographic measurements were undertaken pretrial and prior to the completion of the treatment period. Laboratory investigations of haematology and clinical chemistry were undertaken pretrial, during weeks 9 and 15 of the treatment period. Determination of methemoglobin and sulfhemoglobin were determined using the method of Evelyn and Malloy [see II A 5.8.2/1, XXXX Evelyn K.A., Malloy H.T. 1938]. This method is based on binding of methemoglobin to cyanide and was used in order to circumvent false-positive findings known to occur with acylurea derivatives such as Flufenoxuron when using the CO-Oxymeter method. Additional hematological assays were also undertaken during weeks 2, 4 and 12 of the treatment period. All the animals were subjected to detailed gross and histopathological examination and organ weight analysis.
5.2	**Results and discussion**	Diets were shown to be acceptably uniform when mixed, and to be stable over a 7-day storage period. Concentrations were close to the target values, except that the 5,000 ppm diet made on 20th October 1986 was formulated, in error, to be 1,000 ppm. Because this diet formulation error resulted in underdosing of the 5,000 ppm animals for the first 2 weeks of treatment, the duration of dietary test substance administration was extended to 15 weeks for all groups. Analyses of all feed samples taken at weekly intervals from all treatment and control groups were performed by the test laboratory. These analyses did not indicate any contamination of control group feed, and treatment group samples showed values that were in the expected range. Selected diets were analysed for Flufenoxuron in parallel by the sponsor. The samples of diet taken from the control formulated on 20th October 1986 contained from 270 to 1,215 ppm of Flufenoxuron. The control diet mixed on 17th November 1986 was satisfactory (the only other diet sample from the control group that was reanalyzed by the sponsor). Thus, by comparison of the analyses from the test laboratory and the sponsor, it appears that contamination of the feed probably occurred during sampling of the probe prior to shipping to the sponsor. For further investigation, renal fat samples were taken from each dog at necropsy and analysed for Flufenoxuron by the sponsor. In control group dogs, Flufenoxuron concentrations in renal fat

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (90-day study) - Dog

ranged from 11–69 ppm, suggesting that the control group dogs were contaminated with Flufenoxuron either during the treatment period or the during sampling of the renal fat specimens at necropsy. Over the range 500–5,000 ppm a tenfold increase in the concentration of Flufenoxuron resulted in an approx. two-fold increase of Flufenoxuron residue in renal fat [see Table 6.4.1/26].

The study authors discussed the possibility that Flufenoxuron residues found in renal fat samples of control group dogs could have been caused indirectly as a result of high-dose treatment (50,000 ppm), which might have led to contamination of the diet mix area and the animal house via high-dose group faeces or via the intestines at post-mortem. Thus, contamination of control-group dogs could either have occurred during the in-life phase of the study or post-mortem (e.g. via working surfaces, dissecting boards, instruments contaminated during necropsy of high-dose group dogs).

Due to the possible contamination of control group dogs, the effects observed in control and test groups of this study should also be compared to historical control values in order to avoid false-negative findings. Reassurance can be obtained by comparison of the results with findings of the 52-week dog study.

Mean intakes of the test substance were calculated on the basis of food consumption and body weight data and are summarized in Table 6.4.1/27.

In this 90-day dog study, no treatment-related mortalities were observed. Clinical signs were limited to pallor of the gums and/or sclera for several 50,000 ppm male and female animals during weeks 13, 14 and 15. These findings are of doubtful clinical significance since they were not observed in the one-year dog study at an identical dietary concentration of test material (see IIIA 6.4.1-4 XXXX). There were no treatment-related effects on food or water consumption or ophthalmoscopic parameters. There were no effects on body weight or body weight gain for males and females at any concentration of test material.

Electrocardiograms revealed a slightly prolonged Q-T interval for all females at 50,000 ppm as compared to controls. Although statistically significant, this finding was not considered toxicologically significant since there were no other related electrocardiographic abnormalities or microscopic changes observed in the heart. Moreover, a similar change was not observed at 26 or 51 weeks in the one-year dog study at an identical dietary concentration (see IIIA 6.4.1-4 XXXX).

Hematological examinations revealed that a mild anemia was apparent for males and females at weeks 9, 12 and 15 of

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (90-day study) - Dog

treatment at all dietary concentrations of the test material [see Table 6.4.1/28 and Table 6.4.1/29]. For males, generally statistically significant and dose-related decreases in erythrocyte count; hemoglobin and hematocrit were noted after 9 weeks of treatment at 500; 5,000 and 50,000 ppm as compared to the control group. After 12 and 15 weeks of treatment, statistically significant effects were confined to the 50,000 ppm group males. Generally dose-related reductions in the majority of the red blood cell parameters were also noted for females at 500; 5,000 and 50,000 ppm; however, these decreases were not statistically significant. A statistically significant increase in reticulocytes was also observed for males at 5,000 and 50,000 ppm, as compared to controls. Non-statistically significant increases in reticulocytes were noted for females at 5,000 and 50,000 ppm and in males at 500 ppm.

Measurement of methemoglobin and sulfhemoglobin revealed dose-related increases in both sexes, which were statistically significant at 5,000 ppm and above after 9 and 15 weeks of treatment (in treatment weak 9 also at 500 ppm in females) [see Table 6.4.1/30].

The presence of methemoglobin was accompanied by small, statistically significant and dose-related increases in sulfhemoglobin in males at 5,000 and 50,000 ppm. Sulfhemoglobin was also increased in females at 5,000 and 50,000 ppm, but these increases were only statistically significant at 50,000 ppm. Similar findings were not observed in rodent studies using the Evelyn and Malloy detection method. However, dogs may be more sensitive to oxidative anemia compared to rodents, given the lower (5- to 10-fold) spontaneous methemoglobin reductase activity in dog erythrocytes compared to rat and mouse erythrocytes (see Smith, R. P. (1991). Toxic responses of the blood. *In* Casarett and Doull's Toxicology (fifth edition); Klaassen, C. D. (editor). McGraw-Hill, pgs. 335 - 354).

Statistically significant increases in serum cholesterol values were observed after 8 weeks of treatment in males and females at 50,000 ppm, as compared to controls. Slight increases in serum cholesterol that attained statistical significance compared to control levels were also noted in the 5,000 and 50,000 ppm group males at study termination [see Table 6.4.1/31].

Absolute liver weights were significantly increased in all treated male groups, however, this was not dose dependent. In contrast, a dose-related and statistically significant increase of relative liver weights (organ to body weight ratio) was observed in males at $\geq$ 5,000 ppm only. There were no other treatment-related

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (90-day study) - Dog

		effects on organ weights [see Table 6.4.1/31]. There were no treatment-related macroscopic changes. Histopathological changes [see Table 6.4.1/31] included bone marrow hyperplasia for all dogs in the 5,000 and 50,000 ppm groups and for 3 males and 2 females in the 500 ppm group. These bone marrow changes likely reflect a compensatory response to the slight anemia observed at all dietary concentrations of test material. Bone marrow hyperplasia was accompanied by increased deposition of yellow pigment in the bone marrow of 4 males and 3 females in the 50,000 ppm group and 3 females in the 5,000 ppm group. This yellow pigment is likely hemosiderin, which is associated with erythrocyte breakdown. Increased yellow pigment deposition (probably hemosiderin) was also observed in the proximal tubules of the kidney in 2 male dogs at 50,000 ppm. In the liver, increased Kupffer cell pigmentation, indicative of mild anemia, was noted in all animals at 50,000 ppm, 7 animals (4 males and 3 females) at 5,000 ppm, and one female at 500 ppm.	
5.3	**Conclusion**	Hematological and histopathological changes indicative of slight anemia were observed at all dose levels tested (500 ppm and above). Therefore, the NOAEL for the 90-day dietary toxicity study in dogs is less than 500 ppm (corresponding to daily intakes of 18 mg/kg bw in males, 21 mg/kg bw in females, and 20 mg/kg bw for the combined sexes).	
5.3.1	LO(A)EL	500 ppm, equivalent to about 18 mg/kg bw in male and 21 mg/kg bw in female dogs	
5.3.2	NO(A)EL	No NOAEL observed	
5.3.3	Other	None	
5.3.4	Reliability	1	X
5.3.5	Deficiencies	No	X

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (90-day study) - Dog

Materials and Methods	**Revisions/Amendments:** In general compliance with OECD 409 with some deviations: (a) contamination of the controls (b) 15 weeks instead of 13 weeks (c) no urinalysis 3.3. Duration of the treatment 15 weeks or 105 days 3.3.4.2. Concentrations 500 ppm (0.05%), 5000 ppm (0.5%) and 50000 ppm (5%) equivalent to *[…]*
Results and discussion	Agree with the applicant's version **Revisions/Amendments:** 4.5.1. Haematology *Mild anemia at all dose levels and* increased levels of MetHb and SHb for all the doses since the week 4 4.6.2. Histopathology *No treatment-related macroscopic changes;* bone marrow hyperplasia observed for all groups, increase of Kupffer cell pigmentation of the liver (in all males from the 2 highest dosage groups and in all female groups) and increase in the pigment deposition in the proximal tubule of the kidneys (for 2 high dose males).
Conclusion	Agree with the applicant's version LO(A)EL: 500 ppm NO(A)EL: no observed **Revisions/Amendments:** 5.3.5. Deficiencies Yes (to be listed)
Reliability	2
Acceptability	Acceptable
Remarks	-

	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (90-day study) - Dog

Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.4.1/26 Flufenoxuron concentrations in dog renal fat sampled at necroscopy

Dose group	**Concentration of Flufenoxuron found in the renal fat [ppm]**		
[ppm]	**Minimum**	**Maximum**	**Average**
0	11	69	40
500	148	208	173
5,000	322	478	407
50,000	580	850	715

Table 6.4.1/27 Test substance intake

Dietary dose level	**Approx. mean test substance intake (mg/kg bw/d)**		
(ppm)	**Males**	**Females**	**Combined**
500	18	21	20
5,000*	163	182	173
50,000	1,961	2,039	1,988

* Animals of the mid-dose group were inadvertently fed 1,000 ppm instead of 5,000 ppm for the first two weeks of treatment; this deviation was taken into account for calculation of Flufenoxuron intake

Table 6.4.1/28 Hematological findings (Weeks 4 and 9)

Test parameter		**Treatment (ppm)**					
		Week 4			**Week 9**		
		0	**5,000[a]**	**0**	**500**	**5,000[a]**	**50,000**
RBC [10^{12}/l]	M	6.61	6.10*	6.75	5.97**	5.80***	5.49***
	F	6.82	6.20	6.89	6.27	6.05	5.90
Hemoglobin[g/dl]	M	14.9	13.3	15.8	13.5**	13.4***	13.1***
	F	15.3	13.8	15.6	14.7	14.2	14.3
Hematocrit [ratio]	M	0.449	0.407	0.467	0.417**	0.420**	0.410**
	F	0.458	0.418	0.472	0.447	0.441	0.437
MCV [fl]	M	67	67	69	69	72*	75**
	F	67	67	69	71	72*	74**
MCHC [g/dl]	M	33.9	33.4	34.7	33.3**	32.5***	32.6***
	F	34.1	33.8	33.6	33.7	32.9	33.3
Reticulocytes [%]	M	0.6	0.6	0.6	1.0	1.8**	1.6**
	F	0.8	0.3	0.8	1.1	2.0	1.6

Statistical evaluation: * = p< 0.05; ** = p<0.01; *** = p<0.001 (F-max test; ANOVA; Student's t-test);
[a] = dosed with 1,000 ppm for the first 2 treatment weeks

Table 6.4.1/29 Hematological findings (Weeks 12 and 15)

		Week 12		**Week 12**				**Week 15**			
		Hist. Ctrl.[b]		**Dose levels (ppm)**				**Dose levels (ppm)**			
		Range	**Mean**	**0**	**500**	**5,000[a]**	**50,000**	**0**	**500**	**5,000[a]**	**50,000**
RBC [10^{12}/l]	M	5.66–7.73	6.70	6.60	6.30	6.14	5.45***	6.53	6.21	6.04	5.41**
	F	5.72–7.89	6.81	6.81	6.21	6.22	6.14	6.82	6.55	6.12	6.33
Hemoglobin [g/dl]	M	12.6–17.6	15.1	15.1	14.4	14.2	13.0***	15.0	13.9	13.7	12.9
	F	12.9–18.1	15.5	15.6	14.5	14.7	14.8	15.6	15.1	14.1	15.3
Hematocrit [ratio]	M	0.39–0.53	0.458	0.461	0.438	0.437	0.406	0.449	0.423	0.421	0.401
	F	0.39–0.55	0.468	0.470	0.442	0.448	0.452	0.470	0.454	0.432	0.460
MCV [fl]	M	63–73	68	69	69	70	74	68	67	68	73*
	F	64–72	68	68	70	71	73	68	68	70	71
MCHC [g/dl]	M	32.2–35.9	34.0	34.8	34.3	34.1	33.4**	34.1	33.5	33.2	33.0
	F	32.6–35.6	34.1	34.6	34.3	34.3	34.2	33.9	34.0	33.2*	34.0
Reticulocytes [%]	M	0.2–1.3	0.7	N.D.	N.D.	N.D.	N.D.	0.4	1.5	1.0	1.5
	F	0.2–1.8	0.8	N.D.	N.D.	N.D.	N.D.	1.0	0.7	1.0	1.3

N.D.= not determined; [a] = dosed with 1,000 ppm for the first 2 treatment weeks

[b] = Historical control data from Inveresk Res. Intl. (included in the report, approx. 200 dogs/sex)

Statistical evaluation: * = $p< 0.05$; ** = $p<0.01$; *** = $p<0.001$ (F-max test; ANOVA; Student's t-test)

Table 6.4.1/30 Methemoglobin and sulfhemoglobin determinations (Weeks 9 and 15)

		Week 9				Week 15			
Treatment (ppm)		**0**	**500**	**5,000[a]**	**50,000**	**0**	**500**	**5,000[a]**	**50,000**
Methemoglobin [%]	M	0.80	1.07	1.42***	1.82***	0.61	0.99	1.46***	1.88***
	F	0.79	1.10**	1.30***	1.80***	0.65	0.87	1.23***	1.69***
Sulfhemoglobin [%]	M	0.12	0.23	0.33*	0.46***	0.10	0.16	0.32***	0.39***
	F	0.28	0.14	0.23	0.35	0.12	0.15	0.25	0.43*

Statistical evaluation: * = p< 0.05; ** = p<0.01; *** = p<0.001 (F-max test; ANOVA; Student's t-test)
[a] = dosed with 1,000 ppm for the first 2 treatment weeks

Table 6.4.1/31 Clinicochemical, organ weight and histopathological changes (Week 15)

Test parameter		**Dose levels (ppm)**			
		0	**500**	**5,000**	**50,000**
Clinicochemical findings					
Cholesterol [mM]	M	3.0	3.5	4.6*	5.0**
	F	3.2	3.3	4.3	4.0
Terminal bw and organ weights					
Terminal body weight [kg]	M	11.1	11.7	11.6	10.7
	F	10.2	9.3	10.3	10.0
Abs. liver weight [g]	M	303.64	356.32*	406.88***	389.21**
	F	323.46	275.17**	331.78	308.71
Rel. liver weight [% bw] (% control level)	M	2.75 (100%)	3.06 (111%)	3.52** (128%)	3.64*** (132%)
	F	3.18 (100%)	2.95 (93%)	3.24 (102%)	3.11 (98%)
Histopathological findings					
Liver, increased Kupffer-cell pigmentation	M	0 / 4	0 / 4	4 / 4$	4 / 4$
	F	0 / 4	1 / 4	3 / 4	4 / 4$
Kidney, increased yellow pigment deposition in proximal tubules	M	0 / 4	0 / 4	0 / 4	2 / 4
	F	0 / 4	0 / 4	0 / 4	0 / 4
Spleen, increased hemosiderin	M	0 / 4	0 / 4	0 / 4	1 / 4
	F	0 / 4	0 / 4	0 / 4	1 / 4
Bone marrow, hyperplasia	M	0 / 4	3 / 4	4 / 4$	4 / 4$
	F	0 / 4	2 / 4	4 / 4$	4 / 4$
Bone marrow, increased yellow pigment deposition	M	0 / 4	0 / 4	0 / 4	4 / 4$
	F	0 / 4	0 / 4	3 / 4	3 / 4

Statistical evaluation: * = p< 0.05; ** = p<0.01 (F-max test; ANOVA; Student's t-test); $ = p < 0.05 (Fisher Exact Test)

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (52-week study) - Dog

			Official use only
		1 REFERENCE 4	
1.1	**Reference**	**11) XXXX** WL 115110: 52 week oral toxicity study in dogs XXXX unpublished XXXX **12) XXXX** Addendum to XXXX - WL 115110: 52 week oral toxicity study in dogs, XXXX, unpublished XXXX **Note:** The addendum contains 3 pages to correct typographical errors of the original study report (page 4; page 38) and to include spleen in list of organs to be weighed (page 26)	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	EPA 83-1; compliant with OECD 452	
2.2	**GLP**	Yes (laboratory certified by the Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch: XXXX	
3.1.2	Specification	As given in section 2	

Section A6.4		**Repeated dose toxicity**	
BPD Annex Point IIA, VI.6.4		6.4.1 Oral administration (52-week study) - Dog	
3.1.2.1	Description	As given in section 2	
3.1.2.2	Purity	98%	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Dog	
3.2.2	Strain	Beagle	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age at study initiation: 4.5–6.5 months	
3.2.6	Number of animals per group	4/sex/group	
3.2.7	Control animals	Yes, 4/sex	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	90 days	X
3.3.2	Frequency of exposure	Daily	
3.3.3	Postexposure period	None	
3.3.4	Oral		
3.3.4.1	Type	In food	
3.3.4.2	Concentration	10; 100;500 and 50,000 ppm equivalent to average daily compound intakes of 0.37; 3.7; 19 and 2018 mg/kg bw in males and 0.39; 3.8; 20 and 1,879 mg/kg bw in females, respectively.	
3.3.4.3	Vehicle	None	
3.3.4.4	Concentration in vehicle	Not applicable	
3.3.4.5	Total volume applied	Not applicable	
3.3.4.6	Controls	Basal diet	
3.4	**Examinations**		
3.4.1	Observations		

Section A6.4		**Repeated dose toxicity**	
BPD Annex Point IIA, VI.6.4		6.4.1 Oral administration (52-week study) - Dog	
3.4.1.1	Clinical signs	Daily	
3.4.1.2	Mortality	Daily	
3.4.2	Body weight	Weekly for the first 13 weeks and thereafter at monthly intervals up until the end of the study period	
3.4.3	Food consumption	Daily	
3.4.4	Water consumption	Quantitative measurements of water consumption over a 24-h period were monitored pretrial and during weeks 12, 25, 38 and 50 of the treatment period.	
3.4.5	Ophthalmoscopic examination	Pretrial and during week 51 of the treatment period	
3.4.6	Haematology	In all animals at weeks 27 and 52; additional examinations were performed at weeks 5, 13, and 40.	X
3.4.7	Clinical Chemisty	In all animals at weeks 27 and 52; additional examinations were performed at weeks 5, 13, and 40.	X
3.4.8	Urinalysis	In all animals at weeks 27 and 52; additional examinations were performed at weeks 5, 13, and 40.	X
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Adrenals, brain, gall-bladder, heart, kidneys, liver, ovaries, testes, thyroid with parathyroid	
3.5.2	Gross and histopathology	On all animals and all tissues collected	
3.5.3	Other examinations	Electrocardiographic measurements were carried out pretrial and during weeks 26 and 51 of the treatment period.	
3.5.4	Statistics	Haematology, clinical chemistry, organ weight data and body weight gain were analysed for homogeneity of variance using the 'F-max' test. If the group variance appeared homogeneous a parametric ANOVA was used and pairwise comparisons made via Student's t-test. If the variances were inhomogeneous, log or square root transformations were used in an attempt to stabilise the variances. For consistency the means presented in summary tables are the untransformed values although the accompanying indicators of levels of significance may have been derived from log or square transformed means. Organ weight data were additionally treated by covariance analysis in order to stabilise values dependent upon body weight.	
3.6	**Further remarks**	None	

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (52-week study) - Dog

		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	No treatment related clinical signs	
4.1.2	Mortality	No treatment related mortality	
4.2	**Body weight gain**	No treatment related effects	
4.3	**Food consumption and compound intake**	No treatment related effects	
4.4	**Ophtalmoscopic examination**	No treatment related effects	
4.5	**Blood analysis**		
4.5.1	Haematology	Mild anemia at ≥ 500 ppm characterized by a decrease of hemoglobin levels and erythrocyte counts with a concomitant increase in reticulocyte count and sulfhemoglobin, as compared to controls. For details see study summary under 5.2	X
4.5.2	Clinical chemistry	No treatment related adverse effects after 6-12 months of treatment	
4.5.3	Urinalysis	No treatment-related urinalysis findings or positive fecal occult blood analyses	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	Increase absolute liver weight and liver weight at 500 and 50,000 ppm, see study summary under 5.2	
4.6.2	Gross and histopathology	No treatment related macroscopic changes; bone marrow hyperplasia with increased deposition yellow/brown pigment, see study summary under 5.2	X
4.7	**Other**	There were no electrocardiographic changes that could be associated with treatment. Heart rate, interval data and waveforms were all considered normal for the animals.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Flufenoxuron was fed to four groups of 4 male and 4 female beagle dogs [source: XXXX; age at study initiation: 4.5–6.5 months] at dietary concentrations of 10; 100; 500 and 50,000 ppm for 52 consecutive weeks. A concurrent control group of 4 males	

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (52-week study) - Dog

and 4 females was fed basal diet.

Due to contamination of control group animals with Flufenoxuron in the 90-day dog study (i.e. Flufenoxuron was found at quantifiable levels in control group feed and in renal fat samples; [see XXXX], the following measures were taken to avoid cross-group contamination:

- Grouping of dogs in pens in treatment-group blocks in ascending dose order
- Food troughs were labelled per dose group (to avoid contamination during routine cleaning procedures)
- Food troughs for the control group were washed, cleaned and allowed to dry in a separate area remote from the animal holding quarters (to avoid any possible transfer of test material from food troughs used with treated diets)
- Pens were cleaned daily in an ascending dose group order using group-specific utensils. Solid faeces were removed with a shovel, followed by washing of the floors
- Diet for the control group was stored in a separate area; all handling procedures such as weighing of the food ration and residues were done remote from the treated groups
- Sampling of whole blood for Flufenoxuron analyses was conducted using disposable gloves between each animal and storing blood samples in separate containers for each dose group. Similar precautions were undertaken when handling samples for analysis.
- Sampling of renal fat for Flufenoxuron analyses was conducted using clean surgical instruments and disposable gloves for each animal. Individual samples were wrapped in foil and bagged separately for each dose group.

The animals were observed daily for any signs of ill health or reaction to treatment. Food consumption was monitored daily and body weight measurements were undertaken weekly for the first 13 weeks and thereafter at monthly intervals up until the end of the study period. Quantitative measurements of water consumption over a 24-h period were monitored pretrial and during weeks 12, 25, 38 and 50 of the treatment period. Ocular examinations were performed pretrial and during week 51 of the treatment period. Electrocardiographic measurements were carried out pretrial and during weeks 26 and 51 of the treatment period. Laboratory investigations of hematology, clinicochemistry and urinalysis were undertaken pretrial and

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (52-week study) - Dog

		during weeks 27 and 52 of the treatment period. Additional hematological investigations were also undertaken during weeks 5, 13 and 40 of the treatment period. Blood samples were also taken at Weeks 13, 29 and 40 during the study and at termination for monitoring Flufenoxuron levels in blood. All the animals were subjected to detailed gross post mortem examination and organ weight analysis. The fixed tissues were dispatched to Sittingbourne Research Centre for detailed histopathologicaI evaluation.
5.2	**Results and discussion**	Acceptable results on dietary concentration of Flufenoxuron, its homogeneity and stability within the diet were obtained, the values remaining within the test protocol specifications. Test substance intakes calculated from weekly mean values for body weight and feed consumption are summarised in the table Table 6.4.1/32. Analyses of whole blood samples taken at study termination revealed evidence of low level contamination of two male dogs from the control group with the test substance [see Table 6.4.1/33]. The authors of the study report explained that contamination of the dogs might have resulted from food trough cleaning procedures that were conducted in a wash-up area close to the two pens where these dogs were located. Furthermore, for 500 ppm dogs, an increase in blood concentration of Flufenoxuron was observed from week 29 to week 40. No further increase in blood concentration was seen in week 52. Results of Flufenoxuron analysis in renal fat performed in all treatment- and control-group dogs at termination of the study are listed in Table 6.4.1/34. With increasing dose level, a corresponding increase in Flufenoxuron concentration in fat was observed. A low level of Flufenoxuron was also detected in the fat of control group animals, which was approx. 2-3fold above the limit of quantitation. Although precautions had been taken to minimise cross-group contamination even exceeding the provisions of Good Animal House standards, the results show that it was not possible to fully avoid low-level contamination of control group dogs under the conditions of the study. There were no treatment-related mortalities or clinical signs of toxicity observed during the study period. There were no treatment-related effects on food or water consumption or on ophthalmoscopic or electrocardiographic parameters. There were no effects on body weight or body weight gain for males and females at any concentration of test material. Hematological data from treatment weeks 13 and 52 are

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (52-week study) - Dog

summarized in Table 6.4.1/35.

In the hematological examinations performed, evidence of a mild anemia was first observed after 5 weeks of treatment in both sexes at 50,000 ppm. By 13 weeks of treatment, mild anemia generally was visible also at 500 ppm in male and female dogs. The anemia was characterized by decreased hemoglobin and decreased erythrocyte count with a concomitant increase in reticulocyte count and sulfhemoglobin, as compared to controls. The changes in the aforementioned parameters were generally dose-related at 500 and 50,000 ppm and were maintained throughout the course of the study (i.e., at 27, 40 and 52 weeks). These changes were more frequently statistically significant in males than in females.

In addition, platelet counts were statistically significantly increased in males at 50,000 ppm from week 13 onwards, while males administered 500 ppm showed statistically significant increases in platelets from week 27 onwards. Platelet counts of females were increased at the high-dose level, albeit not statistically significant. Only a few sporadic changes in hematology parameters were noted at 10 and 100 ppm, but these incidental changes were not considered to be treatment-related because they were observed at only one time point of investigation.

Methemoglobin increases over controls reaching statistical significance and considered biologically meaningful were observed at 50,000 ppm in both sexes and to a very slight degree also at 500 ppm in females. Statistical significance obtained at lower dose levels in isolated cases were considered to be incidental.

Examination of the bone marrow revealed no significant morphological changes at 10 and 100 ppm, while at 500 and 50,000 ppm increased cellularity, increased numbers of erythrocyte precursers (normoblasts) and increased numbers of macrophages were noted. These changes in bone marrow were indicative of compensatory erythropoiesis and elevated erythrocyte turnover at 500 and 50,000 ppm.

There were no treatment-related adverse effects on clinical chemistry parameters after 6 or 12 months of treatment. Minimal increases in cholesterol that were seen in males of the 90-day oral feed study at 5,000 and 50,000 ppm were also observed in treatment group males at 50,000 ppm after 6 or 12 months of treatment. However, no statistically significant difference to control group levels could be substantiated [see Table 6.4.1/36]. There were no treatment-related urinalysis findings or positive

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (52-week study) - Dog

fecal occult blood analyses.

Organ weight findings are summarised in Table 6.4.1/37 below. Absolute liver weights and liver weights adjusted for terminal body weights were statistically significantly increased in males at 500 and 50,000 ppm when compared to control values. There were no other treatment-related effects on organ weights.

There was an increase in male liver weights at 10 ppm and 500 ppm ($p<0.05$), and 50,000 ppm ($p<0.001$) which was statistically significant for absolute values. After covariance analysis the differences were statistically significant only at 500 ppm ($p<0.05$) and 50,000 ppm ($P<0.01$) compared to controls. Female liver weights did not show any treatment-related changes that were statistically significant compared to control. However, the increased liver weight at the highest dose level of 50,000 ppm was considered to represent a treatment-related effect in females.

no treatment-related macroscopic changes were observed in this study at gross necropsy examination following 52 weeks of treatment. Histopathological changes [see Table 6.4.1/38] included bone marrow hyperplasia for all dogs at 50,000 ppm and for one female at 500 ppm. These bone marrow changes likely reflect a compensatory response to the slight anemia observed at 500 and 50,000 ppm. Bone marrow hyperplasia was accompanied by increased deposition of yellow/brown pigment (probably hemosiderin) in the bone marrow of all dogs in the 50,000 ppm group. Brown pigmentation was also noted in the proximal tubular cells of the kidneys in 4 males and 1 female at 50,000 ppm and in 1 female at 500 ppm. Fat-like vacuoles in hepatocytes were found in all dogs receiving 50,000 ppm. In addition, increased Kupffer cell pigmentation, which was identified as hemosiderin, was observed in all 50,000 ppm animals, and in 3 dogs at 500 ppm (2 females and 1 male). Increased hemosiderin deposition was also noted in the spleens of 2 males and 3 females at 50,000 ppm and 1 female at 500 ppm (with associated extramedullary hematopoiesis). The histopathological findings in the bone marrow, kidney and liver were likely related to the anemia observed at 50,000 ppm and to a lesser degree at 500 ppm. Hemosiderin deposition in the spleen was noted for 1 male at 100 ppm and for 1 male at 10 ppm. The study pathologist concluded that the isolated findings of slightly increased splenic hemosiderin in one male dog in both 100 and 10 ppm groups were not treatment-related. The amount of splenic hemosiderin in individual dogs was variable, and no increases were seen in the 500 ppm level. Moreover, there were no consistent corresponding findings of anemia at 100 or 10 ppm.

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (52-week study) - Dog

		Discussion of control group contamination with test substance The results of Flufenoxuron in blood and perirenal fat indicated a low level of contamination for the control group. This did not influence the outcome of the study results. In control group dogs, the hematology parameters (representing the most sensitive indicators of Flufenoxuron exposure) remained within normal reference ranges. In addition, no pathological changes such as hemosiderin deposition in liver or kidney were observed in control group dogs that could be related to Flufenoxuron exposure.
5.3	**Conclusion**	Based on hematological and histopathological changes indicative of mild anemia in both sexes at 500 ppm, and increased absolute liver weights and liver weights adjusted for terminal body weights in males at 500 ppm, the NOAEL for this study was 100 ppm (equivalent to an average daily intake of 3.5 mg/kg bw/d in males and 3.7 mg/kg bw/d in females).
5.3.1	LO(A)EL	500 ppm, equivalent to average daily compound intakes of about 19 mg/kg bw in males and 20 mg/kg bw in females.
5.3.2	NO(A)EL	100 ppm, equivalent to average daily compound intakes of about 3.5 mg/kg bw/d in males and 3.7 mg/kg bw/d in females dog
5.3.3	Other	None
5.3.4	Reliability	1
5.3.5	Deficiencies	No

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (52-week study) - Dog

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	In general compliance with OECD 452 **Revisions/Amendments:** 3.3.1. Duration of treatment 52 weeks (or 1 year) 3.4.6. Haematology In all animals during pretrial and at weeks 27 and 52; additional examinations were performed at weeks 5, 13, and 40. 3.4.7. Clinical chemistry In all animals during pretrial and at weeks 27 and 52 3.4.8. Urinalysis In all animals during pretrial and at weeks 27 and 52
Results and discussion	Agree with the applicant's version **Revisions/Amendments:** 4.5.1. Haematology […] increase in reticulocyte count, sulfhemoglobin and methemoglobin, as compared to controls. 4.6.2. Gross and Histopathology […] hepatic fatty vacuolation and bone marrow hyperplasia with increased deposition of yellow/brown pigment
Conclusion	Agree with the applicant's version LO(A)EL: 500 ppm NO(A)EL: 100 ppm
Reliability	1
Acceptability	Acceptable
Remarks	-
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*

Section A6.4	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.1 Oral administration (52-week study) - Dog

Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.4.1/32 Test substance intake

Dietary dose level	**Approx. mean test substance intake (mg/kg bw/d)**		
(ppm)	**Males**	**Females**	**Combined**
Wk 1-13			
10	0.37	0.39	0.38
100	3.7	3.8	3.7
500	19	20	20
50,000	2,018	1,984	2,001
Wk 1-52			
10	0.36	0.36	0.36
100	3.5	3.8	3.6
500	19	19	19
50,000	1,898	1,879	1,888

Table 6.4.1/33 Results of Flufenoxuron determination in whole blood samples

Dose levels		**Flufenoxuron concentration in whole blood (ng/ml) of individual dogs**											
(ppm)		**Week 29**				**Week 40**				**Week 52**			
0	M	–	–	–	–	–	–	–	–	39.4	46.4	N.D.	N.D.
	F	–	–	–	–	–	–	–	–	N.D.	N.D.	N.D.	N.D.
10	M	–	–	–	–	–	–	–	–	32.5	44.1	48.7	39.4
	F	–	–	–	–	–	–	–	–	9.3*	18.5	27.8	32.5
100	M	–	–	–	–	–	–	–	–	164.8	178.7	211.2	160.1
	F	–	–	–	–	–	–	–	–	153.2	208.9	157.8	125.3
500	M	665	976	1,794	1,779	705	1,244	3,668	3,657	790	1,170	2,470	3,250
	F	–	630	935	682	–	914	1,018	1,069	–	680	620	900
50,000	M	–	–	–	–	–	–	–	–	6310	7,990	9,000	6,610
	F	–	–	–	–	–	–	–	–	12,700	8,270	8,690	8,990

–: not measured; N.D. below limit of reliable determination (approx. 15 ng/ml);

* below limit of reliable determination but quantifiable on this occasion

Table 6.4.1/34 Results of Flufenoxuron determination in perirenal fat samples

Dose levels (ppm)		**Flufenoxuron concentration in renal fat (ppm); week 52**				
		Data from individual dogs				**Mean**
0	M	3.53	1.58	1.32	1.37	1.95
	F	3.07	1.28	2.30	1.43	2.02
10	M	4.83	4.80	7.15	5.65	5.61
	F	3.63	5.56	5.30	3.38	4.47
100	M	28.46	23.14	37.19	27.52	29.08
	F	22.20	30.26	11.42	15.62	19.88
500	M	102.53	140.59	156.96	312.86	178.24
	F	–	88.54	76.79	100.07	88.47
50,000	M	1,309.1	809.1	878.2	830.0	956.6
	F	1,020.8	826.3	754.8	865.3	866.8

–: not measured (female dog killed on humane grounds during week 25)

Table 6.4.1/35 Methemoglobin levels (% of total hemoglobin)

Test parameters	Males					Females				
	0	10	100	500	50,000	0	10	100	500	50,000
Pretrial	0.48	0.54	0.52	0.50	0.54	0.64	0.58	0.59	0.56	0.57
Week 5	0.75	0.58	0.69	0.90	1.96***	0.66	0.97	0.58	0.87	1.48***
Week 13	0.73	0.77	0.76	0.99	1.52**	0.63	0.88	0.97	0.99	1.61
Week 27	0.54	0.63	0.53	0.86*	1.60***	0.56	0.68	0.68	0.84	1.96***
Week 40	0.75	0.71	0.75	1.05	1.87***	0.69	0.75	0.80**	1.53***	2.18***
Week 52	1.16	1.27	1.04	1.14	1.95	0.71	1.08	0.68	0.98	2.39***

Statistical evaluation: * = p< 0.05; ** = p<0.01; *** = p<0.001 (F-max test; ANOVA; Student's t-test)

Table 6.4.1/36 Clinicochemical findings (Week 27 and Week 52)

Test parameter		Dose levels (ppm)				
		0	10	100	500	50,000
Wk 27: Cholesterol [mM]	M	2.8±0.3	3.1±0.5	3.4±0.7	3.5±0.6	3.8±0.5
	F	3.4±0.7	3.6±1.3	3.7±0.6	2.8±0.5	4.1±1.1
Wk 52: Cholesterol [mM]	M	2.8±0.1	2.7±0.5	2.9±1.1	3.2±0.7	4.0±0.9
	F	4.2±1.6	3.2±1.0	4.8±2.2	3.1±1.0	4.0±0.2

Statistical evaluation: * = p< 0.05; ** = p<0.01; otherwise p>0.05 (F-max test; ANOVA; Student's t-test)

Table 6.4.1/37 Organ weight changes

Test parameter		**Dose levels (ppm)**				
		0	**10**	**100**	**500**	**50,000**
Terminal body weight [kg] (% control)	M	10.1 (100%)	11.0 (109%)	11.3 (112%)	10.1 (100%)	11.3 (110%)
	F	9.7 (100%)	11.0 (113%)	10.1 (104%)	10.8 (109%)	9.7 (100%)
Abs. liver weight [g] (% control)	M	285.3 (100%)	350.1* (123%)	319.1 (112%)	340.7* (119%)	387.7*** (136%)
	F	269.8 (100%)	334.0 (124%)	292.7 (108%)	295.9 (114%)	328.3 (122%)
Liver weight, adjusted for bw [g] (% control)	M	302.2 (100%)	343.8 (114%)	306.6 (101%)	356.4* (118%)	374.0** (124%)
	F	281.3 (100%)	317.9 (113%)	295.5 (105%)	283.7 (101%)	339.3 (121%)

Statistical evaluation: * = p< 0.05; ** = p<0.01 *** = p< 0.001 (F-max test; ANOVA; Student's t-test)

Table 6.4.1/38 Histopathological changes

Test parameter		**Dose levels (ppm)**				
		0	**10**	**100**	**500**	**50,000**
Dogs examined	M	4	4	4	4	4
	F	4	4	4	4	4
Bone marrow, hyperplasia, - moderate/severe -	M	0	0	0	0	4$
	F	0	0	0	1	4$
Bone marrow, increased yellow pigment deposition	M	0	0	0	0	4$
	F	0	0	0	0	4$
Kidney, increased proximal tubular pigment deposition	M	0	0	0	0	4$
	F	0	0	0	1	1
Liver, fatty vacuolation, - slight -	M	0	0	0	0	4$
	F	0	0	0	0	0
Liver, fatty vacuolation, - moderate -	M	0	0	0	0	0
	F	0	0	0	0	4$
Liver, increased hemosiderin in Kupffer-cells, - slight -	M	0	0	0	3	4$
	F	0	0	0	0	4$
Liver, increased hemosiderin in Kupffer-cells, - moderate -	M	0	0	0	1	4$
	F	0	0	0	2	4$
Spleen, increased hemosiderin	M	0	1	1	0	2
	F	0	0	0	1	3

Statistical evaluation: $ = $p < 0.05$; (Fisher's Exact Test)

Section A6.4.2	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.4	6.4.2 Dermal

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Acute toxicity studies did not indicate any adverse findings when Flufenoxuron was tested by the dermal route at limit dose levels. Therefore, no repeated (sub-chronic) toxicity studies by the dermal route were conducted.	
Undertaking of intended data submission [ ]	Not applicable	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Evaluation of applicant's justification	Agree with applicant's justification
Conclusion	Waiving accepted
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.4	**Subchronic toxicity**
BPD Annex Point IIA, VI.6.4	6.4.3 Inhalation

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Acute toxicity studies did not indicate any adverse findings when Flufenoxuron was tested by the inhalation route. Furthermore, short-term inhalation toxicity studies are not necessary due to the low volatility of Flufenoxuron. Therefore, no subchronic toxicity studies by the inhalation route were conducted.	X
Undertaking of intended data submission [ ]	Not applicable	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Evaluation of applicant's justification	Discussions with the notifiant took place during the evaluation phase. As the formulation to be assessed is a liquid, no exposure to the neat Flufenoxuron powder will occur and the waiving for inhalation repeated dose toxicity was hence accepted. In addition, the formulation has a low volatility and spraying aqueous dilution is not expected to generate substantial amounts of droplets < 50 µm. During risk characterisation, exposition levels will be particularly checked and margin of safety will be determined taking into account this lack of data.
Conclusion	Waiving accepted
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*

Evaluation of applicant's justification	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.5	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.5	6.5 Chronic Toxicity - 2-year feeding study in rat

Official use only

1 REFERENCE

1.1	**Reference**	**1) XXXX** WL115110: A two year chronic toxicity feeding study in rats XXXX unpublished XXXX **2) XXXX** Addendum to XXXX - Volume 4 of 5: WL115110: A 2 year chronic toxicity feeding study in rats XXXX unpublished XXXX **Note:** This addendum provides exchange pages (pp. 1968-1970) of individual pathology data to be inserted in the report. **3) XXXX** Corrigenda/addenda to XXXX - WL115110: A 2 year chronic toxicity feeding study in rats XXXX unpublished XXXX **Note:** This corrigendum corrects two typing errors on pages 46 and 49 of the report
1.2	**Data protection**	No
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	XXXX
1.2.3	Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Not reported; in general compliance with OECD 452
2.2	**GLP**	Yes (laboratory certified by the Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
2.3	**Deviations**	No

Section A6.5	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.5	6.5 Chronic Toxicity - 2-year feeding study in rat

3 MATERIALS AND METHODS

3.1	**Test material**		
3.1.1	Lot/Batch number	Batch: XXXX	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	As given in section 2	
3.1.2.2	Purity	97.6%	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fischer 344	
3.2.3	Source	XXXX.	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	About 6 weeks old	X
3.2.6	Number of animals per group	20/sex/group + 10/sex as satellite group for interim sacrifice	
3.2.7	Control animals	Yes, 40/sex + 20/sex as satellite group for interim sacrifice	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	24 months	
3.3.2	Frequency of exposure	Daily	
3.3.3	Postexposure period	None	
3.3.4	**Oral**		
3.3.4.1	Type	In food	
3.3.4.2	Concentration	1; 5; 50; 500; 5,000 and 50,000 ppm, equivalent to a mean daily compound intake of 0.044, 0.23, 2.2, 22, 233 and 2,471 mg/kg bw in males and 0.055, 0.28, 2.8, 28, 301 and 3,206 mg/kg bw in females	
3.3.4.3	Vehicle	None	

Section A6.5		**Repeated dose toxicity**	
BPD Annex Point IIA, VI.6.5		6.5 Chronic Toxicity - 2-year feeding study in rat	
3.3.4.4	Concentration in vehicle	Not applicable	
3.3.4.5	Total volume applied	Not applicable	
3.3.4.6	Controls	Basal diet	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Twice daily on week days and once daily on weekends and public holidays.	
3.4.1.2	Mortality	At least once a day	
3.4.2	Body weight	Weekly during the first 13 weeks and every 4 weeks thereafter.	
3.4.3	Food consumption	Weekly during the first 13 weeks and every 4 weeks thereafter.	
3.4.4	Water consumption	No	
3.4.5	Ophthalmoscopic examination	On rats from carcinogenicity study see 6.7-1	
3.4.6	Haematology	At 3, 6, 12, 18 and 24 months	
3.4.7	Clinical Chemisty	At 6, 12, 18 and 24 months.	
3.4.8	Urinalysis	At 6, 12, 18 and 24 months.	X
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes, brain, heart, liver, kidneys, spleen, adrenals and testes or ovaries	
3.5.2	Gross and histopathology	Yes, histopathological examination of all organs and tissues was performed on all control and top dose animals as well as from all animals which died or were sacrificed moribund; and on kidneys, liver, lungs and spleen at all doses	
3.5.3	Other examinations	None	

Section A6.5	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.5	6.5 Chronic Toxicity - 2-year feeding study in rat

3.5.4	Statistics	Analysis of variance was performed on the data from all variates, except for those variates for which there were serious doubts about the validity of the analysis. Where analysis of variance was suitable, a two-way analysis (Snedecor and Cochran, 1968) with treatments and blocks as factors was used for a variate provided that both of the following were true: (i) less than 20% of the possible observations were missing and (ii) each treatment group had at least 50% of its possible observations. If this was not the case, then a one-way analysis (Snedecor and Cochran, 1968), ignoring blocks, was used. When there were serious doubts about the validity of the assumptions required for the analysis of variance, the Wilcoxon two-sample rank sum test (Hill and Peto, 1971; Lehman, 1961) was used. In this case, the data from each treated group in turn were compared with those from the control group. The significance levels of the differences_between the control and treated means were determined using the Williams' t test (Williams, 1971; 1972) whenever justifiable. Dunnett's test (Dunnett, 1964) was used if a monotonic dose response could not be assumed. When a covariate was used, the standard error of differences took account of the adjustment made to the means (Snedecor and Cochran, 1968). Fisher's exact test (Fischer 1950) was used for the analysis of leukaemia data, and for comparison of the incidences of histopathology findings. The proportion of rats with a particular finding in each treated group was compared with the proportion in the control group. The analysis was performed separately for samples from animals which survived to terminal kill, and for samples from intercurrent deaths. All comparisons were one sided. The log rank test (Kalbfleisch and Prentice, 1980) was used to investigate the effect of treatments on survival. Animals which were killed at the scheduled necropsies, or which died as a result of anaesthetic overdose etc. were considered to have censored survival times. (References see study report)
3.6	**Further remarks**	None

Section A6.5	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.5	6.5 Chronic Toxicity - 2-year feeding study in rat

		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	Survival of rats was not affected by treatment. During the last six months, a number of spontaneous, age-related changes (poor condition, soiled fur, unkempt appearance and low food intake) were, when compared to the control, observed at a lower incidence in rats at dose levels ≥ 5,000 ppm	
4.1.2	Mortality	Mortality was not affected by treatment	
4.2	**Body weight gain**	Body weight development was impaired at dose levels ≥ 5,000 ppm	
4.3	**Food consumption and compound intake**	Food consumption was slightly higher in both sexes at the top dose level.	
4.4	**Ophtalmoscopic examination**	No treatment-related effects	X
4.5	**Blood analysis**		
4.5.1	Haematology	Slight anemia at dose levels ≥ 5,000 ppm; details given under 5.2	X
4.5.2	Clinical chemistry	Increased bilirubin and decreased triglyceride levels at ≥ 5,000 ppm. Changes were consistent with the observed anemia and the decreased body weights at the affected dose levels. Details given under 5.2	
4.5.3	Urinalysis	No adverse treatment-related effects	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	No adverse treatment-related effect, details given under 5.2	
4.6.2	Gross and histopathology	No adverse treatment-related effect, details given under 5.2	
4.7	**Other**	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Groups of 20 male and 20 female Fischer 344 (F344) rats (XXXX.) were administered Flufenoxuron at dietary dose levels of 1; 5; 50; 500; 5,000 and 50,000 ppm for 24 months. The rats used in this study were from the same batch/delivery of animals used also for the concurrently conducted carcinogenicity study	

Section A6.5	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.5	6.5 Chronic Toxicity - 2-year feeding study in rat

[see IIIA 6.7, XXXX]. Additional satellite groups of 10 male and 10 female F344 rats used for an interim sacrifice after one year were fed the same dietary concentrations for 12 months. The control groups consisted of 40 male and 40 female F344 rats for the main (chronic toxicity) group and of 20 male and 20 female F344 rats for the satellite (interim sacrifice) group. The animals were housed individually and were about 6 weeks old at commencement of treatment.

Clinical signs were recorded twice daily on week days and once daily on weekends and public holidays. In order to avoid any interferences of the orbital sinus blood sampling procedure, ophthalmoscopy was performed on 20 males and 20 females of the concurrent carcinogenicity study. As the animals of the carcinogenicity study were from the same batch of animals, were housed in the same animal unit and were fed the same formulated diets, the results of these examinations are valid for the chronic toxicity study, too. The main reason for this was to avoid damage to the eyes in the chronic toxicity study due to the orbital sinus bleeding procedure. Body weights and food consumption were determined weekly during the first 13 weeks and every 4 weeks thereafter. Hematology examinations were conducted at 3, 6, 12, 18 and 24 months, whereas clinical chemistry and urinalysis were performed at 6, 12, 18 and 24 months. Blood was sampled from all chronic toxicity group animals but not from interim sacrifice rats. Methemoglobin concentrations were measured spectrophotometrically by the reference method of Evelyn and Malloy (1938). Urine was sampled from 10 male and 10 female rats one to three weeks prior to blood sampling at 6, 12, 18 and 24 months by the means of placing the animals in metabolism cages overnight (16 hours).

All animals - regardless whether surviving till scheduled sacrifice or found dead/sacrificed moribund - were subjected to a detailed necropsy. Organ weights were determined in surviving animals (brain, heart, liver, kidneys, spleen, adrenals and testes or ovaries). A full range of organs or representative section thereof from all animals were sampled, fixed, blocked, sliced and stained. Histopathological examination of all organs and tissues was performed on all control and top dose animals as well as from all animals which died or were sacrificed moribund. Kidneys, liver, lungs and spleen were examined from all groups. The laboratory chemistry and histopathological examinations included at least the parameters and organs listed in OECD Guideline 452.

5.2 Results and discussion

Flufenoxuron at dietary concentrations of 1 to 50,000 ppm was shown to be stable in the diet for at least 42 days when stored at room temperature. Accordingly, formulated diets were prepared

Section A6.5	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.5	6.5 Chronic Toxicity - 2-year feeding study in rat

	at two to 4 week intervals. The diet preparation technique resulted in homogenous diets as was demonstrated for the first batch of formulated diets used in this study. All samples except of the 1 ppm dietary dose level taken at the top, middle or bottom of the diet container were within 10% of the nominal value indicating a homogenous distribution of the test article in the diet. The maximum deviation from the nominal concentration for the 1 ppm diets was 12%. However, if one calculates the relative standard deviation (RSD) of the concentrations in the top, middle, and bottom samples ((S.D./Mean) x 100) the RSD values were 2.5, 3.6, 1.5, 3.1, 0.8 and 2.0% at 1, 5; 50; 500; 5,000 and 50,000 ppm, respectively. Although the concentration at the lowest dose level was somewhat lower than intended, the diet preparation was sufficiently homogenous. Twelve of the diet preparations at 50,000; 5,000; 500 and 50 ppm were analyzed for test article content, whereas all preparations at 0, 1, and 5 ppm were analyzed. The average concentrations were 100.5 ± 5.3, 97.2 ± 4.1, 97.8 ± 4.0, 100.7 ± 2.9, 100.3 ± 2.4 and 100.0 ± 3.3 percent of the nominal concentrations at 1; 5; 50; 500; 5,000 and 50,000 ppm, respectively. Except for two cases the levels of Flufenoxuron in the control diet were ≤ 0.1 ppm. One of the control diet preparations apparently contained 0.14 and 0.29 ppm Flufenoxuron. However, based on chromatographic peak shape, this peak was considered to represent some other compound which interfered with the analysis for the test substance. Based on food consumption the average daily compound intake was 0.044, 0.23, 2.2, 22, 233 and 2,471 mg/kg bw in males and 0.055, 0.28, 2.8, 28, 301 and 3,206 mg/kg bw in females at dietary dose levels of 1; 5; 50; 500; 5,000 and 50,000 ppm. Survival of rats was not affected by treatment as indicated by survival rates of 60, 45, 45, 55, 60, 65, and 65% in males and 75, 75, 70, 75, 65 70, and 85% in females at doses ranging from 0 to 50,000 ppm. During the last six months, a number of spontaneous, age-related changes (poor condition, soiled fur, unkempt appearance and low food intake) were, when compared to the control, observed at a lower incidence in rats at dose levels ≥ 5,000 ppm. Body weight development was impaired at dose levels ≥ 5,000 ppm. This was especially evident from week 28 onwards [see Table 6.5/39]. Food consumption was slightly higher in both sexes at the top dose level. Hematology investigations revealed a number of significant differences of red blood cell parameters [see Table 6.5/40]. In	X

Section A6.5	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.5	6.5 Chronic Toxicity - 2-year feeding study in rat

females at ≥ 5,000 ppm a very slight "anemia" characterized by lower erythrocyte counts (RBC) and hemoglobin concentrations (Hb) was observed throughout the study. Lower hemoglobin values were also observed in top dose males at 3 and 12 months. A slight increase of erythropoietic activity was observed in the bone marrow smears from the 12 months satellite groups at 50,000 ppm which is in line with the slight anemia observed in this group. It is however important to note that no clinical signs of anemia were observed at any dose level.

