Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

4-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}furan-2(5*H*)-one; flupyradifurone

EC Number: -
CAS Number: 951659-40-8

CLH-O-0000001412-86-228/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

14 September 2018

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: 4-[(6-chloro-3-pyridylmethyl)(2,2-difluoroethyl)amino]furan-2(5H)-one; flupyradifurone

EC Number: **not allocated**

CAS Number: **951659-40-8**

Index Number: **not allocated**

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ANNEX 1 – BACKGROUND DOCUMENT TO RAC OPINION ON 4-[(6-CHLORO-3-PYRIDYLMETHYL)(2,2-DIFLUOROETHYL)AMINO]FURAN-2(5H)-ONE; FLUPYRADIFURONE

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***4-[(6-chloro-3-pyridylmethyl)(2,2-difluoroethyl)amino]furan-2(5H)-one; flupyradifurone***
EC number:	***not allocated***
CAS number:	***951659-40-8***
Annex VI Index number:	***not allocated***
Degree of purity:	***≥960 g/kg***
Impurities:	***Confidential***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	None
Current proposal for consideration by RAC	Acute Tox. 4, H302 STOT RE 2 (muscle), H373 Repr. 2, H361 Aquatic Acute 1, H400 (M=10) Aquatic Chronic 1, H410 (M=10)
Resulting harmonised classification (future entry in Annex VI, CLP	Acute Tox. 4, H302

Regulation)	STOT RE 2 (muscle), H373 Repr. 2, H361 Aquatic Acute 1, H400 (M=10) Aquatic Chronic 1, H410 (M=10)

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
Explosives	None		None	conclusive but not sufficient for classification
Flammable gases	None		None	conclusive but not sufficient for classification
Flammable aerosols	None		None	conclusive but not sufficient for classification
Oxidising gases	None		None	conclusive but not sufficient for classification
Gases under pressure	None		None	conclusive but not sufficient for classification
Flammable liquids	None		None	conclusive but not sufficient for classification
Flammable solids	None		None	conclusive but not sufficient for classification
Self-reactive substances and mixtures	None		None	conclusive but not sufficient for classification
Pyrophoric liquids	None		None	conclusive but not sufficient for classification
Pyrophoric solids	None		None	conclusive but not sufficient for classification
Self-heating substances and mixtures	None		None	conclusive but not sufficient for classification
Substances and mixtures which in contact with water emit flammable gases	None		None	conclusive but not sufficient for classification
Oxidising liquids	None		None	conclusive but not sufficient for classification
Oxidising solids	None		None	conclusive but not sufficient for classification
Organic peroxides	None		None	conclusive but not sufficient for classification

Substance and mixtures corrosive to metals	None		None	conclusive but not sufficient for classification
Acute toxicity - oral	Acute tox. 4, H302			
Acute toxicity - dermal	None		None	conclusive but not sufficient for classification
Acute toxicity - inhalation	None		None	conclusive but not sufficient for classification
Skin corrosion / irritation	None		None	conclusive but not sufficient for classification
Serious eye damage / eye irritation	None		None	
Respiratory sensitisation	None		None	Data lacking
Skin sensitisation	None		None	conclusive but not sufficient for classification
Germ cell mutagenicity	None		None	conclusive but not sufficient for classification
Carcinogenicity	None		None	conclusive but not sufficient for classification
Reproductive toxicity	Repr. 2, H361		None	
Specific target organ toxicity –single exposure	None		None	conclusive but not sufficient for classification
Specific target organ toxicity – repeated exposure	STOT RE 2 (muscle), H373		None	
Aspiration hazard	None		None	conclusive but not sufficient for classification
Hazardous to the aquatic environment	Aquatic Acute 1, H400 Aquatic Chronic 1, H410	(M=10) (M=10)		
Hazardous to the ozone layer				

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Warning
Hazard statements: H302: Harmful if swallowed
H373: May cause damage to organs through prolonged or repeated exposure
H361: Suspected of damaging fertility or the unborn child
H410: Very toxic to aquatic life with long lasting effects
Precautionary statements: Not stated as they are not included in Annex VI of CLP

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Proposed notes assigned to an entry:

: none

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Flupyradifurone has not previously been assessed for harmonised classification by RAC or TC C&L. Flupyradifurone is a systemic insecticide intended for agricultural uses.

In accordance with Article 36(2) of the CLP Regulation, flupyradifurone should be considered for harmonised classification and labelling. Therefore, this proposal considers all human health and environmental endpoints. This dossier presents a classification and labelling proposal based mainly on the information presented in the assessment of flupyradifurone under Directive 91/414/EEC (DAR January 2014 plus final addendum January 2015). All information in this proposal is taken over from the DAR without review of the original study reports. Detailed information is included for the key studies used to derive the classification. For more details the reader is referred to the DAR and its addenda. The DAR is publicly available via the EFSA web site (http://dar.efsa.europa.eu/dar-web/provision). There is currently no registration (April 2015). A search for available data on flupyradifurone in the public literature was performed by the applicant. No new relevant information was identified.

2.2 Short summary of the scientific justification for the CLH proposal

In accordance with the criteria of the CLP regulation, flupyradifurone should be classified as Acute Tox 4 (H302) based on mortality at 2000 mg/kg bw but not at 300 mg/kg bw/day in an acute oral toxicity test according to the acute toxic class method (OECD 423).

Flupyradifurone induced consistent, severe effects in dogs in repeated dose studies, below or around the guideline values for STOT RE Category 2. These effects include weight loss, minimal myofiber atrophy/degeneration, changes in haematology (> 20% Hb reduction) and clinical chemistry. According to Regulation (EC) 1272/2008, flupyradifurone (BYI 02960) needs to be classified as STOT RE Cat. 2 H373 (muscle).

The following reproductive effects were observed in a 2-generation study: reduced number of estrous cycles of the F1 females at the high dose and reduced numbers of implantation sites and pups. These effects were observed in dams, which showed reduced body weight (16%) in the pre-mating period. Such effects on oestrus cycle, implantation sites and pups were not observed in the parental generation, which had a 5% lower exposure during the pre-mating period. The body weight was also reduced compared to the controls but only for 10%. It is unclear whether the observed effects on the F1 females are secondary to the reduced body weights. As a result, we consider that the criteria for category 2 are met. As it is unclear whether this should be considered an effect on fertility or development, no specification is proposed (H361). In accordance with Regulation (EC) 1272/2008, Flupyradifurone (BYI 02960) needs to be classified as Reproductive toxicant Cat. 2 H361.

Flupyradifurone is not considered rapidly degradable. The bioaccumulation potential is low based on the log Kow of 1.2, which is below the cut-off value of log Kow = 4.

As there is adequate acute and chronic toxicity data available for all three trophic levels, classification is carried out according to table 4.1.0(b)(i) (according to CLP guidance V4.0 nov 2013, p. 524 & Table on page 525). The most sensitive species acute and chronically is *Chironomus*

riparius, with an EC50 = 0.0617 mg a.s./L (48h, nominal) and a NOEC = 0.01 mg a.s./L (28 days, m.m.). Flupyradifurone is therefore classified as Aquatic Acute 1, M factor 10 and Aquatic Chronic 1, M factor 10.

2.3 Current harmonised classification and labelling

Not applicable

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Not applicable

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

2.4 Current self-classification and labelling

Not applicable

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Flupyradifurone is not included in the inventory of self-classified substances.

2.4.2 Current self-classification and labelling based on DSD criteria

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

RAC general comment
Flupyradifurone is approved in the EU as an insecticide for plant protection products. Flupyradifurone interacts with insect nicotinic acetylcholine receptors, a target also known for neonicotinoid insecticides. EFSA (2015) informally proposed flupyradifurone to be classified as Acute Tox. 4; H301 and as Aquatic Acute 1 and Aquatic Chronic 1 (with no M-factors) in accordance with the provisions of Regulation (EC) No 1272/2008 (CLP).