The higher erythrocyte counts at 18 and 24 months and the higher hemoglobin concentration and hematocrit (Hct) at 24 months in males at ≥ 5,000 ppm in conjunction with the decline of these parameters in control males are indicative of a "healthier" state of these males during the last quarter of the study.

Microcytosis (quotient of hematocrit and erythrocyte count) was observed fairly persistently throughout the study at dose levels ≥ 50 ppm and was even seen at the 5 ppm dose group at 24 months for both sexes. Generally, the degree of microcytosis was small. As there were no consistent decreases of the hematocrit over time at dose levels ≤ 500 ppm and considering the small magnitude of change the microcytosis was not considered to be of toxicological significance. In addition to the significant changes listed in Table 6.5/40 a number of other red blood cell parameters (mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW) or erythrocyte mean diameter) displayed significant differences to the control. Consistent changes over time were only observed at dose levels ≥ 5,000 ppm. Significant changes at ≤ 500 ppm were observed sporadically only or were of small magnitude and within the physiological range. Therefore these changes were not considered to be toxicologically significant. The latter holds true for the decrease of the red cell distribution width which was observed in male at dose levels down to dose levels of 5 ppm at 6 months and in females down to dose levels of 50 ppm at 18 months. A decrease of the red cell distribution width indicates a more homogenous erythrocyte population and is not an adverse finding.

In addition to the significant changes of red blood cell parameters there were a number of statistically significant changes of white blood cell parameters. However, these changes were not consistent over time and between sexes, were of small magnitude or displayed no dose-response relationship. These changes were therefore not considered to be of toxicological relevance.

Changes of clinical chemistry parameters consistent over time and between sexes were only observed at the two highest dose

Section A6.5	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.5	6.5 Chronic Toxicity - 2-year feeding study in rat

	levels. They pertained total bilirubin and triglycerides levels [see Table 6.5/41]. The changes of bilirubin levels are consistent with the changes of erythrocyte numbers. The lower triglyceride levels are consistent with the lower body weight of high dose animals. Calcium levels tended to be lower in both sexes at dose levels ≥ 5,000 ppm. In contrast to the former changes, changes of calcium levels were not strictly dose dependent or entirely consistent over time and therefore of questionable toxicological relevance. Statistically significant changes of total protein and cholesterol levels were not consistent between sexes. Decreased values were observed in males whereas increased values were observed in females. Therefore, a relation to treatment is unlikely. In addition, a number of isolated, statistically significant changes were observed at dose levels ≥ 50 ppm. These included for example decreased cholesterol levels for 50 and 500 ppm males at 12 months, decreased triglyceride levels for 50 and 500 ppm males at 6 months, decreased urea nitrogen for 50 and 500 ppm females at 6 months (significant increases were observed for 5,000 ppm females at 12 months and for top dose females at 12 and 18 months), or decreased alkaline phosphatase for 50; 500 and 50,000 ppm at 12 months. The latter findings were neither dose-related nor in a direction which is toxicologically meaningful. Therefore, these isolated, statistically significant changes were considered to be incidental. There were a number of statistically significant differences of urinary parameters at all dose levels. However, the changes were neither dose-related, nor consistent over time. Some changes even indicated an improved state of the kidney like decreased urinary protein or glucose levels. Therefore, none of these changes were considered to be adverse. Organ weight analysis at interim sacrifice revealed lower mean spleen, liver and kidney weights in males at ≥ 5,000 ppm. This was no longer the case when the organ weights were analyzed using the terminal body weight as covariate. Therefore these changes were reflecting the decreased body weight of these groups [see Table 6.5/42]. Slightly increased absolute and body weight adjusted spleen and adrenal weights were observed in females at ≥ 5,000 ppm at the interim sacrifice. The increased spleen weights in 5,000 and 50,000 ppm females at 12 months are likely related to the anemia observed in females at these dietary concentrations of test material. In addition body weight adjusted heart, kidney and liver weights were increased. There were no corroborative histopathological changes at the interim sacrifice and no comparable organ weight changes were observed at the terminal sacrifice. Therefore these changes were considered to be	X

Section A6.5	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.5	6.5 Chronic Toxicity - 2-year feeding study in rat

at least partially secondary to the lower terminal body weight and as a total to be of no toxicological relevance.

At the 2-year terminal sacrifice, decreased absolute and body weight adjusted spleen weights were observed in males at ≥ 50 ppm (1.12/1.10 g (abs./adj.) at 50 ppm and 1.16/1.12g (abs./adj.) at 500 ppm, values at ≥ 5,000 ppm see Table 6.5/42). Considering the high variability of the spleen weights and taking in account that 78 and 62% of the spleen weights at 50 and 500 ppm were within the range of the controls, no biological relevance is attached to this finding in these male groups. The lower absolute and body weight adjusted spleen weights at 5,000 and 50,000 ppm are probably due to the remarkable decrease in the incidence of spleen enlargement when compared to the controls [see Table 6.5/43]. In this chronic toxicity study as well as in the rat carcinogenicity study the decreased incidence of macropathological spleen enlargement was accompanied by a decreased incidence of splenic mononuclear cell leukemia [see Table 6.5/46 and Table 6.5/45]. Mononuclear cell leukemia in the spleen results in increased spleen weights. The spleen weight effects are therefore not considered to be of adverse nature. This view is supported by the absence of adverse, treatment-related histopathological findings in the spleen. The spleen weight effects are therefore not considered to be of adverse nature.

Although the absolute and body weight adjusted brain weights of females at ≥ 500 ppm were significantly lower when compared to the controls, the absent dose-response relationship and the small magnitude of the difference renders this effect not to be related to treatment. The higher weight adjusted adrenal weights in 5,000 and 50,000 ppm females at the terminal sacrifice were not accompanied by significant changes of the absolute weights and therefore probably secondary to the lower body weight of these groups.

Macropathology of interim kill animals revealed no treatment-related findings. In males administered Flufenoxuron for up to 24 months a decreased incidence of liver and spleen findings was observed at ≥ 5,000 ppm [see Table 6.5/43]. These changes are in line with the generally healthier state of males at ≥ 5,000 ppm.

The type and incidence of histopathological lesions at interim sacrifice were typical for F344 rats of this age. There were a few statistically significant differences between male treated and control rats. The only apparent treatment-related increase of basophilic parenchymal foci was observed at dose levels ≥ 500 ppm [see Table 6.5/44]. However, the incidence of this finding was comparable between all groups in the 24-months

Section A6.5	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.5	6.5 Chronic Toxicity - 2-year feeding study in rat

group [see Table 6.5/45]. In addition, no treatment-related increase in the incidence of hepatocellular tumors was observed in the 2-year oncogenicity study [see 6.7/5]. Thus, the increased incidence of basophilic foci of cellular alteration in the livers of males exposed to dietary concentrations of 500 ppm or above for 12 months was not considered to be related to any pre-neoplastic process.

Some of the remaining statistically significant findings indicated a lower incidence and/or severity at higher dose levels (e.g. chronic nephropathy or pigmented macrophages in lymph nodes) and others displayed no dose-response relationship (e.g. periportal microvascular vacuolation of the liver).

The incidence of tumors at interim sacrifice was low. A total of 5, 0, 1, 2, 3, 0, and 1 tumors were observed in males at dose levels from 0 to 50,000 ppm, respectively. In females 2, 3, 0, 0, 1, 0, and 2 tumors were observed at the respective dose levels. Neither the incidence nor type of tumors indicated a relation to treatment.

Histopathological examination of rats fed Flufenoxuron for up to 2 years revealed a number of statistically significant, potentially treatment-related findings in the liver of male and female rats [see Table 6.5/45]. In male rats an increased incidence of zonal (periportal) fatty vacuolation was observed at dose levels 50; 500 and 5,000 ppm. However, at the 10 fold higher concentration (50,000 ppm) the incidence was identical to the control incidence. In females this finding tended to occur at a lower incidence at higher dose levels. The toxicological relevance of this finding is highly questionable.

Furthermore, similar to the interim sacrifice a number of liver findings occurred at a decreased incidence at dose levels ≥ 5,000 ppm. This included, for example, Kupffer-cell hypertrophy and pigmentation or spongiosis hepatis. Other findings like the incidences of hyperplastic nodules in males and females or the incidence of small granulomata and metastatic mineralization in females displayed no (strict) dose-response relationships. Therefore, findings are of questionable toxicological relevance. In addition, metastatic mineralization - like the periportal fatty vacuolation - is a common observation in ageing F344 rats. These slight changes in incidence therefore are not considered to be adverse effects.

There were no treatment-related increases in the incidence of any neoplastic lesion for males or females.

A variety of spontaneous tumors were observed in all groups including controls. A total of 291 rats (91%; 155 males and 136 females) developed tumors. Of the neoplasms 428 were benign

Section A6.5	**Repeated dose toxicity**
BPD Annex Point IIA, VI.6.5	6.5 Chronic Toxicity - 2-year feeding study in rat

		and 114 were malignant. Distant metastases were observed in 8 animals (6 males and 2 females). The most common tumors were pituitary adenoma (190) and benign and malignant testicular interstitial cell tumors (124). As indicated in Table 6.5/46 a few tumor incidences attained statistical significance. For mononuclear cell lymphoma of the hematopoietic tissues in males a dose dependent decrease of this tumor was observed, as compared to controls. In all other cases the incidence of tumors displayed no dose-response relationship. Therefore, these incidences were not indicative for a relation to treatment.
5.3	**Conclusion**	In a 24 month chronic toxicity study the administration of Flufenoxuron to Fischer 344 rats at dietary dose levels of 0; 1; 5; 50; 500; 5,000 and 50,000 ppm resulted in decreased body weight gain and a slightly higher food consumption in males and females at ≥ 5,000 ppm. A slight anemia characterized by lower red blood cell counts, hemoglobin concentrations, hematocrit and slightly increased reticulocyte counts was observed at the two highest dose levels. Macro- and micropathological changes at higher dose levels were largely related to a general reduction of age-related pathology and therefore were not considered adverse. There were no treatment-related increases in the incidence of any neoplastic lesion for males or females. Based on the results of this study the NOAEL for chronic toxicity was 500 ppm, which is equivalent to a mean daily dose of 22 mg/kg bw in males and 28 mg/kg bw in females. Given the absence of any carcinogenic effects, the NOAEL for oncogenicity was 50,000 ppm, the highest concentration tested, which is equivalent to an mean daily intake of 2,470 mg/kg bw in males and 3,205 mg/kg bw in females.
5.3.1	LO(A)EL	5,000 ppm, equivalent to about 233 mg/kg bw/d for male and 301 mg/kg bw/d for female rats
5.3.2	NO(A)EL	500 ppm, equivalent to about 22 mg/kg bw/d for male and 28 mg/kg bw/d for female rats
5.3.3	Other	None
5.3.4	Reliability	1
5.3.5	Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 3.2.5. Age range between 6 and 8 weeks 3.4.8. Urinalysis no sample realised at 3 months
Results and discussion	Applicant's version acceptable **Revisions/Amendments:** 4.5.1 Haematology First signs of slight anemia are visible from 50 ppm, for the females (decreased levels of hemoglobin and hematocrit), but are really consistent with other parameters at 5000 ppm 4.4. Ophthalmoscopic examination see carcinogenicity study 6.7 / 1 for details 5.2. According to the two previous remarks (slight anemia and decreased spleen weight observed from 50 ppm), the corresponding paragraphs had to be changed accordingly, taking into account these observations.
Conclusion	Agree with the applicant's version, NOAEL = 500 ppm (equivalent to about 22 mg/kg bw/d for male and 28 mg/kg bw/d for female rats)
Reliability	1
Acceptability	Acceptable provided all the modifications mentioned above are integrated in the new version of the study summary
Remarks	-
	COMMENTS FROM ... (specify)
Date	Give date of comments submitted
Materials and Methods	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state
Results and discussion	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Table 6.5/39 Body weight development of rats fed Flufenoxuron for 2 years - Chronic toxicity study

Dose level [ppm]	0	1	5	50	500	5,000	50,000
Males							
Week 0	126.6	125.4	127.8	125.5	126.8	124.5	124.3
Week 13	323.2	324.0 (1.0)[a]	325.4 (0.5)	322.5 (0.2)	326.2 (1.4)	320.9 (-0.1)	315.9* (-2.5)
Week 28	377.7	374.6 (-0.8)	377.5 (-0.6)	373.4 (-1.3)	378.4 (0.2)	363.9* (-4.7)	350.3** (-10.0)
Week 52	426.0	419.8 (-1.7)	425.6 (-0.5)	424.7 (-0.1)	430.3 (1.4)	395.1** (-9.6)	385.9** (-12.6)
Week 76	456.0	446.3 (-2.6)	453.0 (-1.3)	457.9 (0.9)	464.2 (2.4)	431.9** (-6.7)	420.4** (-10.1)
Week 104	426.1	414.9 (-3.3)	428.1 (0.3)	436.1 (3.7)	453.6 (9.1)	418.7 (-1.8)	411.0 (-4.3)
Females							
Week 0	100.9	100.4	100.4	104.0*	99.6	99.8	98.0
Week 13	183.2	184.7 (2.4)	186.6 (4.7)	185.0 (-1.6)	180.6 (-1.6)	181.6 (-0.6)	175.9** (-5.3)
Week 28	208.1	211.6 (3.7)	209.1 (1.4)	207.6 (-3.4)	204.3 (-2.3)	200.9* (-5.7)	197.2** (-7.5)
Week 52	245.1	247.7 (2.1)	243.4 (-0.8)	238.2 (-6.9)	239.1 (-3.3)	227.2 (-11.7)	222.9 (-13.4)
Week 76	296.9	308.8 (6.3)	298.0 (0.8)	291.8 (-4.2)	290.5 (-2.6)	276.7 (-9.7)	267.7 (-13.4)
Week 104	326.2	341.3 (6.9)	327.8 (0.9)	327.7 (-0.7)	315.5 (-4.2)	303.4* (-9.6)	291.1** (-14.3)

* $p < 0.05$; ** $p < 0.01$ (Williams test)

[a] Cumulative body weight gain expressed as percent difference from control

Table 6.5/40 Selected hematology of rats fed Flufenoxuron for 2 years - Chronic toxicity study

Dose [ppm]		0	1	5	50	500	5,000	50,000
Males								
RBC	3	10.18	10.17	10.19	10.37	10.31	10.16	9.99
[10^{12}/l]	6	10.16	9.97	10.17	10.17	10.16	10.21	10.32
	12	10.19	10.23	10.19	10.45**	10.44**	10.23	10.29
	18	9.52	9.56	9.45	9.76	9.92	**10.03***	**10.01****
	24	7.26	7.38	7.51	8.31	7.31	**8.48****	**8.78***
Hb	3	16.2	16.3	16.3	16.5**	16.4	16.1	**15.8***
[g/dl]	6	15.6	15.7	15.5	15.6	15.6	15.6	15.7
	12	16.6	16.5	16.6	16.9	16.9	16.5	**16.3****
	18	15.4	15.5	15.1	15.8	16.1	15.7	15.2
	24	13.5	13.8	13.5	14.9	13.4	**15.1****	**15.4****
Hct	3	48.7	48.7	48.8	49.2	49.0	**47.6***	**47.0***
[%]	6	48.7	48.1	48.4	48.4	48.4	48.8	48.6
	12	51.7	51.2	51.2	52.0	51.8	**50.5***	**50.3***
	18	49.6	49.9	48.3	49.9	50.2	49.5	47.3
	24	42.3	42.7	41.7	45.8	41.4	45.9	**46.9****
MCV	3	48.0	47.9	47.9	47.4*	47.5**	46.8*	47.0*
[fl]	6	48.0	48.3	47.6	47.6	47.7	47.8	47.1*
	12	50.8	50.6	50.3	49.8*	49.5*	49.4*	49.0*
	18	52.2	52.2	51.7	51.1**	50.6*	49.4*	48.8*
	24	57.0	57.4	55.6**	54.6*	55.5*	54.2*	53.5*
Females								
RBC	3	9.83	9.95	9.86	9.69	9.68	**9.37***	**9.03***
[10^{12}/l]	6	9.33	9.51	9.50	9.51	9.39	9.38	9.13
	12	9.62	9.62	9.63	9.54	9.49	9.51	**9.30***
	18	9.60	9.67	9.61	9.61	9.58	9.55	**9.30****
	24	8.25	7.79	8.25	8.56	8.25	8.45	8.33
Hb	3	16.8	17.0	16.7	16.5**	16.4**	**15.7***	**15.5***
[g/dl]	6	15.5	15.6	15.6	15.6	15.3	15.4	**14.9***
	12	17.0	17.0	16.8	16.9	16.8	16.6*	**16.4***
	18	16.3	16.5	16.4	16.4	16.1	16.1	**15.7****
	24	15.6	15.0	15.3	15.8	15.4	15.6	15.5
Hct	3	50.1	50.6	50.1	49.1**	48.8*	**47.9***	**46.5***
[%]	6	47.8	48.8	48.5	48.4	47.9	47.5	**46.4****
	12	52.1	51.9	51.7	51.3	51.1**	**51.1****	**50.3***
	18	51.0	51.0	50.4	49.9	50.0	49.7**	48.9*
	24	46.2	44.8	45.8	47.0	45.3	45.9	45.5
MCV	3	51.0	50.9	50.8	50.7	50.4*	51.1	51.5*
[fl]	6	51.2	51.3	51.0	50.9	51.0	50.7**	50.9**
	12	54.0	53.9	53.7	53.8	53.9	53.7	54.2
	18	52.9	52.7	52.5	52.1*	52.2*	52.0*	52.2*
	24	56.2	56.5	54.8**	55.0**	54.9**	54.3*	54.6**

* $p < 0.05$; # $p < 0.01$ (Williams' or Dunnett test)

Table 6.5/41 Selected clinical chemistry parameters of rats fed Flufenoxuron for 2 years - Chronic toxicity study

Sex		Male			Female		
Dose [ppm]	month	0	5,000	50,000	0	5,000	50,000
Bilirubin	6	2.8	3.1*	3.2**	3.1	3.4*	3.8**
[µM]	12	2.4	2.8**	2.6	2.9	3.5**	3.7**
	18	2.9	2.4**	2.5*	2.7	3.0*	3.2**
	24	4.0	2.6*	2.8*	2.3	2.9**	3.0**
Triglycerides	6	2.56	1.93**	1.46**	0.71	0.66	0.62
[mM]	12	3.46	2.30**	1.71**	2.35	1.06**	0.82**
	18	2.93	2.28	1.87**	2.48	1.49**	1.40**
	24	2.75	2.50	2.29	3.10	2.64	2.29
Calcium	6	2.88	2.72**	2.70**	2.91	2.80**	2.77**
[mM]	12	2.88	2.66**	2.63**	3.03	2.98	2.96
	18	2.73	2.38**	2.47**	2.87	2.64**	2.72**
	24	2.82	2.77	2.69**	2.78	2.82	2.75
Protein	6	76.4	77.7	77.3	76.8	79.4**	79.2**
[g/L]	12	76.3	75.8	74.6*	81.7	84.1**	84.3**
	18	73.9	74.5	74.9	81.7	84.8*	84.1*
	24	62.8	65.8	64.3	71.3	74.5*	72.3
Cholesterol	6	2.28	2.16	2.15	3.10	3.24	3.53**
[mM]	12	2.80	2.36**	2.77**	3.86	3.90	4.35**
	18	5.02	3.60**	3.60**	4.62	4.77	5.26**
	24	5.61	6.16	5.95	4.77	4.83	4.97

* $p < 0.05$; ** $p < 0.01$ (Williams' or Dunnett test)

Table 6.5/42 Organ weights of rats fed Flufenoxuron for 1 or 2 years - Chronic toxicity study

Sex	Males						Females					
Dose [ppm]	0	5,000	50,000	0	5,000	50,000	0	5,000	50,000	0	5,000	50,000
	Absolute organ weights			Terminal body weight (TBW) adjusted organ weights			Absolute organ weights			Terminal body weight adjusted organ weights		
1 Year interim sacrifice												
TBW	428.5	394.2**	387.8**				248.8	231.0**	216.8**			
Spleen	0.79	0.69**	0.70**	0.77	0.72	0.74	0.46	0.49	0.53**	0.45	0.50*	0.55**
Heart	1.17	1.16	1.19	1.15	1.21**	1.26**	0.79	0.81	0.80	0.77	0.83**	0.84**
Brain	1.96	1.94	1.93	1.94	1.96	1.96	1.76	1.77	1.77	1.76	1.77	1.77
Kidneys	2.71	2.55*	2.48**	2.65	2.66	2.62	1.56	1.60	1.57	1.53	1.62**	1.64**
Liver	12.13	10.79**	10.61**	11.8	11.38	11.38	6.68	6.75	6.67	6.41	6.93**	7.21**
Testes/Ovary	3.20	3.23	3.18	3.16	3.30	3.27	0.10	0.11	0.11	0.10	0.11	0.11
Adrenals	0.051	0.048	0.049	0.05	0.05	0.051	0.062	0.063	0.067*	0.061	0.063	0.068**
2 Year terminal sacrifice												
TBW	399.4	394.6	384.5				306.9	286.6*	273.9**			
Spleen	1.47	0.96**	0.92**	1.47	0.97**	0.95**	0.62	0.56	0.60	0.62	0.57	0.62
Heart	1.27	1.24	1.26	1.27	1.24	1.27	0.94	0.91	0.91	0.93	0.93	0.95
Brain	1.99	1.97	1.98	1.99	1.96	1.98	1.80	1.76*	1.78**	1.80	1.76*	1.80
Kidneys	2.94	3.03	2.89	2.94	3.05	2.94	2.03	1.93	1.91	2.00	1.98	2.02
Liver	12.4	11.72	11.73	12.40	11.77	11.87	8.35	7.97	7.78	8.20	8.31	8.44
Testes/Ovary	4.30	3.93	4.77	4.30	3.92	4.77	0.09	0.09	0.09	0.09	0.09	0.09
Adrenals	0.068	0.065	0.071	0.068	0.066	0.072	0.064	0.067	0.067	0.063	0.068*	0.070**

* $p < 0.05$; ** $p < 0.01$ (Williams test)

Table 6.5/43 Selected macropathological findings in male rats fed Flufenoxuron for 2 years - Chronic toxicity study

Dose [ppm]	0	1	5	50	500	5,000	50,000
No. of animals examined	40	20	20	20	20	20	20
Liver							
- Dark focus(i)/area(s)	13	5	4	4	5	3	-
- Roughened surface	14	10	4	2	3	-	1
- Enlargement	10	3	5	2	3	2	
Spleen							
- Enlargement	19	13	5	8	4	2	1
Mean severity[a]	3.1	3.2	3.8	2.8	2.8	2.0	2.0

[a] Findings were graded as very slight (Grade 1), slight (Grade 2), moderate (Grade 3), severe (Grade 4) and very severe (Grade 5). The mean severity is the average of the severity grades observed for the respective lesion

Table 6.5/44 Selected histopathological findings of male rats fed Flufenoxuron for 1 year - Chronic toxicity study

Dose [ppm]	0	1	5	50	500	5,000	50,000
Liver (# examined)	(20)	(10)	(10)	(10)	(10)	(10)	(10)
- biliary epithelial hyperplasia	20	10	10	10	10	10	10
Mean severity	2.5	2.8	2.5	2.8	2.8	2.9*	2.6
- basophilic parenchymal foci	3	5	3	3	7**	7**	9***
- Kupffer-cell pigmentation	7	5	2	5	5	0	0
- Periport. microvasc. vacuolation	2	5*	0	1	1	0	0
Kidney (# examined)	(20)	(10)	(10)	(10)	(10)	(10)	(10)
- chronic nephropathy	20	10	9	10	10	7*	6**
Mean severity	1.7	1.7	1.8	2.1	2.1	1.3	1.5
- papillary mineralization	0	0	2	0	4**	2	1
- protein casts	0	0	0	0	0	3*	2
Lymph nodes (# examined)	20	2	1	-	1	-	10
- Pigmented macrophages	13	0	0	-	0	-	0**

[a] Histopathology findings were grades as very slight (Grade 1), slight (Grade 2), moderate (Grade 3), severe (Grade 4) and very severe (Grade 5). The mean severity is the average of the severity grades observed for the respective lesion

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 6.5/45 Selected histopathological findings of rats fed Flufenoxuron for up to 2 years - Chronic toxicity study

Dose [ppm]	0	1	5	50	500	5,000	50,000
Males							
Liver (# examined)	(40)	(20)	(20)	(20)	(20)	(20)	(20)
- zonal fatty vacuolation	12	8	9	13*	14**	14**	12
- hyperplastic nodules, few (2-5)	2	3	6*	4	6*	7**	3
- hyperplastic nodules, multifocal (> 5)	-	-	-	-	-	-	-
- basophilic parenchymal foci	34	11*	12	14	17	19	18
- parenchymal hypertrophy	6	8	3	1	1	0	0
- spongiosis hepatis	15	4	8	8	8	4	0**
- Kupffer-cell hypertrophy	12	5	6	4	7	2	0*
- Kupffer-cell hypertrophy, centrilob.	4	4	2	3	2	0	0
- Kupffer-cell pigmentation, centrilob.	6	8	3	3	2	0	0
- mononuc. cell leukemia, infiltration by	12	10	4	4	3	0*	1
- leukocytosis	8	3	0	2	1	0	0
Females							
Liver (# examined)	(40)	(20)	(20)	(20)	(20)	(20)	(20)
- zonal fatty vacuolation	29	15	13	17	11	15	9
- hyperplastic nodules, few (2-5)	17	12	9	13	11	12	13
- hyperplastic nodules, multifocal (> 5)	1	3	3	1	4*	1	0
- Kupffer-cell pigmentation	13	8	9	7	4	3	2
- extramedullary hematopoiesis	10	4	3	3	4	1	1
- lymphocyte infiltration, perivascular	3	2	4	4	0	1	6*
- mineralization, metastatic	0	4**	4**	1	1	1	1
- granulomata, small	8	4	7	10*	7	9	7

[a] Histopathology findings were grades as very slight (Grade 1), slight (Grade 2), moderate (Grade 3), severe (Grade 4) and very severe (Grade 5). The mean severity is the average of the severity grades observed for the respective lesion

* $p < 0.05$; ** $p < 0.01$ (Fisher's Exact Test)

Table 6.5/46 Neoplastic findings in rats fed Flufenoxuron for up to 2 years - Chronic toxicity study

Sex	Males							Females						
Dose [ppm]	0	1	5	50	500	5,000	50,000	0	1	5	50	500	5,000	50,000
Animals														
- examined	40	20	20	20	20	20	20	40	20	20	20	20	20	20
- with benign tumors	38	19	20	17	19	17	17	27	14	15	17	15	19	14
- with malignant tumors	16	11	11	7	7	6	6	14	7	4	2	3	4	7
- with metastatic tumors	0	0	2	1	2	0	1	1	0	0	0	1	0	0
- with single tumors	9	5	0	5	5	7	10	14	12	15	12	12	14	8
- with multiple tumors	31	14	20	14	15	11	9	17	5	3	5	5	5	9
Liver	(40)	(20)	(20)	(20)	(20)	(20)	(20)	(40)	(20)	(20)	(20)	(20)	(20)	(20)
- mononuc. cell lymphoma	12	10	4	4	3	0*	1	2	3	1	1	2	0	1
Skin	(40)	(17)	(12)	(15)	(13)	(11)	(20)	(40)	(8)	(7)	(6)	(11)	(8)	(20)
- basal cell carcinoma	0	0	1	0	0	2*	0	-	-	-	-	-	-	-
- squamous cell carcinoma	0	0	2*	0	0	0	0	-	-	-	-	-	-	-
Spleen	(40)	(20)	(20)	(20)	(20)	(20)	(20)	(40)	(20)	(20)	(20)	(20)	(20)	(20)
- mononuc. cell lymphoma	12	10	4	3	2	0*	1	2	3	1	1	2	0	1
Testes	(40)	(20)	(20)	(20)	(18)	(18)	(20)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
- interstitial cell tumor (B)	33	17	16	15	13	15	14							
- interstitial cell tumor (M)	0	0	1	0	0	0	0							
Pituitary4	(40)	(17)	(19)	(16)	(18)	(15)	(20)	(40)	(18)	(19)	(18)	(18)	(20)	(20)
- adenoma	26	7	12	9	14	8	8	22	13	12	16*	14	18*	11
Mammary gland	(40)	(13)	(15)	(11)	(12)	(7)	(20)	(40)	(13)	(12)	(14)	(10)	(10)	(20)
- adenocarcinoma	-	-	-	-	-	-	-	0	0	0	0	0	2*	0
Thyroid	(40)	(14)	(13)	(10)	(8)	(7)	(20)	(40)	(6)	(7)	(5)	(8)	(6)	(20)
- Parafollic. cell adenoma	4	1	3	2	0	0	4	1	1	3**	0	1	0	0

() Number of organs/tissues examined; (B) benign, (M) malignant

* $p < 0.05$; ** $p < 0.01$ (Fisher's Exact Test); - no tumors of this type; n.a. not applicable

Section A6.6.1	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.1	6.6.1 In-Vitro gene mutation study in bacteria

			Official use only
		1 REFERENCE 1	
1.1	**Reference**	**1) Brooks T. M., Wiggins D. E. 1986** Microbial mutagenicity studies with WL115110 XXXX unpublished XXXX **2) Brooks T. M. 1991** Addendum to SBGR.86.026: Microbial mutagenicity of WL115110 XXXX unpublished XXXX **Note**: The 2-page addendum gives supplemental information concerning Material and Methods (i.e. on the experimental design and selection of doses); an update of the GLP compliance statement was issued.	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not reported; in general compliance with OECD 471	
2.2	**GLP**	No (at the time the study was conducted GLP was not compulsory. However the study was conducted according to the principle of Good Laboratory Practices)	
2.3	**Deviations**	No	X
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch: XXXX	
3.1.2	Specification	As given in section 2	

Section A6.6.1		**Genotoxicity in vitro**	
BPD Annex Point IIA, VI.6.VI.6.1		6.6.1 In-Vitro gene mutation study in bacteria	
3.1.2.1	Description	As given in section 2	
3.1.2.2	Purity	99%	
3.1.2.3	Stability	Stable	
3.2	**Study Type**	Bacterial reverse mutation assay	
3.2.1	Organism/cell type	S. typhimurium: TA 100, TA 1535, TA 1537, TA 1538 and TA 98 E. coli: WP2 uvr A pKM 101	
3.2.2	Deficiencies / Proficiencies	None	
3.2.3	Metabolic activation system	S9	
3.2.4	Positive control	benzo(a)pyrene, sodium azide or potassium dichromate	X
3.3	**Administration / Exposure; Application of test substance**		
3.3.1	Concentrations	Doses ranging from 31.25 - 4,000 µg/plate (standard plate test)	
3.3.2	Way of application	Test substance dissolved in dimethyl sulphoxide (DMSO); volumes of solutions of Flufenoxuron dissolved in DMSO were added to top agar mix and plated.	X
3.3.3	Pre-incubation time	None	
3.3.4	Other modifications	None	
3.4	**Examinations**		
3.4.1	Number of cells evaluated	Not applicable examination based on plate concentration (see results below)	
		4 RESULTS AND DISCUSSION	
4.1	**Genotoxicity**		
4.1.1	without metabolic activation	No; For details see study summary under 5.2	
4.1.2	with metabolic activation	No; For details see study summary under 5.2	
4.2	**Cytotoxicity**	No; For details see study summary under 5.2	

Section A6.6.1	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.1	6.6.1 In-Vitro gene mutation study in bacteria

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Flufenoxuron was tested for its mutagenic potential based on the ability to induce back mutations in selected gene loci of several bacterial strains in the Ames reverse mutation assay. The *Salmonella typhimurium* strains TA 100, TA 1535, TA 1537, TA 1538 and TA 98 and the *Escherichia coli* strain WP2 uvrA pKM 101 were exposed to the test substance dissolved in dimethyl sulphoxide (DMSO) at doses ranging from 31.25 - 4,000 µg/plate (standard plate test). The dose levels for use in the bacterial assays were selected on the basis of cytotoxicity and solubility. In the first experiments, a range of doses up to 4,000 µg per plate were selected. Because of precipitation of the test compound, the maximum test concentration was reduced to 2,000 µg per plate for subsequent experiments. The bacteria were exposed to Flufenoxuron both in the presence and absence of a liver microsomal activation system (S-9 mix) using the liver homogenate from Arochlor-treated rats.

20 µl volumes of solutions of Flufenoxuron dissolved in DMSO were added to top agar mix and plated. The cultures were incubated at 37°C for 48 - 72 hours

Three plates were used per dose for each strain and test condition. Two independent experiments were carried out on different days in order to confirm the reproducibility of the results.

For control purposes and to demonstrate the sensitivity of the test system, positive controls included:

20 µg/plate of benzo(a)pyrene with and without metabolic activation administered to TA 98, TA 100 and TA 1538; 20 µg/plate of neutral red with and without metabolic activation administered to TA 1537; 5 µg/plate of sodium azide with and without metabolic activation administered to TA 1535 of *S. typhimurium*; and 20 µg/plate of potassium dichromate with and without metabolic activation administered to *E. coli* strain WP2 uvrA pKM 101. All test strains also received DMSO as the negative control.

5.2 Results and discussion

The stability of formulations of the test substance in DMSO was assessed using high performance liquid chromatography (HPLC). Solutions stored for 20 hours and solutions freshly prepared gave the same chromatographic results. On this basis, the basis solutions for the test substance were demonstrated to be stable for at least 20 hours.

Flufenoxuron formed a fine suspension in the top agar at a dose of 31.25 µg/plate, the lowest amount tested. This suspension

Section A6.6.1	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.1	6.6.1 In-Vitro gene mutation study in bacteria

became denser as the concentration increased. At 1,000 µg/plate a milky suspension was present. At 1,000 and 4,000 µg/plate, lumps of precipitate were observed in the top agar. At 4,000 µg/plate, the pH of the medium was slightly increased from pH 7.31 to pH 7.39. Therefore, the highest dose to be tested in experiment (2) was 2,000 µg/plate.

In the bacterial assays no cytotoxicity was observed in any of the tester strains exposed to the test compound at amounts up to 4,000 µg/plate either in the presence or in the absence of rat liver S-9 mix.

The addition of Flufenoxuron to agar layer cultures of *S. typhimurium* or *E. coli* did not increase the reverse mutation frequency in any of the strains either in the presence or in the absence of rat liver S–9 fraction [see Table 6.6.1/47].

The activity of the S–9 mix and the sensitivities of the strains TA 1538, TA 98 and TA 100 were monitored by treating cultures with a known positive control compound, benzo(a)pyrene, which requires metabolic activation before it is able to induce gene mutation. The sensitivity of TA 1537 was monitored by use of the indirect mutagen, Neutral Red; the sensitivities of *E. coli* WP_2 uvrA pKM101 and TA 1535 were monitored by testing of the direct-acting mutagens potassium dichromate or sodium azide, respectively.

Discussion regarding the use of positive control substances in the study:

Since the positive control substance benzo(a)pyrene requires metabolic activation in order to induce gene mutations, the reverse mutation counts were not increased when benzo(a)pyrene was tested without metabolic activation in the test strains TA 1538, TA 98 and TA 100. For the same reason, no positive response was obtained when the indirect mutagen Neutral Red was tested without metabolic activation in the test strain TA 1537. However, as can be seen from Table 6.6.1/1, TA 1537, TA 1538, TA 98 and TA 100 did give positive results when the same positive control substances were tested in the presence of S–9 mix, demonstrating that

(1) it was possible to reliably induce mutations in the test strains

(2) the S–9 mix had sufficient metabolic capacity to transform mutagens requiring metabolisation.

Therefore, it can be concluded that the choice of positive controls provided sufficient evidence that the test system was able to reliably detect substance-induced mutagenicity under the

X

Section A6.6.1	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.1	6.6.1 In-Vitro gene mutation study in bacteria

		conditions of the study.	
5.3	**Conclusion**	Flufenoxuron did not induce reverse gene mutation in the selected bacterial tester strains under the study conditions.	
5.3.1	Reliability	2	X
5.3.2	Deficiencies	No	X

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	2.3. Deviations *Yes: examination based on plate concentration instead of counting revertant cell ; no precisions about experimental conditions* (tested substance /plate, volumes of metabolic activation system-DMSO….not provided*), 20 µl of test substance dissolved in DMSO added instead of 0.05 or 0.1ml required* 3.2.4. Positive control *Add Neutral red* 3.3.2. Way of application volumes of solution not given (deviation)
Results and discussion	Positive controls, without S9-mix were not valid for TA 1537, TA 1538, TA 98 and TA 100. Although TA 1537, TA 1538, TA 98 and TA 100 show their ability to mutate in presence of metabolic activation, the positive controls are necessary to confirm that the strains conserved their ability to mutate all along the study.
Conclusion	Without S-9 mix, the test is not valid, excepted for TA 1535 and E coli. For the other strains, as the positive controls were invalid, no conclusion could be drawn.
Reliability	3
Acceptability	Accepted only for tested strains in presence of metabolic activation and for TA 1535 and E coli in absence of metabolic activation. No definitive conclusion for the other strains without S9 mix. Another test was provided (Engelhardt & Leibold, 2005) and retained as key study.
Remarks	-

Section A6.6.1	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.1	6.6.1 In-Vitro gene mutation study in bacteria

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.6.1/47 Mean reverse mutation counts from two independent experiments (1) and (2)

		Plate concentration [µg/plate]									Pos. Ctrl
		0	**31.25**	**62.5**	**125**	**250**	**500**	**1,000**	**2,000**	**4,000**	
With S-9 mix											
E. coli	(1)	45.0	48.0	49.0	44.0	53.3	45.0	37.0	27.3	34.3	326.0* [c]
	(2)	64.0	67.0	61.0	62.3	65.0	71.3	77.0	72.0	–	435.0* [c]
TA 1535	(1)	12.7	13.3	10.7	11.0	10.3	10.7	8.7	8.7	9.7	705.3* [a]
	(2)	13.3	11.3	12.0	14.0	13.7	11.0	11.3	12.0	–	733.0* [a]
TA 1537	(1)	14.3	13.7	9.3	11.7	11.3	18.7	13.0	9.3	9.7	211.7* [d]
	(2)	12.3	8.7	7.0	12.7	10.0	11.3	10.0	10.0	–	238.0* [d]
TA 1538	(1)	19.0	21.0	17.3	18.0	14.3	12.7	10.7	13.7	13.7	158.3* [b]
	(2)	28.0	28.7	28.3	26.0	29.3	27.0	25.0	19.7	–	165.0* [b]
TA 98	(1)	19.0	16.0	15.7	16.0	12.0	14.0	13.3	10.0	11.3	586.3* [b]
	(2)	19.0	13.3	11.7	12.3	10.7	9.7	10.7	8.0	–	387.3* [b]
TA 100	(1)	100.7	81.7	89.7	104.0	117.3	100.0	93.7	94.0	100.7	557.3* [b]
	(2)	88.0	92.7	86.7	95.0	81.7	90.0	77.7	82.3	–	590.0* [b]
Without S-9 mix											
E. coli	(1)	42.7	45.3	46.0	40.7	45.0	45.7	42.0	42.0	45.7	700.7* [c]
	(2)	61.7	69.3	66.7	53.3	60.3	56.0	63.7	61.7	–	562.0* [c]
TA 1535	(1)	22.3	23.3	22.0	22.7	21.7	22.7	19.7	18.3	13.7	947.0* [a]
	(2)	21.0	21.0	22.3	21.0	22.3	28.3	16.3	19.0	–	864.0* [a]
TA 1537	(1)	12.0	10.0	11.7	10.3	19.3	15.0	8.0	10.7	7.7	16.3 [d]
	(2)	9.7	8.0	5.3	6.3	9.7	5.3	6.3	5.3	–	11.7 [d]
TA 1538	(1)	18.7	20.0	13.3	14.7	16.3	12.7	16.3	13.0	11.0	21.7 [b]

Table 6.6.1/47 Mean reverse mutation counts from two independent experiments (1) and (2)

		Plate concentration [µg/plate]									Pos. Ctrl
		0	**31.25**	**62.5**	**125**	**250**	**500**	**1,000**	**2,000**	**4,000**	
	(2)	28.3	25.0	25.3	26.7	30.0	28.3	24.7	23.0	–	30.0 [b]
TA 98	(1)	11.0	10.0	15.7	13.7	9.7	9.0	10.3	11.7	8.0	17.7 [b]
	(2)	12.3	14.7	13.0	13.3	10.0	7.7	10.3	9.0	–	11.0 [b]
TA 100	(1)	96.5	86.3	90.7	81.0	100.0	103.7	113.0	84.0	79.3	91.0 [b]
	(2)	77.7	75.7	74.3	83.7	78.3	72.0	80.3	70.0	–	81.3 [b]

* Reproducible values of 2.5 × control value or greater were considered to indicate mutagenic response.
[a]: Sodium azide (5 µg/plate): Positive control substance suitable for testing TA 1535 or TA 100
[b]: Benzo(a)pyrene (20 µg/plate): Positive control substance suitable for testing strains in the presence of S-9 mix
[c]: Potassium dichromate (20 µg/plate): Positive control substance suitable for *E. coli* without S-9 mix
[d]: Neutral Red (20 µg/plate): Positive control substance suitable for testing strains in the presence of S-9 mix

Section A6.6.1	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.1	6.6.1 In-Vitro gene mutation study in bacteria

			Official use only
		1 REFERENCE 2	
1.1	**Reference**	**3) Brooks T. M., Wiggins D. E. 1986** Microbial mutagenicity studies with WL115110 XXXX unpublished XXXX	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not reported; in general compliance with OECD 481	
2.2	**GLP**	No (at the time the study was conducted GLP was not compulsory. However the study was conducted according to the principle of Good Laboratory Practices)	
2.3	**Deviations**	No	X
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch: XXXX)	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	As given in section 2	
3.1.2.2	Purity	99%	
3.1.2.3	Stability	Stable	
3.2	**Study Type**	DNA damage and repair	
3.2.1	Organism/cell type	*Saccharomyces cerevisiae*, JD1 strain	

Section A6.6.1	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.1	6.6.1 In-Vitro gene mutation study in bacteria

3.2.2	Deficiencies / Proficiencies	None
3.2.3	Metabolic activation system	S9
3.2.4	Positive control	4-Nitroquinoline-N-oxide without metabolic activation and Cyclophosphamide with metabolic activation
3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	0.01; 0.1; 0.25; 0.5 and 1.0 mg/ml (final concentrations) with and without S-9 metabolic activation (S–9 mix from livers of rats treated with Arochlor 1254)
3.3.2	Way of application	Test substance dissolved in dimethyl sulphoxide (DMSO); 20 µl of Flufenoxuron dissolved in DMSO were added to liquid suspensions of *Saccharomyces cerevisiae*. After incubation (see below) the cells were seeded onto the appropriate culture media for the selection of prototrophic colonies. The plates were incubated for 3 days at 30 °C.
3.3.3	Pre-incubation time	The liquid *Saccharomyces cerevisiae* suspensions were incubated for 18-hours at 30 °C in the absence of S–9 fraction and for 2 hours at 37 °C followed by 16 hours at 30 °C in the presence of S–9 fraction
3.3.4	Other modifications	None
3.4	**Examinations**	
3.4.1	Number of cells evaluated	Not applicable

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	
4.1.1	without metabolic activation	No; For details see study summary under 5.2
4.1.2	with metabolic activation	No; For details see study summary under 5.2
4.2	**Cytotoxicity**	No; For details see study summary under 5.2

Section A6.6.1	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.1	6.6.1 In-Vitro gene mutation study in bacteria

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Flufenoxuron was examined for the ability to induce mitotic gene conversion, reciprocal crossing over, mutation and/or aneuploidy in the JD1 strain of *Saccharomyces cerevisiae*. Liquid suspension cultures of log phase cells received the test material in DMSO at dose levels of 0.01; 0.1; 0.25; 0.5 and 1.0 mg/ml with and without S-9 metabolic activation (S–9 mix from livers of rats treated with Arochlor 1254). Three replicate plates were used for each dose condition. Positive controls included: 0.25 µg/ml of 4-Nitroquinoline-N-oxide without metabolic activation and 1.25 mg/ml of Cyclophosphamide with metabolic activation. Negative control cultures received the vehicle DMSO only. After 18-hour incubation at 30 °C in the absence of S–9 fraction or 2 hours at 37 °C followed by 16 hours at 30 °C in the presence of S–9 fraction, the cultures were seeded onto the appropriate culture media for the selection of prototrophic colonies. After 3-day incubation at 30 °C the numbers of prototrophic colonies were counted. Two independent experiments were conducted.	
5.2	**Results and discussion**	The stability of the test substance in DMSO has been demonstrated analytically. Flufenoxuron did not have any effect on cell viability, and the results obtained from both trials showed no increase in the rate of mitotic gene conversion in the presence or absence of metabolic activation [see Table 6.6.1/48]. The positive control materials induced mitotic gene conversion indicating that the test organism and metabolic activation system were functioning properly. Flufenoxuron was, therefore, judged to be negative for inducing mitotic gene conversion in the yeast *Saccharomyces cerevisiae* JD1.	
5.3	**Conclusion**	Flufenoxuron did not induce genetic damage in *Saccharomyces cerevisiae*.	X
5.3.1	Reliability	2	
5.3.2	Deficiencies	No	X

Section A6.6.1	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.1	6.6.1 In-Vitro gene mutation study in bacteria

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 2.3. Deviations Yes: examination based on plate concentration instead of counting revertant cell ; no precisions about experimental conditions (tested substance /plate, volumes of metabolic activation system-DMSO….not provided) No precision about the strain of bacteria used in this study; Strain used not in accordance with the strains recommended by the guideline
Results and discussion	Agree with applicant's version for genotoxicity
Conclusion	Flufenoxuron did not induce genetic damage in *Saccharomyces cerevisiae* under the experimental conditions described
Reliability	3
Acceptability	Acceptable despite deficiencies: scientific information provided on in vitro genotoxicity is relevant for the hazard evaluation
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.6.1/48 Results of mitotic gene conversion test in *S. cerevisiae* JD1

Flufenoxuron			**Histidine locus**			**Tryptophan locus**		
[mg/ml]	Exp	% cell viability	Prototrophs per plate	Prototrophs per 10^6 survivors	Ratio over control[a]	Prototrophs per plate	Prototrophs per 10^6 survivors	Ratio over control[a]
Without S–9 mix								
0	1	100	11.3	3.3	–	60.8	17.8	–
	2	100	10.8	3.7	–	45.5	15.6	–
0.01	1	93	12.3	3.9	1.2	59.5	18.9	1.1
	2	108	12.8	4.1	1.1	55.3	17.6	1.1
0.1	1	105	17.0	4.7	1.4	58.8	16.4	0.9
	2	97	14.5	5.1	1.4	44.0	15.5	1.0
0.25	1	120	12.3	3.0	0.9	69.5	17.0	1.0
	2	133	10.8	2.8	0.8	60.3	15.5	1.0
0.5	1	80	16.3	6.0	1.8	61.3	22.6	1.3
	2	110	11.0	3.4	0.9	55.0	17.1	1.1
1.0	1	90	11.0	3.6	1.1	67.8	22.1	1.2
	2	99	15.5	5.4	1.5	55.8	19.3	1.2
0.00025 NQO[b]	1	56	39.3	20.6	6.2*	168.0	88.1	4.9*
	2	55	45.8	28.7	7.8*	195.5	122.7	7.9*
With S–9 mix								
0	1	100	5.8	1.6	–	36.8	10.1	–
	2	100	8.5	2.8	–	40.0	13.1	–
0.01	1	98	3.3	0.9	0.6	36.8	10.4	1.0
	2	89	7.5	2.8	1.0	32.5	12.0	0.9
0.1	1	102	6.0	1.6	1.0	45.0	12.2	1.2
	2	81	5.8	2.3	0.8	23.8	9.6	0.7
0.25	1	98	3.0	0.8	0.5	39.8	11.2	1.1
	2	86	7.3	2.8	1.0	33.5	12.8	1.0
0.5	1	89	6.5	2.0	1.3	44.8	13.9	1.4
	2	87	10.0	3.8	1.4	29.3	11.0	0.8
1.0	1	96	3.0	0.9	0.6	40.0	11.5	1.1
	2	79	8.0	3.3	1.2	28.5	11.8	0.9
1.25 CP[c]	1	80	18.3	6.3	3.9*	132.8	45.9	4.5*
	2	86	24.3	9.3	3.3*	109.0	41.7	3.2*

* Values greater than twice the control value are considered to indicate a mutagenic response

[a] Ratio between mean number of revertants per 1 million survivors per treated plate vs. the same per control plate

[b] NQO = 4-nitroquinoline-N-oxide; [c] CP = Cyclophosphamide (both positive controls)

EXP: experiment 1 or 2

		Official use only
1 REFERENCE 3		
1.1 Reference	**3) Engelhardt G., Leibold E.** Salmonella typhimurium / Escherichia coli - Reverse mutation assay (standard plate test and preincubation test) with BAS 307 I (Flufenoxuron) XXXX unpublished XXXX	X
1.2 Data protection	Yes	
1.2.1. Data owner	BASF	
1.2.2. Companies with letter of access	XXXX	
1.2.3. Criteria for data protection	New key study	
2. GUIDELINES AND QUALITY ASSURANCE		
2.2. Guideline study	EEC 2000/32 B.13/B.14; OECD 471, EPA/OPPTS 870.5100	
2.3. GLP	Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)	
2.4. Deviations	No	
3. MATERIALS AND METHODS		
3.2. Test material		
3.2.1. Lot/Batch number	XXXX	
3.2.2. Specification	As given in the chemical glossary	
3.2.2.1. Description	As given in the chemical glossary	
3.2.2.2. Purity	99.1%	
3.2.2.3. Stability	Stable	
3.3. Study Type	Bacterial reverse mutation assay	
3.3.1. Organism/cell type	S. typhimurium: TA 100, TA 1535, TA 1537, and TA 98 E. coli: WP2 uvr A	
3.3.2. Deficiencies / Proficiencies	None	
3.3.3. Metabolic activation system	S9	

3.3.4. Positive control

Without metabolic activation:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

With metabolic activation:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

X

3.4. Administration / Exposure; Application of test substance

3.4.1. Concentrations: Doses ranging from 20 - 5,000 µg/plate in the standard plate test and 4 - 2,500 µg/plate in the pre-incubation assay

3.4.2. Way of application: Standard plate test: Test substance dissolved in dimethyl sulphoxide (DMSO); volumes of solutions of Flufenoxuron dissolved in DMSO were added to top agar mix and plated.