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Flupyradifurone is an active substance in the meaning of Regulation (EC) No 1107/2009 and therefore subject to harmonised classification and labelling (CLP, article 36.2).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

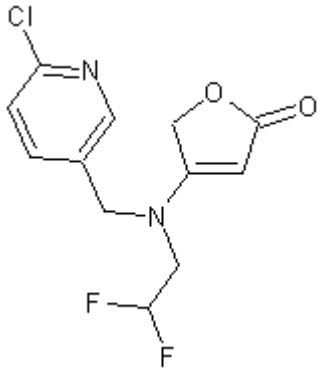
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	-
EC name:	-
CAS number (EC inventory):	-
CAS number:	951659-40-8
CAS name:	4-[[(6-chloro-3-pyridinyl)methyl](2,2-difluoroethyl)amino]-2(5H)-Furanone
IUPAC name:	4-[(6-chloro-3-pyridylmethyl)(2,2-difluoroethyl)amino]furan-2(5H)-one
ISO name:	flupyradifurone
CLP Annex VI Index number:	Not allocated
Molecular formula:	$C_{12}H_{11}ClF_2N_2O_2$
Molecular weight range:	288.68 g/mol

Structural formula:



1.2 Composition of the substance

See Confidential Annex

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Flupyradifurone	98.4%	≥96%	mono constituent substance

Current Annex VI entry:

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
confidential			

Current Annex VI entry:

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
confidential				

Current Annex VI entry:

1.2.1 Composition of test material

During the development of BYI 02960 (= flupyradifurone), some changes occurred which lead to a slightly different impurity profile of the final technical product compared to the technical batches that were used to characterize the toxicological profile of BYI 02960. A number of impurities are present in excess of 0.1 % in the proposed specification, which were not present or not present at sufficient levels in the key toxicological batch. None of these impurities has an Annex VI entry. An Ames test (4.9.1.1. Study 2) and an *in vivo* micronucleus test in females (4.9.1.2. study 2) were conducted with a batch with an impurity profile closer to the final technical product. None of the batches were genotoxic. Considering this result and the low concentrations of the impurities (<1%), it is expected that the final technical material will not present an increased hazard compared to the material used in the toxicity studies.

1.3 Physico-chemical properties

Flupyradifurone is an insecticide. Its vapour pressure and volatility are very low. The solubility in water is about 3.2 g/L at a range of pH between 4 and 9. With a log Kow of 1.2 it has no bioaccumulative potential in lipophilic matrices. Flupyradifurone is hydrolytically stable under acidic, neutral and under alkaline conditions at ambient temperature. The hydrolytic degradation is negligible under acidic, neutral and alkaline conditions. Flupyradifurone is rather susceptible for reactions with hydroxyl radicals with photochemical degradation process to contribute significantly to the overall degradation of the substance in case of its occurrence in the atmosphere. Its flammability, explosive and oxidizing properties are not critical.

Table 8: Summary of physico - chemical properties

Endpoint	purity	method	results	comment	reference
Melting point	99.4% 97.6%	EC A.1, OECD 102 GLP:Y	The melting point of Flupyradifurone, pure at atmospheric pressure is 69.0 °C. The melting point of Flupyradifurone, technical at atmospheric pressure is 67.1 °C.	Acceptable	Smeykal, H.; 2010 Smeykal, H.; 2011
Boiling point	99.4% 97.6%	EC A.2, OECD 103 GLP:Y	The test item had no boiling point at atmospheric conditions; the test item decomposed first at a temperature of 270 °C The test item had no boiling point; the test item decomposes first starting at a temperature of 245 °C	Acceptable	Smeykal, H.; 2010 Smeykal, H.; 2011

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Endpoint	**purity**	**method**	**results**	**comment**	**reference**
Thermal stability	99.4% 97.6%	EC A.2, OECD 103 GLP:Y	The test item showed an exothermal decomposition in the temperature range 270 – 355 °C with a mean decomposition energy of 895 J/g. The test item Flupyradifurone (BYI 02960), technical substance, showed an exothermal effect in the temperature range of 245 – 400 °C (245 – 355 °C respectively) with an energy of 836 to 938 J/g.	Acceptable. Decomposition temperature by DSC; visual observations (capillary method) showed change of colour to brown at 240 °C. Test performed under helium atmosphere.	Smeykal, H.; 2010 Smeykal, H.; 2011
Relative density	99.4% 97.6%	EC A.3, OECD 109, OPPTS 830.7300	Active substance, pure: D_4^{20} = 1.43 Active substance, technical: D_4^{20} = 1.52	Acceptable	Bogdoll, B.; Strunk, B.; 2011 Eyrich, U.; Bogdoll, B.; 2011
Vapour pressure	99.9%	EC A.4, OECD 104	Extrapolated: 9.1 x 10^{-7} Pa for 20 °C 1.7 x 10^{-6} Pa for 25 °C 2.6 x 10^{-5} Pa for 50 °C	Acceptable	Smeykal, H.; 2008
Volatility, Henry's law constant		Calculation.	Henry's law constant at 20 °C in distilled water (pH: 7.0) 8.2 x 10^{-8} Pa.m^3.mol^{-1} Henry's law constants at 20 °C at different pH values: at pH 4: 8.2 x 10^{-8} Pa.m^3.mol^{-1} at pH 9: 8.8 x 10^{-8} Pa.m^3.mol^{-1} A vapour pressure of 9.1 x 10^{-7} Pa (20 °C) and the follwing water solubility values at • pH 4: 3.2 g/L pH 9: 3.0 g/L were used to calculate the Henry's law constant.	Acceptable	Bogdoll, B.; Eyrich, U.; 2011

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Endpoint	**purity**	**method**	**results**	**comment**	**reference**
Appearance: physical state	99.4% 97.6%	Visual	Active substance, pure (at 21°C): white powder Active substance, technical (at 23°C): beige powder	Acceptable	Bogdoll, B.; Strunk, B.; 2011 Eyrich, U., Bogdoll, B.; 2011
Appearance: colour	99.4% 97.6%	visual	Active substance, pure (at 21°C): white powder Active substance, technical (at 23°C): beige powder	Acceptable	
Appearance: odour	99.4% 97.6%	OPPTS 830.6304	active substance, pure (at 21°C): weak, not characteristic Active substance, technical (at 23°C): distinct, solvent-like odour		Bogdoll, B.; Strunk, B.; 2011; Eyrich, U., Bogdoll, B.; 2011;
Solubility in water	99.4%	EC A.6, OECD 105 GLP:Y	pH 4 (buffer) 3.2 g/L at 20°C pH 9 (buffer) 3.0 g/L at 20°C In distilled water: pH 7 3.2 g/L at 20°C	Acceptable	Wiche, A., Bogdoll, B.; 2011
Solubility in organic solvents	99.4%	EC A.6, OECD 105	[g/L at 20 °C] methanol > 250 n-heptane 0.0005 toluene 3.7 dichloromethane > 250 acetone > 250 ethylacetate > 250 dimethyl sulfoxide > 250	Acceptable	Eyrich, U., Bogdoll, B.; 2011;

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Endpoint	**purity**	**method**	**results**	**comment**	**reference**
Partition co-efficient	99.4%	ECA.8 OECD 117; High Performance Liquid Chromatography (HPLC) Method.	Determination of the partition coefficient of Flupyradifurone in 1-octanol / water (25 °C) Kow log Kow pH 7 16 1.2	Acceptable	Bogdoll, B.; Stunk, B.; 2011;
Hydrolysis rate	Radio chemical purity 99%	Furanone-4-^{14}C] BYI 02960, pure Vial No. C-1116	BYI 02960 is hydrolytically stable at ambient temperature in the range of pH 4 to pH9. 3 minor components were observed during 5 days at 50°C which accounted for a total of 4.9%, none were more than 2.7% of the applied activity in any of the pH	**See fate section**	Mislankar, S.; Woodard, D., 2011
Photochemical degradation	Radio chemical purity 99.3%	[Furanone-4-^{14}C] BYI 02960, pure Vial No. C-1116A	1st order half-life for photolytic degradation of BYI 02960 in sterile phosphate buffer (pH 7) was 13.8 experimental hrs.	See fate section	Hall, L.R., 2011
Quantum yield	purity 99.4 % w/w	BYI 02960, pure NLL 7780-47-4	A mean quantum yield of **<D** = 0.000138 was calculated on the basis of UV absorption data and the degradation kinetics determined from 2 degradation experiments.	Environmental half-lives of sunlight exposed top surface water layers containing BYI 02960 can be estimated using respective models (GC SOLAR or Kloepffer Model) dependent on season and location of exposure.	Heinemann, O. 2011
Dissociation constant (pKa)	purity 99.4 % w/w	OECD 112	No dissociation occurs in aqueous solutions in the pH-range 1< pH <12		Wiche, A., Bogdoll, B.; 2011

Endpoint	purity	method	results	comment	reference
Stability in air, photochemical oxidative degradation		Atkinson calculation by AOPWIN™ version 1.92a	The half-life time ($t_{1/2}$) was estimated within a range of 4.4 hours (short-term scenario) to 13.1 hours (long-term scenario), depending on the mean concentration of hydroxyl radicals present in the troposphere. In addition, BYI 02960 is susceptible to reactions with ozone, however, that attack and its resulting chemical half-life is considered to be more slowly by a factor of 2 to 10. As a consequence of the short half life in air, no long-range transport BYI 02960 in the atmosphere is likely to occur nor an accumulation of BYI02960 in the environmental compartment air.	See fate section	Hellpointner, E.;
Flammability and auto-flammability (technical active substance)	Tech. a.s. 97.6%	EEC A.10 GLP:Y	Technical substance is not a highly flammable solid in the sense of the EC guideline (EC) No. 440/2008 Method AIO	Acceptable	Smeykal, H, 2011
Flash point (technical active substance)		Statement	Not applicable. The active substance as manufactured is a solid; its melting point is > 40 °C.		
Explosive properties (technical active substance)	Tech. a.s. 97.6%	EC A.14 OECD 113	Not explosive in the sense of EC guideline A.14	Acceptable	Smeykal, H.; 2011;
Surface tension (technical active substance)	Tech. a.s. 97.6%	EC A.5, OECD 115	Technical substance: 69.1 mN/m at 20°C	Acceptable	Eyrich, U., Bogdoll, B.; 2011;
Oxidizing properties of the active substance as manufactured	BYI 02960, technical PFV107N004 purity 97.6 % w/w	EC A.17	Flupyradifurone (BYI 2960) has no oxidizing properties in the sense of EC guideline A.17.	Acceptable	Smeykal, H.; 2011;

Endpoint	purity	method	results	comment	reference
Storage stability (technical active substance)	BYI 02960, technical 2009-000239 purity 96.6% PFV087N002 purity 98.7% PFV087N007 purity 98.6%	OPPTS 830.6317	The results from 24 months stability study at ambient temperature (22.1°C) and 30°C showed Flupyradifurone to be stable in polypropylene and polyethylene containers. Even at elevated temperature no decomposition of the test substance and the storage vessels could be established.	Not applicable	Wagner, S.; 2011

2 MANUFACTURE AND USES

2.1 Manufacture

2.2 Identified uses

Flupyradifurone is a systemic insecticide intended for agricultural uses. The active substance Flupyradifurone according to the applicant, exhibits high activity especially against sucking insects like aphids and whiteflies.

Flupyradifurone interacts with insect nicotinic acetylcholine receptors, a class of neurotransmitter-gated cation channels which are involved in excitatory neurotransmission, a target also known for neonicotinoid insecticides. Blockage of these receptors leads to paralysis and death of the target insects.

Control of aphids in lettuce and hop were chosen as representative uses. The representative formulation for flupyradifurone is Sivanto SL 200 g/L, this is a soluble concentrate (SL) containing 200 grams per litre (g/L) of Flupyradifurone. Flupyradifurone 200SL (Sivanto) is applied with traditional foliar sprayer.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

3.1 *Physico-chemical properties*

3.1.1 Summary and discussion of physico-chemical properties

Flupyradifurone has no explosive properties as shown in the EEC A.14 study, is a solid and has no auto-ignition properties, is not flammable in contact with water and the molecular structure does not indicate oxidizing properties (*Table 8*). Therefore, no classification of flupyradifurone for physico-chemical properties is required.

3.1.2 Comparison with criteria

Flupyradifurone does not meet the criteria for classification on its physico-chemical properties. Therefore, no classification is proposed under the CLP Regulation.

3.1.3 Conclusions on classification and labelling

No classification is proposed for physico-chemical properties under the CLP Regulation.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The purified substance is a white powder at room temperature. The odour is weak, not characteristic. The melting point is 69.0 °C, the boiling point is 270 °C. No decomposition was observed prior to boiling. The vapour pressure of purified flupyradifurone is 9.1×10^{-7} Pa at 20 °C, which, combined with a low and pH dependent water solubility of 3.2 g/L at pH 7 in distilled water, results in a Henry´s law constant of 8.2×10^{-8} $Pa.m^3.mol^{-1}$.

Flupyradifurone has no bioaccumulative potential in lipophilic matrices (log K_{ow}: 1.2 at pH 7, 25 °C) and it is hydrolytically stable in aqueous solutions in the pH-range 1 < pH < 12 at ambient temperature. The substance is susceptible to reactions with hydroxyl radicals and to a lower extent with ozone in the troposphere.

The surface tension of technical flupyradifurone with a purity of 97.6 % is 69.1 mN/m at 20 °C. The material is not highly flammable, does not self-ignite and does not have oxidising or explosive properties. The chemical structure of the substance does not indicate any potential for self-reactivity.

In summary, no classification for physical hazard properties is proposed by the Dossier Submitter (DS).

Comments received during public consultation

One Member State Competent Authority (MSCA) commented on the classification for explosive properties. The appropriateness of the test battery for classification was contested, namely that the EU A.14 method for explosive properties does not entirely correspond to the CLP requirements. In order to validate the proposed classification, at least a "BAM Trauzl Test" should have been performed according to the specifications of test methods under the United Nation scheme.

Assessment and comparison with the classification criteria

The CLP Regulation (Section 2.1) and the CLP Guidance state that the classification for explosive properties is almost entirely adopted based on Part I of the UN Recommendations on the Transport of Dangerous Goods (UN RTDG; Manual of Tests and Criteria), which are appropriate for transport and also storage of packaged explosives. The classification of substances, mixtures and articles in the class of explosives and further allocation to a division is a very complex procedure.

Flupyradifurone was investigated under a test battery which cannot be directly related to the CLP regulatory text. However, the results proved negative in three relevant key areas: behaviour to heat, shock and friction.

In conclusion, RAC considers that there are sufficient data to conclude that flupyradifurone should not be classified for Explosive properties under the CLP Regulation although the exothermic decomposition energy of the substance is 895 J/g between 270 °C and 355 °C.

Consequently, the DS's proposal of **no classification for physical hazards** is supported.

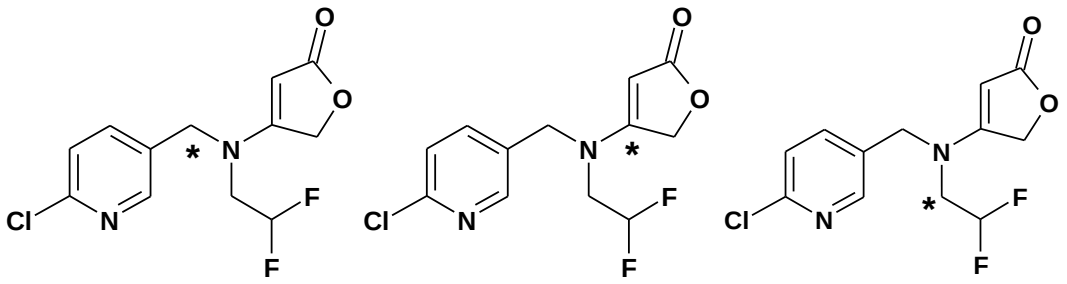
4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Toxicokinetic studies – single dose, oral route in rats

Seven studies are summarised in this chapter; two with the pyridinylmethyl-^{14}C label (study 1 and 2), three with the furanone-^{14}C label (study 3-5), and two with the ethyl-1-^{14}C label (study 6 and 7). All are single dose studies.