3.4.3. Pre-incubation time: Pre-incubation assay: for about 20 minutes at 37°C.

3.4.4. Other modifications: None

3.5. Examinations

3.5.1.	Number of cells evaluated	Not applicable

4. RESULTS AND DISCUSSION

4.2.	**Genotoxicity**	
4.2.1.	without metabolic activation	No; For details see study summary under 5.2
4.2.2.	with metabolic activation	No; For details see study summary under 5.2
4.3.	**Cytotoxicity**	Weak cytotoxicity at ≥ 2500 µg/plate; For details see study summary under 5.2

5. APPLICANT'S SUMMARY AND CONCLUSION

5.2. Materials and methods

The possible mutagenic activity of BAS 307 I (Flufenoxuron; Batch: XXXX, Purity: 99.1%) in bacteria was investigated in 4 strains of S. typhimurium and in E. coli in the presence and absence of S9 activation. Two independent sets of experiments were performed using Dimethylsulfoxide (DMSO) as solvent. Triplicate plates were used per dose and per condition. Vehicle and positive controls were included in each experiment.

Plate incorporation assay: To test tubes containing 2-ml portions of warm soft agar 0.1 ml test solution or vehicle, 0.1 ml fresh bacterial culture, 0.5 ml S-9 mix (in tests with metabolic activation) or 0.5 ml phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Vogel Bonner agar plates (minimal glucose agar plates). In the experiments with E. coli the Vogel Bonner agar plates were replaced by plates containing a SA1 selective agar according to Green and Muriel.

Pre-incubation assay: 0.1 ml of test solution or vehicle, 0.1 ml bacterial suspension and 0.5 ml S 9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 ml of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated of mutation assay data

5.3. Results and discussion

The stability of the test substance at room temperature in the vehicle DMSO or water over a period of 4 hours was verified analytically.

Neither in the plate incorporation assay (original experiment) nor in the pre-incubation assay (confirmatory experiment) with and without metabolic activation an increased number of revertants was observed in any strain tested [see Table 6.6.1/3]. The positive controls yielded increases of revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system.

Precipitates were observed at concentrations ≥ 100 µg/plate in the original and confirmatory experiments. A weak bacteriotoxic effect (slight decrease in the number of his+ or trp+ revertants, slight reduction in the titer) was observed in the standard plate test depending on the strain and test conditions at doses ≥ 2500 µg/plate. In the preincubation assay a weak bacteriotoxicity (slight decrease in the number revertants) was occasionally observed.

5.4. Conclusion

Flufenoxuron did not induce reverse gene mutation in the selected bacterial tester strains under the study conditions.

5.4.1. Reliability 1

5.4.2. Deficiencies No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	03/02/2006
Materials and Methods	1.1 The date should be indicated 3.2.4. Positive control With metabolic activation, the only positive control use is the 2-aminoanthracene (2-AA). The table should be amended accordingly (2.5 µg/plate in DMSO)
Results and discussion	Agree with the applicant's version
Conclusion	According to this new key study, Flufenoxuron did not induce reverse gene mutation in the selected bacterial tester strains, under the study conditions.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.6.1/49 Bacterial gene mutation assay with BAS 307 I (Flufenoxuron) - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Experiment 1 (original): Plate incorporation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	35	30	109	108	17	16	10	9	38	31
BAS 307 I										
20 µg/plate	35	29	108	110	16	17	7	8	33	29
100 µg/plate	32	26	105	105	15	14	8	8	27	31
500 µg/plate	32	20	102	102	13	13	5	6	25	28
2500 µg/plate	29	19	99	72	12	10	4	4	25	22
5000 µg/plate	14	15	76	38	8	12	6	3	19	16
Pos. control[§]	836	610	869	789	134	914	148	488	212	654
Experiment 2 (confirmatory): Pre-incubation assay										
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg.control (DMSO)	37	29	110	105	19	18	11	10	37	34
M320I02										
4 µg/plate	33	26	110	107	18	18	10	8	32	31
20 µg/plate	24	28	104	108	16	20	10	7	29	31
100 µg/plate	28	27	105	113	15	17	7	7	30	26
500 µg/plate	28	22	103	103	12	18	5	6	28	30
2500 µg/plate	22	23	90	93	13	13	4	6	25	29
Pos. control[§]	768	842	836	1012	139	726	103	473	227	618

[§] Positive control compounds and concentrations see 3.2.4. above

Section 6.6.2	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.2	6.6.2 In Vitro cytogenicity study in mammalian cells

Official use only

		1 REFERENCE 1
1.1	**Reference**	**1) Meyer A. L. 1987** Genotoxicity studies with WL115110: in vitro chromosome studies with WL115110 XXXX unpublished XXXX **2) Meyer A. L. 1991** Addendum to XXXX: Genotoxicity studies with WL115110: in vitro chromosome studies with WL115110 XXXX unpublished XXXX **Note:** The Addendum consists of 4 pages with revised summary tables 2a and 3a and contains an updated GLP Compliance statement
1.2	**Data protection**	No
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	XXXX
1.2.3	Criteria for data protection	No data protection claimed
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Not reported, in general compliance with OECD 473
2.2	**GLP**	No (at the time the study was conducted GLP was not compulsory. However the study was conducted according to the principle of Good Laboratory Practices)
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	
3.1.1	Lot/Batch number	Batch: XXXX
3.1.2	Specification	As given in section 2

Section 6.6.2		**Genotoxicity in vitro**	
BPD Annex Point IIA, VI.6.VI.6.2		6.6.2 In Vitro cytogenicity study in mammalian cells	
3.1.2.1	Description		X
3.1.2.2	Purity	98.3%	
3.1.2.3	Stability	Stable	
3.2	**Study Type**	In vitro mammalian chromosome aberration test	
3.2.1	Organism/cell type	Chinese Hamster Ovary (CHO) (CHO-K1) cells	
3.2.2	Deficiencies / Proficiencies	None	
3.2.3	Metabolic activation system	S9 mix	
3.2.4	Positive control	methyl methanesulphonate without metabolic activation and cyclophosphamide with metabolic activation	
3.3	**Administration / Exposure; Application of test substance**		
3.3.1	Concentrations	Concentrations of up to 250 µg/ml without S-9 and 300 µg/ml with S-9 mix.	X
3.3.2	Way of application	Test substance dissolved in dimethyl sulphoxide (DMSO) and then diluted in culture medium to give the desired final concentration.	
3.3.3	Pre-incubation time	None	
3.3.4	Other modifications	None	
3.4	**Examinations**		
3.4.1	Number of cells evaluated	300 metaphases (100 per culture) were assessed where possible; only those cells showing the modal chromosome number (20) ± 2 centromeres were analyzed for chromosomal damage. The mitotic index at 24 hours was determined by screening 500 cells for metaphases.	
		4 RESULTS AND DISCUSSION	
4.1	**Genotoxicity**		
4.1.1	without metabolic activation	No	

Section 6.6.2	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.2	6.6.2 In Vitro cytogenicity study in mammalian cells

4.1.2	with metabolic activation	A non-dose dependent increase in the number of chromosomal aberrations (with and without gaps) was observed.
4.2	**Cytotoxicity**	The top dose level of 150 µg/ml in the main experiment was based on a 50% reduction of the mitotic index (MI) in the cytotoxicity experiment.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Flufenoxuron was assessed for its potential to cause chromosomal damage in Chinese Hamster Ovary (CHO-K1) cells. Doses used in this assay were based on results from cytotoxicity assays with Flufenoxuron (endpoints: cell confluence and cell counts) conducted at concentrations of up to 250 µg/ml without S-9 and 300 µg/ml with S-9 mix. Cultured cells were exposed to Flufenoxuron for 3 h either in the presence or in the absence of S-9 metabolic activation mix, and then harvested for chromosome preparations at 8, 12 and 24 hours after the start of Flufenoxuron treatment. The S-9 mix was used at 10% and comprised a buffered solution of 10% Aroclor-induced rat-liver homogenate (S-9 fraction), $MgCl_2$, KCl, glucose-6-phosphate and nicotinamide adenine dinucleotide phosphate. A concurrent untreated control, a solvent control (DMSO), and positive controls (methyl methanesulphonate at 60 µg/ml without metabolic activation and cyclophosphamide at 110 µg/ml with metabolic activation) were also tested. Two hours before each sample time, colcemid was added to each culture (final concentration: 0.2 µg/ml) to arrest cycling cells in metaphase. In each experimental group, 300 metaphases (100 per culture) were assessed where possible; only those cells showing the modal chromosome number (20) ± 2 centromeres were analyzed for chromosomal damage. The mitotic index (MI) was assessed at the 24-h sample time by counting the number of metaphases in a total of 500 cells from each slide.
5.2	**Results and discussion**	The solubility and stability of Flufenoxuron in DMSO has been demonstrated analytically for another Flufenoxuron batch [see IIIA 6.6.1/1, XXXX; see also XXXX]. The stability is considered to be independent of the batch. The highest doses (250 and 300 µg/ml) were limited by Flufenoxuron solubility. At 150 µg/ml, 50% inhibition of cell growth was observed with and without metabolic activation. Thus, the doses evaluated for chromosomal aberrations in the main assay were 15, 75 and 150 µg/ml with and without

Section 6.6.2	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.2	6.6.2 In Vitro cytogenicity study in mammalian cells

metabolic activation.

The results of metaphase analysis are summarized in Table 6.6.2/50.

In the absence of S-9 mix, there was no evidence of induced chromosome damage in any treatment group. An apparent mean increase in % polyploid cells at 75 µg/ml resulted from incidences of polyploid cells or endoreduplicated cells in one of three cultures (incidence in three cultures: 11/82; 4/94; 4/93 or 7/82; 2/94; 3/93, respectively). The findings in the other two cultures were similar to control incidences. There was no dose-related response in percentage of polyploid cells. The apparent increase in polyploid cells without metabolic activation was considered to be incidental and not related to Flufenoxuron treatment. Cultures exposed to the positive control Methyl methanesulphonate showed the expected increased incidence of chromatid-type aberrations.

In the presence of S-9 mix, cultures treated with Flufenoxuron showed an increase in chromosomal aberrations at the 24-hour harvest for all three doses tested, as compared with the solvent controls. The chromosomal aberrations, however, were not dose-related (7.3%, 15.6% and 7.5% cells with aberrations [excluding gaps] at 15; 75 and 150 µg/ml, respectively, compared with 2.2% cells in the solvent control group, and 13.9%, 23.6%, and 15.4% cells with aberrations [including gaps] at 15; 75 and 150 µg/ml, respectively, compared with 5.8% cells in the solvent control group). Cultures exposed to the positive control Cyclophosphamide showed substantial chromatid damage. Because of the observed chromosomal damage at 24 hours, no analyses were performed at the 8 and 12 hour sample times. In addition, due to the clear results no statistical evaluation of the data was conducted.

5.3	**Conclusion**	Flufenoxuron did not induce chromosome damage in cultured CHO cells in the absence of S-9 mix even when administered at cytotoxic concentrations. With S-9 mix, substantial chromosome damage was observed in CHO cells exposed to Flufenoxuron concentrations up to 150 µg/ml (not dose-dependent). In subsequent investigations, it was shown that the apparent clastogenic activity observed in this assay in the presence of S-9 mix was not expressed when Flufenoxuron was tested in the presence of glutathione [see IIIA 6.6.2/2 XXXX].
5.3.1	Reliability	2
5.3.2	Deficiencies	No

Section 6.6.2	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.2	6.6.2 In Vitro cytogenicity study in mammalian cells

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	Agree with the applicant's version **Revisions/Amendments:** 3.1.2.1. Description <u>White powder</u> 3.3.1. Concentrations <u>Concentrations of up to 250 µg/ml without S-9 and 300 µg/ml with S-9 mix during the cytotoxicity assays. For the chromosome aberration assay, concentrations were 15, 75 and 150 µg/ml with and without S9 mix.</u> The choice of the concentrations is not sufficiently validated (no data on solubility in the test, concentrations lower than that is recommended in the OECD guidelines, no sufficient toxicity). Exposure for 3 hours and then metaphase cells were collected 8-12-24 hours after the initiation of exposure No precisions given about the cell strain used (modal chromosome number, doubling time, no mycoplasma contamination)
Results and discussion	Agree with the applicant's version
Conclusion	Partially agree with the applicant's version (see comments on the choice of the concentrations)
Reliability	2
Acceptability	Acceptable
Remarks	-

Section 6.6.2	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.2	6.6.2 In Vitro cytogenicity study in mammalian cells

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.6.2/50 Metaphase chromosome analysis of CHO cells in the absence or presence of S-9 mix; 24-hour sample period

Group	Dose level [µg/ml]	MI	Numerical aberrations	Structural Aberrations					
				Including gaps			Excluding gaps		
			% polyploid cells	Cells with aberrations		Mean no. of aberrations per cell	Cells with aberrations		Mean no. of aberrations per cell
				No.	%		No.	%	
Without S-9 mix									
Untreated	0	0.015	3.0	9	3.1	0.034	0	0.0	0.000
DMSO	0	0.015	4.7	7	2.4	0.028	1	0.3	0.003
Flufenoxuron	15	0.019	8.7	6	2.2	0.023	1	0.4	0.004
	75	0.026	10.3	6	2.2	0.022	0	0.0	0.000
	150	0.025	5.0	4	1.4	0.014	0	0.0	0.000
MMS	60	0.037	2.3	92	31.4	0.573	88	30.0	0.532
With S-9 mix									
Untreated	0	0.042	6.3	3	1.1	0.011	0	0.0	0.000
DMSO	0	0.052	8.7	16	5.8	0.066	6	2.2	0.022
Flufenoxuron	15	0.052	8.7	38	13.9	0.157	20	7.3	0.077
	75	0.038	3.8	59	23.6	0.352	39	15.6	0.180
	150	0.032	6.7	43	15.4	0.207	21	7.5	0.096
CP	100	0.010	2.7	64	59.3	1.111	59	54.6	0.954

MI = Mitotic Index (fraction of mitotic cells per 500 cells counted); MMS = Methyl methanesulphonate;
CP = Cyclophosphamide

Section 6.6.2	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.2	6.6.2 In vitro cytogenicity study in mammalian cells

			Official use only
		1 REFERENCE 2	
1.1	**Reference**	**3) Meyer A. L. 1988** Genotoxicity studies with WL115110: in vitro chromosome studies with WL115110 and glutathione using Chinese hamster ovary (CHO) cells XXXX unpublished XXXX **4) Meyer A. L. 1991** Addendum to XXXX: Genotoxicity studies with WL115110: in vitro chromosome studies with WL115110 and glutathione using Chinese hamster ovary (CHO) cells XXXX unpublished XXXX **Note:** The Addendum consists of 3 pages with revised summary table 1 and contains an updated GLP Compliance statement.	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	Janssen Pharmaceutica	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not reported, in compliance with OECD 473	
2.2	**GLP**	No (at the time the study was conducted GLP was not compulsory. However the study was conducted according to the principle of Good Laboratory Practices)	
2.3	**Deviations**	Yes, only one test concentration tested and only in the presence of metabolic activation, therefore the study is considered to represent supplemental information	X
		3 MATERIALS AND METHODS	

Section 6.6.2		**Genotoxicity in vitro**	
BPD Annex Point IIA, VI.6.VI.6.2		6.6.2 In vitro cytogenicity study in mammalian cells	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	Batch: XXXX	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description		X
3.1.2.2	Purity	98.3%	
3.1.2.3	Stability	Stable	
3.2	**Study Type**	In vitro mammalian chromosome aberration test	
3.2.1	Organism/cell type	Chinese Hamster Ovary (CHO) cells	X
3.2.2	Deficiencies / Proficiencies	None	
3.2.3	Metabolic activation system	S9 mix	
3.2.4	Positive control	Cyclophosphamide with metabolic activation	
3.3	**Administration / Exposure; Application of test substance**		
3.3.1	Concentrations	A single concentration of 150 µg/ml	
3.3.2	Way of application	Test substance dissolved in dimethyl sulphoxide (DMSO) and then diluted in culture medium to give the desired final consentration. The final concentration of DMSO in the culture medium was 0.5%.	
3.3.3	Pre-incubation time	None	
3.3.4	Other modifications	The test was performed in presence and in absence of gluthathione (5 mM)	
3.4	**Examinations**		
3.4.1	Number of cells evaluated	On about 300 metaphases per group. For the determination of the mitotic index, 500 cells were scored for mitotic figures.	

4 RESULTS AND DISCUSSION

Section 6.6.2	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.2	6.6.2 In vitro cytogenicity study in mammalian cells

4.1	**Genotoxicity**	
4.1.1	without metabolic activation	Not applicable
4.1.2	with metabolic activation	An increased number of aberrant cells was only observed in absence of glutathione. When glutathione was present, the incidence of aberrant cells was comparable to the controls.
4.2	**Cytotoxicity**	At the concentration tested the mitotic index was reduced by about 27%

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In order to examine the effect of glutathione (GSH) on the apparent clastogenic activity of Flufenoxuron observed in the preceding study (see IIIA 6.6.2/1, XXXX), Flufenoxuron was tested for its potential to induce chromosome aberrations in CHO cells in the presence of Glutathione at physiological concentrations. Glutathione, the most abundant biological thiol peptide found in animals, plays a crucial role in the detoxification of xenobiotics, including carcinogens. A single concentration of 150 µg/ml was used in this study, based on a 50% inhibition in cell growth observed at this dose in the previous study (see IIIA 6.6.2/1, XXXX). Moreover, a dose level of 150 µg/ml was the highest dose that produced an apparent positive response in the previous study. Triplicate cell cultures were exposed to Flufenoxuron with S-9 mix for 3 hours in the presence or absence of glutathione (5 mM). Negative controls containing phosphate buffer and DMSO, or glutathione in phosphate buffer with DMSO, were also tested along with the positive control, cyclophosphamide, at 100 µg/ml with metabolic activation. Cultured cells were harvested for chromosome preparations 24 hours after the start of the treatment. Approximately 300 metaphases were scored per experimental group.
5.2	**Results and discussion**	The solubility and stability of Flufenoxuron in DMSO has been demonstrated analytically for another Flufenoxuron batch [see IIA 6.6.1/1, XXXX; see also XXXX]. The stability is considered to be independent of the batch. Results from this study are summarized in Table 6.6.2/51. When glutathione was not supplemented with the culture medium, Flufenoxuron induced statistically significant increases in the number of cells with chromosomal aberrations in the

Section 6.6.2	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.2	6.6.2 In vitro cytogenicity study in mammalian cells

		presence of S-9 mix as compared with the negative controls. However, in the presence of phosphate-buffered glutathione at the physiological concentration of 5 mM, the extent of the chromosomal damage was substantially reduced to levels comparable with the negative controls. The positive control (cyclophosphamide) showed significant increases in chromosomal aberrations, validating the test system. **Discussion:** The results of the study suggest that in the presence of S-9 mix activation, a reactive metabolic intermediate is generated from the parent molecule (Flufenoxuron), which is clastogenic to CHO cells. This putative metabolite is likely subject to detoxification by glutathione at the physiological concentration of 5mM used in this study. Thus, clastogenic effects would not be expected in mammals following an *in vivo* exposure to Flufenoxuron, since glutathione is an endogenous thiol peptide. The lack of any clastogenic effects following *in vivo* exposure to Flufenoxuron is confirmed in both the rat bone marrow chromosomal aberration assay and mouse micronucleus assay described below.	
5.3	**Conclusion**	*In vitro c*lastogenicity of Flufenoxuron observed in CHO cells in the presence of S-9 mix was completely abolished when glutathione was added to the culture medium at physiological concentrations. Flufenoxuron was not clastogenic *in vitro* under these study conditions.	
5.3.1	Reliability	2	X
5.3.2	Deficiencies	No	X

Section 6.6.2	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.2	6.6.2 In vitro cytogenicity study in mammalian cells

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 2.3. Deviations Yes, *only one test concentration tested, only in the presence of metabolic activation* and no cytotoxicity assay performed in this test (concentration based on data issued from the previous study in CHO cells). The study is considered to represent supplemental information 3.1.2.1. Description White powder 3.2.1. Organism/cell type Chinese Hamster Ovary (CHO-K1) cells
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version 5.3.1. Reliability 4 5.3.2. Deficiencies Yes (to be listed)
Reliability	4
Acceptability	Not acceptable as a study report: it is only considered to represent additional information about cytogenicity of a flufenoxuron metabolite and mechanism involved.
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 6.6.2	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.2	6.6.2 In vitro cytogenicity study in mammalian cells

Table 6.6.2/51 Metaphase chromosome analysis of CHO cells after 3-h exposure and harvested after 24 hours in the presence of S-9 mix with or without Glutathione (GSH) supplementation

Group	Dose level [µg/ml]	MI	Numerical aberrations	Structural Aberrations					
				Including gaps			Excluding gaps		
			% polyploid cells	Cells with aberrations		Mean no. of aberrations per cell	Cells with aberrations		Mean no. of aberrations per cell
				No.	%		No.	%	
Without S-9 mix									
Buffer	0	0.06	1.33	9	3.04	0.031	2	0.68	0.007
Glutathione	0	0.07	0.00	5	1.67	0.017	0	0.00	0.000
Flufenoxuron without buffer	150	0.04	1.90	51	19.77	0.302	35	13.57	0.171
Flufenoxuron with Buffer	150	0.05	0.46	40	18.52	0.310	28	12.96	0.204
Flufenoxuron + Gluthathione	150	0.05	1.33	8	2.70	0.027	1	0.34	0.003
CP	100	0.05	0.67	52	17.45	0.201	31	10.40	0.114

Buffer: Phosphate buffer plus DMSO in culture medium; Glutathione: 5 mM glutathione plus "Buffer" in culture medium MI = Mitotic Index (fraction of mitotic cells per 500 cells counted); CP = Cyclophosphamide

Section 6.6.2	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.2	6.6.2 In vitro cytogenicity study in mammalian cells

			Official use only
		1 REFERENCE 3	
1.1	**Reference**	**5) Meyer A. L. 1988** Genotoxicity studies with WL115110: in vitro chromosome studies with WL115110 using a rat liver (RL4) cell line XXXX unpublished XXXX **6) Meyer A. L. 1991** Corrigendum/Addendum to XXXX: Genotoxicity studies with WL115110: in vitro chromosome studies with WL115110 using a rat liver (RL4) cell line XXXX unpublished XXXX **Note** : The Addendum consists of 10 pages with revised summary tables 7a, 7b and 7c (changes do not alter conclusions of original report) and an updated GLP Compliance statement.	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not reported; similar to OECD 473; The study was performed according to method published by Dean B.J. and Hodson-Walker G. (1979): "An in-vitro chromosome assay using cultured rat-liver cells", Mutat. Res. 64, 329-337).	
2.2	**GLP**	No (at the time the study was conducted GLP was not compulsory. However the study was conducted according to the principle of Good Laboratory Practices)	
2.3	**Deviations**	No	X

Section 6.6.2	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.2	6.6.2 In vitro cytogenicity study in mammalian cells

		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	Batch: XXXX	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description		X
3.1.2.2	Purity	98.3%	
3.1.2.3	Stability	Stable	
3.2	**Study Type**	In Vitro mammalian chromosome aberration test	
3.2.1	Organism/cell type	Rat liver (RL4) cells	
3.2.2	Deficiencies / Proficiencies	None	
3.2.3	Metabolic activation system	S9 mix	
3.2.4	Positive control	Methyl methanesulphonate without activation and cyclophosphamide with activation	
3.3	**Administration / Exposure; Application of test substance**		
3.3.1	Concentrations	45; 225 and 450 µg/ml in the absence of metabolic activation, and 16; 80 and 160 µg/ml in the presence of metabolic activation.	
3.3.2	Way of application	Test substance dissolved in dimethyl sulphoxide (DMSO), diluted in culture medium and applied to the culture dishes for 3 hours.	
3.3.3	Pre-incubation time	None	
3.3.4	Other modifications	None	
3.4	**Examinations**		
3.4.1	Number of cells evaluated	300 metaphases (100 per culture) were assessed where possible in each experimental group. Only those cells showing the modal chromosome number ± 2 centromeres were analyzed for structural chromosomal damage.	

Section 6.6.2	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.2	6.6.2 In vitro cytogenicity study in mammalian cells

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	
4.1.1	without metabolic activation	No evidence for chromosome damage. For details see study summary under 5.2.
4.1.2	with metabolic activation	No evidence for chromosome damage. For details see study summary under 5.2.
4.2	**Cytotoxicity**	In a cytotoxicity assay, total cell counts were reduced to 45.9 and 61.7% of solvent control at the highest dose tested, i.e. 450 µg/ml without S9 and 160 µg/ml with S9, respectively. For details see study summary under 5.2. In the mutagenicity experiment the evaluation of mitotic indices at the 24-hour sampling time revealed a slight reduction at the highest dose level tested (approx. -30% without S-9 and -10% with S-9 mix).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Flufenoxuron was tested for its ability to induce chromosomal aberrations in cultured rat liver (RL4) cells. The epitheloid RL4 cell line has a modal chromosome number per cell of 45 and a doubling time of approx. 14 hours. The passage number of cell cultures used in this assay was 30. A representative sample of these cells was shown to be free of mycoplasma contamination. Cultured cells were exposed to Flufenoxuron for 3 hours in the presence or absence of S-9 metabolic activation and harvested for chromosome preparations at 8, 12 and 24 hours after the start of the treatment. Selection of test concentrations were based on the results of growth inhibition assessment. Initially, cell confluency was visually assessed after exposure of the cultures to concentrations ranging from 0.1-300 µg/ml with S-9 mix and 0.1-3,400 µg/ml without S-9 mix. Cells were exposed for three hours to the test concentrations and were harvested after further 21 hours. A quantitative assessment of growth inhibition was subsequently performed in a further assay over a narrower range of concentrations in order to determine the top concentration level to be tested in the chromosome assay. The concentration of Flufenoxuron that reduced the number of cells significantly (approx. 50% compared to the controls) was used as the top concentration in the subsequent chromosome assay. The doses evaluated in the definitive chromosomal aberration assay were: 45; 225 and 450 µg/ml in the absence of metabolic activation, and 16; 80 and 160 µg/ml in the presence of metabolic

Section 6.6.2	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.2	6.6.2 In vitro cytogenicity study in mammalian cells

activation. A concurrent, untreated, solvent control (DMSO) and positive controls (methyl methanesulphonate at 50 µg/ml without activation and cyclophosphamide at 2 µg/ml with activation) were also tested. Colcemid was added to each culture two hours before sampling (final concentration 0.2 µg/ml) in order to arrest dividing cells in metaphase. 300 metaphases (100 per culture) were assessed where possible in each experimental group. Only those cells showing the modal chromosome number (20) ± 2 centromeres were analyzed for structural chromosomal damage.

As a measure of cytotoxicity in the chromosome assay, the mitotic index (MI) was assessed at the 24-h sample time by counting the number of metaphases in a total of 500 cells from each slide.

The data were statistically analyzed using a generalized linear model with a logit link and binomial error structure. This analysis was carried out for the number of cells with aberrations excluding gaps, the number of cells with aberrations including gaps, the number of cells with isogaps or chromatid gaps or both ("gaps"), and the number of cells with polyploidy or endoreduplication or both. Each of the treated groups and the positive control were compared with the corresponding control group. There was little evidence for any extra-binomial random variation between the proportions. Adjustment of the variances in those cases where it was appropriate to allow for extra-binomial variation in the model made little change to any of the interpretations drawn from the original analysis. In some cases where no aberrations were seen for one or more treatment groups, the above described analysis was replaced by Fisher's Exact test.

5.2 Results and discussion

The solubility and stability of Flufenoxuron in DMSO has been demonstrated analytically for another Flufenoxuron batch [see IIIA 6.61, XXXX; see also XXXX]. The stability is considered to be independent of the batch.

In the cytotoxicity test, the degree of cell confluency was reduced by approximately 50% at a Flufenoxuron concentration of 500 µg/ml in the absence of S9 mix and at a concentration of between 100 and 200 µg/ml in the presence of S9 mix. The total cell counts were reduced to 45.9% of solvent control values at a Flufenoxuron concentration of 450 µg/ml in the absence of S-9 mix and reduced to 61.7% of solvent control values at a test substance concentration of 160 µg/ml in the presence of S-9 mix. As significant levels of toxicity were observed at these two test compound concentrations, 160 µg/ml and 450 µg/ml Flufenoxuron were used as the highest test concentrations in the

Section 6.6.2	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.2	6.6.2 In vitro cytogenicity study in mammalian cells

		chromosome assay with and without S-9 mix, respectively. The results of the metaphase chromosome analysis are shown in Table 6.6.2/52. Flufenoxuron did not induce any increases in structural chromosomal aberrations at any dose level, at any harvest time, when tested up to 450 µg/ml without activation and up to 160 µg/ml with activation. Evaluation of mitotic indices at the 24-hour sampling time showed a slight reduction at the highest dose level (approx. -30% without S-9 and -10% with S-9 mix) confirming Flufenoxuron cytotoxicity at the top dose level. The number of polyploid cells present at the 45 and 225 µg/ml Flufenoxuron groups were statistically increased over untreated control group levels. No toxicological significance was attached to this finding since the values were comparable to solvent control levels. The positive controls induced statistically significant increases in chromosomal aberrations, validating the test system.
5.3	**Conclusion**	Flufenoxuron does not possess clastogenic activity in cultured rat liver RL4 cells in vitro under the study conditions
5.3.1	Reliability	2
5.3.2	Deficiencies	No

Section 6.6.2	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.2	6.6.2 In vitro cytogenicity study in mammalian cells

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 2.3. Deviations Yes, maximal test dose not validated, not toxic enough and below the concentration recommended 3.1.2.1. Description White powder
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version
Reliability	2
Acceptability	Acceptable as supportive information
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.6.2/52 Metaphase chromosome analysis of RL4 cells in the absence or presence of S-9 mix; 24-hour sample period

Group	Dose level [µg/ml]	MI	Numerical aberrations	Structural Aberrations					
				Including gaps			Excluding gaps		
			% polyploid cells	Cells with aberrations		Mean no. of aberrations per cell	Cells with aberrations		Mean no. of aberrations per cell
				No.	%		No.	%	
Without S-9 mix									
Untreated	0	6.2	1.33	11	3.72	0.037	2	0.68	0.007
DMSO	0	5.7	4.33	10	3.48	0.038	1	0.35	0.003
Flufenoxuron	45	8.1	4.67*	5	1.75	0.017	1	0.35	0.003
	225	7.2	5.33*	7	2.46	0.028	0	0.00	0.000
	450	4.5	1.67	8	2.71	0.027	1	0.34	0.003
MMS	50	4.0	7.00**	76**	27.2	0.505	53**	19.00	0.305
With S-9 mix									
Untreated	0	6.80	2.67	14	4.8	0.050	3	1.0	0.010
DMSO	0	7.07	0.67	8	2.7	0.030	3	1.0	0.013
Flufenoxuron	16	6.33	3.33	21	7.2	0.076	5	1.7	0.017
	80	7.20	4.67	10	3.5	0.035	3	1.0	0.010
	160	6.07	1.33	3*	1.0	0.014	2	0.7	0.006
CP	2	5.20	1.33	39**	13.2	0.160	33**	11.1	0.138

MI = Mitotic Index (fraction of mitotic cells per 500 cells counted); MMS = Methyl methanesulphonate; CP = Cyclophosphamide; Statistical analysis: * = $p < 0.05$; ** = $p < 0.01$ compared to untreated control (generalized linear model; Fisher's Exact test)

Section 6.6.2	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.2	6.6.2 In vitro cytogenicity study in mammalian cells

			Official use only
		1 REFERENCE 4	
1.1	**Reference**	**7) McEnaney S. 1992** Study to evaluate the chromosome damaging potential of WL115110 by its effects on cultured human lymphocytes using an in vitro cytogenetics assay XXXX unpublished XXXX	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD 473; JMAFF; JMHW	
2.2	**GLP**	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)	
2.3	**Deviations**	No	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	XXXX	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description		X
3.1.2.2	Purity	98.1%	
3.1.2.3	Stability	Stable	
3.2	**Study Type**	In-vitro mammalian chromosome aberration test	
3.2.1	Organism/cell type	In cultured human lymphocytes from peripheral blood	

Section 6.6.2		**Genotoxicity in vitro**	
BPD Annex Point IIA, VI.6.VI.6.2		6.6.2 In vitro cytogenicity study in mammalian cells	
3.2.2	Deficiencies / Proficiencies	None	
3.2.3	Metabolic activation system	S9 mix	
3.2.4	Positive control	4-Nitroquinoline 1-oxide without S-9, and Cyclophosphamide, with S-9 mix	
3.3	**Administration / Exposure; Application of test substance**		
3.3.1	Concentrations	3.164 – 160 µg/ml (solubility limit in culture medium)	X
3.3.2	Way of application	Test substance dissolved in dimethyl sulphoxide (DMSO) and 0.1 ml of the respective dilutions were added to 10 ml culture medium	
3.3.3	Pre-incubation time	None	
3.3.4	Other modifications	None	
3.4	**Examinations**		
3.4.1	Number of cells evaluated	One hundred metaphases from each culture were analyzed for chromosome aberrations using coded slides. Only cells with 44 (2n-2) chromosomes were considered acceptable for chromosome aberration analysis although any cell with more than 46 chromosomes (i.e., polyploid, endoreduplicated and hyperdiploid cells) observed during this search was noted and recorded separately.	
		4. RESULTS AND DISCUSSION	
4.1	**Genotoxicity**		
3.4.2	without metabolic activation	No evidence for chromosome damage. For details see study summary under 5.2.	
3.4.3	with metabolic activation	No evidence for chromosome damage. For details see study summary under 5.2.	
3.5	**Cytotoxicity**	At the highest dose tested (i.e. at the solubility limit) a reduction of mitotic indices of 0 to 42% were observed.	

Section 6.6.2	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.2	6.6.2 In vitro cytogenicity study in mammalian cells

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Flufenoxuron was tested for its ability to induce chromosomal aberrations in cultured human lymphocytes from peripheral blood, in the presence and absence of an S-9 metabolic activation system. The S-9 fraction used for metabolic activation was prepared from male Sprague-Dawley rats induced with Aroclor 1254. Flufenoxuron was tested at a range of 3.164 – 160 µg/ml in the assay. A concurrent solvent control (DMSO) and positive controls (4-Nitroquinoline 1-oxide, from 1.25 to 5.0 µg/ml without S-9, and Cyclophosphamide, from 12.5 to 25 µg/ml with S-9 mix) were also utilized in this assay. The cells were treated continuously, in the absence of S-9 mix, for 20 or 44 hours. In contrast, in the presence of S-9 mix, the cells were treated for only 3 hours, followed by a 17- or 41-hour recovery period prior to harvest. A pulse treatment of 3 hours followed by a recovery period of 17 hours, in the absence of S-9 mix, was also included. A tabular summary of the study design is given in Table 6.6.2/53.

One hundred metaphases from each culture were analyzed for chromosome aberrations using coded slides. Only cells with 44 (2n-2) chromosomes were considered acceptable for chromosome aberration analysis although any cell with more than 46 chromosomes (i.e., polyploid, endoreduplicated and hyperdiploid cells) observed during this search was noted and recorded separately. Aberrations were classified according to the International System for Human Cytogenetic Nomenclature (ISCN).

The human lymphocyte assay was considered to be valid if the following criteria were met:

- heterogeneity between replicate cultures are within acceptable limits (statistically tested using the Binomial Dispersion Test)
- proportion of cells with structural aberrations (without gaps) in negative control cultures were within historical control range
- at least 160 cells of intended 200 were analysable at each treatment level
- the positive control chemicals induced statistically significant increases in the number of cells with structural aberrations (statistically tested using the Fisher's Exact Test)

The test chemical was considered to be clearly positive if …

- statistically significant increases in the proportion of

Section 6.6.2	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.2	6.6.2 In vitro cytogenicity study in mammalian cells

		structurally aberrant cells (without gaps) occurred at one or more concentrations (statistically tested using the Fisher's Exact Test) • the proportion of aberrant cells at such data points exceeded the normal range Increases in numbers of cells with gaps or increases in numbers of cells with structural aberrations not exceeding the normal range or occurring only at very high or very toxic concentrations were likely to be concluded as "equivocal" or "probably of no biological importance". Cells with exchange aberrations (chromosome and/or chromatid) or cells with greater than one aberration were to be considered of greater biological significance.	
5.2	**Results and discussion**	The stability of Flufenoxuron solutions in DMSO has been repeatedly demonstrated in previous investigations. The stability of Flufenoxuron is considered to be independent of the batch used. In a preliminary solubility test, limited precipitation, which redissolved on agitation of the cultures, was observed at the top 3 doses tested (78.4; 112; 160 µg/ml) indicating that a concentration close to the solubility limit of Flufenoxuron in culture medium had been achieved. Because only minimal mitotic inhibition was achieved at the highest dose, chromosomal aberrations were analyzed at the 3 highest dose levels (160; 112 and 78.4 µg/ml) at the 20-h sampling time, and at the 160 µg/ml dosage at the delayed (44-h) sampling time [see Table 6.6.2/53]. The assay was considered to be valid as all of the acceptance criteria cited above were met. The results of the metaphase analysis are summarized in Table 6.6.2/54. Flufenoxuron did not increase structural chromosomal aberrations compared to concurrent solvent control cultures at any harvest time, either in the presence or absence of metabolic activation. Acceptable frequencies of cells with numerical aberrations, within normal range, were observed in all treated cultures under all treatment/sampling conditions.	X
3.6	**Conclusion**	Flufenoxuron did not induce chromosomal aberrations in cultured human peripheral blood lymphocytes when tested to the limit of its solubility in the absence and presence of S-9 mix.	
3.6.1	Reliability	1	X
3.6.2	Deficiencies	No	X

Section 6.6.2	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.2	6.6.2 In vitro cytogenicity study in mammalian cells

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 2.3. Deviations Yes, highest concentration not validated, not toxic enough and below the recommended concentration 3.1.2.1. Description White powder. 3.3.1. Concentrations 3.164 – 160 µg/ml (solubility limit in culture medium) for cytogenetic assay and 78.4-112 and 160 µg/ml for chromosome aberration assay
Results and discussion	Agree with the applicant's version At the highest dose tested, a reduction of mitotic indices of 0 to 9% (with S9-mix) and 25 to 42% (without S9-mix) was observed, depending on duration of exposure and post-exposure.
Conclusion	Agree with the applicant's version
Reliability	2
Acceptability	Acceptable as supportive information
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.6.2/53 Exposure/harvest regimen and results of mitotic inhibition analysis

Flufenoxuron exposure period	Post exposure period until harvest	S-9 mix	Concentrations tested [µg/ml]			Mitotic inhibition at 160 µg/ml
20 h	0 h	Without	78.4	112	160	42%
44 h	0 h	Without			160	25%
3 h	17 h	Without	78.4	112	160	29%
3 h	17 h	With	78.4	112	160	9%
3 h	41 h	With			160	0%

Table 6.6.2/54 Metaphase chromosome analysis of human peripheral lymphocytes in the absence or presence of S-9 mix

Group	Dose level [µg/ml]	MI	Numerical aberrations	Structural Aberrations					
				Including gaps			Excluding gaps		
			% polyploid cells	Cells with aberrations		Historical control range[a]	Cells with aberrations		Historical control range
				No.	%		No.	%	
Without S-9 mix, 20-h exposure, 0-h recovery									
DMSO	0.0	4.2	1.0	1	0.5	2.3 (0 – 7)	1	0.5	0.9 (0 – 4)
Flufenoxuron	78.4	3.6	0.0	1	0.5		0	0.0	
	112.0	3.2	0.0	2	1		1	0.5	
	160.0	2.4	0.5	4	2		0	0.0	
NQO	2.5	-	0.0	11	22		9***	18***	
Without S-9 mix, 44-h exposure, 0-h recovery									
DMSO	0.0	3.0	0.0	4	2.0	1.7 (0 – 5)	3	1.5	0.6 (0 – 3)
Flufenoxuron	160.0	2.3	0.0	2	1.0		1	0.5	
Without S-9 mix, 3-h exposure, 17-h recovery									
DMSO	0.0	4.5	1.0	0	0.0	2.3 (0 – 7)	0	0.0	0.9 (0 – 4)
Flufenoxuron	78.4	3.5	0.0	0	0.0		0	0.0	
	112.0	3.3	0.0	0	0.0		0	0.0	
	160.0	3.2	0.0	0	0.0		0	0.0	
With S-9 mix, 3-h exposure, 17-h recovery									
DMSO	0.0	3.8	0.0	5	2.5	3.0 (0 – 9)	1	0.5	0.9 (0 – 4)
Flufenoxuron	78.4	4.3	0.5	7	3.5		3	1.5	
	112.0	3.8	0.0	1	0.5		0	0.0	
	160.0	3.4	0.0	3	1.5		3	1.5	
CPA	25	-	0.0	13	26		9***	18***	
With S-9 mix, 3-h exposure, 41-h recovery									
DMSO	0.0	4.9	0.5	2	1.0	1.5 (0 – 5)	0	0.0	0.7 (0 – 4)
Flufenoxuron	160.0	5.5	0.5	3	1.5		1	0.5	

MI = Mitotic Index (fraction of mitotic cells per 500 cells counted); NQO = 4-Nitroquinoline 1-oxide

CPA = Cyclophosphamide; Statistical analysis: *** = $p < 0.001$ compared to untreated control (Fisher's Exact Test)

[a] = Historical control data calculated on the basis of 35 most recent conducted experiments in the test laboratory (5400 cell scored)

- = not determined

Section A6.6.3	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.3	6.6.3 In vitro gene mutation assay in mammalian cells

			Official use only
		1 REFERENCE	
1.1	**Reference**	**1) Clare M. G., Wiggins D. E. 1986** In vitro mutagenicity studies with WL115110 (insecticide) using cultured Chinese hamster V79 cells XXXX unpublished XXXX **2) Brooks T. M. 1991** Corrigendum/Addendum to XXXX: In vitro mutagenicity studies with WL115110 (insecticide) using cultured Chinese hamster V79 cells XXXX unpublished XXXX **Note**: An addendum was issued to correct some minor errors, which do not affect the conclusions given in the original report. An updated GLP compliance statement was included in the addendum.	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not reported; study compliant with OECD 476 in all important aspects	
2.2	**GLP**	No (at the time the study was conducted GLP was not compulsory. However the study was conducted according to the principle of Good Laboratory Practices)	
2.3	**Deviations**	No	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	

Section A6.6.3	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.3	6.6.3 In vitro gene mutation assay in mammalian cells

3.1.1	Lot/Batch number	Batch: XXXX
3.1.2	Specification	
3.1.2.1	Description	As given in section 2
3.1.2.2	Purity	99%
3.1.2.3	Stability	Stable
3.2	**Study Type**	In vitro mammalian cell gene mutation test
3.2.1	Organism/cell type	Chinese hamster (V79) cells
3.2.2	Deficiencies / Proficiencies	None
3.2.3	Metabolic activation system	S9 mix
3.2.4	Positive control	7,12-Dimethylbenz[α]anthracene (DMBA) with S-9 metabolic activation and Ethyl methanesulphonate (EMS) without S-9 metabolic activation
3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	50; 150; 450; 900 and 1,350 µg/ml with S-9 metabolic activation, and 50; 150; 450 and 1,350 µg/ml without S-9 metabolic activation
3.3.2	Way of application	Test substance dissolved in dimethyl sulphoxide (DMSO) and added to the culture medium.
3.3.3	Pre-incubation time	None
3.3.4	Other modifications	None
3.4	**Examinations**	
3.4.1	Number of cells evaluated	Not applicable

4 RESULTS AND DISCUSSION

4.1 Genotoxicity

Section A6.6.3	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.3	6.6.3 In vitro gene mutation assay in mammalian cells

4.1.1	without metabolic activation	No increase of the number of mutant cells. For details see study summary under 5.2.
4.1.2	with metabolic activation	No increase of the number of mutant cells. For details see study summary under 5.2.
4.2	**Cytotoxicity**	In the cytotoxicity test cloning efficiency at 1,000 µg/ml was reduced to 19% with metabolic activation and 48% without metabolic activation.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Flufenoxuron was tested for its potential to induce mutations at the HGPRT locus in Chinese Hamster V79 cells *in vitro* by measuring resistance to the base analogue 6-thioguanine. Dose levels of 50; 150; 450; 900 and 1,350 µg/ml with S-9 metabolic activation, and 50; 150; 450 and 1,350 µg/ml without S-9 metabolic activation were used in the definitive study. The S-9 mix was used at 10% and comprised a buffered solution of 10% Aroclor-induced rat-liver homogenate (S-9 fraction), glucose-6-phosphate and nicotinamide adenine dinucleotide phosphate. The selection of these dose levels was based on results from two preliminary cytotoxicity tests.

The definitive assay was performed in two independent trials. In each trial, two 125 cm² culture flasks were used of each dilution to be tested. Approx. 750,000 cells were seeded into the culture flasks. Twenty-four hours later the medium was replaced by fresh medium containing the appropriate dilution of the test-article. After four hours of exposure to different Flufenoxuron concentrations the media were replaced by fresh medium and the cells were incubated for further 24 hours. Subsequently, for each test concentration and flask, 100,000 cells were plated into each of six 90 mm Petri dishes (mutation assay dishes). Further 4 dishes per test concentration and flask were plated with cells for determination of plating efficiency. Approx. 750,000 cells were re-cultured in 125 cm² flasks and sampled as described above 3 and 7 days after exposure to the chemicals.
After two hours, 6-thioguanine was added to all mutation assay dishes to a final concentration of 10 µg/ml. Colonies on all dishes were stained and counted when visible to the naked eye, usually after an incubation period of 6 days.

The solvent control was DMSO, used at 0.5% in the first experiment and at 1% in the second experiment. The positive controls were 7,12-Dimethylbenz[α]anthracene (DMBA) with S-9 metabolic activation (5 µg/ml in the first trial and 10 µg/ml in

Section A6.6.3	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.3	6.6.3 In vitro gene mutation assay in mammalian cells

the second trial) and Ethyl methanesulphonate (EMS) without S-9 metabolic activation (200 µg/ml in the first trial and 400 µg/ml in the second trial).

5.2 Results and discussion

The solubility and stability of Flufenoxuron in DMSO has been demonstrated analytically for another Flufenoxuron batch [see IIIA 6.6.1, XXXX; see also XXXX]. The stability is considered to be independent of the batch.

Results of cytotoxicity assays

In the first cytotoxicity test, dose levels ranging from 2.5 to 250 µg/ml with and without S-9 metabolic activation showed no cytotoxicity (i.e., approx. 100% cloning efficiency) in the presence of S-9, and a 50% reduction in the cloning efficiency in the absence of S-9 at a concentration of 250 µg/ml. Therefore, a second cytotoxicity test was performed at a dose range of 200 to 1,000 µg/ml. In this test, at the highest tested dose of 1,000 µg/ml, the cloning efficiency was reduced to 19% with metabolic activation and 48% without metabolic activation. Therefore, to achieve higher toxicity, a wider range of concentrations including the high dose of 1,350 µg/ml, was chosen for the definitive mutagenicity assay.

Results of mutagenicity assay

Results of the definitive mutation assay are summarized in Table 6.6.3/56 and Table 6.6.3/57. The spontaneous mutation frequency per surviving 100,000 cells was on average 4.22 in the first experiment and 1.24 in the second experiment.

Treatment of cultures with positive controls EMS nor DMBA resulted in statistically significant an increased incidences of mutations on day 7 post-treatment, demonstrating the ability of the test system to detect mutations. However, since the mutations were not induced very effectively on day 3 post-treatment (data not shown), the concentrations of EMS and DMBA were doubled in the second experiment. In the second experiment, the positive controls induced mutations at statistically significant incidences when cultures were evaluated 3 or 7 days after treatment: EMS raised the frequency per 100,000 survivors by between 6.5 and 27 times and DMBA by between 5.4 and 16 times of the control values.

In cultures exposed to Flufenoxuron, small, sporadic increases in the frequency of gene mutation were observed. These increases in mutation frequency relative to the solvent control means were not

Section A6.6.3	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.3	6.6.3 In vitro gene mutation assay in mammalian cells

		dose-related and were usually confined to a single flask and not reproducible from one experiment to another. Statistical analysis did not reveal any significant increases in the frequency of gene mutation attributable to exposure to Flufenoxuron. The variations were within the range normally encountered in this assay.	
5.3	**Conclusion**	Flufenoxuron is judged to be negative for inducing gene mutations in mammalian cells.	X
5.3.1	Reliability	2	
5.3.2	Deficiencies	No	X

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 2.3. Deviations Yes, the highest test concentration was not toxic enough and below the recommended maximum of 2 mg/ml; no negative control No precision about pH and osmolarity evolutions
Results and discussion	Agree with the applicant's version
Conclusion	**Revisions/Amendments:** 5.3.2. Deficiencies Yes: no negative control Flufenoxuron is judged to be negative for inducing gene mutations in mammalian cells, in these experimental conditions.
Reliability	2
Acceptability	Acceptable despite the deficiencies
Remarks	**Revisions/Amendments:** Table 6.6.3/55 and 6.6.3/2: Concentrations in µg/ml Results reported in these tables are not in accordance with those reported in the study report.