Pyridinylmethyl-^{14}C Flupyradifurone	Furanone-4-^{14}C flupyradifurone	Ethyl-1-^{14}C flupyradifurone

Study 1 is an ADME-study in which male and female rats were orally administered with a single low (2 mg/kg/bw) or high dose (200 mg/kg bw) of flupyradifurone. Additionally, male rats were administered intravenously with flupyradifurone (2 mg/kg bw). The excretion via urine and faeces was investigated, as well as the distribution in the plasma and the radioactivity concentration in organs and tissues after sacrifice. The metabolism was investigated in urine and faeces.

Study 2 is a quantitative whole body autoradiography study following a single oral dose of flupyradifurone (5 mg/kg bw) to male and female rats. At various time points the excretion of radioactivity was determined in urine, faeces and expired air, as well as the distribution in the plasma and the radioactivity concentration in the organs and tissues.

Study 3 is an ADME-study in which male and female rats were orally administered with a single dose of flupyradifurone (2 mg/kg bw). The excretion via urine and faeces was investigated, as well as the distribution in the plasma and the radioactivity concentration in organs and tissues after sacrifice. The metabolism was investigated in urine and faeces.

Study 4 is a quantitative whole body autoradiography study following a single oral dose of flupyradifurone (5 mg/kg bw) to male and female rats. At various time points the excretion of radioactivity was determined in urine, faeces and expired air, as well as the distribution in the plasma and the radioactivity concentration in the organs and tissues.

Study 5 is an organ metabolism study. Male and female rats were orally administered with a single dose of flupyradifurone (3 mg/kg bw) and sacrificed after 6 hours. The metabolism was investigated in urine, plasma, and in extracts of liver, kidney, muscle and fat tissues.

Study 6 is an ADME-study in which male rats were orally administered with a single dose of flupyradifurone (2 mg/kg bw). The excretion via urine and faeces was investigated, as well as the distribution in the plasma and the radioactivity concentration in organs and tissues after sacrifice. The metabolism was investigated in urine and faeces.

Study 7 is an organ metabolism study. Male and female rats were orally administered with a single dose of flupyradifurone (3 mg/kg bw) and sacrificed after 1, 6 or 24 hours. The metabolism was investigated in urine, plasma, and in extracts of liver, kidney, muscle and fat tissues.

As all single dose experiments revealed no indication of a potential for retention, accumulation and/or persistence of the administered radioactivity in organs or tissues. In line with paragraph 26 of

the OECD Test Guideline 417, a repeated dose study was not considered necessary. This observation is supported by the low log Kow of flupyradifurone of 1.2.

The different studies and their results are described in more detail below.

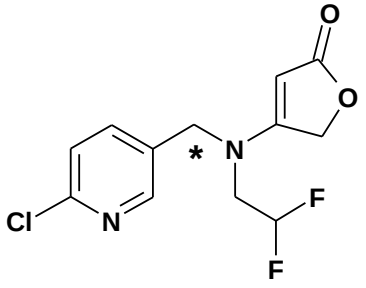
Study 1

Characteristics

Reference	:	Klempner, A. 2012	exposure	:	Single exposure by gavage or iv
type of study	:	Toxicokinetic study (ADME in rat)	dose	:	2 mg/kg or 200 mg/kg bw (gavage) 2 mg/kg bw (iv)
year of execution	:	2009-2011	vehicle	:	0.5% aqueous Tragacanth® suspension (gavage), water (iv)
test substance	:	[pyridinylmethyl-^{14}C]-flupyradifurone, batch no. KATH 6264 (test 1-4) and KATH 6351 (test 5), chemical purity >99%, radiochemical purity >99%, 4.37 MBq/mg	GLP statement	:	yes
Route	:	oral, iv	guideline	:	OECD 417
Species	:	Rat Wistar Hsd/Cpb: WU	acceptability	:	acceptable
group size	:	4/dose/sex (oral), 4 males (iv)			

Study design

The study was performed according to OECD Guideline 417. Male and female rats in groups of four rats/sex were administered orally by gavage a single dose of flupyradifurone suspended in 0.5% aqueous Tragacanth® (2 mL) at target dose levels of 2 mg/kg bw or 200 mg/kg bw. In addition, a group of four male rats was administered intravenously with a single dose of flupyradifurone in water (0.5 mL) at a dose level of 2 mg/kg bw (Table 9). Flupyradifurone was radiolabelled with ^{14}C in the pyridinylmethyl bridge of the molecule.



* denotes the ^{14}C-label position

Urine and faeces were collected for the time range between dosing and sacrifice and the animals were sacrificed after 72 hours. Plasma levels of radioactivity were followed from each animal for 17 timepoints. Total radioactivity which included the test compound and metabolites was determined in excreta and in organs and tissues collected at sacrifice. Metabolism was investigated in urine and faeces.

Samples were analyzed by radio HPLC, radio TLC, LC-MS and NMR methods. Toxicokinetic parameters were calculated with the software TOPFIT version 2.0 where the dose normalized values were used for better comparison of the low tests among themselves and to the high dose tests.

Table 9: Experimental groups for each dose level of [pyridinylmethyl-^{14}C]-flupyradifurone

Group no.	**No. of animals**	**Route**	**Dose**	**Sacrifice time (hours after last dose)**
1	4 M	Oral (gavage)	2 mg/kg bw	72
2	4 F	Oral (gavage)	2 mg/kg bw	72
3	4 M	Oral (gavage)	200 mg/kg bw	72
4	4 F	Oral (gavage)	200 mg/kg bw	72
5	4 M	I.v.	2 mg/kg bw	72

Results

Recovery

The total recovery for the orally administered test was between approximately 97% and 103% of the administered dose in the excreta and the body of male and female rats. The total recovery was approximately 91% of the administered dose in the low dose intravenous administrated male rats.

Table 10: Recovery of radioactivity in urine, gastrointestinal tract and the body following oral or intravenous dosing of [pyridinylmethyl-^{14}C] flupyradifurone

	% of the administered radioactivity				
	Male 2 mg/kg bw (oral)	**Female 2 mg/kg bw (oral)**	**Male 200 mg/kg bw (oral)**	**Female 200 mg/kg bw (oral)**	**Male 2 mg/kg bw (i.v.)**
Faeces	23.09	7.49	26.14	10.32	14.64
Urine	75.45	90.07	76.26	85.95	76.24
Sum of excreta	**98.55**	**97.56**	**102.40**	**96.26**	**90.88**
Body without GIT	0.119	0.064	0.128	0.241	0.141
GIT	0.069	0.010	0.086	0.064	0.086
Total in body	**0.188**	**0.074**	**0.214**	**0.306**	**0.227**
Balance	**98.73**	**97.63**	**102.60**	**96.57**	**91.11**

Absorption

The bioavailability factor was determined from plasma kinetics of the oral and i.v. low dose test with male rats (group 1 and 5). A bioavailability factor of F = 0.93 was obtained demonstrating that the orally administered test compound was almost completely bioavailable. This high

bioavailability factor was confirmed by the high radioactivity (76% to 90%) detected in the urines and the bodies without GIT of male and female rats. The absorption started immediately after dosing as could be seen from the quick increase of radioactivity in plasma.