Section A6.6.3	**Genotoxicity in vitro**
BPD Annex Point IIA, VI.6.VI.6.3	6.6.3 In vitro gene mutation assay in mammalian cells

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.6.3/56 The effect of Flufenoxuron or ethyl methanesulphonate (EMS) on forward mutation rates to 6-thioguanine resistance in V79 cells in absence of metabolic activation on day 7.

Concentration [mg/ml]	Number of mutant colonies per 10^5 cells			Mean plating efficiency %		
	Flask A	Flask B	Mean (% control)	Flask A	Flask B	Mean (% control)
Experiment 1 (without S-9 mix)						
0	2.10	3.75	2.93 (100%)	103	129	116 (100%)
50	2.36	2.08	2.22 (76%)	134	112	123 (106%)
150	1.58	3.97	2.78 (95%)	95	84	90 (78%)
450	2.76	3.14	2.95 (101%)	139	70	105 (91%)
1,350	4.13	7.99	6.06 (207%)	117	73	95 (82%)
EMS (200)	22.34	17.82	20.08 (685%)	47	58	53 (46%)
Experiment 2 (without S-9 mix)						
0	1.58	1.47	1.53 (100%)	190	159	175 (100%)
50	1.70	1.33	1.52 (99%)	137	125	131 (75%)
150	0.00	0.12	0.06 (4%)	295	144	220 (126%)
450	0.26	1.10	0.68 (44%)	127	91	109 (62%)
1,350	0.89	0.00	0.45 (29%)	188	112	150 (86%)
EMS (400)	22.73	26.06	24.40 (1,595%)	132	126	129 (74%)

Table 6.6.3/57 The effect of Flufenoxuron or dimethyl benzanthracene (DMBA) on forward mutation rates to 6-thioguanine resistance in V79 cells in the presence of metabolic activation on day 7.

Concentration [mg/ml]	Number of mutant colonies per 10^5 cells			Mean plating efficiency %		
	Flask A	Flask B	Mean (% control)	Flask A	Flask B	Mean (% control)
Experiment 1 (with S-9 mix)						
0	3.29	8.10	5.70 (100%)	71	60	66 (100%)
50	15.00	2.84	8.92 (157%)	20	35	28 (42%)
150	12.00	21.10	16.55 (291%)	50	53	52 (79%)
450	7.80	6.11	6.96 (122%)	62	60	61 (93%)
900	9.89	9.26	9.58 (168%)	59	81	70 (107%)
1,350	13.16	4.80	8.98 (158%)	57	66	62 (94%)
DMBA (5)	31.18	26.67	28.93 (508%)	62	60	61 (93%)
Experiment 2 (with S-9 mix)						

Table 6.6.3/57 The effect of Flufenoxuron or dimethyl benzanthracene (DMBA) on forward mutation rates to 6-thioguanine resistance in V79 cells in the presence of metabolic activation on day 7.

Concentration [mg/ml]	Number of mutant colonies per 10^5 cells				Mean plating efficiency %			
	Flask A	Flask B	Mean (% control)		Flask A	Flask B	Mean (% control)	
0	0.71	1.57	1.14	(100%)	47	85	66	(100%)
50	1.00	0.62	0.81	(71%)	67	81	74	(112%)
150	0.00	1.73	0.87	(76%)	60	77	69	(104%)
450	1.83	0.50	1.17	(102%)	73	66	70	(105%)
900	2.22	1.56	1.89	(166%)	90	85	88	(133%)
1,350	2.61	2.08	2.35	(206%)	83	96	90	(136%)
DMBA (10)	21.62	20.80	21.21	(1,861%)	74	75	75	(113%)

Section A6.6.4	**Genotoxicity in vivo**
BPD Annex Point IIA, VI.6.VI.6.4	6.6.4 In vivo cytogenitic test

Official use only

1 REFERENCE 1

1.1	**Reference**	**1) XXXX** Genotoxicity studies with WL115110: In vivo chromosome studies with rat bone marrow cells XXXX unpublished XXXX **2) XXXX** Report amendment no. 1 - Genotoxicity studies with WL115110: In vivo chromosome studies with rat bone marrow cells XXXX unpublished XXXX **Note:** Amendment no.1 contains updated GLP compliance and QAU statements, and revised Table No. 6 (to include two footnotes); the amendments do not alter the conclusion of the original study report. **3) XXXX** Report amendment no.2 - Genotoxicity studies with WL115110: In vivo chromosome studies with rat bone marrow cells XXXX unpublished XXXX **Note:** Two-page amendment of the GLP compliance statement prepared to fulfill US EPA wording requirements.
1.2	**Data protection**	No
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	XXXX
1.2.3	Criteria for data protection	No data protection claimed

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, OECD 475, EEC 79/831, Part B
2.2	**GLP**	No, at the time the study was conducted GLP was not compulsory. However the study was conducted according to the principle of Good Laboratory Practices.

Section A6.6.4	**Genotoxicity in vivo**
BPD Annex Point IIA, VI.6.VI.6.4	6.6.4 In vivo cytogenitic test

2.3	**Deviations**	No	X
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch: XXXX	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	As given in section 2	
3.1.2.2	Purity	99%	
3.1.2.3	Stability	Stable	
3.1.2.4	Maximum tolerable dose	4000 mg/kg bw representing maximum attainable dose; this dose is twice the limit dose of 2000 mg/kg!	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley	
3.2.3	Source	XXXX	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	Not reported; the report indicates that the animals weighed 40 to 45 g at delivery indicating that these animals were just weaned, i.e. were about 3 weeks old. Furthermore, it is mentioned in the report that the animals were acclimatized to laboratory conditions for at least 10 days. Accordingly, the animals had a minimum age of 5 weeks at study initiation.	
3.2.6	Number of animals per group	5m + 5f per dose and sampling time	X
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Number of applications	1	
3.3.2	Interval between applications	Not applicable	
3.3.3	Postexposure period	6, 24 and 48 h after treatment	
3.3.4	Vehicle	Corn oil	

Section A6.6.4	**Genotoxicity in vivo**	
BPD Annex Point IIA, VI.6.VI.6.4	6.6.4 In vivo cytogenitic test	
3.3.5 Concentration in vehicle	25 to 400 mg/ml	
3.3.6 Total volume applied	10 ml/kg	X
3.3.7 Dose applied	4000 mg/kg bw representing maximum attainable dose	X
3.3.8 Substance used as Positive Control	Cyclophophamide; 40 mg/kg bw (dissolved in 0.9% NaCl, intraperitoneal injection)	
3.3.9 Controls	Vehicle (corn oil)	
3.4 Examinations		
3.4.1 Clinical signs	Yes	
3.4.2 Tissue	Bone marrow	
	Number of animals: 5 animals/sex/dose	
	Number of cells: 50 cells/animal, i.e. 500 cells per dose	
	Time points: 6, 24, and, 48 h after treatment	
	Type of cells bone marrow cells	
	Parameters: numbers and types of structural aberrations	
3.5 Further remarks	None	
	4 RESULTS AND DISCUSSION	
4.1 Clinical signs	Piloerection, hunched posture and ptosis.	
4.2 Haematology / Tissue examination	Not investigated	
4.3 Genotoxicity	No	
4.4 Other	No	X

Section A6.6.4	**Genotoxicity in vivo**
BPD Annex Point IIA, VI.6.VI.6.4	6.6.4 In vivo cytogenitic test

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Flufenoxuron was tested for its ability to cause chromosomal damage under *in vivo* conditions in male and female Sprague-Dawley rats. The dose of 4,000 mg/kg bw, which was the maximum attainable dose based on its suspension limitations in corn oil, was used in the definitive study as limit dose, and based on the results of a preliminary MTD test. Suspensions of Flufenoxuron were prepared in corn oil just prior to use using a high-speed mixer. The concurrent vehicle control group received an oral administration of corn oil, and the positive control group received Cyclophosphamide at 40 mg/kg bw by intraperitoneal injection. Five rats/sex/dose group from the vehicle and Flufenoxuron treated groups were sacrificed at 6, 24 and 48 hours following dosing for collection of bone marrow cells and preparation of slides for metaphase analysis. Positive control animals were sacrificed at 24 hours following dosing. Rats were treated with Colchicine (4 mg/kg bw in 0.9% saline via i.p. injection) three hours before they were killed. For each animal, 50 metaphases were evaluated for chromosomal aberrations.

The statistical significance of the observed difference in the incidence of aberrant cells between control and treatment groups was assessed using the "Wilcoxon's sum of rank test".

5.2 Results and discussion

In a dose-range finding test, at doses of 250 to 4,000 mg/kg bw administered by gastric intubation using corn oil as the vehicle, Flufenoxuron induced no mortalities; clinical signs of toxicity comprised slight or moderate piloerection and hunched posture which were observable for up to 4 h post-treatment at 250 - 500 mg/kg bw and for 24 h postreatment at 1,000 – 4,000 mg/kg bw post-treatment.

No mortality was observed in the main study. Clinical signs observed in rats that were considered related to Flufenoxuron treatment at 4,000 mg/kg bw were piloerection, hunched posture and ptosis. Results are summarized in Table 6.6.4/58. There were no statistically significant increases in the proportion of cells with chromosomal aberrations in male or female treated animals for any sampling time at 4,000 mg/kg bw, compared to the vehicle control animals. Administration of cyclophosphamide resulted in a significant increase in the number of cells showing aberrant chromosomes, demonstrating the validity of the assay.

5.3 Conclusion

Flufenoxuron was considered to be negative in the *in vivo* chromosomal aberration assay conducted at the dose of 4,000 mg/kg bw (limit dose based on solubility). Therefore, it was

Section A6.6.4	**Genotoxicity in vivo**	
BPD Annex Point IIA, VI.6.VI.6.4	6.6.4 In vivo cytogenitic test	
	concluded that Flufenoxuron did not cause chromosomal damage *in vivo.*	X
5.3.1 Reliability	1	X
5.3.2 Deficiencies	No	X

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 2.3 Deviations Yes: number of cells analysed lower than required (50), no data on mitotic indices and on frequency of changes in cell ploidy What about rat age, humidity and food? 3.2.6. Number of animals per group Preliminary toxicity test 2m + 2f per dose, Metaphase test 5m + 5f per dose and sampling time 3.3.6. Total volume applied 10 ml/kg bw of WL 115110 and 20 ml/kg of cyclophophasmide 3.3.7. Dose applied Preliminary toxicity test 250 to 4000 mg/kg, Metaphase test 4000 mg/kg bw representing maximum attainable dose (limit test).
Results and discussion	Agree with the applicant's version 4.4. Other No mortality Although there was no evidence that the target cells were exposed (no data on mitotic indices, no ratio PCE/NCE), kinetic data show that flufenoxuron was well distributed in carcass and organs.
Conclusion	*Flufenoxuron was considered to be negative in the in vivo chromosomal aberration assay conducted at the dose of 4,000 mg/kg bw (limit dose based on solubility). Therefore, it was concluded that Flufenoxuron did not cause chromosomal damage in vivo,* under the experimental conditions described Deficiencies Yes
Reliability	2
Acceptability	Acceptable
Remarks	-

Section A6.6.4	**Genotoxicity in vivo**
BPD Annex Point IIA, VI.6.VI.6.4	6.6.4 In vivo cytogenitic test

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.6.4/58 Summary of in-vivo chromosome aberration assay results in rats

Sampling time (h)	Treatment	Dosage	Incidence of aberrant cells (%)	
		(mg/kg bw)	Including gaps	Excluding gaps
6	Corn oil	0	0.6	0.6
	Flufenoxuron	4,000	0.2	0.2
24	Corn oil	0	0.2	0.2
	Flufenoxuron	4,000	0.2	0.2
	Cyclophosphamide	40	21.9***	21.7***
48	Flufenoxuron	0	0.0	0.0
	Corn oil	4,000	0.6	0.6

Statistical evaluation: *** = $p<0.001$; otherwise $p>0.05$ (Wilcoxon's sum of ranks test)

Section A6.6.4	**Genotoxicity in vivo**
BPD Annex Point IIA, VI.6.VI.6.4	6.6.4 In vivo cytogenitic test

			Official use only
		1 REFERENCE 2	
1.1	**Reference**	**4) XXXX** Micronucleus test on WL115110 in mice XXXX unpublished XXXX	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, JMHW (1989); in general compliance to OECD 474	
2.2	**GLP**	Yes (Ministry of Agriculture, Forestry and Fisheries of Japan, Japan)	
2.3	**Deviations**	No	X
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch/lot number: XXXX	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	As given in section 2	
3.1.2.2	Purity	98.2%	
3.1.2.3	Stability	Stable	
3.1.2.4	Maximum tolerable dose	The limit dose of 2000 mg/kg was selected as highest dose level	
3.2	**Test Animals**		
3.2.1	Species	Mice	
3.2.2	Strain	ICR	
3.2.3	Source	XXXX.	

Section A6.6.4		**Genotoxicity in vivo**	
BPD Annex Point IIA, VI.6.VI.6.4		6.6.4 In vivo cytogenitic test	
3.2.4	Sex	Males	X
3.2.5	Age/weight at study initiation	8 weeks	
3.2.6	Number of animals per group	6 animals per dose and sampling time	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Intraperitoneal	
3.3.1	Number of applications	2	
3.3.2	Interval between applications	24-hours intervals	
3.3.3	Postexposure period	24 h	
3.3.4	Vehicle	Olive oil	
3.3.5	Concentration in vehicle	50 to 200 mg/ml	
3.3.6	Total volume applied	10 ml/kg	
3.3.7	Dose applied	Two times 500; 1,000 and 2,000 mg/kg bw	
3.3.8	Substance used as Positive Control	Cyclophophamide; 4 mg/ml in distilled water, 10 ml/kg applied once resulting in a dose 40 mg/kg	
3.3.9	Controls	Olive oil	

Section A6.6.4	**Genotoxicity in vivo**
BPD Annex Point IIA, VI.6.VI.6.4	6.6.4 In vivo cytogenitic test

3.4	**Examinations**		
3.4.1	Clinical signs	Yes	
3.4.2	Tissue	Bone marrow preparation	
		Number of animals:	all animals
		Number of cells:	1000 cells per animal were scored for micronuclei
		Time points:	24 h after treatment
		Type of cells	bone marrow cells
		Parameters:	polychromatic erythrocytes (PCE's) for presence of micronuclei
			Erythrocytes to determine cytotoxicity
3.5	**Further remarks**	None	

4 RESULTS AND DISCUSSION

4.1	**Clinical signs**	Marginal reduction of bodyweight in males at 500 and 2000 mg/kg bw and in positive control males.
4.2	**Haematology / Tissue examination**	The ratio of poly- (PCE) to normochromatic erythrocytes (NCE) was not affected by treatment with Flufenoxuron. In positive control males a slightly increased PCE/NCE ratio was observed. The incidence of micronucleated PCEs was comparable between treated groups and the control. The positive control displayed a significant increase in the incidence of micronuleated PCEs. For details see study summary under 5.2
4.3	**Genotoxicity**	No
4.4	**Other**	No

Section A6.6.4	**Genotoxicity in vivo**
BPD Annex Point IIA, VI.6.VI.6.4	6.6.4 In vivo cytogenitic test

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Flufenoxuron was tested in the *in vivo* micronucleus assay using ICR male mice (XXXX.) at dose levels of 500; 1,000 and 2,000 mg/kg bw. The test material was administered twice by intraperitoneal injection, in an olive oil suspension, at two consecutive 24-hour intervals. Dose levels were selected based on an acute toxicity test in ICR mice, which showed no mortalities following an intraperitoneal injection of Flufenoxuron at 2,000 mg/kg bw (limit dose according to OECD 474) within a 7-day post-treatment period. The vehicle control group received two consecutive intraperitoneal injections of olive oil, at 24-hour intervals, and the positive control group received 40 mg/kg bw Cyclophosphamide by a single intraperitoneal injection.

Mice were observed for their mortality and clinical signs at 24 and 48 hours after the first administration. Body weights of mice were recorded at the first administration and the bone marrow preparation.

Six mice per dose group were sacrificed 24 hours following the second dosing for collection of bone marrow cells. For each animal, 1,000 polychromatic erythrocytes (PCE's) were evaluated for the presence of micronuclei. In addition, 1,000 erythrocytes per animal were evaluated for classification of polychromatic erythrocytes and normochromatic erythrocytes in order to determine cytotoxicity.

With regard to statistical analyses, the Student's t-test was applied in case of the frequency of PCE's in 1,000 erythrocytes, while the tables of Kastenbaum and Bowman were used for assessment of the total number of micronucleated PCE's encountered in 1,000 PCE's.

5.2 Results and discussion

Groups of rats administered Flufenoxuron at 500 or 2000 mg/kg bw or the positive control showed a slight reduction in body weights. No mortalities or clinical signs were noted in any group.

Results [see Table 6.6.4/59] showed no statistically significant increases in the number of micronucleated polychromatic erythrocytes or in polychromatic to normochromatic erythrocyte ratios, for any dose of Flufenoxuron tested. Administration of the positive control material resulted in a significant increase in the number of micronucleated polychromatic erythrocytes, demonstrating the validity of the assay. No mortalities or notable clinical signs of toxicity were observed in the test material-treated, vehicle control, or positive control groups.

Section A6.6.4	**Genotoxicity in vivo**
BPD Annex Point IIA, VI.6.VI.6.4	6.6.4 In vivo cytogenitic test

5.3	**Conclusion**	Flufenoxuron did not cause chromosomal damage *in vivo* based on the results of the *in vivo* mouse micronucleus assay, conducted with Flufenoxuron up to the limit dose of 2,000 mg/kg bw.	X
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	X

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	Agree with the applicant's version **Revisions/Amendments:** 2.3. Deviations Yes: number of polychromatic erythrocytes scored 1000 instead of 2000 per animal required by OECD guideline 474.
Results and discussion	Agree with the applicant's version Athough there was no evidence that target cells were exposed (no significant decrease in PCE/NCE ratio), the kinetic data show that flufenoxuron was well distributed in carcass and organs.
Conclusion	**Revisions/Amendments:** *Flufenoxuron did not cause chromosomal damage in vivo based on the results of the in vivo mouse micronucleus assay, conducted with Flufenoxuron up to the limit dose of 2,000 mg/kg bw*, under the experimental conditions
Reliability	1, Deficiencies Yes
Acceptability	Acceptable despite the deficiency
Remarks	-

Section A6.6.4	**Genotoxicity in vivo**
BPD Annex Point IIA, VI.6.VI.6.4	6.6.4 In vivo cytogenitic test

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.6.4/59 Results of in-vivo micronucleus test in mice

Treatment group	Dose level	Micronucleated PCE		PCE / (PCE + NCE)
	(mg/kg bw)	(Mean ± S.D.)	No. / 6,000 cells	(Mean ± S.D.)
Olive oil	0 x 2	0.10 ± 0.09	6	39.2 ± 4.98
Flufenoxuron	500 x 2	0.08 ± 0.12	5	40.6 ± 1.16
	1,000 x 2	0.07 ± 0.12	4	39.2 ± 2.05
	2,000 x 2	0.10 ± 0.13	6	40.6 ± 4.92
Cyclophosphamide	40 x 1	2.63 ± 0.62	158** (1)	50.4 ± 3.45** (2)

PCE = Polychromatic Erythrocytes; NCE = Normochromatic Erythrocytes

Statistical evaluation: ** = $p < 0.01$ [(1) Kastenbaum and Bowman; (2) Student's t-test)

Section 6.6.5	**Genotoxicity in vivo**
BPD Annex Point IIA, VI.6.VI.6.5	6.6.5 DNA damage and repair

Official use only

		1 REFERENCE
1.1	**Reference**	**1) XXXX** Mutagenicity test on WL115110 in the in vivo/in vitro rat primary hepatocyte unscheduled DNA synthesis assay - Revised final report XXXX unpublished XXXX
1.2	**Data protection**	No
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	XXXX
1.2.3	Criteria for data protection	No data protection claimed
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Not reported, in compliance with OECD 486 in all important aspects
2.2	**GLP**	No (at the time the study was conducted GLP was not compulsory. However the study was conducted according to the principle of Good Laboratory Practices)
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	
3.1.1	Lot/Batch number	Batch: XXXX
3.1.2	Specification	As given in section 2
3.1.2.1	Description	As given in section 2
3.1.2.2	Purity	97.4%
3.1.2.3	Stability	Stable
3.1.2.4	Maximum tolerable dose	The top dose was based on ability to prepare useable suspensions, (maximum=1500 g/kg bw)
3.2	**Test Animals**	

Section 6.6.5		**Genotoxicity in vivo**	
BPD Annex Point IIA, VI.6.VI.6.5		6.6.5 DNA damage and repair	
3.2.1	Species	Rat	
3.2.2	Strain	Fisher 344	
3.2.3	Source	XXXX	
3.2.4	Sex	Males	
3.2.5	Age/weight at study initiation	150 to 300g	
3.2.6	Number of animals per group	3	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Number of applications	1	
3.3.2	Interval between applications	Not applicable	
3.3.3	Postexposure period	4 hours	
3.3.4	Type	Gavage	
3.3.5	Concentration	188; 375; 750 and 1,500mg/kg bw	
3.3.6	Vehicle	Corn oil	
3.3.7	Concentration in vehicle	37.6; 75; 107 and 304 mg/ml	X
3.3.8	Total volume applied	Approx. 5 ml	
3.3.9	Controls	Vehicle: corn oil and positive control: aqueous N,N'-Dimethylnitrosamine (DMN) solution by intraperitoneal injection	

Section 6.6.5	**Genotoxicity in vivo**
BPD Annex Point IIA, VI.6.VI.6.5	6.6.5 DNA damage and repair

3.4	**Examinations**		
3.4.1	Clinical signs	No	
3.4.2	Tissue	Liver tissue	
		Number of animals:	all animals
		Number of cells:	150 nuclei per animal
		Time points:	4 h
		Type of cells	Hepatocytes
		Parameters:	Net nuclear grain count
3.5	**Further remarks**	None	
		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	Not applicable	
4.2	**Haematology / Tissue examination**	No increase in the nuclear grain or net nuclear grain counts	
4.3	**Genotoxicity**	No	
4.4	**Other**	None	

Section 6.6.5	**Genotoxicity in vivo**
BPD Annex Point IIA, VI.6.VI.6.5	6.6.5 DNA damage and repair

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Flufenoxuron was tested in the *in vivo* / *in vitro* unscheduled DNA synthesis (UDS) assay using F344 male rats at dose levels of 188; 375; 750 and 1,500 mg/kg bw. The test material was suspended in corn oil and administered by oral gavage. The high dose level utilized in this study was based on the ability to prepare useable suspensions of the test substance in corn oil and also on the F344 rat oral LD_{50} value of greater than 3,000 mg/kg bw for Flufenoxuron (suspended in DMSO).

In a preliminary study following gavage dosing of 1,500 mg/kg bw of Flufenoxuron in two rats, one rat was sacrificed approximately 4 hours after treatment, and the second rat was sacrificed approximately 15 hours after treatment. Liver tissues were dissected, and hepatocytes were harvested, cultured, exposed to tritiated Thymidine, prepared for autoradiography and evaluated under a microscope.

In the main study, groups of 3 rats/dose group were administered the test compound, and hepatocytes were harvested 4 hours following treatment, cultured, and then exposed to tritiated Thymidine. Vehicle control rats received corn oil by oral gavages, and positive controls received 10 mg/kg bw of aqueous N,N'-Dimethylnitrosamine (DMN) solution by intraperitoneal injection. After a labeling period of 18–20 hours, hepatocytes were processed for attachment to coverslips and subsequent audioradiography. UDS was determined microscopically (1,500x magnification under oil immersion) for 50 randomly selected cells on each of three coverslips per animal by counting nuclear grains and subtracting the average number of grains in three nuclear-sized areas adjacent to each nucleus. The mean net nuclear grain count was determined from the triplicate coverslips (150 total nuclei) for each treatment condition. In addition, 1,500 nuclei per animal were scored to determine the percentage of nuclei in replicative DNA synthesis.

5.2 Results and discussion

In the preliminary study, the distribution of labeling between the nucleus and cytoplasm was similar at 4 h and 15 h following gavage dosing of 1,500 mg/kg bw of Flufenoxuron. Therefore, the definitive UDS assay was performed utilizing a sacrifice time of 4 hours post treatment.

Results of this assay are summarized in Table 6.6.5/60. The

Section 6.6.5	**Genotoxicity in vivo**
BPD Annex Point IIA, VI.6.VI.6.5	6.6.5 DNA damage and repair

		minimum criteria for UDS in this area were - a mean net nuclear grain count exceeding 4.15, or - at least 12.0% of the nuclei containing 6 or more grains, or - at least 2% of the nuclei containing 20 or more grains. None of the treatments with Flufenoxuron caused nuclear labeling that was significantly different from the vehicle control. Furthermore no dose-related trend was evident. Treatment of animals with the positive control material (DMN) induced large increases in nuclear labeling that greatly exceeded all three criteria used by the test facility to indicate UDS, thus demonstrating the validity of the assay. In addition, no treatment-related effect was noted for the replicative DNA synthesis assay (only 140 cells, i.e. 0.52% among the 27,000 cells screened in the entire assay were heavily labeled), indicating that the negative UDS response was not due to inhibition of DNA synthesis.	
5.3	**Conclusion**	Flufenoxuron did not induce unscheduled DNA synthesis in the *in vivo* / *in vitro* rat primary hepatocyte UDS assay over a dose range of 188 – 1,500 mg/kg bw.	X
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Section 6.6.5	**Genotoxicity in vivo**
BPD Annex Point IIA, VI.6.VI.6.5	6.6.5 DNA damage and repair

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	Agree with the applicant's version **Revisions/Amendments:** 3.3.7. Concentration in vehicle 38-76-152-304 mg/ml
Results and discussion	Agree with the applicant's version
Conclusion	**Revisions/Amendments:** *Flufenoxuron did not induce unscheduled DNA synthesis in the in vivo / in vitro rat primary hepatocyte UDS assay over a dose range of 188 – 1,500 mg/kg bw,* under the experimental conditions.
Reliability	1
Acceptability	Acceptable
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.6.5/60 Results of in-vivo / in-vitro rat hepatocyte UDS assay

Treatment	Dose level (mg/kg bw)	Animal no.	Perfusion viability (%)	Attachment efficiency (%)[b]	Attachment viability (%)[b]	MI	UDS (Mean)	% nuclei with ≥ 6 grains †	% nuclei with ≥ 20 grains †
Corn oil	0	1209	84.3	63.9	86.6	6	-1.14	3.3	0.0
		1193	87.2	89.8	89.1	6	-3.77	1.3	0.0
		1186	90.0	78.6	90.4	0	-0.65	1.3	0.0
Flufenoxuron	188	1208	94.9	78.2	95.0	11	-1.12 [c]	5.8	0.0
		1206	91.3	68.9	93.7	7	-3.26 [c]	2.7	0.0
		1203[a]	81.4	35.9	77.0	-	-	-	-
	375	1213	93.0	75.3	92.4	8	-3.77	0.0	0.0
		1211	93.0	75.1	94.7	5	-0.74	4.7	0.0
		1185	93.5	77.6	95.9	13	-2.60	0.7	0.0
	750	1191	92.0	82.4	96.3	5	-3.81	2.0	0.0
		1183	92.9	76.1	90.8	9	-1.77	3.3	0.0
		1197	90.5	72.5	92.1	2	-2.23	4.0	0.0
	1,500	1188	93.2	72.0	94.4	4	-3.72	0.7	0.0
		1210	92.5	76.9	94.1	5	-1.50	10.7	0.0
		1198	93.2	68.2	95.4	2	-4.29	4.0	0.0
DMN	10	1212	93.8	71.2	96.5	11	34.72	99.3	91.3
		1200	95.2	79.1	93.2	4	33.71	97.3	80.7
		1199	91.5	77.8	93.3	32	30.85	95.3	81.3

MI = Mitotic index (number of S-phase per 1,500 cells scored); DMN = Dimethylnitrosamine

UDS = Average of net nuclear grain counts on triplicate coverslips (150 total cells)

† Average values for triplicate coverslips

[a] = Cultures were terminated due to poor attachment efficiency

[b] = Results based on viability assessment (Trypan Blue dye exclusion) of 4 randomly selected areas on each of 2 plates

[c] = 2,250 cells per animal were scored for S-phase; number adjusted for 1,500 cells counted

Section 6.6.7 Further testing
BPD Annex Point III-0§

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	No further testing was triggered by the outcome of available genotoxicity assays performed with Flufenoxuron.	
Undertaking of intended data submission [ ]	Not applicable	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Evaluation of applicant's justification	Genotoxicity of relevant metabolites was evaluated in the section 6.10
Conclusion	Waiving accepted
Remarks	-

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (rat)

			Official use only
		1 REFERENCE 1	
1.1	**Reference**	**1) XXXX** WL115110: A two year oncogenicity feeding study in rats XXXX unpublished XXXX **2) XXXX** Addendum to XXXX - WL115110: A two year oncogenicity feeding study in rats XXXX unpublished XXXX **Note**: This addendum certifies that Antony James Harvey Basford was involved in the study as ophthalmologist **3) XXXX** Corrigenda/addenda to XXXX - WL115110: A 2 year oncogenicity feeding study in rats XXXX unpublished XXXX **Note:** This corrigendum replaces pages 34, 758, 870, and 872 of the report	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	Janssen Pharmaceutica	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not reported; in general compliance with OECD 451	
2.2	**GLP**	Yes (laboratory certified by the Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)	
2.3	**Deviations**	No	

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (rat)

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	Batch: XXXX
3.1.2	Specification	As given in section 2
3.1.2.1	Description	As given in section 2
3.1.2.2	Purity	97.6 %
3.1.2.3	Stability	Stable
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Fischer 344
3.2.3	Source	XXXX
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	About 6 weeks old
3.2.6	Number of animals per group	50/sex/dose
3.2.6.1	at interim sacrifice	Not applicable
3.2.6.2	at terminal sacrifice	50/sex/dose
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral – feeding study
3.3.1	Duration of treatment	24 months
3.3.2	Interim sacrifice(s)	Not applicable
3.3.3	Final sacrifice	Not applicable
3.3.4	Freqency of exposure	Daily
3.3.5	Postexposure period	None
3.3.6	Type	In food

Section A6.7		**Carcinogenicity**
BPD Annex Point IIA, VI.6.7		6.7 Carcinogenicity – Oral administration (rat)
3.3.7	Concentration	0; 500; 5,000 and 50,000 ppm, equivalent to a mean daily compound intake of 21.57, 217.5 and 2,289.8 mg/kg bw in males and 25.91, 276.4 and 2,900.9 mg/kg bw in females, respectively.
3.3.8	Vehicle	None
3.3.9	Concentration in vehicle	Not applicable
3.3.10	Total volume applied	Not applicable
3.3.11	Controls	Basal diet
3.4	**Examinations**	
3.4.1	Body weight	Weekly during the first 13 weeks and every 4 weeks thereafter
3.4.2	Food consumption	Weekly during the first 13 weeks and every 4 weeks thereafter
3.4.3	Water consumption	No
3.4.4	Clinical signs	Twice daily on week days and once daily on weekends and public holidays
3.4.5	Macroscopic investigations	Palpable masses, skin tumours
3.4.6	Ophthalmoscopic examination	Yes
3.4.7	Haematology	Yes Number of animals: all animals Time points: 12, 18, 24 months and at study termination Parameters: Blood smears were prepared, stained and examined of differential leukocyte count, erythrocyte morphology and for the presence of any leukemias and anemias.
3.4.8	Clinical Chemistry	No
3.4.9	Urinalysis	No

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (rat)

3.4.10	(Macro) Pathology	Yes	
3.4.10.1	Organ Weights	Yes	
		From:	all surviving
		Organs:	brain, heart, liver, kidneys, spleen, adrenals and testes or ovaries
3.4.11	Histopathology	Yes	
		From:	controls and high dose groups and all animals which died or were sacrificed moribund
			other dose groups: Kidneys, liver, lungs and spleen
		Organs:	at least the organs and tissues listed in OECD Guideline 451
3.4.12	Other examinations	Not applicable	
3.5	**Statistics**	(i) Survival: The log rank test (Kalbfleisch and Prentice, 1980) was used to emrestimate the effects of treatment on survival. Animals which were killed at the scheduled necropsy were considered to have censored survival times. (ii) Bodyweights, food intakes, haematology and organ weights: Analysis of variance was performed on the data from all variates, except for those variates for which there were serious doubts about the validity of the analysis. Where analysis of variance was suitable, a two-way analysis (Snedecor and Cochran, 1968) with treatments and blocks as factors was used for a variate provided that both of the following were true: (i) less than 20% of the possible observations were missing and (ii) each treatment group had at least 50% of its possible observations. If this was not the case, then a one-way analysis (Snedecor and Cochran, 1968) ignoring blocks was used. The significance levels of the differences between the control and treated means (adjusted if necessary) were determined using the William's t test (Williams, 1971; 1972) whenever justifiable. Dunnett's test (Dunnett, 1964) was used if a monotonic dose response could not be assumed. When a covariate was used, the standard error of differences took account of the adjustment made to the means (Snedecor and Cochran, 1968). (iii) Histopathology: The Cochran-Armitage trend test (Armitage 1971) followed by a Peto analysis (Peto et al. 1980) was applied to non-neoplastic	

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (rat)

		findings of interest. Fischer's Exact Test was also applied to these findings. Tumour types were only combined where the pathologist indicated that it was valid to do so, following the National Cancer Institute Guidelines (McConnell et al., 1986). (References see study report)	
3.6	**Further remarks**	None	
		4 RESULTS AND DISCUSSION	
4.1	**Body weight**	Decreased body weight gain at dietary dose levels ≥ 5,000 ppm, see study summary under 5.2	
4.2	**Food consumption**	Slightly higher in both sexes at 50,000 ppm, see study summary under 5.2	
4.3	**Water consumption**	Not applicable	
4.4	**Clinical signs**	No adverse treatment-related clinical signs, see study summary under 5.2	
4.5	**Macroscopic investigations**	No adverse treatment-related clinical signs, see study summary under 5.2	
4.6	**Ophthalmoscopic examination**	No treatment-related findings, see study summary under 5.2	
4.7	**Haematology**	No adverse treatment-related clinical signs, see study summary under 5.2	X
4.8	**Clinical Chemistry**	Not applicable	
4.9	**Urinalysis**	Not applicable	
4.10	**Pathology**	No adverse treatment-related clinical signs, see study summary under 5.2	
4.11	**Organ Weights**	No adverse treatment-related clinical signs, see study summary under 5.2	
4.12	**Histopathology**	No adverse treatment-related clinical signs, see study summary under 5.2	
4.13	**Other examinations**	Not applicable	
4.14	**Time to tumours**	In absence of treatment related effects on tumor incidence: Not applicable	

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (rat)

4.15	**Other**	Not applicable

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Groups of 50 male and 50 female Fischer 344 (F344) rats (XXXX) were fed Flufenoxuron at dietary dose levels of 0; 500; 5,000 and 50,000 ppm for 24 months. The animals were from the same delivery as those for the chronic toxicity study described above [see IIIA 6.5, XXXX]. The animals were housed individually and were about 6 weeks old at commencement of treatment. Clinical signs were recorded twice daily on week days and once daily on weekends and public holidays. Ophthalmoscopy was performed on 20 males and 20 females per dose group prior to study start. Top dose and control rats were examined again at the end of the study. Body weights and food consumption were determined weekly during the first 13 weeks and every 4 weeks thereafter. Blood was sampled from all rats from the tip of the tail at 12, 18 and 24 months and from all surviving rats at terminal sacrifice. Blood smears were prepared, stained and examined of differential leukocyte count, erythrocyte morphology and for the presence of any leukemias and anemias. All animals - regardless whether surviving till scheduled sacrifice or found dead/sacrificed moribund - were subjected to a detailed necropsy. Surviving rats were anesthetized by intraperitoneal injection of sodium phenobarbitone and killed by exsanguination by cardiac puncture. Terminal body weight and organ weights were determined in surviving animals (brain, heart, liver, kidneys, spleen, adrenals and testes or ovaries). A full range of organs or representative section thereof from all animals were sampled, fixed, blocked, sliced and stained. Histopathological examination of all organs and tissues was performed on all control and top dose animals as well as from all animals which died or were sacrificed moribund. Kidneys, liver, lungs and spleen were examined from all groups. Sampling of organs and histopathological examinations included at least the organs and tissues listed in OECD Guideline 451.
5.2	**Results and discussion**	Diets preparations and dietary analyses were conducted conjointly with the chronic toxicity study in rats described above. Twelve of the 27 diet preparations were analyzed for test article content. All batches of unformulated diet were analyzed. The results of diet analyses were already provided in the rat chronic toxicity study [see IIIA 6.5, XXXX]. Based on food consumption the average daily compound intake

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (rat)

was 21.57, 217.5 and 2,289.8 mg/kg bw in males and 25.91, 276.4 and 2,900.9 mg/kg bw in females at dietary dose levels of 500; 5,000 and 50,000 ppm, respectively.

There was no adverse effect on survival. On the contrary, there was a statistically significant trend for increased survival in males and females. Specifically, at study termination, survival rates were 21/50 (42%), 26/50 (52%), 29/50 (58%), and 33/50 (66%) for males and 28/50 (56%), 35/50 (70%), 37/50 (74%), and 38/50 (76%) for females at 0; 500; 5,000 and 50,000 ppm, respectively. The improved survival in treated groups was probably due to lower body weights resulting in a reduced incidence of age related pathology (see below).

There were no adverse treatment-related clinical signs. In fact, the incidence of age-related clinical signs was reduced in treated rats when compared to controls, particularly in males at the high dose. Amongst these rats there was a lower incidence of poor condition, soiled fur, unkempt appearance and reduced food intake. There was also an apparent reduction in the incidence of pale eyes or skin in male rats at the two upper dose levels. Treated females had a reduced incidence of subcutaneous masses.

Ophthalmology revealed no treatment-related findings.

Body weight development of rats fed Flufenoxuron at dietary dose levels ≥ 5,000 ppm was impaired [see Table 6.7/61]. The effect in females was more pronounced than in males. No treatment-related effect on body weight was observed at 500 ppm.

Food consumption tended to be slightly higher in both sexes at the high dose level. Cumulative food consumption was increased by 3.4 and 1.3% in high dose males and females, respectively. The increased food consumption was considered to be due to the reduced caloric value of the diet at 50,000 ppm as the nutritionally inert test substance constituted 5% of the diet. Cumulative food consumption in mid dose rats was essentially comparable to that of the controls although there were occasional statistically significant increases of weekly food consumption values. Food efficiency (gram food consumed per gram body weight gained) was not calculated in the study. However, if calculated based on mean weekly body weight gains and food consumption, cumulative food efficiency for Weeks 1 to 13 was decreased by 6.6% in high dose males and by 8.5 and 14.0% in mid and high dose females, respectively.

Examination of blood smears revealed no treatment-related findings. The incidence of leukemias as identified by blood smear

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (rat)

analysis tended to be lower in treated groups as there were 11, 3, 1 and 1 cases in males and 8, 3, 1 and 0 cases in females at dose levels of 0; 500; 5,000 and 50,000 ppm, respectively. Significant changes of differential white blood cell counts were only observed at 18 months in males and consisted of decreased polymorphonuclear neutrophils and increased lymphocytes at ≥ 5,000 ppm and increased monocytes at 50,000 ppm. Due to the isolated occurrence of this finding, no toxicological significance is attached to these changes.

A number of absolute and terminal body weight adjusted ('relative') organ weights were statistically significant different from controls [see Table 6.7/62]. Some of these changes (i.e. the decreased absolute liver and ovary weights in high dose females) were probably secondary to the lower terminal body weight as the differences were no longer statistically significant after adjustment for terminal body weight. In addition, ovary weight changes were not dose related and of small magnitude. Likewise, the slightly higher adrenal weights in all treated female groups were also not considered to be adverse since the magnitude of change was small (+6.6 to +16.4%), was not strictly dose related and was not accompanied by treatment-related histopathological findings. The lower spleen weights in treated groups probably correlated with the decreased incidence of spleen enlargement [see Table 6.7/63]. This decrease was accompanied by a decreased incidence of splenic mononuclear cell leukemia in treated groups [see Table 6.7/65]. As mononuclear cell infiltration results in increased spleen weights, the spleen weight effects are therefore not considered to be of adverse nature. This view is supported by the absence of adverse, treatment-related histopathological findings in the spleen.

At necropsy most of the macropathological findings were evenly distributed between control and treated groups and of a type and incidence commonly observed in rats of this strain and age. For a few findings numerical differences were observed [see Table 6.7/63]. With the exception of an exaggerated lobular pattern of the liver in treated male rats all of these findings were observed at a lower incidence in treated groups. The incidences of exaggerated lobular pattern in the liver for males at 5,000 ppm and 50,000 ppm (18/50) are not considered to be treatment-related because a very similar incidence (17/50) was observed in control females. These changes were therefore not considered to be adverse.

Histopathology revealed a range of non-neoplastic pathological findings commonly observed in ageing F344 rats. An increased

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (rat)

incidence of basophilic foci of cellular change was observed in the livers of high dose males (Fisher Exact Test ($p < 0.05$) [see Table 6.7/64]. However, this apparent increased incidence of basophilic foci of cellular alteration in the livers of high dose males was neither considered to be treatment-related nor related to any pre-neoplastic process for the following reasons: (1) As suggested by the study pathologist, "it is possible that the higher incidence of mononuclear cell leukemia, which infiltrates the liver," (observed in 22 of 50 controls but only in 3 of 50 males at 50,000 ppm), "could have obscured the diagnosis of this focal change in controls, leading to an apparent increase" in males at 50,000 ppm. (2) Moreover, for the subset of male nonleukemic rats (i.e. males without mononuclear cell leukemia) sacrificed at termination, no statistically significant difference ($p < 0.05$) was observed for the incidence of basophilic foci between controls (10/13 or 77%) versus males at 50,000 ppm (29/32 or 91%). (3) In addition, although a positive trend ($p < 0.05$) was noted for the incidence of basophilic foci for all males on study (i.e., 25/50, 26/50, 33/50, and 42/50 at 0, 500, 5,000, and 50,000 ppm, respectively), no statistically significant positive trend was seen for the subset of male nonleukemic rats sacrificed at termination with relatively comparable incidences (i.e., 10/13, 18/24, 24/29, and 29/32 at 0, 500, 5,000, and 50,000 ppm, respectively). (4) Furthermore, in the 2-year chronic toxicity study with F344 rats, for all animals on study, comparable incidences of basophilic foci were observed in controls males (34/40 or 85%) and males at 50,000 ppm (18/20 or 90%). (5) Finally, similar to the 2-year chronic rat study, no treatment-related hepatocellular tumors were observed for males and females that were sacrificed at termination, or died on study, or for all animals combined. Thus, for the reasons given above, the increased incidence of basophilic foci of cellular alteration in the livers of 50,000 ppm males was neither considered to be treatment-related nor related to any pre-neoplastic process.

Other non-neoplastic findings tended to occur at lower incidences in treated groups. Such findings were observed in the liver of both sexes and lymph nodes and pituitary of males.

There were no treatment-related increases in the incidence of any neoplastic lesion for males or females. The incidence of neoplastic findings was generally comparable between control and treated groups [seeTable 6.7/65]. A decreased incidence of lymphatic mononuclear cell leukemia was observed in dosed male and female groups. In top dose females the incidence of mammary fibroadenoma was statistically significant reduced.

Section A6.7	**Carcinogenicity**	
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (rat)	
	Overall, there was a significant decrease of primary malignant tumors in high dose males and of multiple primary benign tumors in high dose males and females.	
5.3 Conclusion	Dietary administration of Flufenoxuron to Fischer 344 rats at dose levels of 0; 500; 5,000 and 50,000 ppm resulted in increased survival of treated groups. This was especially obvious at 50,000 ppm with 57% and 35% better survival in male and females, respectively. The better survival is thought to be due to the slightly to moderately lower body weights of rats at the high dose level. The few statistically significant organ weight changes were not accompanied by any treatment-related histopathological findings. These changes were therefore of questionable toxicological relevance. No treatment-related effects on the incidence of non-neoplastic lesions was observed in treated males or females. There was no evidence for an oncogenic effect of Flufenoxuron in rats at dose levels up to 50,000 ppm. On the contrary, there was a significant decrease of multiple primary benign tumors in males and females and of malignant primary tumors in males at 50,000 ppm. Based on the results of this study the NOEL for oncogenicity was 50,000 ppm, the highest concentration tested, which is equivalent to a mean daily dose of about 2,290 mg/kg bw in males and 2,900 mg/kg bw in females. The NOAEL for systemic toxicity in this study was 500 ppm (21.57 mg/kg in males and 25.91 mg/kg in females) based mainly on body weight effects observed at 5,000 ppm.	
5.3.1 Reliability	1	X
5.3.2 Deficiencies	No	

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (rat)

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	Agree with the applicant's version
Results and discussion	**Revisions/Amendments:** 4.7. Haematology: Haematological data were not provided for red blood parameters like hemoglobin, hematocrit or number of red blood cells. These parameters represent early anemia signs and all the previous chronic or subchronic studies performed mentioned the effect of Flufenoxuron on these variables. So, with regard to haematological data provided in the study report, we can only conclude that Flufenoxuron does not induce leukaemia. The decreased spleen weight need to be discussed
Conclusion	Based on increased incidence of basophilic foci in the livers of high dose males and decreasing female body weight at the two highest doses, the NOAEL for chronic toxicity was 500 ppm (25.91 mg/kg bw/day) for females and 5,000 ppm (217.5 mg/kg bw/day) for males. NOAEL carcinogenicity = 50000 ppm (2289.8-2900.9 mg/kg bw/day)
Reliability	2, due to the lack of haematological data
Acceptability	Acceptable
Remarks	-

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (rat)

	COMMENTS FROM ...
Date	Give date of comments submitted
Materials and Methods	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state
Results and discussion	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Table 6.7/61 Body weight development of rats fed Flufenoxuron for 2 years - Carcinogenicity study

Sex	Males				Females			
Dose [ppm]	0	500	5,000	50,000	0	500	5,000	50,000
Body weight [g]								
Week 0	151.7	151.0	152.7	151.9	112.7	113.3	115.6*	115.1*
Week 13	329.5	328.3 (-0.3)a	325.4 (-2.9)	322.4* (-4.1)	187.5	190.3 (2.9)	185.1 (-7.1)	182.5** (-9.9)
Week 28	384.5	381.4 (-1.0)	379.4 (-2.6)	371.4** (-5.7)	218.1	218.1 (-0.6)	209.0** (-11.4)	208.6** (-11.3)
Week 52	426.3	424.4 (-0.4)	420.3 (-2.5)	409.9** (-6.0)	253.1	252 (-1.2)	242.8** (-9.4)	236.6** (-13.5)
Week 76	455.7	451.3 (-1.2)	448.2 (-2.8)	435.2** (-6.8)	304.4	300 (-2.6)	291.3** (-8.3)	279.6** (-14.2)
Week 104	414.9	424.9 (4.1)	428.6 (4.8)	422.7 (2.9)	325.4	324.4 (-0.8)	316.9 (-5.4)	300.1** (-13.0)

* $p < 0.05$; ** $p < 0.01$; [a] Cumulative body weight gain expressed as percent difference from control

Table 6.7/62 Selected organ weights of rats fed Flufenoxuron for 24 months - Carcinogenicity study

	Absolute organ weights [g]				Terminal body weight adjusted organ weights [g]			
Dose [ppm]	0	500	5,000	50,000	0	500	5,000	50,000
Males								
Terminal body weight	388.7	404.0	394.4	388.4	388.7	404.0	394.4	388.4
Spleen	1.38	1.20*	0.99**	0.95**	1.39	1.20*	0.99**	0.95**
Kidneys	3.09	2.97	2.76*	2.87*	3.09	2.97	2.76*	2.87*
Liver	12.98	12.56	11.73*	11.81*	12.98	12.56	11.73	11.81*
Testes	4.78	5.69	5.23	5.15	4.79	5.67	5.23	5.17
Adrenals	0.074	0.071	0.070	0.071	0.074	0.071	0.070	0.071
Females								
Terminal body weight	308.3	307.4	294.8	277.7**	308.3	307.4	294.8	277.7**
Spleen	0.68	0.64	0.62	0.63	0.67	0.63	0.62	0.64
Kidneys	2.08	2.06	2.09	2.01	2.05	2.04	2.09	2.05
Liver	9.00	8.71	8.76	8.31*	8.76	8.49	8.78	8.67
Ovary	0.10	0.09	0.10	0.09*	0.10	0.09	0.10	0.09
Adrenals	0.062	0.068	0.065*	0.07**	0.061	0.067*	0.065*	0.07**

* $p < 0.05$; ** $p < 0.01$ (William's test)

Table 6.7/63 Selected macropathological findings in rats fed Flufenoxuron for 2 years - Carcinogenicity study

Sex	Males				Females			
Dose [ppm]	0	500	5,000	50,000	0	500	5,000	50,000
No. of animals examined	50	50	50	50	50	50	50	50
Brain								
- ventral compression	6	5	3	4	12	8	8	4
Liver								
- exaggerated lobular pattern	4	8	14	18	17	7	10	3
- dark focus(i)/area(s)	13	9	8	3	7	12	8	11
- rough/pitted/granular surface	15	3	4	1	13	4	-	1
- enlargement	13	6	6	6	4	2	-	1
Lungs								
- dark discoloration	14	4	7	7	11	4	6	3
Mammary gland								
- enlargement	4	-	4	2	13	6	7	5
- nodule/mass(es)	1	-	1	-	4	1	-	-
Pituitary								
- nodule/mass(es)	16	18	15	20	27	27	24	18
Spleen								
- enlargement	24	5	4	7	11	3	1	2
Subcutis								
- nodule/mass(es)	4	6	9	8	13	13	4	4

- finding not observed at this dose level

Table 6.7/64 Selected histopathological findings of rats fed Flufenoxuron for up to 2 years – Carcinogenicity study

Sex	Males				Females			
Dose [ppm]	0	500	5,000	50,000	0	500	5,000	50,000
Liver (# examined)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
- basophilic parenchymal foci	25	26	33	42**	30	28	27	29
- spongiosis hepatis	21	13	7**	2**	0	0	1	0
- necrosis, focal	2	0	4	1	7	0*	1	1
Lymph node, mesenteric (# examined)	(50)	(26)	(23)	(50)	(50)	(15)	(14)	(49)
- pseudocystic degeneration	9	2	2	1	1	0	0	1
Pituitary gland (# examined)	(50)	(40)	(38)	(50)	(49)	(45)	(41)	(48)
- cyst(s)/cystic degeneration	10	2	3	3	15	15	10	12

* $p < 0.05$; ** $p < 0.01$ (Fisher's exact test)

Table 6.7/65 Incidence of neoplastic lesions in rats administered Flufenoxuron for 24 months

Sex	Males				Females			
Dose [ppm]	0	500	5,000	50,000	0	500	5,000	50,000
Overview (# examined)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Primary tumors								
- benign	22	39	40	33	29	31	31	24
- malignant	28	11	10	17**	19	11	6	14
Primary tumors, multiple								
- benign	40	35	28	34*	26	18	16	16*
- malignant	3	0	1	1	1	2	0	2
Lymphatic leukemia	22	5	2	3***	14	3	2	2***
Histiocytic sarcoma	0	0	0	3	0	0	0	0
Multicentric, other	3	1	2	1	0	0	0	0
Individual tumors								
Number of animals examined	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Adrenal cortex	(50)	(26)	(22)	(50)	(49)	(19)	(19)	(50)
Hyperplastic focus/foci	0	1	0	0	3	1	0	2
Adenoma, one	2	1	1	0	2	0	0	0
Adenoma, two					0	0	0	1
Adrenal, medulla	(50)	(26)	(21)	(50)	(49)	(19)	(18)	(50)
Hyperplastic focus	16	6	3	17	3	1	1	6

Table 6.7/65 Incidence of neoplastic lesions in rats administered Flufenoxuron for 24 months

Sex	Males				Females			
Dose [ppm]	0	500	5,000	50,000	0	500	5,000	50,000
Benign phaeochromocytoma	9	1	3	4	1	1	0	2
Ganglioneuroma	0	0	1	0	0	0	0	1
Bone	(50)	(24)	(21)	(50)	(50)	(15)	(17)	(50)
Osteosarcoma	0	0	0	1	0	0	0	0
Brain	(50)	(25)	(21)	(50)	(50)	(18)	(15)	(50)
Oligodendroglioma	1	0	0	0	0	0	0	0
Colon	(49)	(24)	(22)	(50)	(49)	(15)	(14)	(50)
Fibrosarcoma (of connective tissue)	0	0	0	1	0	0	0	0
Duodenum	48	24	22	50	(50)	(15)	(14)	(50)
Leiomyoma (of connective tissue)	0	0	0	0	1	0	0	0
Epididymides	(50)	(46)	(37)	(50)	NA	NA	NA	NA
Mesothelioma	3	1	1	1	-	-	-	-
Eyes	(50)	(32)	(32)	(50)	(50)	(46)	(42)	(49)
Benign Schwannoma	0	1	0	0	0	0	0	0
Head/nasal cavity	(50)	(23)	(21)	(50)	(50)	(15)	(14)	(49)
Squamous cell papilloma	0	0	0	2	0	0	0	0
Squamous cell carcinoma	2	1	0	1	1	0	0	1
Adenocarcinoma	0	0	0	0	0	1	0	0
Heart	(50)	(27)	(24)	(50)	(50)	(17)	(16)	(50)
Schwannoma, benign	0	0	0	0	1	0	0	0
Jejunum	(49)	(24)	(21)	(50)	(50)	(15)	(14)	(50)
Fibrosarcoma (of connective tissue)	1	0	0	0	0	0	0	0
Malignant Schwannoma (dto)	0	0	0	1	0	0	0	0
Kidney	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Hyperplastic focus/foci	1	0	0	0	0	0	0	0
Adenoma(s)	0	1	0	0	0	0	0	0
Carcinoma(s)	0	0	0	0	1	0	0	0
Fibrosarcoma (of connective tissue)	0	0	0	0	0	1	0	0
Liver	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Adenoma(s)	0	2	1	0	0	0	1	0
Carcinoma(s)	0	1	0	0	0	0	0	0
Lymphatic leukemia, mononuclear cell	22	5	2	3	14	3	2	2
Histiocytic sarcoma	0	0	0	2	0	0	0	0

Table 6.7/65 Incidence of neoplastic lesions in rats administered Flufenoxuron for 24 months

Sex	Males				Females			
Dose [ppm]	0	500	5,000	50,000	0	500	5,000	50,000
Mesothelioma	0	0	0	1	0	0	0	0
Lung	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Hyperplastic focus, bronchiolar/alveolar	0	1	0	0	2	0	0	1
Adenoma, pulmonary	1	0	1	0	0	1	0	0
Mammary gland	(48)	(23)	(21)	(49)	(50)	(26)	(19)	(50)
Fibroadenoma, one	0	0	2	0	8	9	3	0[#]
Fibroadenoma, two	0	0	0	0	2	0	1	0
Adenocarcinoma	0	0	0	1	1	1	1	2
Ovaries	NA	NA	NA	NA	(50)	(18)	(18)	(50)
Tubulostromal hyperplasia	-	-	-	-	0	0	0	1
Benign Granulosa cell tumor	-	-	-	-	0	0	0	1
Malig. Granulosa cell tumor	-	-	-	-	0	0	0	1
Haemangioma	-	-	-	-	0	0	0	1
Pancreas	(49)	(25)	(23)	(50)	(50)	(16)	(15)	(50)
Hyperplastic focus/foci, Islet cell	4	0	0	0	1	0	0	1
Islet cell adenoma	3	2	3	4	1	0	0	2
Hyperplastic focus/foci, exocrine pancreas	0	0	0	0	0	0	1	0
Pituitary gland	(50)	(40)	(38)	(50)	(49)	(45)	(41)	(48)
Hyperplastic focus/foci, Pars anterior	9	5	5	13	10	5	9	5
Adenoma(s), Pars anterior	23	27	23	27	25	27	27	20
Hyperplastic focus/foci, Pars intermedia	0	0	0	0	1	0	0	0
Adenom(s), Pars intermedia	0	0	0	0	0	0	1	0
Craniopharyngioma	0	0	0	0	1	0	0	0
Prostate gland	(49)	(24)	(25)	(50)	NA	NA	NA	NA
Hyperplastic focus/foci	2	1	3	6	-	-	-	-
Seminal vesicles	(50)	(32)	(31)	(49)	NA	NA	NA	NA
Hyperplastic focus/foci	0	0	0	1	-	-	-	-
Mesothelioma	1	0	0	1	-	-	-	-
Skeletal muscle	(50)	(24)	(21)	(50)	(50)	(15)	(14)	(50)
Sarcoma NOS	0	0	0	1	0	0	0	0
Spleen	(50)	(29)	(24)	(50)	(50)	(18)	(14)	(50)
Haemangiosarcoma (of connective tissue)	0	0	1	0	0	0	0	0
Lymphatic leukemia Mononuclear cell	17	4	2	3	8	3	1	2

Table 6.7/65 Incidence of neoplastic lesions in rats administered Flufenoxuron for 24 months

Sex	Males				Females			
Dose [ppm]	0	500	5,000	50,000	0	500	5,000	50,000
Mesothelioma	0	0	0	1	0	0	0	0
Stomach, non glandular	(50)	(28)	(24)	(50)	(50)	(17)	(18)	(50)
Hyperplastic focus/foci, Basal cell	1	0	0	0	0	0	0	0
Squamous papilloma	0	0	0	0	0	0	1	1
Mesothelioma	1	0	0	0	0	0	0	0

Table 6.7/66 Incidence of neoplastic lesions in rats administered Flufenoxuron for 24 months

Testes	(50)	(50)	(48)	(50)	NA	NA	NA	NA
Hyperplastic foci: Leydig cell (1-6)	20	12	12	16	-	-	-	-
Hyperplastic foci: Leydig cell (7-10)	4	1	4	5	-	-	-	-
Hyperplastic foci: Leydig cell (>10)	1	0	2	3	-	-	-	-
Benign Leydig cell Tumor(s)	44	43	40	40	-	-	-	-
Mesothelioma	3	0	1	1		-	-	-
Thymus	(45)	(18)	(21)	(44)	(49)	(15)	(14)	(42)
Benign thymona	1	0	0	0	1	0	0	0
Thyroid gland	(50)	(28)	(22)	(50)	(50)	(17)	(18)	(50)
Hyperplastic focus/foci, follicular cell	2	2	0	3	0	1	1	3
Adenoma(s), follicular cell	0	0	0	1	0	0	0	0
Adenocarcinoma(s), follicular cell	0	1	1	0	1	0	0	0
hyperplastic focus/foci, C-cell	8	4	2	7	5	1	3	5
Adenoma(s), one, C-cell	9	5	1	4	6	2	6	5
Adenoma(s), two, C-cell	0	1	0	1	0	0	0	1
Adenocarcinoma(s), one, C-cell	0	0	1	0	0	2	0	1
Tongue	(48)	(24)	(21)	(49)	(50)	(15)	(15)	(49)
Basal cell, Hyperplastic focus/foci	0	0	0	1	0	0	0	0
Papilloma, squamous	0	0	0	0	1	0	0	0
Uterus	NA	NA	NA	NA	(50)	(27)	(24)	(50)
Endometrial stromal polyp	-	-	-	-	12	10	8	8
Endometrial stromal sarcoma	-	-	-	-	0	0	0	1
Endometrial adenocarcinoma	-	-	-	-	2	2	3	5
Urinary bladder	(50)	(24)	(22)	(50)	(50)	(15)	(14)	(50)
Transitional cell papilloma	0	0	1	0	0	0	0	0
Non-protocol organs								
Buccal cavity								
Squamous cell carcinoma	0	0	0	0	0	1	0	0
Mesentery								
Mesothelioma	0	0	0	1	0	0	0	0
Sarcoma NOS	0	0	0	0	0	0	0	1
Preputial gland								
Sebaceous-squamous carcinoma	0	0	0	1	0	0	0	0
Sebaceous adenoma	0	0	0	0	1	0	1	1
Sebaceous carcinoma	0	1	0	0	0	0	0	0

Table 6.7/67 Incidence of neoplastic lesions in rats administered Flufenoxuron for 24 months

Skin								
Hyperplastic focus - epidermal	1	0	2	0	0	0	0	0
Sebaceous adenoma	0	1	0	0	0	0	0	0
Squamous papilloma	0	1	0	0	0	1	0	0
Squamous cell carcinoma	0	0	0	1	0	0	0	0
Basal cell carcinoma	0	0	0	0	0	0	0	1
Basosquamous carcinoma	0	0	0	0	0	0	0	1
Malignant Schwannoma	0	0	1	1	0	0	0	0
Skull								
Osteosarcoma	0	0	0	0	0	1	0	0
Subcutaneous tissues								
Fibroma	2	4	3	1	0	2	0	0
Fibrosarcoma	1	1	2	1	0	0	0	0
Malignant Schwannoma	1	0	0	1	0	0	0	0
Lipoma	0	0	1	1	0	0	0	0
Osteosarcoma	0	0	1	0	0	0	0	0
Zymbal gland								
Kerato-acanthoma	0	1	0	1	0	0	0	0
Squamous cell carcinoma	0	0	0	0	0	1	0	0

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Chi square test, Trend test); NA: Not applicable
NOS (not other specified)

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

			Official use only
		1 REFERENCE 2	
1.1	**Reference**	**4) XXXX** WL115110: A 2 year oncogenicity feeding study in mice XXXX unpublished XXXX **5) XXXX** Corrigenda/addenda to XXXX - WL115110: A 2 year oncogenicity feeding study in mice XXXX unpublished XXXX **Note:** This corrigendum/addendum corrects typing errors on pages 22 and 544 of the report **6) XXXX** Corrigenda/addenda to XXXX - WL115110: A 2 year oncogenicity feeding study in mice XXXX unpublished XXXX **Note**: This corrigendum/addendum provides some exchange text and a total of 38 pages to be replaced **7) XXXX** Corrigenda/addenda to XXXX - WL115110: A 2 year oncogenicity feeding study in mice XXXX unpublished XXXX **Note**: This corrigendum/addendum provides a revision of the pathology report including individual pathology data and comprises of 478 pages **8) Haseman J. K. et al. 1985** Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57BL/6N x C3H/HeN)F1 (B6C3F1) mice Journal National Cancer Institute, Vol. 75, No.5, 975-984 XXXX	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	

Section A6.7		**Carcinogenicity**	
BPD Annex Point IIA, VI.6.7		6.7 Carcinogenicity – Oral administration (mouse)	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not reported; but study is in general compliance with OECD 451	
2.2	**GLP**	Yes (laboratory certified by the Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	Batch: XXXX	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	As given in section 2	
3.1.2.2	Purity	97.6 %	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Mice	
3.2.2	Strain	C57/C3H (=B6C3) F_1 hybrid	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	About 6 weeks old	
3.2.6	Number of animals per group	60/sex/dose	X

Section A6.7		**Carcinogenicity**
BPD Annex Point IIA, VI.6.7		6.7 Carcinogenicity – Oral administration (mouse)
3.2.6.1	at interim sacrifice	10/sex/dose
3.2.6.2	at terminal sacrifice	50/sex/dose
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral – feeding study
3.3.1	Duration of treatment	24 months
3.3.2	Interim sacrifice(s)	12 months
3.3.3	Final sacrifice	24 months
3.3.4	Frequency of exposure	Daily
3.3.5	Postexposure period	None
3.3.6	Type	In food
3.3.7	Concentration	0; 500; 5,000 and 50,000 ppm, equivalent to a mean daily compound intake of 56; 559 and 7,356 mg/kg bw in males and 73; 739 and 7,780 mg/kg bw in females, respectively
3.3.8	Vehicle	None
3.3.9	Concentration in vehicle	Not applicable
3.3.10	Total volume applied	Not applicable
3.3.11	Controls	Basal diet

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

3.4	**Examinations**		
3.4.1	Body weight	Weekly during the first 13 weeks and every 4 weeks thereafter	
3.4.2	Food consumption	Weekly during the first 13 weeks and every 4 weeks thereafter	
3.4.3	Water consumption	No	
3.4.4	Clinical signs	Twice daily on week days and once daily on weekends and public holidays	
3.4.5	Macroscopic investigations	Palpable masses, skin tumours	
3.4.6	Ophthalmoscopic examination	No	
3.4.7	Haematology	Yes	
		Number of animals:	All
		Time points:	At 12, 18 and 24 months blood was taken from the tip of the tail.
		Parameters:	Blood smears were prepared, stained and examined of differential leukocyte count, erythrocyte morphology and for the presence of any leukemias and anemias.
3.4.8	Clinical Chemistry	No	
3.4.9	Urinalysis	No	
3.4.10	(Macro) Pathology	Yes	
3.4.10.1	Organ Weights	Yes	
		From:	All surviving
		Organs:	Brain, heart, liver, kidneys, spleen and testes or ovaries
3.4.11	Histopathology	Yes	
		From:	All animals on study
		Organs:	At least the organs and tissues listed in OECD Guideline 451
		Other:	

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

3.4.12	Other examinations	Not applicable
3.5	**Statistics**	(i) Bodyweights, food intakes, and organ weights: Analysis of variance was performed on the data from all variates, except for those variates for which there were serious doubts about the validity of the analysis. Where analysis of variance was suitable, a two-way analysis (Snedecor and Cochran, 1968) with treatments and blocks as factors was used for a variate provided that both of the following were true: (i) less than 20% of the possible observations were missing and (ii) each treatment group had at least 50% of its possible observations. If this was not the case, then a one-way analysis (Snedecor and Cochran, 1968) ignoring blocks was used. Bodyweights were analysed with initial bodyweight as a covariate. Where the covariance relationship was significant ($p<0.05$), the corresponding means were reported as adjusted for initial bodyweight; otherwise means unadjusted for this covariate were reported. Food intake data were considered by the analysis of variance. In order to adjust for differences in terminal bodyweight, organ weights were also considered with terminal bodyweight as a covariate. This procedure is preferable to the analysis of relative organ weights, which is intrinsically misleading. The advantages of the analysis of covariance are well documented in the toxicological literature (Shirley, 1977a; Takizawa, 1978). The significance levels of the differences between the control and treated means (adjusted if necessary) were determined using the Williams' test (Williams, 1971; 1972) whenever justifiable. Dunnett's test (Dunnett, 1964) was used if a monotonic dose response could not be assumed. When a covariate was used, the standard error of differences took account of the adjustment made to the means (Snedecor and Cochran, 1968). ii) Haematology. Where the relative frequency of the mode was less than 75%, the method of Bartlett (1937) was used with transformations where appropriate. Kruskal-Wallis analysis of ranks (Kruskal and Wallis 1952) was used where no satisfactory transformation was found. Williams' test (Williams 1971) and Students' t test or its non-parametric equivalent (Shirley, 1977b) was used to test for a dose response. For parameters with a relative frequency of the mode of over 75%, Fisher's exact test (Fisher, 1950) and Mantel's test (Mantel, 1963) were used. iii) Histopathology: Fisher's exact test (Fisher, 1950) was used to compare the

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

proportion of animals in each group which were diagnosed as having a lesion or abnormality. The proportion in each treated group was compared with the proportion in the control group. The analysis was performed separately for samples from animals which survived to terminal kill, and for samples from intercurrent deaths. All comparisons were one sided.

(iv) Neoplasia and Survival:

The log rank test (Kalbfleisch and Prentice, 1980) was used to investigate the effect of treatments on survival. Animals which were killed at the scheduled necropsies or which died accidentally were considered to have censored survival times. The method of Peto et al. (1980) was used for the analysis of tumour data. Tumour types were only combined where the pathologist indicated that it was valid to do so, following the National Cancer Institute Guidelines (McConnell et al., 1986).

(References see study report)

3.6	**Further remarks**	None

4 RESULTS AND DISCUSSION

4.1	**Body weight**	Impaired body weight development at 50,000 ppm, equivocal effects in females at 5,000 ppm; details see study summary under 5.2
4.2	**Food consumption**	Not affected by treatment
4.3	**Water consumption**	Not applicable
4.4	**Clinical signs**	No adverse treatment-related clinical signs; details see study summary under 5.2
4.5	**Macroscopic investigations**	At the terminal sacrifice findings were observed in high dose animals in liver and stomach; details see study summary under 5.2
4.6	**Ophthalmoscopic examination**	Not applicable
4.7	**Haematology**	No adverse treatment-related findings, see study summary under 5.2
4.8	**Clinical Chemistry**	Not applicable

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

4.9	**Urinalysis**	Not applicable
4.10	**Pathology**	
4.11	**Organ Weights**	Increased liver weights at 50,000 ppm; details see study summary under 5.2
4.12	**Histopathology**	Findings in the liver (single cell necrosis, hepatocellular hypertrophy, Kupffer cell aggregates), spleen (aggregation of syncytical macrophages) and glandular stomach (ulceration); details see study summary under 5.2. For neoplastic findings see study summary under 5.2
4.13	**Other examinations**	Not applicable
4.14	**Time to tumours**	Not explicitly investigated
4.15	**Other**	Not applicable

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Groups of 50 male and 50 female C57/C3H (=B6C3) F_1 hybrid mice (XXXX) were administered Flufenoxuron at dietary dose levels of 0; 500; 5,000 and 50,000 ppm for 24 months. Additional groups of 10 mice per dose and sex were employed as a satellite group for an interim kill at 12 months. The animals were housed individually and were about 6 weeks old at commencement of treatment.

Clinical signs were recorded twice daily on week days and once daily on weekends and public holidays. All animals were palpated at each weighing. Body weights and food consumption were determined weekly during the first 13 weeks and every 4 weeks thereafter. Blood was sampled from all mice scheduled for the 24-months necropsy from the tip of the tail at 12, 18 and 24 months and at terminal sacrifice. Blood smears were prepared, stained and examined of differential leukocyte count, erythrocyte morphology and for the presence of any leukemias and anemias.

All animals - regardless whether surviving till scheduled sacrifice or found dead/sacrificed moribund - were subjected to a detailed necropsy. Surviving mice were anesthetized by intraperitoneal injection of sodium phenobarbitone and killed by exsanguinations by cardiac puncture. Terminal body weight and organ weights were determined in surviving animals (brain, heart, liver, kidneys, spleen and testes or ovaries). A full range of organs or representative section thereof from all animals were sampled, fixed, blocked, sliced and stained. Histopathological examination of all organs and tissues was initially performed on all control and top dose animals as well as from all animals which died or were sacrificed moribund. Kidneys, liver, lungs form both sexes and spleens from females were examined from all groups. For the Pathology Addendum to this study [see XXXX] the remaining organs of the intermediate dose levels were histopathologically examined, too. Sampling and histopathological examinations included at least the organs and tissues listed in OECD Guideline 451.

5.2 Results and discussion

The stability of the test article in the diet was determined in the course of the chronic toxicity study in rats [see IIIA 6.5, XXXX] and revealed that Flufenoxuron was stable in the diet at room temperature for at least 42 day. The diet preparation procedures resulted in homogenous preparations as was determined by analyzing each two samples at the top, middle and bottom of the first diets prepared for this study. Eight of the 23 diet preparations were analyzed for test-article content. All samples were within

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

	the specification (no more than 10% deviation from nominal concentration). The average concentrations in percent of the nominal concentration were 99.4 ± 2.4, 97.9 ± 3.1 and 98.1 ± 1.5 at dietary concentrations of 500; 5,000 and 50,000 ppm, respectively. Based on mean food consumption and mean body weight data, the average daily intake of Flufenoxuron was 56; 559 and 7,356 mg/kg bw in males and 73; 739 and 7,780 mg/kg bw in females at 500; 5,000 and 50,000 ppm, respectively. Importantly, the highest dietary concentration of 50,000 ppm represents an extremely high dietary concentration of technical material equivalent to 5% of the diet. This 50,000 ppm treatment level is approximately 7.5 times higher that the current limit dose (7,000 ppm / 1,000 mg/kg bw/day) recommended for testing in mice. Survival was not affected by treatment. At the end of the study 39/50 (78%), 35/50 (70%), 34/50 (68%), and 39/50 (78%) male and 35/50 (70%) 36/50 (72%), 31/50 (62%), and 30/50 (60%) female survivors were counted at dietary dose levels of 0; 500, 5,000 and 50,000 ppm, respectively. There were no definitive treatment-related clinical observations in male or female mice. In females an apparently higher incidence of eye damage and subcutaneous masses was observed at 50,000 ppm [see Table 6.7/68]. The former finding was probably an indirect effect related to the increased incidence of food spillage (see below) since these animals may have been pushing their heads further down into the food hopper and thus causing damage to the eye. An increased incidence of lordotic episodes was observed in females at ≥ 5,000 ppm. This term was used to describe a characteristic posture change occasionally seen in some mice when they were stressed, for example when the lid was removed from their cage. The mice held their head raised and back, usually with open jaws; the forelegs were outstretched and the back was curved downwards. The animals remained standing in this tensed posture for a few seconds and then relaxed. The mice were completely normal after 10 to 20 seconds. (Note: In the individual data this finding was described using the term 'convulsion' which however is recognized to be an incorrect description of the condition.) It is important to note that the incidence of lordotic events in high dose females was roughly comparable to the incidence in control males, indicating that this ‘condition’ is frequently observed in rats. The toxicological relevance of this finding is therefore unclear. Body weight development was impaired in both sexes at	X

Section A6.7	**Carcinogenicity**	
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)	
	50,000 ppm [see Table 6.7/69]. Whereas in high dose males a lower body weight was consistently observed throughout the study, this was seen in high dose females generally from week 16 onwards. A small and often not statistically significant lower body weight was observed in mid dose females (5,000 ppm) from Week 60 onwards. The toxicological relevance of this decrease is uncertain. No effects on body weights or body weight gains were noted for 5,000 ppm males or for males and females at 500 ppm.	
	Determination of food consumption was complicated by the fact that for all groups (including controls) a high incidence of food spillage was observed. The overall incidence of food spillage was significantly higher in high dose males (31.6, 35.7, 35.4 and 38.6%** at 0; 500; 5,000 and 50,000 ppm, respectively) and mid and high dose females (38.5, 42.6, 48.3** and 57.1%** at 0 to 50,000 ppm).	X
	Significant differences in food consumption were observed in all treated groups. However, there were no consistent trends as increased as well as decreased values were observed in all groups. Overall, there was no convincing evidence for a treatment-related effect on food consumption.	
	There were no treatment-related hematological effects. Differential white blood cell counts revealed some minor, but statistically significant changes in high dose males and females at week 52 and in mid and high dose males at Week 78 [see Table 6.7/70]. However, no significant differences were observed at Week 104. The magnitude of change was low and no atypical cells were observed. Therefore, these changes are not considered to be of toxicological relevance.	X
	Organ weight analysis at interim and terminal sacrifice revealed a number of slight but statistically significant differences between treated and control groups [see Table 6.7/71]. However, only the increased absolute and/or terminal body weight adjusted liver weights at 50,000 ppm were considered to be related to treatment. In contrast, the changes of absolute and relative heart and kidney weights in treated females displayed no dose-response relationship, were of small magnitude and were not accompanied by corroborative histopathological finding. These changes were therefore not considered to be due to treatment.	X
	Macropathology revealed no treatment-related findings at the interim sacrifice. At study termination a number of treatment-related macroscopic findings were observed in various organs [see Table 3.7/72]. Most of these findings were observed in the liver of high dose males and females. These included an increased incidence of enlargement, pallor, pale areas or foci, exaggerated	

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

lobular pattern and dark areas/foci. An increased incidence of pale areas or foci was also observed in mid dose males. A higher incidence of ulcera in the forestomach of high dose males was noted. The lower incidence of high dose females with excessive body fat is due to the lower body weight at this dose level. The slight increase of focal alopecia in females at ≥ 5,000 ppm was considered to result from hair plucking and to be due to the increased stress of these animals as was evident from the increased incidence of lordotic events (see clinical observations above). The incidence of seminal vesicle enlargement in high dose males and of parovarian cysts in the ovary of high dose females was decreased.	X
Other macroscopic changes present at high frequency did not show any association with treatment.	
In the following text the histopathological data provided in the report addendum dated 23.11.1993 are presented [see XXXX]. This addendum extended the histopathological examination to all organs and tissues of the low and mid dose levels. For the original report only selected organs of low and mid dose were examined histopathologically. Both, the original and revised pathology report were generated XXXX.	X
Treatment-related histopathological findings at the interim sacrifice were restricted to the liver of high dose mice, and consisted of an increased incidence of Kupffer cell aggregates in 9 of 10 animals of both sexes, as compared to none in controls [see Table 6.7/73]. This may indicate an accumulation or storage of parent compound and/or metabolites in the liver. Additionally, individual cell necrosis and hypertrophy of hepatocytes was noted in single animals.	X
In high dose animals scheduled to be terminated after two years, treatment-related non-neoplastic findings were observed in the liver, spleen and stomach [see Table 6.7/74]. In the liver, this included individual cell necrosis, Kupffer cell aggregates and hepatocellular hypertrophy in both sexes and inflammation in males. These findings attained statistical significance mainly at 50,000 ppm with the exception of Kupffer cell aggregates, which were also significantly increased in mid dose females. Individual cell necrosis and inflammation are indicative of cellular damage, an effect which may be interpreted as resulting from long-term exposure to Flufenoxuron at a dietary concentration exceeding the MTD. The hypertrophy may also have resulted from long-term oral exposure to an excessively toxic concentration of 50,000 ppm.	X
In the spleen of high dose rats an increased incidence of	

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

aggregation of syncytial macrophages was noted. This, like the aggregation of Kupffer cells in the liver, is indicative for a compound accumulation in the spleen. The glandular stomach of high dose males displayed a slightly increased incidence of ulcerative changes (inflammation), which may represent a direct irritant effect on the gastric squamous epithelium at this extremely high dietary concentration of technical material representing 5% of the diet.

There were a few histopathological findings which occurred at a lower incidence in high dose groups. These included splenic lymphoid hyperplasia and seminal vesicle enlargement in high dose males. None of these changes are considered to be adverse. In addition there were some statistically significant differences which however displayed no dose-response relationship and therefore are considered to be incidental. These included the decreased incidence of renal tubule vacuolation in the kidneys of low dose females and the increased incidence of renal cortical inflammation in the right kidney of females at 500 and 5,000 ppm.

For neoplastic findings, the results of this oncogenicity study in the B6C3F1 mouse showed that the total incidence of liver tumors (hepatocellular adenomas and carcinomas combined, as based on similar cell of origin) was not affected by treatment [see Table 6.7/75]. The statistically increase in the incidence of hepatocellular carcinoma in all treated male groups and in low dose females was accompanied by concomitant decrease of hepatocellular adenoma. The morphological appearance and biological behavior of the hepatocellular tumors were similar between treated and control groups. Specifically, the majority of the hepatic tumors developed late in the study, and there was no evidence that the liver tumors developed earlier in treated animals compared to control animals. The earliest hepatocellular tumor, an adenoma, was diagnosed for a control male at the 12-month sacrifice.

Compared to historical control data the incidence of hepatocellular tumors in male mice (36 to 52%, i.e. 18/50 in control males and 26/50 in mid dose males) is well within the historical control (Haseman et al, Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57BL/6N x C3H/HeN)F1 (B6C3F1) mice, Journal of the National Cancer Institute, 75 (5), 975-984, 1985 [see XXXX]; data from NTP studies completed between 01.01.1979 and 01.08.1984, i.e. conducted several (i.e. 6 to 11) years before the conduct of the Flufenoxuron study). The incidence of

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

hepatocellular carcinoma in control males (6%) was unusually low in this study. Haseman reports a mean for hepatocellular carcinoma in $B6C3F_1$ mice of 21% with a range of 8 - 32%. The maximum incidence of hepatocellular carcinoma (38%) observed in low dose males (500 ppm) was slightly outside the historical control range. Historical control data considering a different time-interval (20 NTP studies started 02.08.1995 to 21.12.1998, i.e. conducted some (i.e. 8 to 11) years after the Flufenoxuron study revealed hepatocellular carcinoma incidences ranging from 8 to 46% with a mean of 22.3% [see (http://ntp-db.niehs.nih.gov/htdocs/HCRS/__Hist_2002_Tumor_Mice.html), thus covering the incidence in low dose males. The historical control data demonstrate the high biological variability of hepatocellular tumor incidences in male $B3C6F_1$ mice. The incidence of hepatocellular carcinoma in low dose females (18%) was slightly outside the historical control range published by Haseman (0 - 15%) as well as the currently published NTP data (3 - 16%). However, the absence of a dose-response relationship for this finding in females makes a relation to treatment unlikely.

In conclusion, the overall tumor incidence in the livers of the males was not affected by treatment; however, the proportion of carcinomas relative to adenomas was increased at all treatment levels. The observed incidence of hepatocellular adenomas and/or carcinomas in treated mice was within the historical background range, and a dose-response relationship was absent. The apparent increase in the incidence of hepatic carcinomas in treated male mice is considered to be associated with the unusually low incidence of these tumors recorded in the control males and is not considered to be directly related to treatment.

In females, the incidence of vascular tumors was increased in high dose female mice. This type of tumor, vascular hemangiomas or hemangiosarcomas, is usually observed at a wide range of sites in mice. In this study, the one predominant location in high-dose females was the spleen, with the incidence of splenic hemangiosarcomas showing a statistical increase ($p < 0.01$) as compared to controls. There were no treatment-related increases in the incidence of vascular tumors, either hemangiomas, hemangiosarcomas, or combined, at any other site in female mice [see Table 6.7/75]. However, because of the increased incidence of splenic tumors, the combined incidence of vascular tumors from all sites was increased in females at 50,000 ppm (1/50, 2/50; 3/49 and 11/49** at 0; 500; 5,000 and 50,000 ppm). In light of this extremely high dietary concentration of Flufenoxuron, these results must be interpreted with caution.

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

Mid dose females (5,000 ppm) showed no increases in the incidence of vascular tumors, neither in the spleen nor at any other site.

In males, the incidence of vascular tumors was similar at all treatment levels, and none of the individual sites showed a statistical increase in these tumors. A statistically significant trend ($p < 0.05$) was noted in the liver for combined hemangiomas and hemangiosarcomas. However, there was no significant increase in the tumor incidence (pairwise comparison) for liver hemangiomas, hemangiosarcomas, or combined hemangiomas and hemangiosarcomas [see Table 6.7/75]. When hemangiomas and hemangiosarcomas from all tissues were combined for this sex, the incidence at 50,000 ppm (24%) was not significantly different from the control incidence (16%). Again, in light of this extremely high dietary concentration, the incidences of vascular tumors in males must be interpreted with caution.

The incidence of all other tumors was comparable between control and treated groups as was the overall incidence of tumors with respect to the number of primary tumors, of animals with tumors etc. [see Table 6.7/75].

5.3 Conclusion

Dietary administration of Flufenoxuron to mice at dose levels of 0; 500; 5,000 and 50,000 ppm resulted in impaired body weight development at 50,000 ppm. The liver, stomach and spleen were identified as target organs. The livers of top dose mice displayed higher absolute and relative weights as well as an increased incidence of single cell necrosis, hepatocellular hypertrophy and aggregation of Kupffer cells. The incidence of the latter finding was also increased in mid dose females. Like in the liver an aggregation of Kupffer cells was observed in the spleen of high dose males and females. In the glandular stomach of high dose males an increased incidence of inflammation was observed.

The combined incidence of hepatocellular tumors was comparable between treated and control groups. An increased incidence of hepatocellular carcinoma was observed in all treated male groups and in low dose females. This increase of hepatocellular carcinoma was paralleled by a decrease of hepatocellular adenoma. The incidence of hepatocellular carcinoma in treated groups was within the US National Toxicology Program (NTP) historical control range for this type of tumor whereas the incidence in control males was below the historical control range. The apparent increase in the incidence of hepatocellular carcinoma in treated male mice is therefore considered to be associated with the unusually low incidence of these tumors recorded in the control males and is not considered

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

		to be directly related to treatment. This view is supported by the results of the second carcinogenicity study in B6C3F1 mice which was conducted some years later [see IIIA 6.7, XXXX]. In this study the incidence of hepatocellular adenoma and carcinoma as well as the combined incidence of hepatocellular tumors was comparable between controls and treated groups.	X
		For female mice at 50,000 ppm, the incidence of vascular tumors was statistically significantly increased. This increase reflected an increase in the incidence of hemangiosarcomas in the spleen. At lower treatment levels of Flufenoxuron (5,000 ppm or less), no differences in the incidence of vascular tumors were observed among females. The 50,000 ppm treatment level, which is about 7.5-fold higher than the limit dose (7,000 ppm), elicited both excessive hepatocellular toxicity and body weight depression and exceeded the maximum tolerated dose for Flufenoxuron. Thus, the vascular tumors observed in the female mouse at 50,000 ppm should not be considered significant for human risk assessment. In male mice, no statistically significant increased incidence of vascular tumors was observed at any treatment level.	X
		Based on the histopathological findings in the liver (Kupffer cell aggregation) as well as the effects on body weight in female mice at 5,000 ppm, the NOAEL for systemic toxicity was 500 ppm. This is equivalent to a mean daily dose of 56 mg/kg bw in males and 73 mg/kg bw in females. Due to the equivocal nature of the oncogenicity results no NOAEL for oncogenic activity is proposed for this study.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	Agree with the applicant's version.

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

Results and discussion

Revisions/Amendments:

5.2 Results and discussion:

Mortality higher mortality in females treated at 5000 and 50000 ppm; at 50000 ppm, mortality rate is 25% higher than in controls (control: 30%, 50000 ppm: 40%). This effect has to be mentioned in the summary

Haematological effects *There were no treatment-related hematological effects.* Only two mice were identified as having lymphocytic leukaemia. *[…]*

Organ weight *[…] In contrast, the changes of absolute and relative heart and kidney weights in treated females displayed no* clear *dose-response relationship, were of small magnitude and were not accompanied by corroborative histopathological finding.* So, their biological significance was doubtful.

Macropathology *revealed no treatment-related findings at the interim sacrifice. At study termination a number of treatment-related macroscopic findings were observed in various organs [see Table 3.7/72]. Most of these findings were observed in the liver of high dose males and females. These included an increased incidence of enlargement, pallor, pale areas or foci, exaggerated lobular pattern and dark areas/foci. An increased incidence of pale areas or foci was also observed* in the liver of treated males at all dose levels. *A higher incidence of ulcera in the forestomach of high dose males was noted. The lower incidence of high dose females with excessive body fat is due to the lower body weight at this dose level. The slight increase of focal alopecia in females at ≥ 5,000 ppm was*

Treatment-related histopathological findings at the interim sacrifice *were restricted to the liver of high dose mice, and consisted of an increased incidence of Kupffer cell aggregates in 9 of 10 animals of both sexes, as compared to none in controls and* a loss of normal hepatocytic periacinar vacuolation in the liver of male mice of the 50 000ppm dosage group *[see Table 6.7/73].[…].*

In the spleen of high dose ~~rats~~ mice *an increased incidence of aggregation of syncytial macrophages was noted.*

There were a number of findings of which treatment appeared to diminish the incidence. *These included splenic lymphoid hyperplasia* in male mice of the 50000 ppm group and seminal vesicle enlargement in the 500 ppm and the 50000 ppm dosage groups.*[…].*

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

Conclusion	We agree with the applicant's conclusion but it is necessary to be very careful with the interpretation of the incidence of hemangiosarcomas in the spleen in treated female mice at the highest dosage (50000 ppm). For female mice at 50000 ppm, the incidence of vascular tumors was statistically significantly increased. This increase reflected an increase in the incidence of hemangiosarcomas in the spleen. At lower treatment levels of Flufenoxuron (5000 ppm or less), no differences in the incidence of vascular tumors were observed among females. The vascular tumors were observed in a toxic context (hepatocellular toxicity and body weight depression) at a very high dose level (7.5 fold higher that the maximal dose recommended in the OECD guidelines for chronic toxicity test). In male mice, no statistically significant increased incidence of vascular tumors was observed at any treatment. *Based on the histopathological findings […]*. Despite equivocal nature of the oncogenicity results related to hepatocellular carcinoma in the male liver, a NOAEL for oncogenic activity could be proposed based on the hemangiosarcomas in spleen observed for female mice: 5000 ppm, equivalent to a mean daily dose of 559 mg/kg bw in males and 739 mg/kg bw in females. NOAEL systemic: 500 ppm (73 mg/kg bw/d) for females and 5000 ppm (559 mg/kg bw/d) for males. **NOAEL oncogenicity females : 5000 ppm** **NOAEL oncogenicity males: 50000 ppm**
Reliability	1
Acceptability	Acceptable
Remarks	-

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

	COMMENTS FROM ...
Date	Give date of comments submitted
Materials and Methods	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state
Results and discussion	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Table 6.7/68 Selected clinical observations in mice fed Flufenoxuron for 2 years - First Carcinogenicity study

Sex	Males				Females			
Dose [ppm]	0	500	5,000	50,000	0	500	5,000	50,000
No. of animals examined	50	50	50	50	50	50	50	50
Damaged eye	1	0	0	0	0	2	0	5
Lordotic event	32	36	43	45	8	15	20	36

Table 6.7/69 Body weight development of mice fed Flufenoxuron for 2 years - First carcinogenicity study

Sex	Males				Females			
Dose [ppm]	0	500	5,000	50,000	0	500	5,000	50,000
Body weight [g]								
Week 0[a]	22.1	22.0	22.1	21.9	18.9	18.7	18.8	18.7
Week 13	33.5	34.1 (6.1)[b]	34.2 (6.1)	32.9 (-3.5)	26.2	27.1* (15.1)	27.1** (13.7)	26.6* (8.2)
Week 28	40.7	40.2 (-2.2)	40.3 (-2.2)	38.8** (-9.1)	34.8	35.2 (3.8)	34.2 (-3.1)	32.3** (-14.5)
Week 52	43.9	44.2 (1.8)	44.3 (1.8)	41.8** (-8.7)	42.9	44.3 (6.7)	41.9 (-3.8)	39.5** (-13.3)
Week 76	46.1	45.0 (-4.2)	46.1 (0.0)	42.9** (-12.5)	48.3	49.3 (4.1)	46.0 (-7.5)	40.4** (-26.2)
Week 104	45.9	45.7 (-0.4)	44.7 (-5.0)	40.6 (-21.4)	47.4	48.1 (3.2)	43.4* (-13.7)	38.6** (-30.2)

* $p < 0.05$; ** $p < 0.01$ (Williams test); [a] Week 0 and 13 means include all 60 animals whereas values for week 28 and later were obtained from the oncogenicity group only. [b] Cumulative body weight gain: Expressed as percent difference from control

Table 6.7/70 Selected hematological findings observed in mice fed Flufenoxuron for 2 years - First carcinogenicity study

Sex		Males					Females			
Dose	[ppm]	Week	0	500	5,000	50,000	0	500	5,000	50,000
PMN	[%]	52	23	--		23	21	--	--	21
		78	33	--	27**	29**	32	--	--	31
		103	34	--	--	33	35	--	--	32
Lymphocytes	[%]	52	72	--	--	71	73	--	--	72
		78	65	--	72**	71**	66	--	--	67
		103	64	--	--	65	64	--	--	68
Eosinophils	[%]	52	2	--	--	2	2	--	--	1*
		78	1	--	1	0**	2	--	--	1
		103	2	--	--	1	1	--	--	1
Monocytes	[%]	52	3	--	--	5**	4	--	--	6**
		78	1	--	0**	0**	0	--	--	1
		103	1	--	--	1	0	--	--	0

* $p < 0.05$; ** $p < 0.01$ (Williams test); PMN = Polymorphonuclear neutrophils; -- not determined

Table 6.7/71 Selected organ weights of mice fed Flufenoxuron - First carcinogenicity study

	Absolute organ weights [g]				Terminal body weight adjusted organ weights [g]			
Dose [ppm]	0	500	5,000	50,000	0	500	5,000	50,000
Males 12 Months								
Terminal body weight	46.2	44.4	43.7*	42.4**	46.2	44.4	43.7*	42.4**
Spleen	0.079	0.082	0.072	0.082	0.076	0.081	0.073	0.086
Heart	0.217	0.217	0.213	0.213	0.21	0.217	0.214	0.219
Kidney	0.73	0.71	0.7	0.71	0.71	0.71	0.7	0.73
Liver	2.47	2.40	2.27	2.53	2.28	2.38	2.32	2.69**
Males 24 Months								
Terminal body weight	46.4	46.7	45.1	40.9**	46.4	46.7	45.1	40.9**
Spleen	0.119	0.100	0.110	0.148*	0.122	0.104	0.118	0.140
Heart	0.225	0.226	0.227	0.218	0.225	0.226	0.227	0.219
Kidney	0.77	0.76	0.74	0.73**	0.76	0.74	0.74	0.77
Liver	2.44	2.52	2.72	3.57**	2.47	2.56	2.72	3.50**
Females: 12 Months								
Terminal body weight	43.9	43.5	40.0	38.5*	44.9	43.5	40.0	38.5*
Spleen	0.112	0.101	0.103	0.160*	0.112	0.101	0.103	0.160*
Heart	0.169	0.164	0.168	0.174	0.169	0.165	0.168	0.174
Kidney	0.44	0.43	0.43	0.47*	0.44	0.42	0.44	0.48**
Liver	1.95	1.93	1.90	2.26**	1.86	1.87	1.95	2.36**
Females: 24 Months								
Terminal body weight	47.5	48.6	42.9**	38.7**	47.5	48.6	42.9*	38.7**
Spleen	0.250	0.240	0.238	0.313	0.256	0.247	0.235	0.300
Heart	0.183	0.204**	0.194**	0.196**	0.182	0.203**	0.195**	0.197**
Kidney	0.49	0.53**	0.51**	0.54**	0.49	0.53**	0.52**	0.55**
Liver	2.08	2.13	2.05	3.24**	2.04	2.08	2.07	3.33**

* $p < 0.05$; ** $p < 0.01$ (Williams test)

Table 3.7/72 Selected macropathological findings in mice fed Flufenoxuron for 2 years - First carcinogenicity study - Terminal Sacrifice

Sex	Males				Females			
Dose [ppm]	0	500	5,000	50,000	0	500	5,000	50,000
General comments								
- excessive body fat	0	1	2	0	19	21	12	4
Liver								
- pallor	11	7	7	33	13	7	13	44
- pale area(s)/focus(i)	10	17	24	19	7	10	4	13
- exaggerated lobular pattern	1	0	1	33	0	1	0	27
- dark area(s)/focus(i)	4	2	5	14	11	10	5	13
- enlargement	1	2	3	11	4	6	4	18
Ovaries								
- parovarian cyst(s)	NA	NA	NA	NA	20	8	10	12
Seminal vesicles								
- enlargement	15	12	18	2	NA	NA	NA	NA
Skin								
- focal alopecia	4	5	7	7	4	4	14	10
Stomach								
- forestomach ulceration	0	4	3	9	3	0	2	2

NA: not applicable

Table 6.7/73 Neoplastic and selected non-neoplastic findings of mice fed Flufenoxuron for 1 year - First carcinogenicity study

Sex	Males				Females			
Dose [ppm]	0	500	5,000	50,000	0	500	5,000	50,000
Non-neoplastic finding								
Liver (# examined)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
- vacuolation, periacinar	8	9	7	0	4	2	0	1
- cell necrosis, individual	0	0	0	1	0	0	0	2
- Kupffer cell aggregates	0	0	0	9***	0	0	0	9***
- hypertrophy, hepatocellular	0	0	0	1	0	0	0	2
Neoplastic findings								
Jejunum (# examined)	(10)	(0)	(0)	(8)	(10)	(1)	(1)	(10)
- adenocarcinoma	1	0	0	0	0	0	0	0
Lachrymal gland (# examined)	(10)	(0)	(0)	(10)	(10)	(0)	(0)	(10)
- adenoma	1	0	0	0	0	0	0	0
Liver (# examined)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
- adenoma, hepatocellular	1	0	0	0	0	0	0	0
Lung (# examined)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
- carcinoma, pulmonary	1	0	0	0	0	0	0	0
Stomach, glandular (# examined)	(10)	(0)	(0)	(10)	(10)	(1)	(2)	(9)
- Mast cell tumor	0	0	0	0	0	0	1	0
Thyroid (# examined)	(10)	(0)	(0)	(10)	(10)	(1)	(1)	(10)
- adenoma, follicular	0	0	0	0	0	0	0	1

*** $p < 0.001$ (Fisher's Exact Test)

Table 6.7/74 Selected non-neoplastic findings of mice fed Flufenoxuron for 2 years - First carcinogenicity study

Sex	Males				Females			
Dose [ppm]	0	500	5,000	50,000	0	500	5,000	50,000
Non-neoplastic findings								
Kidney (# examined)	(50)	(50)	(49)	(49)	(50)	(50)	(50)	(49)
- vacuolation, tubular (left)	48	49	48	46	26	7***	15	15
- vacuolation, tubular (right)	46	49	46	46	13	5***	12	13
- inflammation, chronic, cortical (left)	12	17	9	10	8	17	17	9
- inflammation, chronic, cortical (right)	5	3	4	3	3	12*	10*	6
Liver (# examined)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
- inflammation	2	3	4	12**	6	5	9	10
- cell necrosis, individual	2	1	1	42***	0	0	1	38***
- Kupffer cell aggregates	0	0	2	47***	0	1	11***	44***
- hypertrophy, hepatocellular	0	0	1	31***	0	0	0	35***
- hyperplasia, focal	11	15	10	8	5	7	4	9
- vacuolation, periacinar	4	11*	5	4	0	1	1	0
Spleen (# examined)	(50)	(50)	(50)	(49)	(50)	(50)	(50)	(50)
- macrophages, syncytial	0	0	0	18***	0	0	0	10***
- hyperplasia, lymphoid	10	6	8	2*	4	7	9	7
Stomach, glandular (# examined)	(50)	(43)	(38)	(47)	(49)	(48)	(49)	(48)
- Inflammation	0	1	0	5*	0	0	0	1
Seminal vesicle (# examined)	(50)	(50)	(49)	(49)	NA	NA	NA	NA
- enlargement	23	13	19	3***	-	-	-	-

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 6.7/75 Incidence of neoplastic findings in mice fed Flufenoxuron for 2-years - First carcinogenicity study

Sex	Males				Females			
Dose [ppm]	0	500	5,000	50,000	0	500	5,000	50,000
Total animals/group	50	50	50	50	50	50	50	50
Total primary tumors	50	52	70	64	70	60	42	74
Total animals with tumors	33	37	45	38	44	37	31	42
Total animals with multiple tumors	14	13	22	21	19	16	11	24
Total benign	30	16	33	25	33	30	16	31
Total malignant	20	36	37	39	37	30	26	43
Total malignant with metastasis	12	10	16	17	32	16	16	32
Individual tumors								
Number of animals examined	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Adrenal	(50)	(50)	(49)	(50)	(49)	(50)	(50)	(50)
- medullary carcinoma	0	1	0	0	0	1	0	0
- medullary adenoma	0	0	0	0	0	0	0	1
Adrenal	(50)	(50)	(49)	(50)	(49)	(48)	(48)	(48)
- cortical carcinoma	0	0	0	0	0	1	0	0
Caecum	(47)	(45)	(47)	(43)	(43)	(47)	(46)	(42)
- haemangioma	0	1	0	0	0	0	0	0
Duodenum	(48)	(44)	(45)	(43)	(44)	(48)	(47)	(41)
- adenocarcinoma	0	0	0	0	1	0	0	0
Hemopoietic tissues	(7)	(4)	(12)	(9)	(29)	(13)	(12)	(23)
- malignant lymphoma	7	3	7	6	23	8	7	19
- granulocytic leukemia	0	1	1	1	0	1	0	0
- histiocytic sarcoma	0	0	3	1	6	4	5	4
Jejumum	(47)	(44)	(45)	(45)	(43)	(49)	(46)	(43)
- adenocarcinoma	0	0	0	1	0	0	0	0
Kidney	(50)	(50)	(49)	(49)	(50)	(50)	(50)	(49)
- adenoma	0	0	0	1	0	0	0	0
- transitional carcinoma	0	0	0	0	0	1	0	0
Lachrymal (Harderian) gland	(50)	(50)	(48)	(49)	(49)	(50)	(49)	(49)
- adenoma	7	5	10	3	6	4	0	1

Table 6.7/76 Incidence of neoplastic findings in mice fed Flufenoxuron for 2-years - First carcinogenicity study

Liver	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
- hepatocellular adenoma	15	3	11	10	10	6	2	13
- hepatocellular carcinoma	3	19***	15**	15**	3	9*	7	5
- hepatocellular tumors, total incidence	18	22	26	25	13	15	9	18
- cholangioma	0	0	0	0	0	0	1	0
- haemangiosarcoma	2	1	0	5	0	0	0	1
- haemangioma	0	0	0	2	0	0	0	0
Lungs	(50)	(50)	(49)	(50)	(50)	(50)	(50)	(50)
- pulmonary adenoma	7	4	8	6	3	3	2	3
- pulmonary carcinoma	2	7	6	6	1	0	1	0
Lymph node mesenteric	(50)	(47)	(45)	(48)	(48)	(50)	(47)	(46)
- haemangiosarcoma	1	0	0	0	0	0	0	0
Mammary gland	(50)	(50)	(50)	(49)	(50)	(47)	(49)	(50)
- adenocarcinoma-type A	-	-	-	-	0	1	0	1
- adenocarcinoma-type B	-	-	-	-	0	1	0	0
Ovary	NA	NA	NA	NA	(50)	(50)	(50)	(50)
- teratoma	-	-	-	-	0	0	1	0
- liposarcoma	-	-	-	-	1	0	0	0
- adenocarcinoma	-	-	-	-	0	1	0	0
Pancreas	(49)	(48)	(48)	(47)	(50)	(49)	(49)	(50)
- haemangiosarcoma	0	0	0	0	1	0	0	0
Pituitary	(48)	(49)	(49)	(50)	(49)	(49)	(49)	(49)
- pars anterior: adenoma	0	0	1	0	11	11	5	3
- pars intermedia: adenoma	0	0	0	0	0	1	0	0
Salivary glands	(50)	(50)	(49)	(50)	(49)	(50)	(49)	(50)
- haemangiosarcoma	0	0	0	0	0	0	0	1
Skin	(50)	(50)	(50)	(50)	(50)	(49)	(50)	(50)
- squamous carcinoma	0	0	1	0	0	0	0	1
- squamous papilloma	1	0	0	0	0	0	0	0
- sarcoma	0	0	0	0	0	0	1	1
- haemangiosarcoma	0	0	0	0	0	0	1	0
- haemangioma	0	0	0	0	0	0	0	1
- fibroma	0	0	0	0	0	0	0	1
- leiomyosarcoma	0	0	0	0	0	0	0	1

Table 6.7/77 Incidence of neoplastic findings in mice fed Flufenoxuron for 2-years - First carcinogenicity study

Spleen	(50)	(50)	(50)	(49)	(50)	(50)	(50)	(50)
- haemangioma	0	0	0	0	0	0	1	0
- haemangiosarcoma	4	3	0	3	0	1	1	7#
Sternum/marrow	(50)	(48)	(49)	(50)	(49)	(50)	(50)	(49)
- haemangioma	0	0	0	0	0	1	0	0
Stomach glandular	(50)	(43)	(38)	(47)	(49)	(48)	(49)	(48)
- sarcoma-undifferentiated	0	1	0	0	0	0	0	0
Stomach keratinized	(50)	(49)	(45)	(48)	(50)	(50)	(50)	(48)
- squamous papilloma	0	1	0	0	1	1	2	3
Testis	(50)	(50)	(50)	(50)	NA	NA	NA	NA
- Leydig cell adenoma	0	0	0	1	-	-	-	-
Thyroid	(50)	(50)	(47)	(48)	(50)	(50)	(50)	(48)
- follicular adenoma	0	1	3	1	0	1	1	0
- follicular carcinoma	0	0	1	0	0	0	0	0
Urinary bladder	(49)	(49)	(49)	(47)	(49)	(50)	(47)	(47)
- haemangioma	0	0	0	1	0	0	0	0
Uterus/cervix	NA	NA	NA	NA	(50)	(50)	(50)	(so)
- fibroma	-	-	-	-	0	1	0	0
- leiomyoma	-	-	-	-	0	0	1	0
- leiomyosarcoma	-	-	-	-	1	1	0	0
- endometrial adenoma	-	-	-	-	2	0	1	3
Vertebrae, lumbar	(50)	(50)	(50)	(50)	(48)	(50)	(50)	(49)
- haemangiosarcoma	0	0	0	1	0	0	0	1
- sarcoma	0	0	0	0	0	0	0	1
Non protocol tissues	(2)	(3)	(3)	(2)	(1)	(2)	(2)	(2)
- mesentery - haemangiosarcoma	1	0	0	0	0	0	0	0
- haemangiosarcoma	0	0	1	0	0	0	0	0
- carcinoma of unknown origin	0	0	2	0	0	0	0	0
- vertebral ganglion: Schwannoma	0	1	0	0	0	0	0	0
- spinal column: osteosarcoma	0	0	0	0	0	0	1	0
- vulva: -squamous carcinoma	0	0	0	0	0	0	1	0
- other sites: lipoma	0	0	0	0	0	0	0	1

* $p < 0.05$; # $p < 0.01$; @ $p < 0.001$ (Fischer's Exact Test)

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

Official use only

		1 REFERENCE 3
1.1	**Reference**	**9) XXXX** WL115110: Oncogenicity study by dietary administration to B6C3F1 mice XXXX, unpublished XXXX
1.2	**Data protection**	No
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	XXXX
1.2.3	Criteria for data protection	No data protection claimed
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	EPA 83-2, OECD 451
2.2	**GLP**	Yes (laboratory certified by the Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Batch: XXXX
3.1.2	Specification	As given in section 2
3.1.2.1	Description	As given in section 2
3.1.2.2	Purity	98.1%
3.1.2.3	Stability	Stable
3.2	**Test Animals**	
3.2.1	Species	Mice
3.2.2	Strain	B6C3F1
3.2.3	Source	XXXX

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	About 5-6 weeks old
3.2.6	Number of animals per group	50/sex/group
3.2.7	Control animals	Yes, 50/sex
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	24 months
3.3.2	Frequency of exposure	Daily
3.3.3	Postexposure period	None
3.3.4	**Oral**	
3.3.4.1	Type	In food
3.3.4.2	Concentration	0, 100, 1000, and 10,000 ppm, equivalent to a mean daily compound intake of 15.3; 152 and 1,592 mg/kg bw in males and 17.4; 187 and 1,890 mg/kg bw in females, respectively
3.3.4.3	Vehicle	None
3.3.4.4	Concentration in vehicle	Not applicable
3.3.4.5	Total volume applied	Not applicable
3.3.4.6	Controls	Basal diet
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Clinical signs were recorded twice daily. At weekly intervals, a more detailed examination including palpation was performed.
3.4.1.2	Mortality	At least twice daily; debilitated animals were observed carefully.
3.4.2	Body weight	Weekly during the first 14 weeks and every 2 weeks thereafter.
3.4.3	Food consumption	Weekly throughout the study.