Table 11: Time course of radioactivity in the plasma following oral or intravenous dosing of [pyridinylmethyl-^{14}C] flupyradifurone

Time [h]	Concentration [mg a.s. equiv. /kg]				
	Male 2 mg/kg bw (oral)	**Female 2 mg/kg bw (oral)**	**Male 200 mg/kg bw (oral)**	**Female 200 mg/kg bw (oral)**	**Male 2 mg/kg bw (i.v.)**
0.17	0.4465	0.5287	32.5100	30.9100	1.6070
0.33	1.0380	1.2010	66.3400	57.2100	1.6970
0.67	1.5690	1.7240	88.9400	74.9300	1.7670
1	1.7130	1.8540	94.3200	84.4500	1.7060
1.5	1.6740	1.7920	96.0300	87.7900	1.5570
2	1.5500	1.7630	96.8600	92.7700	1.3850
3	1.3410	1.6100	95.6200	98.3200	1.1180
4	1.1990	1.4500	96.3100	99.9900	0.8976
6	0.7990	1.1350	80.6500	91.7700	0.6250
8	0.5281	0.8420	69.2600	81.9800	0.4357
24	0.0200	0.0354	6.4460	22.4000	0.0193
28	0.0134	0.0228	3.3800	17.8300	0.0157
32	0.0100	0.0157	2.1660	10.1800	0.0103
48	---	0.0052	0.6626	1.4800	0.0062
52	---	---	0.6165	1.2700	0.0053
46	---	---	0.5762	0.9593	0.0038
72	---	---	0.3602	0.5697	0.0044

Distribution and plasma kinetics

Flupyradifurone was quickly absorbed and distributed. The C_{max} was reached at approximately 1 hour after administration at the low dose tests. In the high dose tests C_{max} was reached approximately 2 hours for the males and at approximately 4 hours for the females. The plasma concentrations declined to 50% of C_{max} within 4 - 8 hours and to 1 - 2% of C_{max} within 24 hours in the low dose test with both male and female rats. In the high dose test the plasma concentrations declined to 50% within 8 to 24 hours and to 1-2% of C_{max} after 48 hours. At time of sacrifice, 72 hours after administration, the plasma concentrations were below or around LOQ for the low dose animals and approximately 0.5% of C_{max} for the high dose animals. An overview of all important results of the TOPFIT analysis is presented in Table 12.

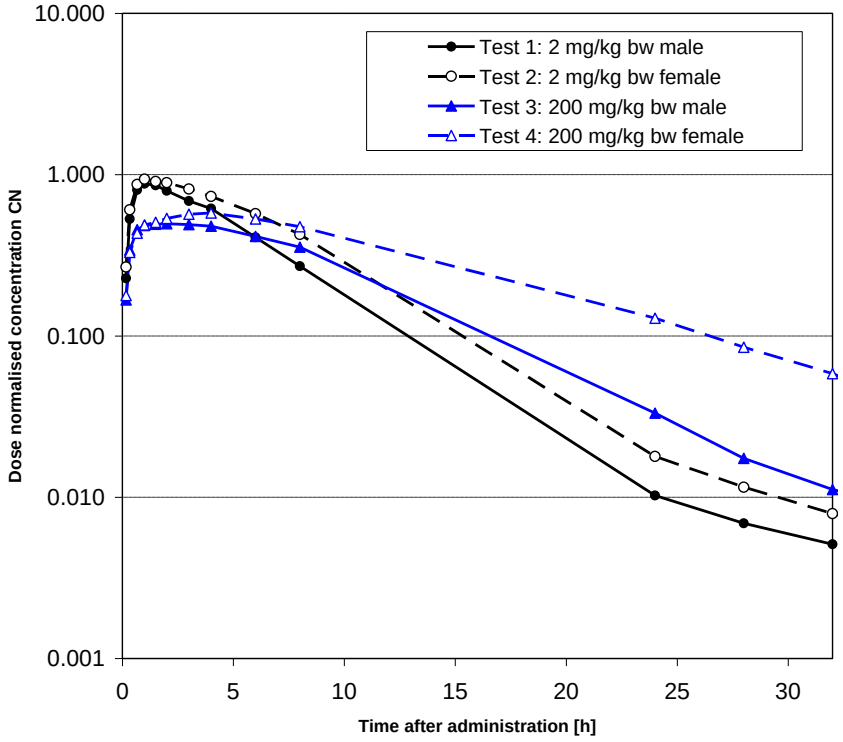


Figure 1: Comparison of dose normalised plasma curves following oral administration of low and high doses of [pyridinylmethyl-^{14}C] flupyradifurone to male and female rats

Table 12: Pharmacokinetic parameters and calculation of the bioavailability of [pyridinylmethyl-^{14}C] flupyradifurone after oral administration to male and female rats, and after i.v. administration to male rats

	Male 2 mg/kg bw (oral)	**Female 2 mg/kg bw (oral)**	**Male 200 mg/kg bw (oral)**	**Female 200 mg/kg bw (oral)**	**Male 2 mg/kg bw (i.v.)**
t_{max} measured [h]	1.0	1.0	2.0	4.0	0.67
t_{max} calculated [h]	1.13	1.15	2.23	3.35	0.38
C_{max} measured values	0.878	0.937	0.497	0.578	1.04
C_{max} calculated (fitted) values	0.880	0.929	0.500	0.582	1.0400
$t_{1/2\ abs}$ [h]	0.21	0.17	0.13	0.17	0.06
$t_{1/2\ elim\ 1}$ [h]	3.9	3.0	3.6	8.1	3.8
$AUC_{0-\infty}$ [mg/L*h]	6.10	7.96	6.16	9.73	6.55
MRT_{tot} [h]	6.07	6.69	8.70	13.10	5.71

The biokinetic parameters were comparable in the three low dose tests. Only the AUC value for females of the low dose test was approx. 1.3 times higher than for males, which is related to the slightly broader peak maximum of the plasma curve of female rats. In the high dose tests, the AUC

value for males was proportional to the dose. For females, the AUC value of the high dose test was approx. 1.2 times higher than for the low dose test with females and approx. 1.6 times higher than for the high dose test with males. According to the broader maximum of the plasma curve of female rats in the high dose test, C_{max} was reached later than in males (approx. 4 hours instead of 2 hours). The biokinetic model confirmed a fast absorption phase in all tests with short half-lives of max. 0.2 hours followed by a short elimination phase with half-lives of about 3 – 4 hours. Only females in the high dose test showed a slightly longer elimination phase with a half-life of about 8 hours. The mean residence times in the low dose tests were in the range of approx. 6 – 7 hours. The mean residence times were slightly longer in the high dose tests and amounted to about 9 hours for males and 13 hours for females.

Excretion

Approximately 76% of the administered dose was detected in the urine of male rats, for low or high oral dose and low i.v. dose. For females 90% and 86% was detected in urine in the low and high dose, respectively. The major route of excretion is therefore by urine. Faecal excretion accounted for ca. 7 - 10% of the given dose in females and was slightly higher for males, where between approximately 15 to 26% was excreted with the faeces. Also the major part of faecal residues, approx. 89 - 92% of the radioactivity in faeces in the low dose tests and approximately 47% (females) and 77% (males) in the high dose tests, was excreted within the first day after treatment and was almost complete 48 hours post administration. The cumulative excretion results are summarized in Table 13.