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

3.4.4	Water consumption	No	
3.4.5	Ophthalmoscopic examination	No	
3.4.6	Haematology	At 50, 76 and 102 week. Blood was sampled from the tail vein and blood smears were prepared, stained and examined.	
3.4.7	Clinical Chemisty	No	
3.4.8	Urinalysis	No	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	In all mice surviving to terminal sacrifice; adrenals, brain, kidneys, liver, ovaries, spleen, testes or uterus were weighed	X
3.5.2	Gross and histopathology	Gross pathology performed on all animals. At least the organs listed in OECD Guideline 451 were sampled, preserved and processed. Histopathology on all organs from control and high dose animals and from animals that died or were killed in extremis. Kidneys, liver and lung were examined in all groups.	
3.5.3	Other examinations	None	
3.5.4	Statistics	Inter-group differences in mortality were analysed by Cox's proportional hazards model and Tarone's partition of the Chi-square statistic into linear trend on dose and deviation from linearity. These tests were two-tailed. For organ weights and bodyweight (weekly values and weight changes), homogeneity of variance was tested using Bartlett's test. Whenever this was found to be statistically significant a Behrens-Fisher test was used to perform pairwise comparisons, otherwise a Dunnett's test was used. Fisher's Exact test was applied as a two-tailed test, where appropriate, to the distribution of macroscopic or microscopic (non-neoplastic) pathological entities. For the distribution of neoplastic microscopic pathological entities a one-tailed test was applied to apparent increases in incidence with treatment. Unless stated, group mean values or incidences for the treated groups were not significantly different from those for the Controls ($p>0.05$).	
3.6	**Further remarks**	None	

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

		4 **RESULTS AND DISCUSSION**	
4.1	**Observations**		
4.1.1	Clinical signs	No treatment-related clinical signs of systemic toxicity were observed throughout the study. Neither the number nor the onset of palpable swellings indicated a relation to treatment.	
4.1.2	Mortality	Survival was not affected by treatment	X
4.2	**Body weight gain**	Body weight development is not considered to be affected by treatment; for details see study summary under 5.2	
4.3	**Food consumption and compound intake**	Food consumption was not affected by treatment*;* for details see study summary under 5.2	
4.4	**Ophtalmoscopic examination**	Not applicable	
4.5	**Blood analysis**		
4.5.1	Haematology	No adverse treatment-related effects; for details see study summary under 5.2	
4.5.2	Clinical chemistry	Not applicable	
4.5.3	Urinalysis	Not applicable	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	No adverse treatment-related effect; for details see study summary under 5.2	
4.6.2	Gross and histopathology	No adverse treatment-related effect; for details see study summary under 5.2	X
4.7	**Other**	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Flufenoxuron was fed to groups of 50 male and 50 female B6C3F1 mice (XXXX) at dietary dose levels of 0; 100; 1000 and 10,000 ppm for 2 years. Upon arrival the animals were assigned to cages (individual housing) and treatment groups by means of computer generated random numbers. Treatment commenced after an acclimatization period of 13 days when the animals had	

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

an age of 5 to 6 weeks. Prior to treatment three males and five females were replaced, with spare animals of the same batch, in order to equalize the group mean body weights.

Clinical signs were recorded twice daily. At weekly intervals, a more detailed examination including palpation was performed. Body weights were determined weekly during the first 14 weeks and every 2 weeks thereafter. Food consumption was determined weekly throughout the study. Blood was sampled from the tail vein of each surviving animals at 50, 76, 102 weeks. Blood smears were prepared, stained and examined. Blood smears were also prepared and examined from animals killed in extremis.

All animals - regardless whether surviving till scheduled sacrifice or found dead/sacrificed moribund - were subjected to a detailed necropsy. For unscheduled and scheduled sacrifice mice were killed by carbon dioxide inhalation. Terminal body weight and organ weights were determined (adrenals, brain, kidneys, liver, ovaries, spleen, testes and uterus). A full range of organs or representative section thereof from all animals were sampled, fixed, blocked, sliced and stained. Histopathological examination of all organs and tissues was performed on all control and top dose animals as well as from all animals which died or were sacrificed moribund. Kidneys, liver and lungs of all groups were subjected to a histopathological examination. Sampling and histopathological examinations included at least the organs and tissues listed in OECD Guideline 451.

5.2 Results and discussion

Diets were prepared weekly during the first 9 weeks and every four weeks from Week 10 onwards. The homogenous distribution of Flufenoxuron in the diet was determined in trial preparations representative for the weekly and 4-weekly preparations. For this purpose 6 samples were taken on regularly spaced positions in the mix of the 100 and 10,000 ppm preparations. As indicated by Relative Standard Deviations of 1.7 to 4.1% for the 100 ppm preparations and of 3.4 and 1.5% for the 10,000 ppm preparations Flufenoxuron was homogenously distributed in the diets. The trial preparations were stored at room temperature for up to 28 days. Analysis for test-article content at Day 28 revealed that Flufenoxuron was stable in the diet for at least 28 days. Ten of diet preparations used in the study were analyzed for test-article content. The average Flufenoxuron content was 104 ± 3.6, 989 ± 33 and 9987 ± 457 ppm at 100; 1,000 and 10,000 ppm, respectively.

Based on nominal test article content, food consumption and body weight data, the average daily nominal test-article intake was 15.3; 152 and 1,592 mg/kg bw in males and 17.4; 187 and

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

	1,890 mg/kg bw in females at 100; 1,000 and 10,000 ppm, respectively. Survival was not affected by treatment. At study termination, survival rates were 42/50 (84%), 39/50 (78%), 38/50 (76%), and 43/50 (86%) for males and 39/50 (78%), 40/50 (80%), 32/50 (64%), and 32/50 (64%) for females at dietary dose levels of 0, 100, 1000, and 10,000 ppm, respectively. Although the Tarone trend test revealed a marginally significant result for female survival at a 5% error level, the pair wise comparison of survival rates (Cox's test and Fisher Exact test) revealed no statistical significance. Specifically, although survival was slightly lower among females receiving 1,000 or 10,000 ppm (both at an incidence of 64%) compared to controls (78%), these inter-group differences were not statistically different from controls as analyzed by pair-wise comparison (Fisher's Exact Test). In addition, there was no dose-response relationship observed for females at the 1,000 and 10,000 ppm levels. Furthermore, in the first mouse oncogenicity study, there were no treatment-related differences in survival rates for females or males at 50,000 ppm, a dietary concentration 5 times greater than the highest dietary concentration tested in the second mouse oncogenicity study. Moreover, there were no other parameters in the second mouse oncogenicity study, including any histopathological lesions, to indicate significant toxicity in males or females at any dietary concentration, including females at 1,000 or 10,000 ppm. Therefore, the slight decrease in survival among females given 1,000 or 10,000 ppm in the second mouse oncogenicity study, was not considered to be treatment-related.	X
	No treatment-related clinical signs of systemic toxicity were observed throughout the study. Neither the number nor the onset of palpable swellings indicated a relation to treatment. Body weight development was not considered to be affected by treatment [see Table 6.7/78 and Figure 6.7/1]. In males statistically significant differences of absolute body weights were mainly observed during the first 3 months of the study. However, body weight gain was statistically different from control during the first week only. This was probably due to a transient palatability problem at commencement of treatment. In high dose females, overall cumulative body weight gain was decreased by 15% whereas at the mid dose level overall body weight gain was decreased by 4%. In contrast to the Study Director's opinion, the author of this summary considers the body weight development in high dose females to be unaffected by treatment, too. As evident from Figure 6.7/1, body weight	

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

development at 1,000 and 10,000 ppm was essentially comparable up to week 96. The differences of 11% in overall body weight gain of mid and high dose females developed during the last 2 weeks only [see Table 6.7/78]. The last two to three months were characterized by substantial variability of body weights in all female groups including controls. Therefore, the body weight changes during this period of time are of no toxicological relevance.

The absence of a dose-response at 1,000 and 10,000 ppm a relation of body weight effects to treatment is questionable. In addition, body weights at 100 ppm were about the same magnitude above control than the body weights at 1,000 and 10,000 ppm were below the controls. This justifies to assume that body weight of all treated groups are within the normal range and thus are unaffected by treatment.

Food consumption was not affected by treatment. Cumulative food consumption (Week 1 to 104) was in the range of 99 to 101% of the control value in males and 98 to 99% of the control value in females.

The evaluation of blood smears of control and high dose animals taken at Weeks 50, 76, and 102 revealed no treatment-related effects.

Organ weight analysis revealed no findings related to treatment. The only statistically significant difference was an increase of absolute adrenal weights in low dose males. Since the difference to controls was only marginal and in absence of a dose-response relationship, this change is considered to be of incidental nature.

Neither the incidence nor the type of macropathological findings indicated a relation to treatment. The findings observed were typical for the age and strain of mice used. There were a few statistically significant differences between control and treated groups, but these involved sporadic decreased incidences of mild gross changes and therefore were not considered to be treatment-related, but rather incidental in nature. These consisted of a lower incidence of pale livers in mid dose males, a reduced number of cystic uteri in females at ≥ 1,000 ppm, a reduced number of distended uteri at ≥ 100 ppm and an increase of fluid distended uteri at ≥ 1,000 ppm. If both types of uterus distension are considered together, the incidences are roughly comparable between all groups (37; 25; 30 and 35 at 100; 1,000 and 10,000 ppm, respectively).

There were no treatment-related microscopic changes (both neoplastic and non-neoplastic) observed at histopathological

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

		examination. For non-neoplastic findings, a slight, but statistically significant increase in the incidence of hepatic extramedullary hematopoiesis was observed in high dose females [see Table 6.7/79]. However, extramedullary hematopoiesis in the liver is not an uncommon finding in mice and since it may have been related to the presence of hematopoietic or other neoplasms in five of the six affected high dosage females, the incidence of 6/49 in females given 10,000 ppm is not considered treatment-related. In addition, there were a few statistically significant differences in the incidence of non-neoplastic histopathological lesions mainly in high dose females which however are considered to have arisen by chance and are of no toxicological significance. These included a decreased incidence of midzonal hepatocytic fatty vacuolation, a decreased incidence of sclerosis of the sternum, a decreased incidence of thyroid lymphoid hyperplasia and a decreased incidence of polyluminal dilatation in the uterus of mid and high dose females. The incidence of neoplastic findings was comparable between all groups [see Table 6.7/80] and thus not indicative of an oncogenic potential of Flufenoxuron when administered at dietary concentrations up to 10,000 ppm for 2 years. There was neither an increased incidence of hepatocellular adenoma or carcinoma nor an increased incidence of splenic hemangiosarcoma.	
5.3	**Conclusion**	Administration of Flufenoxuron at dietary dose levels up to 10,000 ppm for up to 2 years did not result in any adverse findings. Nevertheless, the study is fully valid as the average daily dose levels exceeded the limit dose of 1,000 mg/kg bw/day by a factor of about 1.6 in males and 1.9 in females. The incidence of neoplastic and non-neoplastic lesions was comparable between all groups and indicated no effect of treatment. The effect on body weight development in high dose females was considered to represent an incidental finding as body weight effects at 10,000 ppm are essentially the same to that at the 10 fold lower dose thus lacking a dose-response relationship. In addition body weights at the low dose level (100 ppm) were about the same magnitude above control level than body weights at the mid and high dose level were below control level. This again indicates the incidental nature of the observed effects. Based on the results of the study the NOAEL for systemic and carcinogenic effects was at least 10,000 ppm. This is equivalent to a mean daily dose of 1,590 mg/kg bw in males and 1,890 mg/kg bw in females.	X

Section A6.7		**Carcinogenicity**	
BPD Annex Point IIA, VI.6.7		6.7 Carcinogenicity – Oral administration (mouse)	
5.3.1	LO(A)EL	No LOAEL observed (highest dose tested was the NOAEL)	X
5.3.2	NO(A)EL	10,000 ppm (systemic and carcinogenic)	X
5.3.3	Other	None	
5.3.4	Reliability	1	
5.3.5	Deficiencies	No	

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** Agree with the applicant's version, with this modification: 3.5.1. Organ weights […] Moreover, weight of heart and lungs were recorded for animals killed or dying during the first 93 weeks only; these weight are not reported but are retained in the archives.
Results and discussion	**Revisions/Amendments:** Agree with the applicant's version with these modifications: 4.1.2. *Survival was not affected by treatment.* The slight increase in mortality among females observed for 1000 and 10000 ppm was attributed to the treatment (without any dose relation) but with a low biological relevance 5.2. **Mortality in the first study in mice:** higher mortality in females treated at 5000 and 50000 ppm; at 50000 ppm, mortality rate is 25% higher than in controls (control: 30%, 50000 ppm: 40%). **Results**: the higher frequency of uterus distension by a fluid in females treated at 1000 or 10000 ppm should be taking into account, despite the absence of any specific histopathological lesions related.
Conclusion	**NOAEL chronic toxicity males = 10000 ppm** **NOAEL chronic toxicity females = 100 ppm (based on uterus distension)** NOAEL oncogenicity = 10000 ppm
Reliability	1
Acceptability	Acceptable
Remarks	-
	COMMENTS FROM ... (specify)
Date	Give date of comments submitted

Section A6.7	**Carcinogenicity**
BPD Annex Point IIA, VI.6.7	6.7 Carcinogenicity – Oral administration (mouse)

Materials and Methods	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state
Results and discussion	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Table 6.7/78 Body weight development of mice fed Flufenoxuron for 2 years - Second carcinogenicity study

Sex	Males				Females			
Dose [ppm]	0	100	1,000	10,000	0	100	1,000	10,000
Body weight [g]								
Week 0	21.5	21.8	22.1	21.5	17.8	17.8	17.8	17.7
Week 13	30.1	30.3	30.5	29.3	25.1	25.0	24.9	24.6
		-1.2	-2.3	-9.3		-1.4	-2.7	-5.5
Week 26	36.3	36.4	36.6	34.9	30.2	31.0	29.1	28.4*
		-1.4	-2.0	-9.5		6.5	-8.9	-13.7
Week 52	42.6	43.6	44.2	42.9	37.9	39.3	36.5	36.6
		3.3	4.7	1.4		7.0	-7.0	-6.0
Week 76	44.8	44.5	46.2	45.1	40.6	42.6	38.8	38.5
		-2.6	3.4	1.3		8.8	-7.9	-8.8
Week 102	44.3	44.7	45.7	45.0	40.5	42.4	39.9	38.7
		0.4	3.5	3.1		8.4	-2.6	-7.5
Week 104	43.3	43.2	43.9	42.9	42.0	42.8	40.9	38.0*
		-1.8	0.0	-1.8		3.3	-4.5	-16.1

* $p < 0.05$

[a] Cumulative body weight gain: Percent difference to control (in contrast to the report, where cumulative body weight was calculated based on individual animals weights that survived, the values given here are based on the mean animal weights)

Figure 6.7/1 Body weight development of mice administered Flufenoxuron for 2 years - Second carcinogenicity study

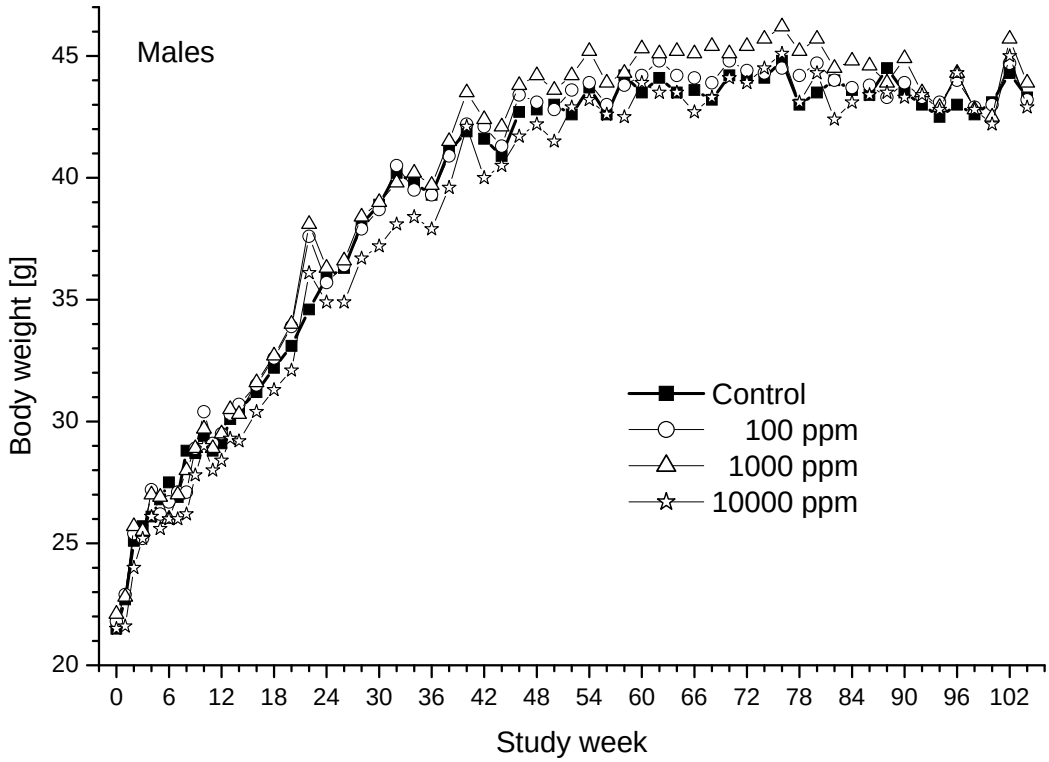


Figure 6.7/1 Body weight development of mice administered Flufenoxuron for 2 years - Second carcinogenicity study

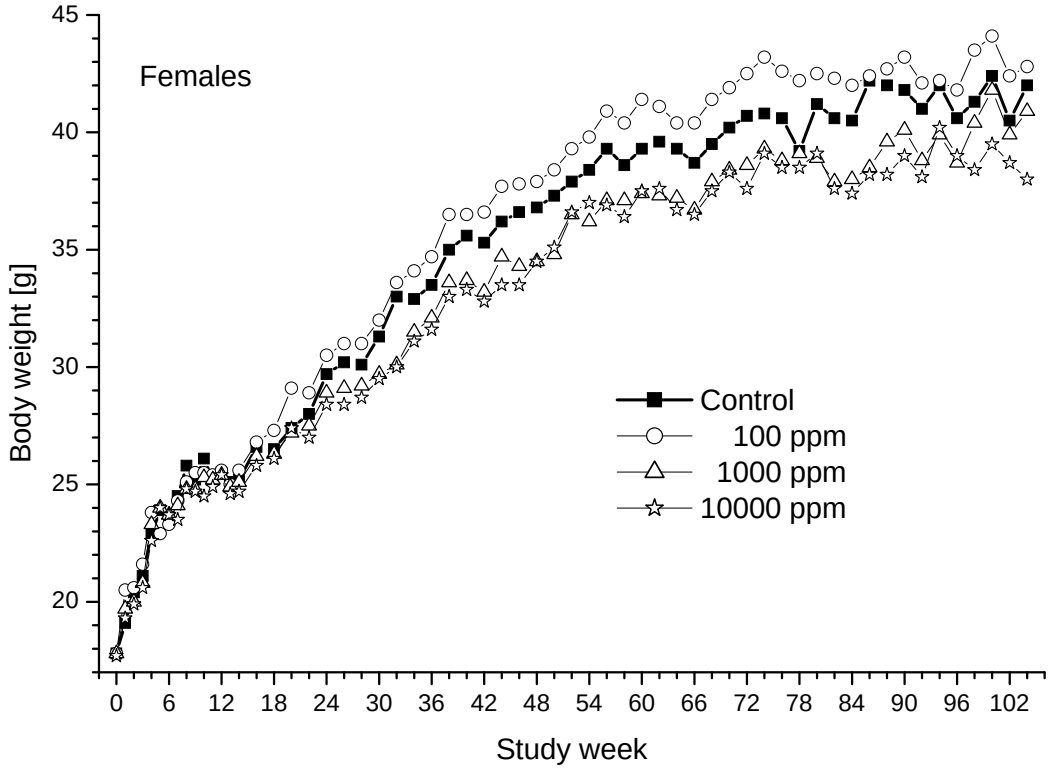


Table 6.7/79 Selected non-neoplastic findings of mice fed Flufenoxuron for 2 years - Second carcinogenicity study

Sex		Males				Females			
Dose [ppm]		0	100	1,000	10,000	0	100	1,000	10,000
Non-neoplastic finding									
Liver	(# examined)	(50)	(49)	(50)	(50)	(50)	(59)	(50)	(49)
- hepatocyte fatty vacuolation, midzonal		9	6	5	4	7	2	2	0*
- hematopoiesis, extramedullary		0	4	1	0	0	1	4	6*
Sternum	(# examined)	(50)	(11)	(12)	(50)	(50)	(10)	(18)	(50)
- sclerosis of the bone		0	0	0	0	39	2	6	28*
Thyroid	(# examined)	(50)	(11)	(12)	(50)	(50)	(10)	(18)	(50)
- Inflammation		12	1	0	9	15	2	1	6*
Uterus	(# examined)	NA	NA	NA	NA	(50)	(49)	(49)	(50)
- dilatation, polyluminal		-	-	-	-	50	49	42**	43*

* $p < 0.05$; ** $p < 0.01$; NA: not applicable

Table 6.7/80 Incidence of neoplastic findings in mice fed Flufenoxuron for 2-years - Second carcinogenicity study

Sex		Males				Females			
Dose [ppm]		0	100	1,000	10,000	0	100	1,000	10,000
Number of animals examined		(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Adrenal, medulla (l&r)	(# examined)	(50)	(10)	(12)	(50)	(50)	(11)	(18)	(49)
- phaeochromocytoma, malignant		0	0	0	0	0	1	1	0
- phaeochromocytoma, benign		0	0	0	0	0	0	0	2
Brain	(# examined)	(50)	(11)	(12)	(50)	(50)	(10)	(20)	(50)
- medulloblastoma		0	0	0	0	0	0	0	1
Duodenum	(# examined)	(47)	(9)	(11)	(47)	(49)	(8)	(16)	(47)
- adenoma		0	0	0	0	0	0	0	1
Kidneys (l&r)	(# examined)	(50)	(49)	(50)	(50)	(50)	(50)	(50)	(50)
- renal adenoma		1	0	0	0	0	0	0	0
Liver	(# examined)	(50)	(49)	(50)	(50)	(50)	(49)	(50)	(49)
- hepatocellular adenoma		12	14	19	13	2	4	1	4
- hepatocellular carcinoma		8	4	3	4	3	0	0	3
- hepatocellular adenoma & carcinoma		20	18	22	17	5	4	1	7
- haemangioma		0	0	1	0	0	0	0	0
- haemangiosarcoma		0	1	0	0	0	0	0	0
- hepatoblastoma		0	0	1	0	0	0	0	0
Lungs	(# examined)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
- pulmonary adenoma		4	9	9	8	4	3	2	4
- pulmonary carcinoma		1	2	4	1	2	5	0	3
Ovaries (l&r)	(# examined)	(0)	(0)	(0)	(0)	(50)	(20)	(28)	(49)
- cystadenoma		0	0	0	0	0	1	0	0
Pancreas	(# examined)	(50)	(10)	(14)	(50)	(50)	(9)	(20)	(49)
- islet cell adenoma		0	1	0	0	0	0	0	0
Pituitary	(# examined)	(50)	(11)	(11)	(49)	(50)	(12)	(24)	(49)
- adenoma		0	0	0	0	6	2	5	4
Spleen	(# examined)	(50)	(20)	(18)	(50)	(50)	(43)	(40)	(49)
- haemangioma		1	1	0	0	1	0	3	0
- haemangiosarcoma		0	0	0	0	1	1	0	0
Stomach	(# examined)	(50)	(10)	(13)	(50)	(49)	(8)	(18)	(49)
- squamous cell carcinoma		0	0	1	0	0	0	0	0
Testes (l&r)	(# examined)	(50)	(12)	(12)	(50)	(0)	(0)	(0)	(0)
- interstitial cell tumor		0	1	0	0	0	0	0	0

Table 6.7/81 Incidence of neoplastic findings in mice fed Flufenoxuron for 2-years - Second carcinogenicity study

Uterus	(# examined)	(0)	(0)	(0)	(0)	(50)	(49)	(49)	(50)
- stromal polyp		0	0	0	0	1	0	1	0
- haemangioma		0	0	0	0	0	0	1	0
- leiomyoma		0	0	0	0	1	0	0	0
- adenocarcinoma		0	0	0	0	0	0	1	0
Hematopoietic tumor	(# examined)	(50)	(14)	(14)	(50)	(49)	(20)	(27)	(50)
- histiocytic sarcoma		5	3	1	3	1	3	5	4
- malignant lymphoma		5	4	3	6	16	13	13	21
Harderian gland, left	(# examined)	(0)	(0)	(3)	(1)	(1)	(1)	(0)	(2)
- adenoma		0	0	3	0	0	1	0	1
- carcinoma		0	0	0	0	1	0	0	1
Harderian gland, right	(# examined)	(3)	(0)	(1)	(1)	(1)	(1)	(1)	(0)
- adenoma		3	0	1	1	0	1	1	0
- carcinoma		0	0	0	0	1	0	0	0
Mammary, other	(# examined)	(0)	(0)	(0)	(0)	(2)	(0)	(0)	(2)
- adenocarcinoma		0	0	0	0	2	0	0	2
Muscle, skeletal	(# examined)	(1)	(0)	(2)	(1)	(1)	(0)	(0)	(1)
- haemangioma		1	0	0	0	0	0	0	0
- sarcoma		0	0	0	1	0	0	0	0
- ameloblastoma		0	0	1	0	0	0	0	0
- osteosarcoma		0	0	1	0	0	0	0	0
Skin, other	(# examined)	(7)	(15)	(4)	(11)	(4)	(2)	(4)	(8)
- basal cell tumor		0	0	0	0	0	1	0	0
- sarcoma		0	0	0	0	0	0	2	0
- haemangioma		0	0	0	0	0	0	0	1
- haemangiosarcoma		0	0	0	0	0	0	1	0

Section A6.8.1	**Teratogenicity Study**
BPD Annex Point IIA, VI.6.8.1	6.8.1 Developmental toxicity - Rat

			Official use only
		1 REFERENCE 1	
1.1	**Reference**	**1) XXXX** Reissued report XXXX: WL115110 Teratogenicity study in rats XXXX unpublished XXXX **2) XXXX** Addendum to XXXX - WL115110: Teratogenicity study in rats XXXX. unpublished XXXX **Note:** This addendum consists of 2 pages, stating that the study was conducted also in accordance to GLP requirements of JMAFF (Japanese authority) and provides a page of signatures of the project contributors. These pages are already included in the revised report. **3) XXXX** Response to BGVV concern regarding variations in branching of the great vessels of the heart in rat fetuses XXXX unpublished XXXX	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not indicated, but in general compliance with OECD 414	
2.2	**GLP**	Yes (laboratory certified by the Department of Health and Social Security, United Kingdom)	
2.3	**Deviations**	Yes, The only deviation from OECD 414 (1981) was the maintenance of the pregnant females in groups of two. However, in OECD 414 (2001) this is considered acceptable. This deviation is not considered to affect the validity of the study.	

Section A6.8.1	**Teratogenicity Study**
BPD Annex Point IIA, VI.6.8.1	6.8.1 Developmental toxicity - Rat

		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch: XXXX);	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	As given in section 2	
3.1.2.2	Purity	96.6%	X
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley	
3.2.3	Source	XXXX	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Approx. 10 weeks	X
3.2.6	Number of animals per group	26/dose group	
3.2.7	Control animals	Yes	
3.2.8	Mating period	Not specified: Presumably pregnant rats were purchased from the breeder	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of exposure	Days 6-16 of gestation (The day sperm was detected in the vaginal lavage or a sperm plug was observed was defined as gestational Day 0)	
3.3.2	Postexposure period	Days 17 to 20 of gestation.	
3.3.3	Type	Gavage	
3.3.4	Concentration	0, 1, 10 and 100 mg/ml	
3.3.5	Vehicle	Aqueous carboxymethylcellulose	
3.3.6	Concentration in vehicle	0.5%	

Section A6.8.1	**Teratogenicity Study**	
BPD Annex Point IIA, VI.6.8.1	6.8.1 Developmental toxicity - Rat	
3.3.7 Total volume applied	10 ml/kg i.e. dose levels were 0; 10; 100 and 1,000 mg/kg bw/day; dosing volume was based on individual animal weights at Day 6 of gestation.	
3.3.8 Controls	Vehicle	
3.4 Examinations		
3.4.1 Body weight	Yes	X
3.4.2 Food consumption	Yes	X
3.4.3 Clinical signs	Yes	X
3.4.4 Examination of uterine content	Uterus weight; number of live and dead fetuses, number coproa-utea for each ovary, number and position of implantation sites, number of early and late resorptions	X
3.4.5 Examination of foetuses		
3.4.5.1 General	Weight, sex & external examination	
3.4.5.2 Skelet	Yes: approx. half of the live foetuses	
3.4.5.3 Soft tissue	Yes: approx. half of the live foetuses	
3.5 Further remarks	None	X
	4 RESULTS AND DISCUSSION.	
4.1 Maternal toxic Effects	No effects	
4.2 Teratogenic / embryotoxic effects	No effects	
4.3 Other effects	None	X
	5 APPLICANT'S SUMMARY AND CONCLUSION	

Section A6.8.1	**Teratogenicity Study**
BPD Annex Point IIA, VI.6.8.1	6.8.1 Developmental toxicity - Rat

5.1	**Materials and methods**	Four groups of 26 presumably pregnant Sprague-Dawley female rats (XXXX) were administered Flufenoxuron by oral gavage (10 ml/kg bw) at dose levels of 0; 10; 100 and 1,000 mg/kg bw during days 6 to 16 of gestation. Aqueous carboxymethylcellulose (0.5%) served as vehicle. The dosing volume was based on the individual body weight on day 6 as determined on the first day of treatment. The dosing suspensions were continuously stirred during administration. The dams were received at the laboratory on day 1 of gestation and were acclimatized to laboratory conditions for 5 days. They were randomly allocated to treatment groups and housed in groups of 2 animals per cage. Survival was checked twice daily and clinical observations were performed once daily. Body weights were recorded on days 2, 6, 9, 13, 17, and 20 of gestation. Food consumption was recorded daily starting on day 3 of gestation. On gestational day 20, the dams were killed by nitrogen asphynxiation. After inspection of the thoracic and abdominal cavities and sampling of any abnormal tissue, the reproductive tract was removed, weighed, opened and examined. The weight of the individual fetuses was recorded and the fetuses were examined externally. Approximately one half of the viable fetuses from each uterus was fixed in methylated ethyl alcohol, examined for gross visceral abnormalities by open dissection and the eviscerated carcasses were subsequently cleared in potassium hydroxide for skeletal examination after staining with Alizarin Red S. The other half of the fetuses was fixed in Bouin's fluid and examined for soft tissue abnormalities by the sectioning technique according to Wilson.
5.2	**Results and discussion**	The homogeneity of the test substance in the preparations was not determined. The test substance concentration was determined for the first two preparations. For this triplicate samples were taken from the center of the preparation. At the high dose (100 mg/ml) the test article content was in good agreement with the intended concentration (97.3 ± 1.2% and 96.0 ± 1.0% of nominal). At the mid and low dose level (10 and 1 mg/ml) there was a considerable deviation from the intended concentrations (10 mg/ml: 80.0 ± 2.6% and 82.3 ± 3.2%; 1 mg/ml: 80.0 ± 8.7% and 78.0 ± 11.3%) and especially at the low dose homogeneity was insufficient. Since the dosing suspensions settled out during the 3 weeks between preparation and analysis and taking into account the difficulties in re-suspending the test substance, the deviation from the nominal concentrations was considered to be due to analytical difficulties rather than to errors during preparation.

Section A6.8.1	**Teratogenicity Study**
BPD Annex Point IIA, VI.6.8.1	6.8.1 Developmental toxicity - Rat

Given the Flufenoxuron hydrolytic half-life of 288 days, it is unlikely that the test substance decomposed within a day and therefore was considered to be stable in the vehicle.

There were no clinical signs of systemic toxicity. Body weight development and food consumption was comparable between all groups [see Table 6.8.1/82].

At necropsy, no treatment-related macroscopical changes were observed. Necropsy findings were restricted to the low dose level and consisted of small dark patches on the lungs of three dams (due to the killing method) and dry scabs on the face of another dam which in addition displayed an enlarged left kidney due to an acute purulent nephritis. These changes are sporadically observed in the laboratory and the strain of rats used.

One, 5, 3, and 3 rats were not pregnant at 0; 10; 100 and 1,000 mg/kg, respectively. Cesarean section revealed no effects on the number of implantations, early and late resorptions, number of live fetuses, fetal weight, fetal sex ratio, or mean uterus weight [see Table 6.8.1/83] . The sex ratios observed in this study were within the historical control range of 1 : 0.85 to 1 : 1.20.

Visceral examination revealed no treatment-related findings. The incidence of ‘major, deleterious abnormalities’ was comparable between all groups (5/5, 3/3, 4/4, 4/3 fetal/litter incidence at 0; 10; 100 and 1,000 mg/kg bw). These major abnormalities consisted of 12 different types of findings. Neither the type nor distribution of these findings indicated a relation to treatment. In addition there were a number of minor abnormalities that again did not indicate a relation to treatment. Compared to the other groups, a few more high dose fetuses displayed variations in the pattern of branching of certain blood vessels from the aortic arch [see Table 6.8.1/84]. If one resolves the lumped incidences of branching abnormalities into individual findings it becomes clear that only the incidences of variants “8” and “10” appear to be increased. Innominate angenesia (Variants “7” and “8”) are quite common findings in Sprague-Dawley rats. Historical control data compiled by MARTA (Middle Atlantic Reproduction and Teratology Association) in 1993 revealed an maximum litter incidence of 18.2% and maximum fetal incidence of 3.4% in control animals (3,240 litters, 22,892 fetuses in 154 studies). The litter (2/23 = 8.7%) and fetal incidences (2/143 = 1.4%) in this study are well within the historical control range. Abnormal origins of innominate (Variant 10) and carotid (Variant 11) are also observed in control animals either in the present study or the MARTA database. As the MARTA database does not always used the same descriptive terms it is difficult to provide exact

Section A6.8.1	**Teratogenicity Study**
BPD Annex Point IIA, VI.6.8.1	6.8.1 Developmental toxicity - Rat

historical control data. Nevertheless, minor position anomalies are not considered to be of toxicological relevance.

The heart vessel branching abnormalities were also evaluated by the expert teratologist, XXXX (see IIIA 6.8.1/3 XXXX). In her review, XXXX confirms the spontaneous nature of these common branching alterations which are detectable in numerous and highly variable positions and patterns for control rat fetuses, indicative of their inherent, fortuitous and idiosyncratic occurrence. Furthermore, XXXX also indicated that the incidences of these minor cardiovascular variants are highly dependent on the particular methods selected for free hand sectioning and for recording procedures and practices used by individual testing laboratories. In fact, she states: "Many laboratories accept these common cardiovascular variants as normal background observations and do not attempt to identify them." FinallyXXXX concludes that regardless of which method or laboratory is used, the "inherent variability of these structures is such that meaningful identification of increases above background levels cannot be identified." Therefore, the slight, non-statistically significant increased incidences of minor variations in branching of the carotid and subclavian arteries from the aortic arch in rat fetuses at 1,000 mg/kg bw/day, were considered to be unrelated to treatment.

Finally, variations of the vessel branching pattern near the heart are also common in humans. Liechty et al. described variations of the aortic arch and its branches based on about 1,000 human autopsies (Liechty J. D., Shields T. W., Anson, B. J., Variations pertaining to the aortic arches and their branches, with comments on surgically important types, Quart. Bull. Northwestern Univ. M. School, 31: 136 - 143, 1975). Only 65% of the cases had the usual arrangement of 3 vessels, i.e. the brachiocephalic trunk branching into the right subclavian and the right common carotid, followed by separate origins of the left common carotid and the left subclavian arteries. In 27% of the cases only two vessels (a common trunk and a separate left subclavian artery) where observed and in the remaining 13% of the cases there were twelve different branching variants.

Skeletal examination revealed a low incidence of 'major, deleterious abnormalities'. Most frequently these major abnormalities consisted of a misalignment of illia and ischia which were observed in 8, 3, 4, and 2 fetuses in 7, 3, 4, and 2 litters at 0; 10; 100 and 1,000 mg/kg bw/day. None of the observed major abnormalities indicated a relation to treatment. Likewise, the incidence of minor skeletal abnormalities was

Section A6.8.1	**Teratogenicity Study**
BPD Annex Point IIA, VI.6.8.1	6.8.1 Developmental toxicity - Rat

		comparable between all groups. Especially, there was no evidence for a treatment-related effect on skeletal ossification.	
5.3	**Conclusion**	Flufenoxuron did not cause any adverse effects in pregnant rats when administered by oral gavage at dose levels up to 1,000 mg/kg bw/day during gestational days 6 to 16. **Based on this, the maternal and developmental NOAELs for Flufenoxuron in the rat are 1,000 mg/kg bw/day (highest dose tested), which correspond to the limit dosage for this type of mammalian toxicity study. Flufenoxuron is neither a developmental toxicant nor a teratogenic agent in the rat.**	
5.3.1	LO(A)EL maternal toxic effects	Not applicable	
5.3.2	NO(A)EL maternal toxic effects	1000 mg/kg bw/day	
5.3.3	LO(A)EL embryotoxic / teratogenic effects	Not applicable	
5.3.4	NO(A)EL embryotoxic / teratogenic effects	1000 mg/kg bw/day	
5.3.5	Reliability	1	
5.3.6	Deficiencies	No	X

Section A6.8.1	**Teratogenicity Study**
BPD Annex Point IIA, VI.6.8.1	6.8.1 Developmental toxicity - Rat

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 3.1.2.2. Purity 94.7% (Ref to Appendix 12) 3.2.5. Age/weight at study initiation Approx. 10 weeks; 198-361g at day 2 of gestation 3.4.1 Body weight Yes, on days 2-6-9-13-17-20 of gestation 3.4.2 Food consumption Yes, everyday 3.4.3 Clinical signs Yes, everyday 3.4.4. Examination of uterine content *Uterus weight; number of live and dead fetuses, number* corpora lutea *for each ovary, number and position of implantation sites, number of early and late resorptions* 3.5. Statistics This part is missing
Results and discussion	Agree with the applicant's version **Revisions/Amendments:** 4.3. Other effects No clinically observed signs of toxicity except some sporadic findings, common in this strain of rats. No significant effect of treatment observed on maternal body weight and on maternal food consumption.
Conclusion	Agree with the applicant's version NOAEL maternal toxic effects 1000 mg/kg/day NOAEL embryotoxic/teratogenic effects 1000 mg/kg/day **Revisions/Amendments:** 5.3.6. Deviations Yes (two animals per cage)
Reliability	1
Acceptability	Acceptable
Remarks	**IUCLID** Change the purity

Section A6.8.1	**Teratogenicity Study**
BPD Annex Point IIA, VI.6.8.1	6.8.1 Developmental toxicity - Rat

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.8.1/82 Maternal data – Prenatal toxicity gavage study in SD rats

Dose (mg/kg bw)	**0**		**10**		**100**		**1,000**	
Body weight gain								
Bw gain (g) day 6-17	85.6	(100%)	89.1	(104%)	92.9	(109%)	94.2	(110%)
Corrected body weight gain (net weight change during the study)	74.8	(100%)	79.6	(106%)	80.1	(107%)	82.8	(111%)
Food consumption (g/animal/day)								
Cumulative (day 6 to 16)	338.8	(100%)	325.6	(96.1%)	339.1	(100%)	337.4	(99.6%)

Table 6.8.1/83 Cesarean data – Prenatal toxicity gavage study in SD rats

Dose (mg/kg bw)	**0**	**10**	**100**	**1,000**
Pregnancy status				
- Number mated	26	26	26	26
- Number pregnant	25	21	23	23
- Number pregnant day 20	25	21	23	23
Cesarean section data				
-Total corpora lutea	365	297	326	341
-Total implantation	351	294	316	331
- Pre-implantation loss (%)	4	1	3	3
- Total live implants	327 (93.2)[a]	279 (94.9)	294 (93.0)	301 (90.9)
- Total early deaths	20 (5.7)	13 (4.4)	12 (3.8)	26 (7.9)
- Total late deaths	4 (1.1)	1 (0.3)	6 (1.9)	4 (1.2)
- Total fetal deaths	0 (0.0)	1 (0.3)	4 (1.3)	0 (0.0)
- Total live male fetuses	152 (46.5)[b]	150 (53.8)	153 (52.0)	160 (53.2)
- Total live female fetuses	175 (53.5)	129 (46.2)	141 (48.0)	141 (46.8)
- Sex ratio	1 : 1.15	1 : 0.86	1 : 0.93	1 : 0.88
Corpara lutea	14.6 ± 2.4[c]	14.1 ± 1.9	14.2 ± 2.5	14.8 ± 2.4
Implants	14.0 ± 3.1	14.0 ± 1.9	13.7 ± 2.4	14.4 ± 2.5
Live implants	13.1 ± 2.9	13.3 ± 1.9	12.8 ± 2.3	13.1 ± 2.3
Early deaths	0.8 ± 1.0	0.6 ± 0.8	0.5 ± 0.7	1.1 ± 1.1
Late deaths	0.4 ± 0.4	0.0 ± 0.2	0.3 ± 1.1	0.2 ± 0.5
Fetal deaths	0.0 ± 0.0	0.0 ± 0.2	0.2 ± 0.4	0.0 ± 0.0
Mean fetal weight	3.69 ± 0.24[c]	3.73 ± 0.24	3.78 ± 0.37	3.67 ± 0.27
Mean uterus weight	77.0 ± 14.3[c]	79.0 ± 9.1	77.7 ± 10.8	78.0 ± 12.6

[a] % of total implantation; [b] % of total fetuses; [c] Mean ± SD on litter basis

Table 6.8.1/84 Incidence of heart vessel branching abnormalities

Dose (mg/kg bw)	**0**	**10**	**100**	**1,000**
Minor variants in pattern of branching if carotid and subclavian arteries from aortic arch (Abnormalities 7 – 11)				
Litter incidence	1/25 (4.0)	1/21 (4.76)	1/23 (4.35)	5/23 (21.7)
Fetal incidence	1/156 (0.64)	2/134 (1.49)	1/142 (0.7)	7/143 (4.9)
Incidence of individual variants				
Variant 7[a]		51/1[§]		
Variant 8[b]				90/1; 97/1
Variant 9[c]		51/1		
Variant 10[d]	8/1		73/1	88/1; 90/2; 94/1
Variant 11[e]				104/1

Values in brackets give percent incidences

[§] Litter affected/No of fetuses affected

[a] No innominate artery. Right acarotid and right subclavian arise separately and lower (more distally) than normal from aortic arch;

[b] No innominate artery. Right carotid and right subclavian arise directly from aortic arch;

[c] Very short innominate artery. Right carotid and right subclavian almost arising directly form aortic arch;

[d] Innominate artery arises low (more proximally) on aortic arch; [e] Innominate artery and left carotid artery arise very close together from aortic arch

Section A6.8.1	**Teratogenicity Study**
BPD Annex Point IIA, VI.6.8.1	6.8.1 Developmental toxicity - Rabbit

			Official use only
		1 REFERENCE 2	
1.1	**Reference**	**4) XXXX** Reissued report XXXX - WL115110: Teratogenicity study in rabbits XXXX unpublished XXXX **5) XXXX** Addendum to XXXX - WL115110: Teratogenicity study in rabbits XXXX. unpublished XXXX **Note:** This addendum consists of 2 pages, stating that the study was conducted also in accordance to GLP requirements of JMAFF (Japanese authority) and provides a page of signatures of the project contributors. These pages are already included in the revised report.	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not indicated, but in general compliance with OECD 414 (1981)	
2.2	**GLP**	Yes (laboratory certified by the Department of Health and Social Security, United Kingdom)	
2.3	**Deviations**	The study employed 15 rabbits/dose groups. According to the current guidelines (OECD 414, adopted 22 January 2001) this is considered to be inappropriate. However, this study was conducted before the update of the OECD 414. At the time the study was conducted (July 1986 to January 1987) OECD Guideline 414 as adopted 12 May 1981 detailed that "At least 20 pregnant rats, mice or hamsters or 12 pregnant rabbits are required at each dose level. The objective is	

Section A6.8.1	**Teratogenicity Study**
BPD Annex Point IIA, VI.6.8.1	6.8.1 Developmental toxicity - Rabbit

		to ensure that sufficient pups are produced to permit an evaluation of the teratogenic potential of the substance. Based on the fact that 15, 13, 15 and 15 dams were pregnant (i.e. had implantations) at 0, 10, 100 and 1000 mg/kg, respectively and considering the fact that the NOAEL in this study was 1000 mg/kg the study is considered to be scientifically fully valid.	
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch: XXXX	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	As given in section 2	
3.1.2.2	Purity	96.6%	X
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	New Zealand	
3.2.3	Source	XXXX	X
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	4.5 to 6 months	X
3.2.6	Number of animals per group	15/dose group	
3.2.7	Control animals	Yes	
3.2.8	Mating period	Each female mated with 2 males; details see under 5.1	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of exposure	Days 6 to 18 of gestation; Day of mating = Day 0 of gestation	
3.3.2	Postexposure period	Days 19 to 28 of gestation; animals were killed at gestational Day 29	
3.3.3	Type	Gavage	
3.3.4	Concentration	0, 2.5, 25 and 250 mg/ml	
3.3.5	Vehicle	Aqueous carboxymethylcellulose	

Section A6.8.1	**Teratogenicity Study**
BPD Annex Point IIA, VI.6.8.1	6.8.1 Developmental toxicity - Rabbit

3.3.6	Concentration in vehicle	0.5%	
3.3.7	Total volume applied	4 ml/kg; applied volume was based on individual animal weight on gestational Day 6	
3.3.8	Controls	Vehicle	
3.4	**Examinations**		
3.4.1	Body weight	Yes	X
3.4.2	Food consumption	Yes	X
3.4.3	Clinical signs	Yes	X
3.4.4	Examination of uterine content	Uterus weight; number of live and dead fetuses, number copora lutea for each ovary, number and position of implantation sites, number of early and late resorptions	
3.4.5	Examination of foetuses		
3.4.5.1	General	Weight, sex & external examination	
3.4.5.2	Skelet	Yes, 2/3 of life fetuses; for details see 5.1	
3.4.5.3	Soft tissue	Yes, 2/3 of the life fetuses by open dissection for gross visceral abnormalities, 1/3 by whole body dissection; for details see 5,1	
3.5	**Further remarks**	None	X
		4 RESULTS AND DISCUSSION.	
4.1	**Maternal toxic Effects**	No effects	
4.2	**Teratogenic / embryotoxic effects**	No effects	X
4.3	**Other effects**	None	X
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Four groups of 15 3½ to 5 month old, presumably pregnant female New Zealand White rabbits (XXXX) weighing 3 to 5 kg were administered Flufenoxuron by oral gavage (4 ml/kg bw) at dose levels of 0; 10; 100 and 1,000 mg/kg during days 6 to 18 of gestation. Aqueous carboxymethylcellulose (0.5%) served as vehicle. In order to assure homogeneity the dosing suspensions were continuously stirred during administration. The dosing	

Section A6.8.1	**Teratogenicity Study**
BPD Annex Point IIA, VI.6.8.1	6.8.1 Developmental toxicity - Rabbit

volume was based on the individual animals weight on day 6. The does were acclimatised to laboratory conditions for 3½ weeks prior to mating and housed individually. Three weeks prior to mating the animals were given chorionic gonadotrophin by intravenous injection. This induced pseudopregnancy and ensured that the animals were in phase, ready for actual mating. Each female was mated with 2 males. After mating each female was again administered chorionic gonadotrophin to ensure ovulation. After mating the females were randomly allocated to treatment groups. Survival was checked twice daily and clinical observations were performed once daily. Body weights were recorded on days 0, 6, 9, 12, 15, 19, 22, 26, and 29 of gestation. Food consumption was recorded daily starting on day 3 of gestation.

On gestational day 29, the does were killed by intravenous injection of sodium phenobarbitone (approx 120 mg/kg bw/day). After inspection of the thoracic and abdominal cavities and sampling of any abnormal tissue, the reproductive tract was removed, weighed, opened and examined. The weight of the individual fetuses was recorded and the fetuses were examined externally. Approx. two thirds of the viable fetuses from each uterus were fixed in methylated ethyl alcohol, examined for gross visceral abnormalities by open dissection and the eviscerated carcasses were subsequently cleared in potassium hydroxide for skeletal examination after staining with Alizarin Red S. The remaining third of the fetuses was fixed in Bouin´s fluid and examined for soft tissue abnormalities by whole-body dissection. The internal head structures were examined by means of a free-hand razor blade sectioning technique.

5.2 Results and discussion

The homogeneity of the test substance in the preparations was not determined. The test substance concentration was determined for the first two preparations. For this triplicate samples were taken from the center of the preparation. The test substance concentration at the high dose (250 mg/ml) was slightly higher than intended (110 ± 6.0% and 109 ± 0.6% of nominal). The mid dose level (25 mg/ml) was in good agreement with the intended concentration (99.7 ± 6.7% and 97.7 ± 2.5% of nominal). At the low dose level (2.5 mg/ml) there was a considerable deviation from the intended concentration (83.3 ± 9.5% and 70.0 ± 14.1%). Homogeneity of the low dose suspension was also not satisfactory. Since the dosing suspensions settled out between preparation and analysis and taking into account the difficulties in re-suspending the test substance, the deviation from the nominal concentrations was considered to be due to analytical difficulties

Section A6.8.1	**Teratogenicity Study**
BPD Annex Point IIA, VI.6.8.1	6.8.1 Developmental toxicity - Rabbit

rather than to errors during preparation. Given the Flufenoxuron hydrolytic half-life of 288 days, the test substance is unlikely to have decomposed within a day and therefore considered to be stable in the vehicle.

There were no clinical signs of systemic toxicity. One control doe was killed in extremis on gestation day 15. At gross necropsy indications for mal-dosing were observed. Three low dose does were killed because of abortion on days 20 (doe #27), 27 (doe #28), and 28 (doe #29). Doe #27 probably aborted due to extreme low food consumption and body weight loss. Abortion of doe #29 may have been due to a mal-dosing around day 18. High dose doe #52 was killed on day 19 because of suspected abortion (blood in tray; red staining around vulva and on forepaws; continuing hemorrhage). In absence of a dose-response relationship none of the deaths or abortions is considered to be related to the test-article administration.

No treatment-related effects on maternal body weight or food consumption were noted. At maternal necropsy no test-substance related findings were noted.

A total of 15, 13, 15, and 15 female rabbits were pregnant at 0; 10; 100 and 1,000 mg/kg, respectively [see Table 6.8.1/85]. Due to mortality in control animals and abortions in 3 low and 1 high dose females 13, 10, 15, and 14 does had life fetuses at cesarean section. The somewhat low number of litters at the 10 mg/kg bw dose level does not affect the validity of the study given the fact that the 1,000 mg/kg bw was identified as NOAEL in this study.

The only noteworthy effect on cesarean section data consisted in slightly lower mean fetus weights in high dose fetuses. Compared to the control fetal weights were reduced by about 7%. This decrease was however not statistically significant. At the high dose the slight decrease in mean fetal weights was accompanied by an increase of the mean litter size by about 7%. As it is known that litter size does affect fetal weight, the pup weight effect is considered to be a non-adverse, secondary effect to the increased litter size and not related to Flufenoxuron administration.

No treatment-related effects were observed on the number of early or late deaths or the mean uterus weights. The fetal sex-ratio appeared to be somewhat high at the mid dose level. However, since the sex-ratio at the low and high dose was essentially comparable to the controls, this is considered be incidental rather than related to treatment.

The incidence of visceral 'major, deleterious abnormalities' was comparable between all groups. A total of 2, 3, 0, and 3 fetuses

Section A6.8.1	**Teratogenicity Study**
BPD Annex Point IIA, VI.6.8.1	6.8.1 Developmental toxicity - Rabbit

from 2, 2, 0, and 3 litters were affected at 0; 10; 100 and 1,000 mg/kg bw/day. Two cases of pre-ductal co-arctation of the aorta were observed in high dose pups. This finding is occasionally observed in the laboratory and strain of animals used, namely one case in a control animal of a study conducted shortly before the Flufenoxuron study. This finding is therefore considered to be incidental.

Like in the rat study, a slight, but not statistically significant increase in the incidence of several minor visceral variations of vascular branching of blood vessels near the heart was observed in high dose pups but not considered to be treatment-related [see Table 6.8.1/86].

These variations in vascular branching in rabbit fetuses are considered to be as inherently spontaneous and idiosyncratic as those observed in the rat teratology study with Flufenoxuron, and also highly dependent on specific procedures employed at individual testing laboratories. Specifically, the rabbit teratology study with Flufenoxuron was conducted by XXXX, when - according to a personal communication of the study director to the American Cyanamid toxicologist T. Wang in 1996 - this laboratory's recognition levels of these minor blood vessel variants were adjusted upwards, "in order to monitor their type and incidence more closely". Shortly thereafter, in 1988, once their natural incidence had been determined, the laboratory's "recognition levels were also adjusted again, with the result that the recorded incidences reduced considerably." Thus the slight, non-statistically significant increases observed for minor variations in vascular branching of vessels for fetuses in the high dose (1,000 mg/kg bw/day) group, as compared with controls, were not considered "to demonstrate an effect of treatment with test material" or "to be detrimental to the animals," but rather "that this pattern of incidence was likely to have been fortuitous." The fortuitous character of the findings becomes also clear by the absence of a dose-response relationship [see Table 6.8.1/86]: the incidences of branching abnormalities in the mid dose is comparable to that of the control whereas increased incidences were observed in the low and high dose groups.

Finally, as already indicated above, variations of the vessel branching pattern near the heart are commonly observed in humans. This indicates that the alterations in the vessel branching pattern observed in this study is of no toxicological concern.

Neither the type nor the distribution of "major, deletorious (or potentially so) skeletal abnormalities" indicated a relation to treatment. A total of 5, 3, 4, and 3 fetuses from 5, 3, 4, and 3

Section A6.8.1	**Teratogenicity Study**
BPD Annex Point IIA, VI.6.8.1	6.8.1 Developmental toxicity - Rabbit

		litters were affected at 0; 10; 100 and 1,000 mg/kg bw The incidence of minor abnormalities was likewise comparable between control and treated groups. Although the individual findings were not statistically significant, a slight retardation of skeletal ossification was noted at the high dose level. This retardation concerned the hyoid arch, the long bone epiphyses, the sternebrae, the olecranon and possibly the pelvic bones. This slight retardation of skeletal ossification parallels the slight, not treatment-related decrease of fetal body weights. Delays of ossification are often observed in fetuses of lower weight and are fully reversible. These findings are therefore considered as non-adverse.
5.3	**Conclusion**	Administration of Flufenoxuron to New Zealand White rabbits at dose levels of 0, 10; 100 and 1,000 mg/kg bw by oral gavage during gestational days 6 to 18 did not result in any maternal toxicity up to the highest dose tested. The slight effects on fetal weights (non significant decrease by 7% when compared to the control) are probably due to a slightly higher mean litter size at the high dose level. Secondary to the lower fetal weights at the high dose level, delays of fetal ossification were observed. These effects are not considered to be of adverse nature. Based on the observed findings the maternal NOEL was 1,000 mg/kg and the developmental NOAEL was 1,000 mg/kg.
5.3.1	LO(A)EL maternal toxic effects	Not applicable
5.3.2	NOEL maternal toxic effects	1000 mg/kg bw/day
5.3.3	LO(A)EL embryotoxic / teratogenic effects	Not applicable
5.3.4	NO(A)EL embryotoxic / teratogenic effects	1000 mg/kg bw/day
5.3.5	Reliability	1
5.3.6	Deficiencies	No

Section A6.8.1	**Teratogenicity Study**
BPD Annex Point IIA, VI.6.8.1	6.8.1 Developmental toxicity - Rabbit

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 3.1.2.2. Purity 94.7% (Ref to Appendix 12) 3.2.3. Source XXXX. 3.2.5. Age/weight at study initiation *4.5 to 6 months;* 3- 5kg on arrival 3.4.1. Body weight Yes, on days 0-6-9-12-15-19-22-26-29 of gestation 3.4.2. Food consumption Yes, *every day since day 2* 3.4.3. Clinical signs Yes, *every day* 3.5. Statistical part is missing
Results and discussion	**Revisions/Amendments:** 4.2. Teratogenic / embryotoxic effects There was a slight reduction of mean fœtal weight in the high dose group about 7%, not significantly different 4.3. Other effects No clinically observed signs of toxicity except few related to dosing accidents . No significant effect of treatment observed on maternal body weight and on maternal food consumption.
Conclusion	Agree with the applicant's version NOEL maternal toxic effects 1000 mg/kg bw/day NO(A)EL embryotoxic / teratogenic effects 1000 mg/kg bw/day
Reliability	1
Acceptability	Acceptable
Remarks	**IUCLID** Change purity 94.7%
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*

Section A6.8.1	**Teratogenicity Study**
BPD Annex Point IIA, VI.6.8.1	6.8.1 Developmental toxicity - Rabbit

Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.8.1/85 Cesarean data – Prenatal toxicity gavage study in NZW rabbits

Dose (mg/kg bw)	**0**	**10**	**100**	**1,000**
Pregnancy status				
- N°mated	15	15	15	15
- N°pregnant	15	13	15	15
- N°pregnant day 29	13	10	15	14
- N°dying/dying pregnant	2/2	3/3	0/0	1/1
- N° aborting	0	3	0	1
Cesarean section data[§]				
-Total corpora lutea	139	97	138	160
-Total implantation	123	91	123	137
- Pre-implantation loss (%)	11.5	6.2	10.9	14.4
- Total live implants	102 (82.9)[a]	81 (89.0)	100 (81.3)	118 (86.8)
- Total early deaths	8 (6.5)	5 (5.5)	13 (10.6)	7 (5.1)
- Total late deaths	7 (5.7)	2 (2.3)	4 (3.3)	5 (3.7)
- Total fetal deaths	6 (4.9)	3 (3.3)	6 (4.9)	7 (5.1)
- Total live male fetuses	54 (52.9)[b]	40 (49.4)	39 (39.0)	57 (48.3)
- Total live female fetuses	48 (47.1)	41 (50.6)	61 (61.0)	61 (51.7)
- Sex ratio	1 : 0.89	1 : 1.03	1 : 1.56	1 : 1.07
Corpara lutea	10.7 ± 2.5[c]	9.7 ± 2.1	9.2 ± 2.9	11.4 ± 2.4
Implants	9.5 ± 2.5	9.1 ± 2.3	8.2 ± 3.3	9.8 ± 2.0
Live implants	7.85 ± 1.4	8.1 ± 2.7	6.7 ± 6.7	8.4 ± 2.2
Early deaths	0.6 ± 1.2	0.5 ± 0.9	0.9 ± 1.1	0.5 ± 0.9
Late deaths	0.5 ± 1.0	0.2 ± 0.4	0.3 ± 0.5	0.4 ± 0.6
Fetal deaths	0.5 ± 1.1	0.3 ± 0.8	0.4 ± 0.8	0.5 ± 0.5
Mean fetal weight	49.3 ± 4.3[c]	50.0 ± 3.1	51.8 ± 7.6	45.9 ± 3.5
Mean uterus weight	571.4 ± 83.5[c]	588.7 ±154.3	523.5 ±176.7	583.0 ±127.5

[§] premature deaths including abortions not included in calculation; [a] % of total imlantation; [b] % of total fetuses; [c] Mean ± SD

on litter basis

Table 6.8.1/86 Incidence of vessel branching abnormalities near the heart

Dose (mg/kg bw)	**0**		**10**		**100**		**1,000**	
Vessel branching abnormalities near the heart (N° 3 – 11, 18, 19)								
Litter incidence	9/13	(69.2)	10/11[§]	(90.9)	9/15	(60.0)	13/14	(92.9)
Fetal incidence	23/102	(22.5)	31/87	(35.6)	29/100	(29.0)	44/118	(37.7)
Pre-ductal co-arctation of aorta								
Litter incidence	0	0	0	0	0	0	2/14	(14.3)
Fetal incidence	0	0	0	0	0	0	2/118	(1.7)

[a] values in brackets give % incidence

[§] includes fetuses from doe #29 which aborted at day 28. The six fetuses from this doe were included into the visceral examination since they were fully assessible. The litter and fetal incidences for branching abnormalities excluding these fetuses were 9/10 (90%) and 29/81 (35.8%)

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat

			Official use only
		1 REFERENCE 1	
1.1	**Reference**	**1) XXXX** The effect of WL 115110 on the reproductive function of two generations in the rat XXXX unpublished XXXX **2) XXXX** Addendum to SLL 138/891394: The effects of WL115110 on the reproductive function of two generations in the rat XXXX unpublished XXXX **Note**: The addendum consists of 1 page, stating that the study was conducted also in accordance to GLP requirements of JMAFF (Japanese authority) **3) XXXX** Amendment no. one: The effects of WL115110 on the reproductive function of two generations in the rat XXXX unpublished XXXX **Note:** This amendment corrects a tying error in Table 10:0 on Page 77 of the report	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD 416, EPA Fed. Reg. 47 - No.100 (1982) = OPP Health Assessment Guidelines, Subdivision F, § 83-4	
2.2	**GLP**	Yes (laboratory certified by the Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)	
2.3	**Deviations**	No	X

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat

		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch: 7016	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	As given in section 2	
3.1.2.2	Purity	97.0%	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	(CrL: CD® /SD) BR VAF/Plus Sprague Dawley	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	About 6 weeks	X
3.2.6	Number of animals per group	28 (P) / 24 (F_1)	
3.2.7	Mating	See 5.1	
3.2.8	Duration of mating	Up to 20-day	
3.2.9	Deviations from standard protocol	A second mating of P and F_1 generations was performed	
3.2.10	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Animal assignment to dosage groups	Random assignment	
3.3.2	Duration of exposure before mating	10 weeks	
3.3.3	Duration of exposure in general P, F1, F2 males, females	From beginning of the study until sacrifice of parent, F1, F2-generation including ensuing gestation, lactation and post-weaning periods.	
3.3.4	Type	In food	
3.3.5	Concentration	0; 50; 190; 710 or 10,000 ppm	X
3.3.6	Vehicle	None	

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**		
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat		
3.3.7	Concentration in vehicle	Not applicable	
3.3.8	Total volume applied	Not applicable	
3.3.9	Controls	Plain diet	
3.4	**Examinations**		
3.4.1	Clinical signs	Yes	
3.4.2	Body weight	Yes	X
3.4.3	Food/water consumption	Yes; food consumption was determined during the pre-mating phases only. Water consumption was determined during the first and last two weeks of the initial pre-mating periods.	X
3.4.4	Oestrus cycle	No; the requirement to determine the estrus cycle was introduced in 2001 only, i.e. more than a decade after the conduct of the study	
3.4.5	Sperm parameters	No; the requirement to determine sperm parameters was introduced in 2001 only, i.e. more than a decade after the conduct of the study	
3.4.6	Offspring	Counted, weighed, sexed and examined for external abnormalities. Pupil reflex was tested in pups of all litters on day 20 p.p. and startle reflex was examined from day 11 p.p. to 100%. In addition, the onset of vaginal opening or balino-preputial cleavage was investigated in female or male F1 pups, respectively	
3.4.7	Organ weights P and F1	Adrenals, brain, heart, kidneys, liver, lungs, ovaries, prostate with seminal vesicles, spleen, testes and thymus	
3.4.8	Histopathology P and F1	Reproductive tract and associated tissues (ovaries, pituitary, prostrate with seminal vesicles, testes with epididymides, uterus with cervix and vagina) of control and top dose groups and apparently infertile males and females. Liver, heart and spleen from control and top dose F_1 parental animals were examined in addition.	
3.4.9	Histopathology F1 not selected for mating, F2	No; the requirement to perform histopathology on selected organs was introduced in 2001 only, i.e. more than a decade after the conduct of the study	
3.5	**Further remarks**	None	

4 RESULTS AND DISCUSSION.

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat

4.1	**Effects**	
4.1.1	Parent males	See study summary under 5.2
4.1.2	Parent females	See study summary under 5.2
4.1.3	F1 males	See study summary under 5.2
4.1.4	F1 females	See study summary under 5.2
4.1.5	F2 males	See study summary under 5.2
4.1.6	F2 females	See study summary under 5.2
4.2	**Other**	None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Flufenoxuron technical was fed to five groups of Crl: CD® (SD) BR VAF/Plus Sprague Dawley rats (XXXX) at dietary concentrations of 0; 50; 190; 710 or 10,000 ppm throughout the entire study. Treatment started at an age of approx. 6 weeks for F_0 and at 4 weeks for the F_1 parental animals.

Study design

The F_0 parental generation consisted of 28 male and 28 female rats per group and was treated for 10 weeks prior to a 20-day mating period on a 1:1 male and female ratio to produce F_{1a} litters. Due to an increased incidence of total litter losses and lower post natal pup survival at the top dose, F_0 parental animals were mated a second time (see below). Twenty-four male and 24 female F_{1a} pups were selected as a contingency to eventually become the F_1 parental generation. These animals were maintained until the outcome of the second F_0 mating was known and selection of F_{1b} was made and were finally killed at an age of about 14 weeks.

Remating of F_0 parental animals took place approximately 10 days after the weaning of the F_{1a} litters. F_0 animals were allowed to mate for up to 20 days to produce F_{1b} litters. Alternative pairings to the first mating were employed, i.e. previously non-pregnant females and males failing to induce pregnancy were mated to animals which were successful at the first mating. Weaned F_{1b} offspring were selected to become the F_{1b} parental generation which consisted of 24 male and 24 female rats per group. The F_{1b} parental generation was treated for 12 weeks prior to a 20-day mating period to produce F_{2a} litters. Approximately 10 days after the weaning of the F_{2a} litters, F_{1b} animals were re-mated as above for a second 20-day mating period to produce F_{2b} litters.

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat

Parental toxicity and fertility

All animals were regularly handled and examined for obvious changes or signs of reaction to treatment. Body weights of the animals were determined on a weekly basis throughout the study period. During mating and up to parturition, body weights of females were determined daily and reported for days 0, 7, 14, 17 and 20 of gestation. For assessment of fertility, the pregnancy rate, mating performance and length of gestation period were recorded. Determination of food consumption was confined to weekly measurements during the pre-mating phases, at which time the food conversion ratios and test substance intake were determined. Water consumption was measured on a daily basis during the initial two and final two weeks of the pre-mating period for each generation.

Parental animals were killed after weaning of F_{1b} and F_{2b} litters, respectively. Organ weight determinations of the adrenals, brain, heart, kidneys, liver, lungs, ovaries, prostate with seminal vesicles, spleen, testes and thymus were carried out on all F_0 and F_{1b} adults. Histopathology of the reproductive tract and associated tissues (ovaries, pituitary, prostrate with seminal vesicles, testes with epididymides, uterus with cervix and vagina) was performed on all adults of the control and the 10,000 ppm dose groups and of apparently infertile males and females from other groups. Finally, for assessment of potential target organs, the heart, liver, and spleen from F_{1b} adults of the control group and 10,000 ppm group were assessed by light microscopy.

Offspring toxicity

At birth, offspring were counted, weighed, sexed and examined for external abnormalities. All litters were examined daily for dead and/or abnormal young. All litters were culled to 8 pups per litter on postnatal day 4 maintaining (if possible) an even sex ratio. Pup body weights were determined on day 4 pre- and post-culling, and on days 8, 12, and 21 post partum (p.p.). For assessment of pre-weaning development, pupil reflex was tested in pups of all litters on day 20 p.p. and startle reflex was examined from day 11 p.p. to 100%. In addition, the onset of vaginal opening or balino-preputial cleavage was investigated in female or male F1 pups, respectively. Organ weight determinations of the adrenals, brain, heart, kidneys, liver, lungs, ovaries, prostate with seminal vesicles, spleen, testes and thymus were carried out on one male and one female from each litter and selected tissues were preserved for histopathological examinations if required. Excess pups not required for formation of a subsequent parental generation were killed and examined for

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat

		external and internal abnormalities. Any tissues showing macroscopic abnormalities were preserved to permit histopathological examination if required.	
5.2	**Results and discussion**	Diet analysis and compound intake As known from the chronic toxicity/oncogenicity studies Flufenoxuron was stable in rodent diets for at least 2 weeks at room temperature in the dark. Therefore the stability of the test substance was not determined in this study. Accordingly, formulated diets were prepared weekly and fed for no longer than 2 weeks. As indicated by Relative Standard Deviations of < 1.8% of the homogeneity samples, the diet preparations were homogenous at test-substance concentrations ranging from 50 to 50,000 ppm. The test-substance content was determined in 8 preparations. The average dietary concentrations were 51.0 ± 1.8; 185.1 ± 2.4; 705.9 ± 6.1 and 9,902 ± 166 ppm at nominal concentrations of 50; 190; 710 and 10,000 ppm, respectively. Compound intake was determined for the first pre-mating periods of the F_0 and F_{1b} generations [see Table 6.8.2/87]. **Parental toxicity** No treatment-related mortality was observed throughout the study period. Four cases of mortality were observed. Three of them occurred during the late phase of the second gestation. This pertained one F_0 control, one low-dose (50 ppm) F_{1b} and one low-intermediate dose (190 ppm) F_{1b} female. The fourth death was observed in another low-intermediate dose F_{1b} female (week 34; post-weaning). In absence of a dose-response relationship none of these deaths is considered to be related to treatment. According to the study director, the only suggestion of a treatment-related clinical sign was an increased incidence of alopecia for F_0 and F_{1b} top dose (10,000 ppm) females. However, in F_0 females 7 low dose females also displayed alopecia [see Table 6.8.2/88]. The lacking dose-response relationship indicates a coincidental occurrence of this finding. Therefore, a relation of this finding to treatment is unlikely. Body weight development was essentially not affected in F_0 males. A statistically lower body weight was noted at the top dose level at Week 20 only [see Figure 6.8.2/2]. In contrast, impaired body weight development was observed in F_{1b} males at dose levels ≥ 190 mg/kg bw from week 8 onwards. At the end of the pre-mating period, body	X

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat

weight gain was reduced in these groups by 10, 10 and 7% at 190; 710 and 10,000 ppm, respectively. Overall body weight gain (Week 4 to 35) was reduced by 12.6, 10.8 and 10.0%, respectively.

Reductions in body weight gain by 8, 8 and 11% were noted for F_0 females at ≥ 190 ppm for the pre-mating period (prior to the first mating). Overall body weight gains for F_0 females for the two gestation and lactation periods were comparable for all groups (219, 221, 214, 208, and 216 g at 0; 50; 190; 710 and 10,000 ppm, respectively). For F_{1b} females, body weight gains during the pre-mating treatment period (prior to the first mating) were slightly reduced by 7, 6 and 7% at ≥ 190 ppm. Again, overall body weight gains of F_{1b} females over the entire study period were essentially comparable for all groups (281; 284; 274; 276 and 269 g at 0; 50; 190; 710 and 10,000 ppm, respectively).

Body weight development for F_0 females for the two gestation and lactation periods were comparable for all groups. This also holds true for the two gestation periods of F_{1b} females. In contrast, for the first lactation period body weight gain for F_{1b} top dose females was statistically significantly decreased (4.4 g versus 20.8 g) when compared to controls. For the second lactation period, high intermediate (710 ppm) and top dose females actually lost weight (2.9 and 2.4 grams, respectively), while controls and low intermediate females gained 9.7 and 8.0 grams, respectively.

Food consumption was - as previously indicated - monitored during the initial pre-mating period only. Sporadically, significant differences were observed between control and treated groups. This was either not dose related (decreased food consumption in F_{1b} males during week 9 at ≥ 190 mg/kg; maximum deviation from control: -5.1%) or only transient (increased food consumption in F_0 top dose males and females during weeks 1 and/or 2; maximum deviation from control: +9.0%). Mean cumulative food consumption during the initial pre-mating period varied from controls in the range of -3.5 to +1.6% in males and -2.7 to +3.5% in female with no apparent dose-response relationship.

Food efficiency (g food consumed/g body weight gain) was generally comparable between controls and treated groups on a weekly basis as well during the entire initial pre-mating period.

Water consumption as determined during the first and last two weeks of the initial pre-mating periods were not affected by treatment. Since the significantly lower values in F_0 females

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat

during Weeks 9 and 10 at ≥ 190 ppm displayed no dose dependency and no comparable effect was observed in F_{1b} females, this deviation from controls is considered of incidental nature. X

Macroscopic examination of parental animals revealed no treatment related findings. The issue of a potential increase of alopecia is discussed above. Microscopical examination of the reproductive tract of control and top dose parental animals did not disclose any indication for a treatment related effect. Likewise, the histopathology of heart, liver and spleen of F_{1b} adults revealed no treatment related findings.

Organ weight analysis revealed statistically significant differences between control and treated groups of either absolute or relative (body weight adjusted) weight for brain, liver and kidney in males and for brain and adrenals in females [see Table 6.8.2/89]. Decreased of adjusted brain weights were also observed in pups killed at weaning [see Table 6.8.2/96]. Altered adjusted liver weights were also seen pups however, in contrast to the decreases seen in F_{1b} males, pups displayed a consistent increase of adjusted liver weight. The increase of absolute adrenal weights in F_0 and F_{1b} females was not paralleled by a comparable change in parental males or male and female pups.

Parental mating and gestation performance

As evident from Table 6.8.2/90 mating and gestation performance was not affected by treatment. The pregnancy rate, the median pre-coital time and the mean gestation length was comparable for all 4 mating between controls and treated groups. The incidences of males failing to induce pregnancy in either female partner or females failing to become pregnant with either male were low and displayed no dose-relationship.

Offspring toxicity X

In the entire study 1, 1, 2, 8, and 13 total litter losses were observed during lactation at 0; 50; 190; 710 and 10,000 ppm, respectively [see Table 6.8.2/91]. The incidences at 710 and 10,000 ppm were considered to be related to treatment, whereas the number of litter losses at 50 and 190 ppm are within the spontaneous range observed in control groups. A general decrease in survival at the top dose level was indicated by significant changes of litter size and cumulative pup loss [see Table 6.8.2/92]. Statistically significant differences were observed at the top dose level from week 8 onwards. If total litter losses are included, significant changes of litter size and cumulative pup loss were already seen at the top dose level at day 12 in 3 of 4

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat

offspring generations (data not shown in Table 6.8.2/92). The decreased survival rate is also evident from the lactation index (pups alive day 21/pups alive day 4 post cull x 100) [see Table 6.8.2/93]. The lactation index also indicates some effect on post-cull survival at dose levels ≥ 190 ppm.	
At birth, mean body weights from pups in treated groups were comparable to, or even slightly higher than that of control pups [see Table 6.8.2/94]. At the end of the lactation period significantly lower pup weights were observed at the top dose level in 3 out of 4 generations. Significantly lower pup weights were also observed at 190 ppm for the F_{1a} and F_{2a} generation and for the high intermediate dose level at 710 ppm for the F_{1a} generation. The toxicological relevance of these findings is unclear since these effects were not dose-dependent. The lacking dose dependency is particularly obvious in the F_{2a} generation, where body weights of 47.3, 42.0*, 46.5 and 41.6** grams were observed in pup at Day 21 at dose levels of 50; 190; 710 and 10,000 ppm, respectively.	X
The pup sex ratio was comparable between controls and treated groups throughout lactation indicating no selective pup mortality in either sex. The mean sex ratios over the 4 matings were 50.5 ± 1.9, 48.9 ± 3.2, 48.5 ± 1.1, 50.0 ± 2.4, and 48.0 ± 1.6 at birth and 50.3 ± 1.0, 49.4 ± 1.1, 49.5 ± 1.2, 51.4 ± 1.3, and 50.2 ± 2.0 at weaning at dose levels of 0; 50; 190; 710 and 10,000 ppm, respectively.	X
Pre-weaning development as assessed by the day of occurrence for startle reflex and successful pupil reflex at day 20 was not affected by treatment [see Table 6.8.2/95]. The statistically significant differences in the occurrence of the startle reflex in F_{1a} pups at 190 and 10,000 ppm were of small magnitude and well within the range observed for control pups.	
Likewise, post-weaning development as assessed in selected animals of the F_{1b} generation by the mean age of vaginal opening and preputial separation was not affected by treatment. Preputial separation was observed at post partum days 43.8 ± 2.0, 44.2 ± 1.3, 45.1 ± 2.7, 45.0 ± 1.8, and 44.2 ± 1.7 at dose levels of 0; 50; 190; 710 and 10,000 ppm, respectively. At the respective dose levels vaginal opening in females was observed at post partum days 33.5 ± 2.1, 34.5 ± 2.0, 34.6 ± 1.4, 35.0 ± 1.4, and 34.4 ± 1.7. Observed intergroup differences were minimal and/or displayed no dose-response relationship.	
<u>Terminal studies in pups</u>	
The incidences of macroscopic findings in pups killed at weaning	

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat

were comparable between all groups and did not indicate a relation to treatment. A number of significant differences of adjusted organ weights were observed in the selected male and female pups killed at weaning [see Table 6.8.2/96]. Like in adult F_0 and F_{1b} rats lower adjusted brain weights were observed at dose levels ≥ 710 ppm. Statistically significant increases of adjusted heart weights were mainly observed in male pups. Female pups were affected only in the F_{1a} generation and adult animals did not show comparable increases. A consistent increase of adjusted liver weights were observed at ≥ 190 ppm in male and female pups. In contrast, adult rat adjusted liver weights were either comparable to the controls or were lower (F_{1b} males). A statistically significant decrease of adjusted kidney weights was only observed in F_{2a} males. In absence of comparable effects in male pups at other generations, in female pups, or in adult males and females this change is considered of incidental nature.

Pup mortality at 10,000 ppm and to a lesser extent at 710 ppm, both in litters totally lost and in litters where dams reared some young to weaning, was associated in many instances with failure to gain weight or actual weight loss in the period prior to death. Deaths at 10,000 ppm included a number of pups which were sacrificed in a moribund condition particularly in the F_{1b} generation. Where it was possible to make an assessment, these dead pups frequently showed absent or minimal stomach content.

5.3 Conclusion

Administration of Flufenoxuron to rats at dietary dose levels of 0; 50; 190; 710 and 10,000 ppm throughout 2 consecutive generations involving 2 matings per parental generation resulted in a decreased parental and pup body weight gain at dose levels ≥ 190 ppm. The ability to induce and maintain gestation as well as the ability to give birth to offspring was not affected by treatment. During lactation an increased incidence of full litter losses was observed at dose levels ≥ 710 ppm. In addition an increase of post cull pup losses was noted at ≥ 190 ppm. The latter was evident by a statistically significant decrease of litter size at 10,000 ppm. A slightly lower lactation index was noted at 190 and 710 ppm whereas a more pronounced effect on the lactation index was observed at 10,000 ppm. No effects on pre- and post-weaning development of parental F_{1b} rats were noted. A number of (mainly body weight adjusted) organ weight changes were observed in weanlings at dose levels ≥ 190 ppm.

Based on the results of this study the NOAEL for systemic effects was 50 ppm for both parental animals and offspring. This is equivalent to a mean daily dose of 4.3 mg/kg bw. The NOAEL for fertility was at least 10,000 ppm (≈ 875 mg/kg

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**	
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat	
	bw/day). The reproductive performance NOAEL for males was likewise 10,000 ppm. Based on the effects on pup survival the NOAEL for female reproductive performance was 50 ppm (4.3 mg/kg bw/day). The developmental NOAEL was determined to be 50 ppm (4.3 mg/kg bw/day).	
5.3.1 LO(A)EL		
5.3.1.1 Parent males	190 ppm; decreased body weight gain	
5.3.1.2 Parent females	190 ppm; decreased body weight gain	
5.3.1.3 F1 males	190 ppm; increased post natal mortality (lower lactation index)	
5.3.1.4 F1 females	190 ppm; increased post natal mortality (lower lactation index)	
5.3.1.5 F2 males	190 ppm; increased post natal mortality (lower lactation index)	
5.3.1.6 F2 females	190 ppm; increased post natal mortality (lower lactation index)	
5.3.2 NO(A)EL		X
5.3.2.1 Parent males	50 ppm	
5.3.2.2 Parent females	50 ppm	
5.3.2.3 F1 males	50 ppm	
5.3.2.4 F1 females	50 ppm	
5.3.2.5 F2 males	50 ppm	
5.3.2.6 F2 females	50 ppm	
5.3.3 Reliability	1	X
5.3.4 Deficiencies	No	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat

Materials and Methods	**Revisions/Amendments:** 2.3. Deviations No in comparison to OECD 416 adopted in 1983 but some in comparison to the new version adopted in 2001 3.2.5. Age/weight at study initiation *About 6 weeks* male weight 100-114g and female weight 79-137g one week before the commencement of treatment 3.3.5. Concentrations add the equivalence between administered doses in ppm and mean daily doses in mg/kg bw / day 3.4.2. Body weight *Yes:* all animals were weighted weekly while females were weighted daily (weights are only reported for days 0-7-14-17-20 of gestation) 3.4.3. Food/water consumption Yes; food consumption was determined weekly during the pre-mating phases only. Water consumption was determined during the first and last two weeks of the initial pre-mating periods.
Results and discussion	Agree with the applicant's version Revisions/Amendments: 5.2. Results and Discussion **Parental toxicity** *No treatment-related mortality was observed throughout the study period. Four cases of mortality were observed. Three of them occurred during the late phase of the second gestation. This pertained one F_0 control, one low-dose (50 ppm) F_{1b} and* two *low-intermediate dose (190 ppm) F_{1b} female. The fourth death was observed in another low-intermediate dose F_{1b} female (week 34; post-weaning). In absence of a dose-response relationship none of these deaths is considered to be related to treatment.* Organ weight: *[…]* Moreover, adjusted weight of heart is significantly higher in weanling males and females F1A and in weanling males F2B, than controls, for the top dose. The higher incidence of minimal luminal dilatation of the uterus of high dose females should be reported Food efficiency was lower for high dose females **Comments:** Where do the data concerning lactation index come from? They are not reported in Doc IV…

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat

Conclusion	Parental toxicity: NOAEL males = 50 ppm NOAEL females = 710 ppm NOAEL pups = 50 ppm NOAEL fertility = 10,000 ppm
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	Give date of comments submitted
Materials and Methods	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state
Results and discussion	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Table 6.8.2/87 Test substance intake

Dose level	**50 ppm**		**190 ppm**		**710 ppm**		**10,000 ppm**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
F0 generation								
Week 1	5.6	5.6	22.3	20.9	85.0	81.1	1,202	1,218
Week 10	2.8	3.3	10.5	12.9	39.2	48.7	578	743
Week 1–10 (mean of means)	**3.8**	**4.3**	**14.3**	**16.0**	**53.6**	**61.0**	**771**	**907**
F1b generation								
Week 5	6.9	7.1	24.7	26.2	99	94	1,372	1,348
Week 16	2.8	3.8	11.4	14.7	43	55	595	769
Week 5–16 (mean of means)	**4.2**	**4.8**	**16.1**	**18.7**	**62.5**	**69.2**	**865**	**956**
Overall mean	**4.3**		**16.3**		**61.6**		**875**	

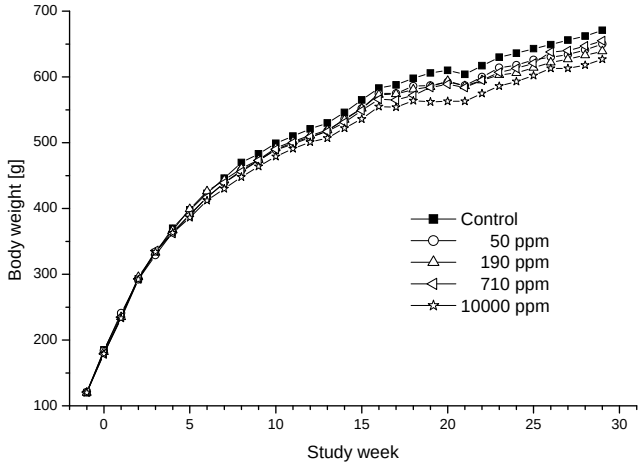
Table 6.8.2/88 Clinical findings observed in parental animals

ALOPECIA	N	0 ppm	50 ppm	190 ppm	710 ppm	10,000 ppm
F_0 males	28	0	0	0	1	(1)[a]
F_0 females	28	0	7	2	2	6
F_{1b} males	24	1	1	0	0	0
F_{1b} females	24	0	0	1	0	5

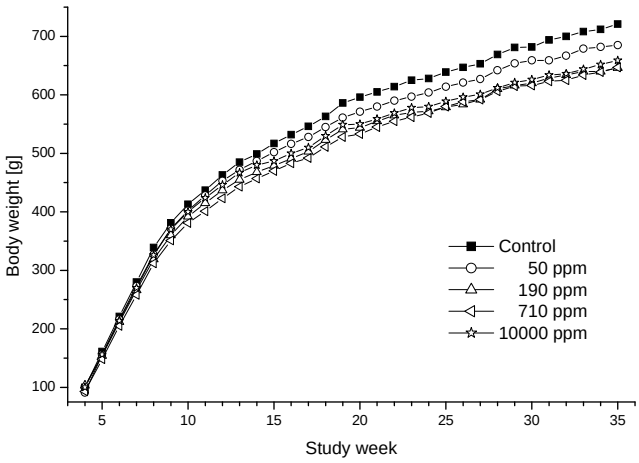
[a] Incidence could not be confirmed in individual data

Figure 6.8.2/2 Body weight development of parental rats

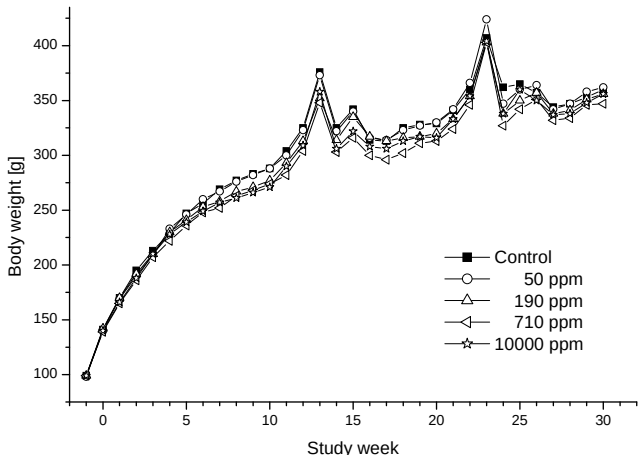
F_0 Males F_{1b} Males



F_0 Females



F_{1b} Females



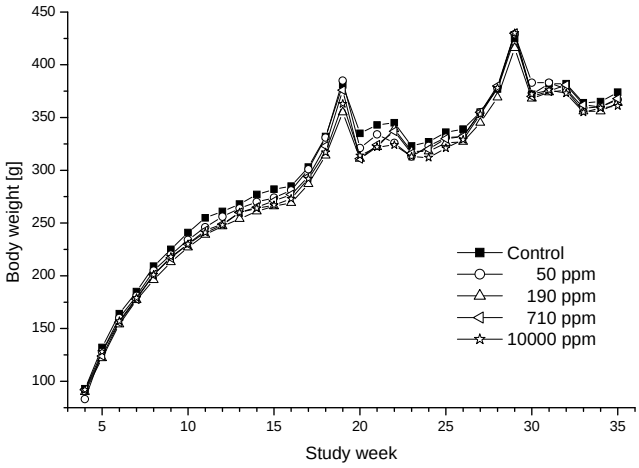


Table 6.8.2/89 Selected organ weights of parental F_0 and F_{1b} rats

	Brain		Heart		Liver		Kidney		Adrenal	
	abs.	adjusted §	abs.	adjusted	abs.	adjusted	abs.	adjusted	abs.	adjusted
Dose	[g]	[g]	[g]	[g]	[g]	[g]	[g]	[g]	[mg]	[mg]
F_0 males										
0	2.16		1.783	1.743	26.79	25.87	4.862	4.742	59.4	58.2
50	2.11		1.754	1.749	25.14	25.04	4.895	4.881	57.6	57.4
190	2.12		1.814	1.838	24.86	25.43	5.109	5.184*	60.2	60.2
710	2.11		1.758	1.750	23.89	23.71	4.940	4.916*	58.5	58.2
10,000	2.13		1.749	1.776	24.37	25.00	4.853	4.936*	56.4	57.2
F_0 females										
0	1.93	1.93	1.247	1.240	14.92	14.76	2.782	2.763	67.0	
50	1.94	1.94	1.250	1.243	14.65	14.47	2.924	2.902	71.0	
190	1.95	1.96	1.229	1.231	14.15	14.20	2.895	2.900	70.6	
710	1.88	1.89	1.238	1.249	13.95	14.22	2.824	2.857	68.3	
10,000	1.91	1.91	1.238	1.238	14.65	14.67	2.800	2.802	74.2**	
F_{1b} males										
0	2.15	2.13	1.892	1.819	28.38	26.52	4.860	4.619	58.20	57.10
50	2.09	2.09	1.875	1.847	25.68	25.16	4.944	4.870	56.10	55.70
190	2.12	2.13	1.837	1.878	23.43	24.44*	4.772	4.903	56.31	56.90
710	2.00	2.01**	1.791	1.83	23.34	24.32*	4.489	4.617	53.80	54.40
10,000	2.06	2.07**	1.884	1.904	25.33	25.77*	4.654	4.712	58.30	58.50
F_{1b} females										
0	1.95	1.94	1.301	1.293	14.58	14.32	2.772	2.738	65.3	
50	1.92	1.92	1.272	1.272	14.24	14.23	2.757	2.756	73.3	
190	1.91	1.91	1.280	1.283	14.33	14.49	2.769	2.782	73.8	
710	1.86	1.86**	1.262	1.261	13.83	13.80	2.706	2.705	69.2	
10,000	1.87	1.87**	1.315	1.322	14.08	14.25	2.582	2.605	75.3**	

§ Provided there was a significant relationship (F-test, P<0.1) between organ weight and bodyweight, organ weights were analysed by analysis of covariance, adjusting for bodyweight at sacrifice as covariate.

* $p < 0.05$; ** $p < 0.01$(Williams' test)

Table 6.8.2/90 Mating and gestation parameters

Parental generation	F_0					F_{1b}				
Dose level [ppm]	0	50	190	710	10,000	0	50	190	710	10,000
1st mating										
Mated	28	28	28	28	28	24	24	24	24	24
- non-pregnant	0	1	0	0	0	2	0	2	0	2
- no young born	0	1	1	0	1	1	2	2	1	2
Pregnancy rate[a] [%]	100.0	92.9	96.4	100.0	96.4	87.5	91.7	83.3	95.8	83.3
Median pre-coital time[c] [d]	2.0	2.0	3.0	3.0	2.0	3.0	2.0	3.5	3.0	2.5
Mean duration of gestation [d]	21.9	22.0	22.3	22.1	22.1	21.8	22.0	22.0	22.2	22.0
2nd mating										
Mated	28	28	28	28	28	24	24	24	24	24
- died/killed (pregnant)	1	0	0	0	0	0	1	1	0	0
- non-pregnant	0	1	0	0	0	2	0	2	0	2
- no young born	1	1	2	2	3	4	4	1	4	2
Pregnancy rate[a] [%]	96.3[b]	92.9	92.9	92.9	89.3	75.0	82.6[b]	87.0[b]	83.3	83.3
Median pre-coital time[c] [d]	3.0	2.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	2.0
Mean duration of gestation [d]	21.9	21.9	21.9	22.0	22.1	22.1	22.1	22.2	22.2	22.2
Failed to conceive/induce pregnancy at both mates										
- females	0	1	0	0	0	2	0	2	0	2
- males	0	1	1	0	1	1	1	1	1	1

a) Number of females mated/Number of females delivering

b) Calculated by excluding dams which died prior to parturition

c) Time by which the median female of the number of females paired had mated

Table 6.8.2/91 Litter data

Parental generation	F_0					F_{1b}				
Dose level [ppm]	0	50	190	710	10,000	0	50	190	710	10,000
1st mating										
Mated	28	28	28	28	28	24	24	24	24	24
Delivering pups	28	26	27	27	27	21	22	20	23	20
Rearing young to weaning	28	26	27	27	25	21	22	19	21	17
Total litter loss:										
- Pre-cull	0	0	0	1	0	0	0	0	1	0
- Post -cull	0	0	0	0	2	0	0	1	1	3
2nd mating										
Mated	28	28	28	28	28	24	24	24	24	24
Delivering pups	26	26	26	26	25	18	19	20	20	20
Rearing young to weaning	25	26	25	23	21	18	18	20	18	16
Total litter loss:										
- pre-cull	1	0	1	3	3	0	1	0	2	0
- post cull	0	0	0	0	1	0	0	0	0	4

Table 6.8.2/92 Litter size during lactation of F_{1a} to F_{2b} litters (total litter losses excluded!)