Table 13: Cumulative excretion of radioactive residues via urine and faeces after oral administration of [pyridinylmethyl-^{14}C] flupyradifurone to male and female rats, and after i.v. administration to male rats

Time [h]	% of dose				
	Male 2 mg/kg bw (oral)	**Female 2 mg/kg bw (oral)**	**Male 200 mg/kg bw (oral)**	**Female 200 mg/kg bw (oral)**	**Male 2 mg/kg bw (i.v.)**
Faeces					
24	20.55	6.86	20.22	4.90	13.31
48	22.82	7.38	25.84	9.64	14.41
72	23.09	7.49	26.14	10.32	14.64
Urine					
4	17.25	5.34	14.89	9.50	26.12
8	40.99	33.80	31.33	28.56	44.02
12	51.67	---	---	---	---
24	72.61	86.95	68.26	72.15	73.80
48	75.15	89.77	75.56	84.15	75.75
72	75.45	90.07	76.26	85.95	76.24
Total excreted	**98.55**	**97.56**	**102.40**	**96.26**	**90.88**

Radioactive residues in organs and tissues at sacrifice

Approximately 0.1 - 0.3% of the total dose was detected in the body of male and female rats at the time of sacrifice 72 hours after oral and i.v. administration; only a trace amount of 0.01 - 0.09% was found in the GIT. The residual concentrations of radioactivity were low in the low dose tests and ranged from 0.0007 to 0.0175 mg/kg. The highest concentrations were detected in blood cells and the GIT, and in the eyes of female rats. But basically, levels for most organs and tissues were very similar and in the range of approx. 0.001 to 0.007 mg/kg. The residual concentrations of the high dose tests were almost dose proportional and ranged from 0.0859 to 2.345 mg/kg. The highest concentrations were also detected in blood cells and the GIT, as well as in the eyes of female rats. For most organs and tissues the residue concentrations in males were slightly higher as compared to females.

The equivalent concentrations of the residual radioactivity in organs and tissues at sacrifice are presented in Table 14.

Table 14: Total radioactive residues in organs and tissues at sacrifice after administration of [pyridinylmethyl-^{14}C] flupyradifurone to male and female rats.

Organ or tissue	**Concentration [mg a.s. equiv. /kg]**				
	Male 2 mg/kg bw (oral)	**Female 2 mg/kg bw (oral)**	**Male 200 mg/kg bw (oral)**	**Female 200 mg/kg bw (oral)**	**Male 2 mg/kg bw (i.v.)**
Blood cells	0.0175	0.0067	2.3450	1.5770	0.0158
Plasma	0.0020	0.0013	0.2989	0.2963	0.0025
Carcass	0.0021	0.0011	0.1794	0.2377	0.0020
Heart	0.0024	n.c.	0.2643	0.3328	0.0023
Brain	0.0008	n.c.	0.0859	0.0922	0.0008
Kidneys	0.0064	0.0033	0.7975	0.6691	0.0067
Liver	0.0068	0.0034	0.8741	0.7720	0.0063
GIT	0.0141	0.0019	1.7290	1.1450	0.0167
Testes	0.0008	---	0.1020	---	0.0011
Ovaries	---	n.c.	---	0.2880	---
Uterus	---	0.0016	---	0.4599	---
Adrenal gland	0.0048	0.0032	0.4436	0.5606	0.0045
Harderian gland	0.0050	0.0022	0.4101	0.7218	0.0034
Thyroid	n.c.	n.c.	n.c.	n.c.	n.c.
Spleen	0.0030	0.0017	0.3586	0.3398	0.0032
Lung	0.0060	0.0035	0.6648	0.5663	0.0054
Eyes	0.0064	0.0133	0.5996	1.3430	0.0066
Skin	0.0018	0.0014	0.2354	0.9710	0.0024
Bone (femur)	n.c.	0.0023	0.1771	0.2794	0.0024
Fat (perirenal)	0.0018	n.c.	n.c.	0.1245	0.0030
Muscle (leg)	0.0008	0.0007	0.1206	0.2098	0.0012

n.c. : not calculated

Metabolites in urine and faeces

Flupyradifurone was metabolized to numerous metabolites, most of them being minor ones. The parent compound was the predominant part of the radioactivity in urine of male and female rats. In faeces samples of male rats, the metabolite BYI 02960-OH was more prominent than the parent compound. Two metabolites, BYI 02960-6-CNA and BYI 02960-hippuric acid were also prominent in male rats but not in females. All other identified and characterised metabolites represented a minor part of the dose. The metabolic profiles in urine and faeces were very similar for both sexes but male rats showed a higher rate of metabolite formation as compared to female animals. The metabolism results are summarized in the Table 15.

Table 15: Identified metabolites in urine and faeces after administration of [pyridinylmethyl-^{14}C] flupyradifurone to male and female rats.

Peak no.	Compound	% of the given dose				
		Male 2 mg/kg bw (oral)	**Female 2 mg/kg bw (oral)**	**Male 200 mg/kg bw (oral)**	**Female 200 mg/kg bw (oral)**	**Male 2 mg/kg bw (i.v.)**
26	Parent compound	40.9	77.7	39.6	65.5	47.3
7	BYI 2960-6-CNA	2.4	0.4	6.3	1.3	2.8
9	Hippuric acid	7.6	1.1	10.5	2.2	5.1
14	BYI 2960-OH-gluA (isomer 1)	1.8	0.4	1.6	0.7	1.6
16	BYI 2960-OH-gluA (isomer 3)	2.4	0.4	2.3	1.1	1.0
18	BYI 2960-des-difluoroethyl	2.2	2.4	1.8	2.7	1.7
24	BYI 2960-OH-SA	0.2	0.3	0.5	0.5	0.2
25	BYI 2960-OH	28.9	10.8	24.0	15.1	22.3
27	BYI 2960-iso-OH	0.4	<0.1	0.4	0.1	0.5
	Total identified	**86.9**	**93.7**	**86.9**	**89.2**	**82.5**
1	unknown 1	0.2	<0.1	0.3	0.1	0.3
2	unknown 2	0.7	0.1	1.1	0.3	0.6
3	unknown 3	0.5	0.1	0.5	0.1	0.4
4	unknown 4	0.3	0.1	0.5	0.3	0.3
5	unknown 5	0.3	0.1	0.2	0.1	0.1
5a	unknown 6	0.2	---	0.1	---	---
6	unknown 7	0.5	0.2	0.2	0.1	0.3
8	unknown 8	0.2	0.2	0.5	0.3	0.3
10	unknown 9	0.4	<0.1	0.3	0.1	0.2
11	unknown 10	0.1	0.1	0.4	0.3	0.2
12	unknown 11	0.9	0.3	0.8	0.6	0.5
13	unknown 12	0.2	0.1	0.2	0.2	0.3
15	unknown 13	0.4	0.3	0.4	0.2	0.3
17	unknown 14	0.2	0.2	0.3	0.2	0.2
19	unknown 15	---	0.1	---	0.2	---

20	unknown 16	0.5	0.1	0.6	0.1	0.5
21	unknown 17	---	---	---	---	0.1
22	unknown 18	---	---	<0.1	---	0.1
23	unknown 19	0.2	0.1	0.2	0.1	0.2
	Total characterized	**5.8**	**2.1**	**6.7**	**3.4**	**4.7**
	Total	**92.7**	**95.9**	**93.7**	**92.7**	**87.2**

Acceptability
The study was considered acceptable.

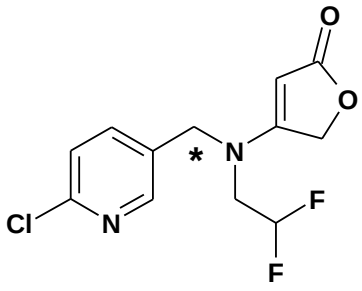
Conclusions
Flupyradifurone was completely absorbed in all test as demonstrated by the high bioavailability after oral administration and by the high amounts of radioactivity detected in urine and the body without GIT at sacrifice. Excretion of radioactivity was fast and mainly renal. Female rats showed slightly higher renal excretion rates of approx. 86% and 90% of the administered dose than males with approx. 76% of the dose. The major part of the dose (>84%) was excreted within 24 hours after treatment. The parent compound, three major and five minor metabolites were identified in all samples. Identification rates were high and amounted to approx. 83% - 94% of the given dose. The metabolic transformation of flupyradifurone was principally oxidative in nature and took place at least at 3 different structural positions.

Study 2

Characteristics

Reference	:	Koester, J; Weber, E. 2011	exposure	:	Single exposure by gavage
type of study	:	Toxicokinetic study (Quantitative whole body autoradiography)	dose	:	5 mg/kg bw
year of execution	:	2009-2011	vehicle	:	0.5% aqueous Tragacanth® suspension
test substance	:	[pyridinylmethyl-^{14}C]-Flupyradifurone, batch no. KATH 6264, chemical purity >99%, radiochemical purity >99%, 4.37 MBq/mg	GLP statement	:	yes
Route	:	oral	guideline	:	OECD 417
Species	:	Rat Wistar Hsd/Cpb: WU	acceptability	:	acceptable
group size	:	8 males + 1 control, 8 females + 1 control			

Study design
The study was performed according to OECD Guideline 417. Male and female rats in groups of eight rats were administered orally by gavage a single dose of [pyridinylmethyl-^{14}C]-flupyradifurone at target dose levels of 5 mg/kg bw after about 16 hours of fasting. A control animal of each gender was treated with 5 mg/kg bw non-radiolabelled flupyradifurone (Table 16).



* denotes the ^{14}C-label position

After administration of [pyridinylmethyl-^{14}C]-flupyradifurone, the rats were kept individually, which allowed for separate and quantitative collection of urine, faeces, and expired air. Urine was collected separately for each animal in intervals of 1h, 4 h, 8 h, 24 h, and every 24 h until 168 h. The radioactivity was determined by LSC. The faeces samples were collected every 24 h separately for each animal. The radioactivity was determined by combustion/LSC. Carbon dioxide and other volatiles from expired air were collected from four male and four female animals for the time ranges 0 - 24 h and 24 - 48 h. At sampling, the exact volume was determined, from which an aliquot was taken for the determination of radioactivity by LSC.
One animal of each gender was sacrificed using carbon dioxide at 1, 4, 8, 24, 48, 72, 120, and 168 hours post administration. The control animals were sacrificed 4 hours after dosing. The distribution of total radioactivity to, and elimination from blood, organs and tissues was analyzed qualitatively and quantitatively by whole-body autoradioluminography.

Table 16: Experimental groups for each dose level of [pyridinylmethyl-^{14}C]-flupyradifurone

Group no.	No. of animals	Route	Dose	Sacrifice time (hoiurs after last dose)
1	8 M	Oral (gavage)	5 mg/kg bw	1, 4, 8, 24, 48, 72, 120, 168
	1 M (control)	Oral (gavage)	5 mg/kg bw	4
2	8 F	Oral (gavage)	5 mg/kg bw	1, 4, 8, 24, 48, 72, 120, 168
	1 F (control)	Oral (gavage)	5 mg/kg bw	4

Results

Distribution

For almost all organs and tissues, maximum concentrations were reached at one hour after administration. At t_{max}, the organ/blood concentration ratios for many organs and tissues were in a range of approx. 0.8 - 1.2, *i.e.* the radioactivity concentration in these organs and tissues was in the range of ± 20% of the concentration in blood. The radioactivity administered with [pyridinylmethyl-^{14}C] flupyradifurone was rapidly cleared from the blood and distributed to the entire body, preferably to those organs or tissues responsible for metabolism (liver), excretion (kidney) and secretion (*e.g.* adrenal, thyroid, Harderian and salivary glands). The lowest values were measured for perirenal fat, the spinal cord, and brain.
The equivalent concentrations in blood, organs and tissues declined rapidly following approximately first order kinetics in the time range between 1 and 48 hours. At 24 hours after dosage, the concentrations in blood and also in most other tissues were below 5% of the maximum concentration, except nasal mucosa and vitreal body with approx. 7 - 11% of CEQ_{max}. At 48 hours after administration, the concentrations had declined to below 1% of the maximum concentration,

except vitreal body and nasal mucosa which exhibited approx. 3 - 8% of $CEQ_{max.}$ After seven days, the equivalent concentrations in almost all organs and tissues had fallen below the limit of quantification. Very low concentrations of radioactivity were still detectable in blood, renal medulla, liver, and lung. For all these organs, the concentration was lower than in the blood. The results are summarized in Table 17 (male rats) and Table 18 (female rats).

Table 17: Distribution of radioactivity in organs and tissues of male rats after a single oral dose of [pyridinylmethyl-^{14}C] flupyradifurone (5 mg/kg bw)

Organ or tissue	**concentration [mg a.s. equiv./kg]**							
	Time of sacrifice [hours after administration]							
	1 h	**4 h**	**8 h**	**24 h**	**48 h**	**72 h**	**120 h**	**168 h**
Blood	3.171	2.376	1.692	0.122	0.026	0.020	0.013	0.009
Liver	5.645	4.326	3.332	0.246	0.027	0.014	0.008	0.005
Renal cortex	4.441	3.267	2.565	0.146	0.016	0.007	0.005	<LOQ
Renal medulla	6.398	4.538	3.680	0.248	0.021	0.013	0.008	0.006
Kidney total	5.420	3.903	3.122	0.197	0.019	0.010	0.006	0.005
Brown fat	3.288	2.479	2.023	0.098	0.007	---	---	---
Perirenal fat	0.446	0.351	0.219	0.013	<LOQ	<LOQ	<LOQ	<LOQ
Skeleton muscle	3.246	2.283	1.608	0.096	0.005	<LOQ	<LOQ	<LOQ
Myocardium	4.426	3.155	2.250	0.142	0.007	<LOQ	<LOQ	<LOQ
Lung	2.450	2.168	1.373	0.082	0.015	0.013	0.005	0.005
Spleen	2.873	2.093	1.557	0.096	0.010	<LOQ	<LOQ	<LOQ
Pancreas	4.089	2.882	2.170	0.129	0.005	<LOQ	<LOQ	<LOQ
Bone marrow	2.770	1.825	1.391	0.083	---	---	---	---
Testis	2.251	2.198	1.466	0.092	<LOQ	<LOQ	<LOQ	<LOQ
Brain	1.214	0.852	0.619	0.036	<LOQ	<LOQ	<LOQ	<LOQ
Spinal cord	1.334	1.004	0.777	0.042	<LOQ	---	---	---
Pituitary gland	3.539	2.435	1.889	0.105	---	---	---	---
Pineal body	3.033	1.488	1.680	0.089	0.005	<LOQ	---	---
Adrenal gland	5.626	4.429	3.338	0.213	0.017	0.007	<LOQ	<LOQ
Thymus	3.173	2.328	1.646	0.096	<LOQ	<LOQ	<LOQ	<LOQ
Thyroid gland	4.252	3.284	2.219	0.143	0.012	<LOQ	---	---
Salivary gland	4.123	3.097	2.195	0.131	0.008	<LOQ	<LOQ	<LOQ
Nasal mucosa	1.409	1.477	1.210	0.250	0.120	0.077	0.043	0.037
Vitreal body	1.575	1.370	1.459	0.273	0.073	0.036	0.011	<LOQ
Harderian gland	4.469	3.083	2.415	0.151	0.014	---	---	---

--- : Organ or tissue was visible in the rat sections but not discernible in the radioluminograms
< LOQ : below limit of quantification

Table 18: Distribution of radioactivity in organs and tissues of female rats after a single oral dose of 5 [pyridinylmethyl-^{14}C] flupyradifurone (mg/kg bw)