	At birth			Day 4 pre-cull		Day 4 post-cull	Day 8		Day 12		Day 21	
Dose [ppm]	litter size total	litter size live	pup loss [%]	litter size	cum. loss [%]	litter size	litter size	cum. loss [%]	litter size	cum. loss [%]	litter size	cum. loss [%]
F_{1a} litter												
0	13.8	13.6	1.3	13.1	4.5	7.9	7.8	1.3	7.8	1.3	7.8	1.3
50	12.6	12.6	0.0	12.2	2.5	7.7	7.7	0.5	7.7	0.5	7.7	0.5
190	13.0	12.9	0.7	12.4	4.5	7.9	7.8	0.9	7.7	1.4	7.7	1.9
710	12.3	12.2	0.6	11.8	3.8	7.8	7.7	0.9	7.7	1.4	7.6	1.9
10,000	12.4	12.4	0.3	11.8	4.7	7.4	7.2	2.5	6.8*	8.0*	6.6#	10.5*
F_{1b} litter												
0	13.2	13.2	0.5	12.8	3.1	7.6	7.5	1.5	7.5	1.5	7.5	1.5
50	14.2	13.8	2.7	13.0	8.4	7.9	7.8	0.5	7.8	0.5	7.8	1.4
190	13.4	13.2	1.5	12.8	4.2	7.9	7.5	5.0	7.4	6.5	7.3	7.5
710	13.6	13.3	2.5	12.6	7.1	8.0	7.7	2.7	7.6	4.3	7.5	5.4
10,000	13.2	12.7	3.5	12.2	6.5	8.0	7.9	1.3	7.7	3.1	7.5	5.4*
F_{2a} litter												
0	13.4	13.2	1.4	12.1	8.9	7.6	7.4	3.0	7.4	3.0	7.4	3.0
50	12.6	12.5	0.6	12.1	3.8	7.6	7.5	1.1	7.5	1.1	7.5	1.1
190	12.2	11.9	2.3	11.4	6.1	7.8	7.5	3.9	7.5	3.9	7.4	4.6
710	12.7	12.3	3.4	12.2	4.1	7.9	7.8	1.2	7.8	1.2	7.7	3.0
10,000	12.1	11.8	2.5	11.0	8.6	7.5	7.2	3.7	6.8	9.6	5.5@	26.2@
F_{2b} litter												
0	14.1	13.2	6.0	12.7	9.4	7.8	7.8	0.7	7.7	1.4	7.7	1.4
50	12.8	12.7	0.9	12.3	3.4	7.9	7.8	1.4	7.7	2.1	7.7	2.1
190	12.3	12.2	1.3	11.9	3.2	7.8	7.5	4.4	7.4	5.0	7.3	6.3
710	12.9	12.6	2.3	12.2	5.2	8.0	7.9	1.4	7.7	4.2	7.4	7.6
10,000	12.8	12.1	4.7	11.8	6.5	7.8	7.4*	5.5*	7.1	9.4*	6.3#	19.5#

* $p < 0.05$; # $p < 0.01$; @ $p < 0.001$ (Kruskal-Wallis or Fischer's exact test)

Table 6.8.2/93 Lactation indices observed in the Flufenoxuron 2-generation study

Dose level	F_{1a}	F_{1b}	F_{2a}	F_{2b}
0 ppm	98.6	98.4	96.9	98.6
50 ppm	99.5	98.5	98.8	97.9
190 ppm	98.1	92.4	90.4	93.6
710 ppm	98.1	94.5	96.4	92.4
10,000 ppm	84.1	90.3	62.3	66.2

Table 6.8.2/94 Body weight development of F_{1a} to F_{2b} pups during lactation

Litter	Dose [ppm]	Lactation Day					
		0	4 pre-cull	4 post-cull	8	12	21
F_{1a}	0	5.7	8.8	8.8	16.9	26.7	51.1
	50	5.9	8.6	8.6	16.0	25.3	48.1
	190	5.9	8.4	8.4	15.5	24.4*	46.4**
	710	5.9	8.8	8.9	16.2	24.8*	46.6**
	10,000	6.1	8.9	9.0	15.1*	24.4*	46.2**
F_{1b}	0	5.9	8.9	8.9	16.4	25.2	49.9
	50	5.4	7.9	7.9	15.0	23.5	47.0
	190	5.6	7.9	7.9	14.1	22.3	44.1
	710	5.5	7.9	7.9	14.2	22.6	44.8
	10,000	5.6	8.6	8.6	15.6	24.3	46.5
F_{2a}	0	5.5	7.8	7.8	14.3	23.4	47.2
	50	5.7	8.7	8.7	15.3	24.3	47.3
	190	5.8*	7.9	7.9	13.5	21.4	42.0*
	710	5.9**	8.7	8.7	15.5	23.8	46.5
	10,000	6.0**	8.3	8.4	14.4	22.2	41.6**
F_{2b}	0	5.6	8.3	8.3	15.5	24.7	50.1
	50	6.0	9.1	9.1	16.9	26.3	52.3
	190	6.1*	8.3	8.4	14.9	23.4	46.2
	710	6.0*	8.2	8.3	14.6	22.5	44.4
	10,000	6.2**	8.6	8.7	15.1	23.4	44.8*

* $p < 0,05$; ** $p < 0.01$ (Kruskal-Wallis and Jonkheere)

Table 6.8.2/95 Pre-weaning development of pups

Dose	Startle reflex [day post coitum]				Pupil reflex [% success at day 20 p.p.]			
[ppm]	F_{1a}	F_{1b}	F_{2a}	F_{2b}	F_{1a}	F_{1b}	F_{2a}	F_{2b}
0	34.5	34.7	35.5	35.7	100	100	100	100
50	34.6	34.9	35.3	35.2	100	100	100	100
190	35.0*	35.4	35.8	36.0	100	100	100	100
710	34.9	35.3	35.4	36.2	100	100	99	100
10,000	35.0**	34.9	35.6	35.8	100	100	99	100

* $p < 0,05$; ** $p < 0.01$ (Kruskal-Wallis and Jonkheere)

Table 6.8.2/96 Selected organ weights of F_{1a} to F_{2b} pups

	Brain		Heart		Liver		Kidney		Adrenal	
	abs.	adjusted[§]	abs.	adjusted	abs.	adjusted	abs.	adjusted	abs.	adjusted
Dose	[g]	[g]	[g]	[g]	[g]	[g]	[g]	[g]	[mg]	[mg]
F_{1a} males										
0	1.43	1.42	0.331	0.317	2.89	2.72	0.781	0.742	20.0	
50	1.42	1.42	0.32	0.315	2.84	2.79	0.797	0.785	19.5	
190	1.42	1.42	0.32	0.323	2.88	2.92**	0.784	0.793	19.4	
710	1.39	1.40	0.317	0.326	2.85	2.96**	0.754	0.778	19.2	
10,000	1.36	1.37**	0.345	0.353**	2.91	3.00**	0.740	0.762	20.2	
F_{1b} males										
0	1.4	1.38	0.326	0.314	2.74	2.59	0.788	0.753	20.6	20.1
50	1.39	1.39	0.317	0.317	2.49	2.49	0.750	0.751	20.7	20.7
190	1.36	1.37	0.307	0.315	2.51	2.61	0.722	0.745	20.7	20.9
710	1.36	1.37	0.316	0.323	2.48	2.56	0.709	0.727	19.6	19.9
10,000	1.37	1.36	0.334	0.332	2.77	2.74*	0.740	0.734	20.8	20.7
F_{2a} males										
0	1.40	1.38	0.330	0.314	2.60	2.41	0.778	0.739	19.2	18.5
50	1.38	1.37	0.313	0.302	2.55	2.42	0.735	0.707	19.5	19.1
190	1.35	1.37	0.305	0.324	2.28	2.51	0.672	0.721	18.5	19.4
710	1.35	1.34	0.341	0.338*	2.60	2.56*	0.710	0.701*	18.7	18.6
10,000	1.33	1.35	0.330	0.345**	2.54	2.73**	0.653	0.693**	17.9	18.6

Table 6.8.2/97 Selected organ weights of F_{1a} to F_{2b} pups

F_{2b} males										
0	1.41	1.41	0.340	0.337	2.63	2.60	0.754	0.746	18.9	18.7
50	1.41	1.38	0.370	0.347	2.86	2.62	0.790	0.740	20.0	19.0
190	1.39	1.39	0.351	0.348	2.69	2.66	0.743	0.737	18.8	18.7
710	1.38	1.39	0.345	0.350	2.66	2.72	0.755	0.767	19.1	19.3
10,000	1.32	1.37	0.340	0.368*	2.61	2.89**	0.707	0.766	18.1	19.2
F_{1a} females										
0	1.39	1.37	0.321	0.302	2.73	2.5	0.775	0.726	20.8	20.2
50	1.36	1.36	0.313	0.308	2.68	2.63	0.753	0.741	20.6	20.4
190	1.35	1.36	0.300	0.304	2.68	2.72**	0.744	0.753	19.5	19.7
710	1.33	1.35	0.309	0.318	2.63	2.74**	0.737	0.762	18.3	18.6
10,000	1.3	1.31**	0.325	0.339**	2.64	2.80**	0.702	0.737	19.0	19.4
F_{1b} females										
0	1.38	1.35	0.311	0.298	2.66	2.49	0.773	0.733	19.2	18.6
50	1.33	1.33	0.298	0.297	2.52	2.52	0.738	0.738	19.8	19.8
190	1.34	1.35	0.297	0.305	2.37	2.47	0.714	0.735	18.0	18.3
710	1.29	1.30**	0.303	0.311	2.36	2.46	0.701	0.723	20.5	20.8
10,000	1.29	1.29**	0.311	0.312	2.57	2.58	0.704	0.706	18.6	18.6
F_{2a} females										
0	1.35	1.33	0.322	0.305	2.53	2.35	0.727	0.688	18.7	18.0
50	1.34	1.32	0.316	0.301	2.57	2.41	0.722	0.687	17.9	17.4
190	1.31	1.34	0.290	0.306	2.15	2.34	0.667	0.707	18.7	19.4
710	1.3	1.30	0.321	0.319	2.41	2.39	0.703	0.699	17.8	17.8
10,000	1.24	1.28*	0.301	0.322	2.34	2.59**	0.641	0.692	17.5	18.4
F_{2b} females										
0	1.38	1.35	0.35	0.329	2.73	2.51	0.777	0.732	19.4	18.4
50	1.36	1.33	0.335	0.315	2.75	2.55	0.781	0.740	18.5	17.7
190	1.34	1.34	0.337	0.342	2.57	2.62	0.718	0.728	19.2	19.5
710	1.29	1.32	0.315	0.335	2.39	2.61	0.687	0.732	18.1	19.0
10,000	1.26	1.29**	0.321	0.341	2.46	2.67*	0.703	0.746	17.9	18.8

§ Provided there was a significant relationship (F-test, P<0.1) between organ weight and bodyweight, organ weights were analysed by analysis of covariance, adjusting for bodyweight at sacrifice as covariate.

* $p < 0.05$; ** $p < 0.01$ (Williams' test)

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat

			Official use only
		1 REFERENCE 2	
1.1	**Reference**	**4) XXXX** Dietary investigative study in pregnant rats rearing young to weaning. Compound: WL 115110 XXXX unpublished XXXX	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	As a range finding/feasibility study this study was not intended to comply with an official guideline.	
2.2	**GLP**	No (The study is considered to be scientifically valid. Considering the limited scope of the experiment the study procedures are adequately described and the results are presented appropriately)	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch: XXXX	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	As given in section 2	
3.1.2.2	Purity	97.4%	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	CrL: CD® /(SD) BR VAF/Plus Sprague Dawley	
3.2.3	Source	XXXX	
3.2.4	Sex	Female	
3.2.5	Age/weight at	8 to 10 weeks old	X

Section A6.8.2		**Multigeneration Reproduction Toxicity Study**	
BPD Annex Point IIA, VI.6.8.2		6.8.2 Multigeneration study - Rat	
	study initiation		
3.2.6	Number of animals per group	15	
3.2.7	Mating	Mated at the breeding station; animals were delivered to the lab on gestational day 2	
3.2.8	Duration of mating	No information available	
3.2.9	Deviations from standard protocol	Not applicable	
3.2.10	Control animals	No	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Animal assignment to dosage groups	Not applicable	
3.3.2	Duration of exposure before mating	Not applicable	
3.3.3	Duration of exposure in general P, F1, F2 males, females	From day 3 of gestation till weaning	
3.3.4	Type	In food	
3.3.5	Concentration	20,000 ppm	
3.3.6	Vehicle	None	
3.3.7	Concentration in vehicle	Not applicable	
3.3.8	Total volume applied	Not applicable	
3.3.9	Controls	Not applicable	
3.4	**Examinations**		
3.4.1	Clinical signs	Yes	X
3.4.2	Body weight	Yes	X
3.4.3	Food/water consumption	Yes	X
3.4.4	Oestrus cycle	Not applicable	
3.4.5	Sperm parameters	Not applicable	

Section A6.8.2		**Multigeneration Reproduction Toxicity Study**	
BPD Annex Point IIA, VI.6.8.2		6.8.2 Multigeneration study - Rat	
3.4.6	Offspring	Counted, sexed and examined for external and internal abnormalities; determination of body weights	
3.4.7	Organ weights P and F1	No	
3.4.8	Histopathology P and F1	No	
3.4.9	Histopathology F1 not selected for mating, F2	No	
3.5	**Further remarks**	None	X
		4 RESULTS AND DISCUSSION.	
4.1	**Effects**		
4.1.1	Parent males	See 5.2	
4.1.2	Parent females		
4.1.3	F1 males	See 5.2	
4.1.4	F1 females		
4.1.5	F2 males	Not applicable	
4.1.6	F2 females	Not applicable	
4.2	**Other**	None	

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The purpose of this study was to investigate whether or not the adverse effects on pup survival and growth could be reproduced when the test substance was administered during gestation and lactation only. Therefore, a group of 15 time-mated, 8 to 10 week old female Crl: CD® (SD) BR VAF/Plus rats (XXXX) were administered Flufenoxuron at a dietary concentration of 20,0000 ppm from day 3 of pregnancy through to weaning of their offspring. No control animals were employed in this study. The dose level of 20,000 ppm was based on results obtained in a range finding study carried out prior to the 2-generation study described above. (This range finding study is part of the report of the 2-generation study (i.e. Addendum 4) [see IIIA 6.8.2 XXXX]). X

During the study maternal bodyweight and food consumption were regularly measured. Offspring were examined daily and weighed regularly through the pre-weaning period. On day 21 or 22 post partum, parents and pups were sacrificed and examined externally and internally for abnormalities. Sex of the pups was confirmed by gonadal inspection. The uterus of each female which gave birth was visually inspected for implantation sites and the numbers of sites were recorded.

5.2 Results and discussion

No mortality or treatment-related clinical signs were observed. Body weight development and food consumption were within the range expected for pregnant and lactation rats of this strain and age.

The mean gestation length of 21.9 days was within the expected range. The live birth index (no. live pups/no. pups born x 100), viability index (pups alive day 4/pups alive at birth x 100) and lactation index (pups alive day 21/pups alive day 4 x 100) were 98.2, 98.2 and 98.8%, respectively and thus not considered to be affected by treatment. Pup weights were comparable to historical control values throughout lactation.

Neither maternal nor offspring necropsy revealed any treatment related findings.

5.3 Conclusion

Dietary administration of Flufenoxuron at a level of 20,000 ppm to rats during gestation and lactation did not result in any adverse findings. Therefore, with the chosen study design, it was not possible to reproduce the adverse effects on pup survival and pup body weight development observed in the 2-generation study. X

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**	
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat	
5.3.1 LO(A)EL		
5.3.1.1 Parent males		
5.3.1.2 Parent females		
5.3.1.3 F1 males	Not applicable	
5.3.1.4 F1 females		
5.3.1.5 F2 males		
5.3.1.6 F2 females		
5.3.2 NO(A)EL		
5.3.2.1 Parent males		
5.3.2.2 Parent females		
5.3.2.3 F1 males	Not applicable	
5.3.2.4 F1 females		
5.3.2.5 F2 males		
5.3.2.6 F2 females		
5.3.3 Reliability	2	X
5.3.4 Deficiencies	No	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 3.2.5. Age/weight at study initiation *8 to 10 weeks old /* weight on arrival 166-200g 3.4.1 Clinical signs *Yes*, daily 3.4.2 Body weight *Yes,* on days 2-3-7-10-14-17-20 of pregnancy and on days 0-7-14-21 post partum 3.4.3 Food/water consumption *Yes*, daily 3.5. Further remarks terminal studies, on days 21 or 22 post partum, on parents and pups
Results and discussion	**Revisions/Amendments:** 5.1. No addendum 4 provided in the study report In the introduction of this study report, authors refer to a previous study realized with exposure to Flufenoxuron at 10000, 20000 et 50000 ppm: this study should have been provided.

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat
Conclusion	**Revisions/Amendments:** Change the conclusion accordingly: *Dietary administration of Flufenoxuron at a level of 20,000 ppm to rats during gestation and lactation did not result in any adverse findings.* Therefore, a short dietary exposure to time-mated females is inappropriate to elucidate the causative mechanism(s) for the effects on pups' ability to thrive seen in previous study using a prolonged maternal exposure period.
Reliability	3
Acceptability	Acceptable: despite major deficiencies (no guideline and not GLP), scientific informations given in this study are relevant to assess and better understand toxicity mechanisms of flufenoxuron
Remarks	No equivalence reported between administered dose (20000 ppm) and mean daily doses in mg/kg bw/day The study, mentioned in the introduction, should have been be provided.

	COMMENTS FROM ...
Date	Give date of comments submitted
Materials and Methods	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state
Results and discussion	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat

			Official use only
		1 REFERENCE 3	
1.1	**Reference**	**5) XXXX** WL115110: A cross-fostering study, supplementary to a previous two generation rat reproduction study XXXX unpublished XXXX	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	There is no official, international accepted guideline for cross-fostering studies	
2.2	**GLP**	Yes (laboratory certified by the Department of Health of the Government of the United Kingdom, United Kingdom)	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	Batch: XXXX	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	As given in section 2	
3.1.2.2	Purity	97.4%	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	CrL: CD® /(SD) BR VAF/Plus Sprague Dawley	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at	6 weeks	X

Section A6.8.2		**Multigeneration Reproduction Toxicity Study**	
BPD Annex Point IIA, VI.6.8.2		6.8.2 Multigeneration study - Rat	
	study initiation		
3.2.6	Number of animals per group	A total of 50 male and 100 female	
3.2.7	Mating	After 10 weeks of treatment	
3.2.8	Duration of mating	2 weeks; in case of failure to mate within 7 days, a previously successful male replaced the unsuccessful one.	
3.2.9	Deviations from standard protocol	Not applicable	
3.2.10	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Animal assignment to dosage groups	Random assignment	
3.3.2	Duration of exposure before mating	10 weeks	
3.3.3	Duration of exposure in general P, F1, F2 males, females	Till parturation, then control diet to day 21 post partum	
		Oral	
3.3.4	Type	In food	
3.3.5	Concentration	20,000 ppm	
3.3.6	Vehicle	None	
3.3.7	Concentration in vehicle	Not applicable	
3.3.8	Total volume applied	Not applicable	
3.3.9	Controls	Basal diet	
3.4	**Examinations**		
3.4.1	Clinical signs	Yes	
3.4.2	Body weight	Yes	X
3.4.3	Food/water consumption	Yes	
3.4.4	Oestrus cycle	Not applicable	
3.4.5	Sperm parameters	Not applicable	

Section A6.8.2		**Multigeneration Reproduction Toxicity Study**	
BPD Annex Point IIA, VI.6.8.2		6.8.2 Multigeneration study - Rat	
3.4.6	Offspring	Counted, sexed and examined for external and internal abnormalities; determination of body weights at post natal days 2, 4, 8, 12, 16, 21	
3.4.7	Organ weights P and F1	No	
3.4.8	Histopathology P and F1	No	
3.4.9	Histopathology F1 not selected for mating, F2	No	
3.5	**Further remarks**	A reciprocal cross-fostering of 26 litters was performed between control and treated dams, i.e. control dams (CD) reared treated pups (TP) from treated dams (TD) and vice versa. Fifteen control and 5 treated dams reared their offspring until weaning (CD/CP and TD/TP) without cross-fostering. Additional 5 control and 12 treated non cross-fostered dams were used to obtain milk and fat samples on days 1, 7, 14, and 21 post partum.	
		4 RESULTS AND DISCUSSION.	
4.1	**Effects**		
4.1.1	Parent males	See 5.2	
4.1.2	Parent females		
4.1.3	F1 males	See 5.2	
4.1.4	F1 females		
4.1.5	F2 males	Not applicable	
4.1.6	F2 females	Not applicable	
4.2	**Other**	None	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In order to investigate the reasons for the increased post partum mortality of pups in the 2-generation study [see Annex II 5.6.1/1 FX-430-001 James P et al. 1990] at dose levels ≥ 190 ppm a cross-fostering study was conducted employing 50 male and 100 female Sprague-Dawley Crl: CD® (SD) BR VAF/Plus rats (XXXX). The rats arrived at the laboratory at an age of four weeks and were acclimatized to laboratory conditions for 11 days. On day 4 of the acclimatization period the rats were randomized into two groups (control and treated) by a computerized method (weight stratification). At an age of 6	X

Section A6.8.2 **Multigeneration Reproduction Toxicity Study**

BPD Annex Point IIA, VI.6.8.2 6.8.2 Multigeneration study - Rat

weeks Flufenoxuron was administered to the treatment group (50 females) at a dietary dose level of 20,000 ppm for 10 weeks. After the 10 week pre-mating period the rats were mated for up to 2 weeks on a 'one male to two females' ratio. Females non-pregnant after 1 week were mated with a previously successful male during the second week. The treated females continued to receive formulated diet until parturition.

After parturition, treated females were fed control diet throughout the lactation period until study termination in order to avoid any direct exposure of pups to Flufenoxuron via the diet. Control females (50) and two groups of each 25 males were administered control diet throughout the study with the exception that the males intended to mate with the treated females were also fed medicated diet one week before mating in order to become accustomed to the treated diet to which they were exposed during mating.

As soon as possible after parturition, the young were counted, individually identified, sexed, weighed and examined for external abnormalities. Thereafter, the litters were culled to a standard litter size of 8 pups consisting - wherever possible - of 4 male and 4 female pups using computer generated random number selection for each sex. Culled pups were subjected to a macroscopic post mortem examination and then frozen.

A reciprocal cross-fostering of 26 litters was performed between control and treated dams, i.e. control dams (CD) reared treated pups (TP) from treated dams (TD) and vice versa. Where practical, cross-fostering 'pairs' were those with a similar litter size prior to the cull in order to take into account any influences of litter size during the pre-natal phase. Additionally, only those litters born within one day of each other were cross-fostered, to prevent possible rejection by the fostering dam. As obvious from the selection criteria above the cross-fostering procedure was not based on a random selection procedure.

Fifteen control and 5 treated dams reared their offspring until weaning (CD/CP and TD/TP) without cross-fostering. Additional 5 control and 12 treated non cross-fostered dams were used to obtain milk and fat samples on days 1, 7, 14, and 21 post partum (1 control and 3 treated dams per occasion) for analysis of residual Flufenoxuron levels in milk and fat.

Section A6.8.2 **Multigeneration Reproduction Toxicity Study**

BPD Annex Point IIA, VI.6.8.2 6.8.2 Multigeneration study - Rat

The study design is visualized below:

10-wk pre-mating		3-wk lactation
2-wk mating		
3-wk gestation		Control diet
		TP (5 litters)
Formulated diet samples	TD	TP (12 litters) → milk and fat
		CP (26 litters) → cross-fostered
		CP (15 litters)
Control diet samples	CD	CP (5 litters) → milk and fat
		TP (26 litters) → cross-fostered

All adult rats and pups were observed for mortality and abnormal clinical signs on a daily basis. Body weights of adult animals were determined on a weekly basis throughout the study period. During mating and up to parturition, body weights of females were determined daily and reported for days 0, 7, 14, 17 and 20 of gestation. Littering dams were weighed on days 0, 7, 14, and 21 post partum (p.p.). Pup weights were determined at birth and days 2, 4, 8, 12, 16, and 21 p.p. Determination of food consumption was performed on a weekly basis during the premating and lactation period. No measurements were taken during mating and gestation. Water consumption was determined on a daily basis for females during the initial and final two weeks of the gestation period.

The dams selected for milk and fat sampling were administered oxytocin by i.p. injection approximately 30 minutes before milking. After milking, the dams were sacrificed and abdominal fat was collected. Milk and fat samples were stored frozen until analysis. For this the samples were extracted with organic solvents. The content of Flufenoxuron was determined by HPLC using optical detection.

At study termination (day 21 p.p.) all (remaining) pups and parental animals were killed and examined for external and internal abnormalities. The uterus of each female which gave birth was visually inspected for implantation sites and the number of sites was recorded. The uteri of apparently non-pregnant females were examined for evidence of implantation using a modified Salewski technique. For males which failed to induce

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**		
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat		
	pregnancy in either female partner, testes and epididymides were weighed and preserved.		
5.2	**Results and discussion**	As known from the chronic toxicity/oncogenicity studies Flufenoxuron was stable in rodent diets for at least 2 weeks at room temperature in the dark. Therefore the stability of the test substance was not determined in this study. Accordingly, formulated diets were prepared weekly and fed for no longer than 2 weeks. The test-substance concentration was determined in 3 preparations (weeks 1, 2, and 14) and revealed a mean concentration of 19,900 ± 100 ppm. Maternal data No treatment-related clinical signs were observed throughout the study. One case of mortality was observed in a control female (#9, non cross-fostered (CD/CP)). This dam was killed at day 9 p.p. because of a hard abdominal swelling. This was due to three dead pups within the uterus. Her litter was killed, too. Body weight development was slightly impaired in treated females during the premating period. Mean body weights were decreased by 3.7% (310 vs. 322 g in controls; $p < 0.05$ (ANOVA followed by Student's ‘t' test used for all statistical tests except litter weight), whereas mean body weight gain was decreased by 7.8% (154 vs. 167 g; $p < 0.01$). There was no adverse effect on body weight development during gestation and lactation. In fact, body weight gain of treated females was higher than that of control females at the majority of determinations. This was statistically significant on gestational days 7, 14, and 17. Food consumption of control and treated females was essentially identical during the pre-mating and lactation period. Based on the slightly lower body weight gain, food efficiency (g food consumed per g body weight gain) was marginally lower in the treated group (mean week 1 - 10: 9.2 vs. 8.4 g). Based on the food intake, the average daily coumpound intake for the pre-mating period was 1,633 mg/kg bw. (ranging from 2,130 mg/kg body weight on week 1 to 1,304 mg/kg bw on week 10). Like food consumption, water consumption was essentially identical between control and treated animals. At necropsy, no treatment-related findings were observed in dams. The most common findings were subpleural foci in the lung and enlargement of cervical lymph nodes. These findings were evenly distributed between the two groups. Reproductive data	X

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat

A total of 46 control (92%) and 43 treated (86%) females were pregnant. The median precoital time was 3 days for control and treated females considering the complete 2 week mating period. (Please note: Table 9 of the report does only give data for the first mate, i.e. data from dams which successfully mated within the first week. If one considers only the first mate the median precoital time was 2 days for both groups). Gestation length was 22.0 ± 0.6 days in control and 21.9 ± 0.5 days in treated females.

Litter data

Litter data at birth are given in Table 6.8.2/98 for the two groups (control and treated) and broken up into subgroups. There were no treatment-related effects on the number of implantations, pre-natal loss, number of dead or live pups per dam, mean pup weight or sex-ratio. There were no biologically meaningful differences of these parameters after splitting in sub-groups.

Pup data

Survival of pups as assessed by the viability and lactation indices were not affected by treatment [see Table 6.8.2/99]. Pup body weight development was comparable between all sub-groups.

Determination of Flufenoxuron in fat and milk

Determination of the test substance in milk and fat revealed a rapid decline of Flufenoxuron levels in both milk and fat. The decline was faster in milk than in fat as indicated by the depletion half-life times of 2.3 and 7.6 days, respectively [see Table 6.8.2/100.

5.3 Conclusion

In a cross-fostering study, administration of Flufenoxuron to female rats at a dietary dose level of 20,000 ppm throughout a 10 week pre-treatment period, a (up to) 2 week mating period and gestation resulted in a slight, but statistically significant decrease of body weight and body weight gain when compared to controls. Reproductive performance was not affected by treatment. Compared to the 2-generation study there was no increased mortality of pups in any of the groups, i.e. in treated females rearing own or control litters or control females rearing own or treated litters under the conditions of this study. The determination of Flufenoxuron levels in milk and fat of treated females revealed a rapid decrease during lactation after cessation of treatment at birth.

Since neither treatment during gestation and lactation alone (see range finding study above; see IIIA 6.8.2/4 XXXX) nor administration of Flufenoxuron throughout a 10 week pre-mating period and gestation resulted in decreased pup survival, a continued exposure to Flufenoxuron throughout pre-mating,

Section A6.8.2		**Multigeneration Reproduction Toxicity Study**	
BPD Annex Point IIA, VI.6.8.2		6.8.2 Multigeneration study - Rat	
		mating, gestation and lactation is apparently required to cause adverse effects on pup survival and body weight development observed in the 2-generation study.	
5.3.1	LO(A)EL	Not applicable	
5.3.1.1	Parent males		
5.3.1.2	Parent females		
5.3.1.3	F1 males		
5.3.1.4	F1 females		
5.3.1.5	F2 males		
5.3.1.6	F2 females		
5.3.2.	NO(A)EL	Not applicable	
5.3.2.1	Parent males		
5.3.2.2	Parent females		
5.3.2.3	F1 males		
5.3.2.4	F1 females		
5.3.2.5	F2 males		
5.3.2.6	F2 females		
5.3.3	Reliability	1	X
5.3.4.	Deficiencies	No	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat
Materials and Methods	**Revisions/Amendments:** 3.2.5 Age/weight at study initiation: precise the weight at study initiation 3.4.2. Body weight *Yes,* at the start of the study, then at weekly intervals and finally on the day of sacrifice
Results and discussion	**Revisions/Amendments:** 5.1. Materials and methods *In order to investigate the reasons for the increased post partum mortality of pups in the 2-generation study [see Annex II 5.6.1/1 FX-430-001 James P et al. 1990] at dose levels* ≥ 710 ppm *[…]* 5.2. Results and discussion Maternal data *No treatment-related clinical signs were observed throughout the study*. However, alopecia was noted in 11 females.*[…]*
Conclusion	Agree with the applicant's version
Reliability	2
Acceptability	Acceptable
Remarks	Based on the presence of flufenoxuron in the milk produced by the dams treated in this study and effects on pup survival and their development during lactation observed in the 2-generation study, a classification R64 "May cause harm to breastfed babies" is proposed.
	COMMENTS FROM ...
Date	Give date of comments submitted
Materials and Methods	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state
Results and discussion	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Table 6.8.2/98 Cross-fostering study: Litter parameters at birth

Observation	Group					
	Control			Treated		
No. of dams delivering	46			43		
Implantations per dam	15.0			14.6		
Pre-natal loss:[a]						
- dams with 1 or less	24			21		
- dams with 2 or more	13			8		
Pups born per dam	14.3			14.8		
Live pups per dam	14.2			14.7		
Pup loss:						
- dams with 1 or less	45			43		
- dams with 2 or more	1			0		
Live birth index[b]	99.2			99.4		
Litter weight [g]	88.2			92.3		
Mean pup weight [g]	6.1			6.2		
No of males [%]	49.1			49.9		
	all CD/CP[c,d]	CD/CP	CD/TP	TD/CP	TD/TP	all TD/TP[d]
No. of dams delivering	20	15	26	26	5	16
Pups born per dam	13.4	12.5	15.0	14.6	10.0	15.2
Live pups per dam	13.2	12.2	15.0	14.5	9.8	15.2
Pup loss:						
- dams with 1 or less	19	14	26	26	5	12
- dams with 2 or more	1	1	0	0	0	0
Live birth Index[b]	98.5	97.9	99.7	98.0	99.6	99.2
Litter weight [g]	88.2	84.9	90.8	91.4	76.8	94.8
Mean pup weight [g]	6.3	6.6	6.2	6.3	6.6	6.0
No of males [%]	53.9	51.4	46.8	48.7	40.8	49.4

[a] Does not include dams which were selected for milk and fat sampling and females which delivered more pups than implantation sites were observed

[b] (Number of pups born alive/Number of pups born) x 100; value determined before cross fostering

[c] Key: C = control, T = treated, D = dam, P = pup

[d] Including dams selected for milk and fat sampling

Table 6.8.2/99 Cross-fostering study: Pup survival and body weight development

Parameter	Group					
	all CD/CP[a,b]	CD/CP	CD/TP	TD/CP	TD/TP	all TD/TP[b]
Viability Index[c]	NA[b]	95.4	97.6	99.5	96.3	NA[b]
Lactation Index[d]	NA	93.2	100.0	100.0	96.2	NA
Pup weight [g]						
- day 0	6.6	6.5	6.2	6.3	6.6	6.0
- day 2	8.1	8.3	8.2	8.2	8.7	7.7
- day 4	11.4	11.7	11.4	11.6	12.1	10.7
- day 8	20.3	20.4	20.2	20.3	21.3	19.0
- day 12	30.6	30.7	30.3	30.7	32.0	29.5
- day 16	42.2	41.8	41.7	41.8	42.4	40.5
- day 21	61.6	61.5	60.1	60.5	62.7	59.8

[a] Key: C = control, T = treated, D = dam, P = pup; NA: Not applicable

[b] Including dams selected for milk and fat sampling; for calculation of lactation indices and body weights only dams and litters were considered which were still alive at the respective day

[c] (No. of pups alive at day 4/No. of pups alive at day 0 after culling and (if applicable) cross fostering) x 100; Mean determined on litter basis

[d] (No. of pups alive at day 21/No. of pups alive at day 4) x 100; Mean determined on litter basis

Table 6.8.2/100 Concentration of Flufenoxuron in milk and fat

Animal Number	Day p.p.	Concentration in Fat [ppm]		Concentration in Milk [ppm]	
		Value	Mean ± SD	Value	Mean ± SD
Control Group					
68	1	ND		NS	
71	1	ND		NS	
96	7	ND		1.58	
99	14	ND		ND	
60	21	ND		U	
Treated Group					
126		896		884	
133	1	1,060	973 ± 82	209	450 ± 377
147		963		256	
108		765		102	
117	7	549	781 ± 240	68.0	91.3 ± 20.2
146		1,030		104	
122		338		11.4	
127	14	286	270 ± 77	14.2	9.4 ± 6.1
128		187		2.59	
105		49.7		9.54	
116	21	77.5	48.5 ± 29.6	U	
118		18.3		U	

ND: Not Detected (below limit of detection (< 1.5 ppm in milk, < 3.0 ppm in fat))

NS: No sample available

U: Fate of sample unknown (not indicated in report)

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat

			Official use only
		1 REFERENCE 4	
1.1	**Reference**	**6) XXXX** WL115110: A CKA embryotoxicity study in rats XXXX unpublished XXXX	
1.2	**Data protection**	No	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	No data protection claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	As a range finding/feasibility study this study was not intended to comply with an official guideline.	
2.2	**GLP**	No	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch: XXXX	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	As given in section 2	
3.1.2.2	Purity	99%	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Fisher F344	
3.2.3	Source	XXXX	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Approx. 10 weeks at mating	X
3.2.6	Number of animals per group	15/dose group	

Section A6.8.2		**Multigeneration Reproduction Toxicity Study**	
BPD Annex Point IIA, VI.6.8.2		6.8.2 Multigeneration study - Rat	
3.2.7	Mating	Mating was performed on a 4:1 female:male ratio on a total of 68 females. Each morning vaginal smears were performed. If sperm was present it was presumed that mating took place. Mating was continued until about 15 presumably pregnant females/group were obtained.	
3.2.8	Duration of mating	Mating was continued until about 15 presumably pregnant females/group were obtained.	
3.2.9	Deviations from standard protocol	Not applicable	
3.2.10	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Animal assignment to dosage groups	Random assignment	
3.3.2	Duration of exposure before mating	Not applicable	
3.3.3	Duration of exposure in general P, F1, F2 males, females	Days 8 to days 17 of gestation	
		Oral	
3.3.4	Type	By gavage	
3.3.5	Concentration	0. 10 and 1000 mg/kg	
3.3.6	Vehicle	Corn oil	
3.3.7	Concentration in vehicle	2.5 and 250 mg/ml	X
3.3.8	Total volume applied	4 ml/kg	
3.3.9	Controls	Corn oil	
3.4	**Examinations**		
3.4.1	Clinical signs	No	X
3.4.2	Body weight	No	X
3.4.3	Food/water consumption	No	
3.4.4	Oestrus cycle	No	
3.4.5	Sperm parameters	No	

Section A6.8.2		**Multigeneration Reproduction Toxicity Study**	
BPD Annex Point IIA, VI.6.8.2		6.8.2 Multigeneration study - Rat	
3.4.6	Offspring	At birth the number of live and dead pups as well as the total weight of the live litter was noted. Each pup was examined for abnormalities.	
3.4.7	Organ weights P and F1	No	
3.4.8	Histopathology P and F1	No	
3.4.9	Histopathology F1 not selected for mating, F2	No	
3.5	**Further remarks**	On the fifth day post partum, the number of surviving pups, the total litter weight, and the dam body weight were recorded again. The dam and pups were then culled and discarded.	X
		4 RESULTS AND DISCUSSION.	
4.1	**Effects**		
4.1.1	Parent males	See 5.2	
4.1.2	Parent females		
4.1.3	F1 males	See 5.2	
4.1.4	F1 females		
4.1.5	F2 males	Not applicable	
4.1.6	F2 females	Not applicable	
4.2	**Other**	None	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The CKA test is an assay based on the method of Chernoff and Kavlock (Chernoff, N. and Kavlock, R. J., A potential in vivo screen for the determination of teratogenic effects in mammals, Teratology 21, 33A, 1981), in which pregnant rodents are exposed to sub-toxic doses of a test substance and the survival and growth of the resultant litters observed. Groups of at least 15 (presumably) pregnant Fisher F344 rats (XXXX) were administered the test substance at a daily dose of 0; 10 and 1,000 mg/kg bw by oral gavage during days 8 to 17 of gestation. The day when sperm was observed in the vaginal lavage fluid was considered to be day 1 of gestation. Corn oil served as vehicle and the dosing volume was 4 ml/kg bw. Dams were weighed daily during the treatment period, on gestational day 21 and at termination on lactational day 5. When a litter was	

Section A6.8.2		**Multigeneration Reproduction Toxicity Study**	
BPD Annex Point IIA, VI.6.8.2		6.8.2 Multigeneration study - Rat	
		born, the day number, number of live pups, the total weight of the live litter and number of still-born pups was noted, and each pup was examined for abnormalities. On the fifth day post partum, the number of surviving pups, the total litter weight, and the dam body weight were recorded again. The dam and pups were then culled and discarded.	
5.2	**Results and discussion**	Seventeen, 13 and 14 female control, low (10 mg/kg bw) and high dose females (1,000 mg/kg bw), respectively were pregnant and delivered offspring. There were no significant effects on maternal body weight, on number and weight of pups born as well as pup body weight development. There was no indication of embryotoxicity. The only significant finding was that four of the high dose dams failed to lactate properly. Two of these dams showed no signs of milk production and their litters died. The other two had reduced milk production; some of the pups from their litters died and the others failed to gain weight properly. In these cases the mammary development was visibly reduced and there was less than average or no milk visible in the stomachs of the neonates. Since reduced lactation was a rare observation in the laboratory, this finding was considered to suggest a relation to treatment. These effects seem to resemble those seen in the two generation study [see Annex IIIA 6.8.2/1 XXXX] and seem to contradict the results of the above described investigative [see Annex IIIA 6.8.2/4 XXXX] and cross-fostering [see Annex IIIA 6.8.2/5 XXXX] studies. However, when interpreting the results of this study one has to consider that the route of administration (gavage) is different from all other studies in this section (dietary administration). Furthermore, the vehicle (corn oil) used in this study probably influenced the extent and kinetics of absorption. Both factors probably influenced the systemic uptake and thus the pharmacodynamics of Flufenoxuron. Finally, this study was conducted without GLP and reporting is rudimentary. Therefore, a final assessment of this study is not possible.	
5.3	**Conclusion**	**Administration of Flufenoxuron to pregnant rats at dose levels of 0; 10 and 1,000 mg/kg bw by oral gavage during days 8 to 17 of gestation in a screening assay did not result in maternal or developmental toxicity. Four out of 14 high dose dams had difficulties to lactate properly which resulted in the complete loss of 2 litters and increased pup mortality and impaired body weight development in the two other litters. Due to substantial differences in study design (route of administration and vehicle used) and its rudimentary reporting, a final assessment of this study is not possible.**	X

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat
5.3.1 LO(A)EL	
5.3.1.1 Parent males	
5.3.1.2 Parent females	
5.3.1.3 F1 males	Not applicable
5.3.1.4 F1 females	
5.3.1.5 F2 males	
5.3.1.6 F2 females	
5.3.2. NO(A)EL	
5.3.2.1 Parent males	
5.3.2.2 Parent females	
5.3.2.3 F1 males	Not applicable
5.3.2.4 F1 females	
5.3.2.5 F2 males	
5.3.2.6 F2 females	
5.3.3 Reliability	4
5.3.4. Deficiencies	See 5.2

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 3.2.5. Age/weight at study initiation: Precise weight at study initiation 3.3.7. Concentration in vehicle: 2.5 and 250 mg/ml: where do these values come from? 3.4.1 Clinical signs Yes, all animals were observed at least once each day for signs of ill health or abnormal behavior or any abnormalities 3.4.2 Body weight Yes, dam weight on days 1-21 and at termination 3.5. Further remarks Number of pregnant females and mean gestation period were also determined. *On the fifth day post partum, the number of surviving pups, the total litter weight, and the dam body weight were recorded again. The dam and pups were then culled and discarded.*
Results and discussion	Agree with the applicant's version
Conclusion	Moderate the conclusion " Due to substantial differences in study design (route of administration and vehicle used) and its rudimentary reporting, a final assessment of this study is not possible." Results obtained were in agreement with the two generation study (absence or minimal stomach content in some dead pups) [see Annex IIIA 6.8.2/1 FX-430-001 James P et al. 1990] realized accordingly OECD 416 and GPL. So these results did not put in evidence teratogenic or reproduction toxicity effects but they may give us relevant informations about one of the effects of flufenoxuron: perturbation of the mammary development and lactation process, which is also important.
Reliability	4
Acceptability	Not acceptable but the results may give us information on effects of Flufenoxuron in rodents.
Remarks	
	COMMENTS FROM ...
Date	Give date of comments submitted
Materials and Methods	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state
Results and discussion	Discuss if deviating from view of rapporteur member state

Section A6.8.2	**Multigeneration Reproduction Toxicity Study**
BPD Annex Point IIA, VI.6.8.2	6.8.2 Multigeneration study - Rat
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Section A6.9	**Delayed Neurotoxicity**
BPD Annex Point IIIA, VI.1	6.9 Acute

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [X]	**Technically not feasible [ ] Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	No studies on acute neurotoxicity have been conducted as, Available studies conducted with Flufenoxuron, including a 28-day oral feed study in rats, do not indicate any neurotoxicity concern [see XXXX].	X
Undertaking of intended data submission [ ]	Not applicable	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Evaluation of applicant's justification	Need to be deleted as a subacute neurotoxicity study is provided in the following pages.
Conclusion	-
Remarks	-

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*

Remarks

Section A6.9	**Delayed Neurotoxicity**
BPD Annex Point IIIA, VI.1	6.9 Subacute

			Official use only
		1 REFERENCE	
1.1		**1) XXXX** BAS 307 I - Subacute neurotoxicity study in Wistar rats; Administration in the diet for 4 weeks XXXX. unpublished XXXX	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	XXXX	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s./b.p. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD 424	
2.2	**GLP**	Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Batch: XXXX	
3.1.2	Specification	As given in section 2	
3.1.2.1	Description	As given in section 2	
3.1.2.2	Purity	99.2%	
3.1.2.3	Stability	Stable	

Section A6.9	**Delayed Neurotoxicity**
BPD Annex Point IIIA, VI.1	6.9 Subacute

3.2	**Reference Substance (positive control)**	Acrylamide Several positive control studies were performed during the decade before the conduct of the study at hand demonstrating the ability to detect signs of neurotoxicity. The latest repeated dose positive control neurotoxicity study was acrylamide and was reported in April 2002	
3.3	**Test Animals**		
3.3.1	Species	Rats	
3.3.2	Strain	Wistar, CrIGIxBrIHan:Wl strain	
3.3.3	Source	XXXX	
3.3.4	Sex	M & F	
3.3.5	Rearing conditions	Single housing in stainless steel wire mesh cages (floor area approx 800 cm^2)	
3.3.6	Age/weight at study initiation	42±1 days old	X
3.3.7	Number of animals per group	10/sex/dose group	
3.3.8	Control animals	Yes	
3.4	**Administration**	In the diet	
3.4.1	Exposure	4 weeks	
3.4.2	Dose Levels	0; 1,000; 5,000 and 20,000 ppm equivalent to a mean daily compound intake of 88, 435 and 1,745 mg/kg in males and 95, 475 and 1,934 mg/kg bw in females, respectively.	X
3.4.3	Vehicle	In diet	
3.4.4	Concentration in vehicle	See 5.2	
3.4.5	Total volume applied	Not applicable	
3.4.6	Postexposure period	None	
3.4.7	Anticholinergic substances used	None	
3.4.8	Controls	Basal diet	

Section A6.9	**Delayed Neurotoxicity**
BPD Annex Point IIIA, VI.1	6.9 Subacute

3.5	**Examinations**			
3.5.1	Body Weight	Once a week and on the days when functional observational batteries were performed		
3.5.2	Signs of Toxicity	Animals were checked for mortality and clinical signs twice daily on working days and once daily on weekends and public holidays. A detailed clinical observation in the open field was conducted on all animals at Days 7, 14, and 21. FOB and motor activity investigations were performed at Days –1 and 27.		
3.5.3	Observation schedule	Food and water consumption were determined once a week		
3.5.4	Clinical Chemistry	No		
3.5.5	Pathology	Yes		
3.5.6	Histopathology	Yes	On 5 animals/sep of control and high dose group	
		Organs:	Peripheral nervous system (dorsal root ganglion, 3 of (C3-C6), dorsal root fiber (C3-C6), ventral root fiber (C3-C6), dorsal root ganglion, 3 of (L1-L4), dorsal root fiber (L1-L4), ventral root fiber (L1-L4), proximal sciatic nerve, proximal tibial nerve (at knee), distal tibial nerve (at lower leg); Gasserian ganglia with nerve, Gastrocnemius muscle9	
			Central nervous system: Brain (cross sections; frontal lobe, parietal lobe with diencephalon, midbrain with occipital and temporal lobe, pons, cerebellum, medulla oblongata), Eyes with retina and optical nerve, Spinal cord (cross and longitudinal sections; Cervical swelling (C3-C6), Lumbar swelling (L1-L49).	X
3.6	**Further remarks**	None		

Section A6.9	**Delayed Neurotoxicity**
BPD Annex Point IIIA, VI.1	6.9 Subacute

4 RESULTS AND DISCUSSION

4.1	**Body Weight**	Body weight change was reduced statistically significantly in male animals of the mid- and high-dose groups on days 14, 21 and 28 (up to -19.4% at 5,000 ppm and -16.6% at 20,000 ppm at the end of the study).
4.2	**Clinical signs of toxicity**	No effects
4.3	**Clinical Chemistry**	Not applicable
4.4	**Pathology**	No test-substance related effects
4.5	**Histopathology**	No test-substance related effects
4.6	**Other**	None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Flufenoxuron was administered to groups of 10 male and 10 female Wistar rats (CrIGIxBrIHan:Wl strain, supplied by XXXX; rats were 42±1 days old at start of test substance administration) in the diet at concentrations of 0; 1,000; 5,000 and 20,000 ppm for 4 weeks. Food and water consumption were determined once a week. Body weight was determined once a week and on the days when functional observational batteries (FOB's) were performed. The animals were examined for clinical signs of toxicity or mortality at least once a day. Detailed clinical examinations in an open field (DCO) were conducted prior to the start of the administration period and weekly thereafter except the week when functional observational batteries were carried out. Functional observational batteries and motor activity measurements were performed on days -1 and 27. At the end of the study the first 5 animals per sex and dose were sacrificed by perfusion fixation and subsequently subjected to neuropathological investigation. The remaining animals were sacrificed without further examination.
5.2	**Results and discussion**	Stability of the test substance was proven by reanalysis performed after the in-life phase of the study. Stability of Flufenoxuron in the diet was demonstrated for the duration of 10 days at room temperature; as the mixtures were stored no longer than this time period, sufficient stability was guaranteed. Homogeneity of the test substance preparations were proved in samples of the low and the high concentration at the start of the administration period.

Section A6.9	**Delayed Neurotoxicity**
BPD Annex Point IIIA, VI.1	6.9 Subacute

Concentration control analyses performed with samples taken at the start of treatment yielded 97 – 97.5% of the nominal value, demonstrating the correctness of the Flufenoxuron concentrations in feed.

The mean daily test substance intake over the entire study period is summarized in Table 6.9/101.

Table 6.9/101 Test substance intake

tance intake (mg/kg	bw/d)	
(ppm)	**Males**	**Females**
1,000	88	95
5,000	435	475
20,000	1,745	1,934

X

General toxicity		
No animal died during the present study. Clinical examinations did not reveal any treatment-related findings. No treatment-related effects on food consumption or water intake were observed in any group.		

In male animal groups administered 5,000 and 20,000 ppm, body weight was reduced statistically significantly on days 14, 21 and 28 (up to -9.6% at 5,000 ppm and -8% at 20,000 ppm). Reduced body weights were also measured during the FOB on day 27.

Body weight change was reduced statistically significantly in male animals of the mid- and high-dose groups on days 14, 21 and 28 (up to -19.4% at 5,000 ppm and -16.6% at 20,000 ppm at the end of the study). These effects were related to the test substance.

No treatment-related effects were observed at the low dose level of 1,000 ppm.

Neurotoxicity investigations

The FOB comprised investigations with regard to feces, grip strength, landing foot-splay test, home cage observations, Open-Field observations, and sensorimotor tests/reflexes. No substance-related findings were revealed in any of these tests. Also regarding the motor activity measurements, no substance-related effects were observed up to the highest dose level tested. Brain weights of treatment-group rats were not significantly different from control group rats. Histopathological examination did not reveal any indication of a neurotoxic potential of Flufenoxuron up to 20,000 ppm.

Section A6.9	**Delayed Neurotoxicity**
BPD Annex Point IIIA, VI.1	6.9 Subacute

5.3	**Conclusion**	In this 28-day dietary neurotoxicity study in rats, no evidence of neurotoxicity was observed up to 20,000 ppm Flufenoxuron, which was the highest feed concentration tested. The no observed adverse effect level (NOAEL) for neurotoxic effects was therefore 20,000 ppm in both sexes (1,775 mg/kg bw/d in males, 1,934 mg/kg bw/d in females).
5.3.1	LOAEL	Not applicable
5.3.2	NOAEL (neurotoxicity)	1,775 mg/kg bw/d in males, 1,934 mg/kg bw/d in females
5.3.3	Reliability	1
5.3.4	Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and Methods	**Revisions/Amendments:** 3.3.6. Age/weight at study initiation: What about the age? 3.4.2. Dose Levels: *0, 1000, 5000 and 20000 ppm equivalent to a mean daily compound intake of 88, 435 and* 1775 *mg/kg in males* […] Change in the table 6.9/1 too 3.5.6. Histopathology […]*Cervical swelling (C3-C6), Lumbar swelling* (L1-L4).
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version
Reliability	1
Acceptability	Acceptable

Section A6.9	**Delayed Neurotoxicity**
BPD Annex Point IIIA, VI.1	6.9 Subacute

Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	