Organ or tissue	**concentration [mg a.s. equiv./kg]**							
	Time of sacrifice [hours after administration]							
	1 h	**4 h**	**8 h**	**24 h**	**48 h**	**72 h**	**120 h**	**168 h**
Blood	4.155	3.211	2.005	0.090	0.016	0.012	0.010	0.007
Liver	7.451	5.878	3.778	0.171	0.016	0.008	0.006	<LOQ
Renal cortex	5.460	4.414	2.834	0.124	0.009	0.005	0.005	<LOQ
Renal medulla	8.212	7.083	4.877	0.222	0.013	0.009	0.007	0.008
Kidney total	6.836	5.748	3.856	0.173	0.011	0.007	0.006	0.006
Brown fat	3.229	3.174	1.811	0.095	<LOQ	---	---	---
Perirenal fat	0.413	0.502	0.315	0.019	<LOQ	<LOQ	<LOQ	<LOQ
Skeleton muscle	3.746	3.140	1.926	0.075	<LOQ	<LOQ	<LOQ	<LOQ
Myocardium	5.198	4.474	2.701	0.105	0.006	<LOQ	<LOQ	<LOQ
Lung	3.230	1.142	1.311	0.054	0.008	0.007	0.005	0.005
Spleen	3.742	2.975	1.932	0.077	<LOQ	<LOQ	<LOQ	<LOQ
Pancreas	5.245	3.961	2.626	0.100	<LOQ	---	<LOQ	<LOQ
Bone marrow	2.843	2.344	1.476	0.062	---	---	---	---
Ovary	3.688	3.101	1.915	0.077	0.006	<LOQ	<LOQ	---
Uterus	3.950	3.262	2.052	0.081	<LOQ	<LOQ	<LOQ	<LOQ
Brain	1.279	1.052	0.626	0.026	<LOQ	<LOQ	<LOQ	<LOQ
Spinal cord	1.348	1.074	0.692	0.029	<LOQ	---	---	---
Pituitary gland	4.306	3.793	2.293	0.082	---	---	---	---
Pineal body	3.427	2.797	1.914	0.075	---	---	---	---
Adrenal gland	7.289	5.669	4.029	0.201	0.017	0.008	<LOQ	<LOQ
Thymus	4.090	3.180	1.971	0.075	<LOQ	<LOQ	<LOQ	<LOQ
Thyroid gland	5.548	4.284	2.809	0.102	0.006	---	---	---
Salivary gland	5.411	4.177	2.642	0.102	0.006	<LOQ	<LOQ	<LOQ
Nasal mucosa	1.585	2.381	1.720	0.164	0.102	0.068	0.047	0.037
Vitreal body	2.303	2.065	1.376	0.248	0.062	0.024	0.009	0.008
Harderian gland	5.008	4.048	2.483	0.107	0.019	<LOQ	---	---

--- : Organ or tissue was visible in the rat sections but not discernible in the radioluminograms
< LOQ : below limit of quantification

Excretion

[Pyridinylmethyl-^{14}C] flupyradifurone was excreted rapidly and completely within ca. 48 hours. The major part of the radioactivity (up to 85% in male and up to 93% in female rats) was excreted with urine and the minor part (about 20% for male and ca. 8% for female rats) with the faeces. One day after dosing >90% of the dose was excreted and the excretion was almost complete after two days.

The expiration of ^{14}C-carbon dioxide and other ^{14}C-labelled volatiles was tested with male and female animals for a test period of 48 hours. Less than 0.1% of the administered dose was expired during the sampling period. This demonstrates the stability of the pyridinylmethyl-^{14}C label with

regard to possible formation of volatile products. The excretion behavior is summarized in Table 19 (male rats) and Table 20 (female rats).

Table 19: Excretion of radioactivity in urine, faeces and expired air of male rats after a single oral administration of [pyridinylmethyl-^{14}C] flupyradifurone (5 mg/kg bw)

	% of the given dose							
	Time of sacrifice [h after administration]							
	1	**4**	**8**	**24**	**48**	**72**	**120**	**168**
Exhaled air								
24 h					0.06	0.06	0.07	0.06
48 h					0.07	0.08	0.09	0.09
Urine								
1 h	2.38							
4 h		22.39	12.55	14.62	2.71	2.16	2.03	11.86
8 h			42.07	45.19	23.14	30.59	44.03	29.61
24 h				78.07	68.52	79.11	76.13	75.12
48 h					72.34	84.54	79.85	79.67
72 h						85.12	80.35	80.05
96 h							80.53	80.19
120 h							80.70	80.29
144 h								80.38
168 h								80.50
Faeces								
24 h	*	*	*	14.93	23.63	17.37	20.26	17.49
48 h					26.75	20.34	22.49	21.59
72 h						20.54	22.74	21.92
96 h							22.82	21.98
120 h							22.85	22.04
144 h								22.05
168 h								22.12
Sum total	**2.38**	**22.39**	**42.07**	**92.99**	**99.17**	**105.74**	**103.64**	**102.71**

* : faeces not collected

Table 20: Excretion of radioactivity in urine, faeces and expired air of female rats after a single oral administration of [pyridinylmethyl-^{14}C] flupyradifurone (5 mg/kg bw)

	Percent of radioactive dose administered							
	Time of sacrifice [h after administration]							
	1	**4**	**8**	**24**	**48**	**72**	**120**	**168**
Exhaled air								
24 h					0.02	0.02	0.02	0.02
48 h					0.03	0.03	0.02	0.03
Urine								
1 h	7.19							
4 h		9.79	9.69	12.35	11.53	3.55	25.51	4.35
8 h			32.74	33.40	27.68	49.84	26.53	4.41
24 h				86.47	84.60	86.82	90.42	78.34
48 h					89.90	88.76	92.95	88.13
72 h						88.94	93.16	89.93
96 h							93.23	91.15
120 h							93.28	91.61
144 h								91.95
168 h								92.46
Faeces								
24 h	*	*	*	10.62	8.61	8.75	6.70	4.54
48 h					10.58	9.75	7.82	6.98
72 h						9.78	7.89	7.36
96 h							7.92	7.47
120 h							7.93	7.49
144 h								7.52
168 h								7.53
Sum total	**7.19**	**9.79**	**32.74**	**97.09**	**100.50**	**98.76**	**101.23**	**100.03**

* : faeces not collected

Acceptability

The study was considered acceptable.

Conclusions

[pyridinylmethyl-^{14}C] flupyradifurone was readily absorbed from the gastrointestinal tract of male and female rats after single oral administration of 5 mg/kg bw. The radioactivity was distributed throughout the body immediately after administration but with a clear preference to liver and kidney. The radioactivity was quickly excreted, mainly with the urine (>80%). More than 90% of the administered dose was excreted within 24 hours, and 95% to 100% after 48 hours. Based on the results of this study, any accumulation or significant retention of [pyridinylmethyl-^{14}C] Flupyradifurone in male and female rats can be excluded.

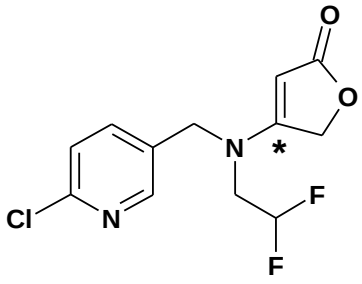
Study 3

Characteristics

Reference	:	Weber, E. 2011	exposure	:	Single exposure by gavage
type of study	:	Toxicokinetic study (ADME in rat)	dose	:	2 mg/kg bw
year of execution	:	2009-2011	vehicle	:	0.5% aqueous Tragacanth® suspension
test substance	:	[Furanone-4-^{14}C]-Flupyradifurone, batch no. KATH 6279, chemical purity >99%, radiochemical purity >99%, 3.94 MBq/mg	GLP statement	:	yes
Route	:	oral	guideline	:	OECD 417
Species	:	Rat Wistar Hsd/Cpb: WU	acceptability	:	acceptable
group size	:	4/sex			

Study design

The study was performed according to OECD Guideline 417. Male and female rats in groups of four rats per sex were administered orally by gavage a single dose of flupyradifurone suspended in 0.5% aqueous Tragacanth® (2 mL) at target dose levels of 2 mg/kg bw (Table 21). Flupyradifurone was radiolabelled with ^{14}C in the 4-position of the furanone ring of the molecule.



* denotes the ^{14}C-label position

Urine and faeces were collected for the time range between dosing and sacrifice and the animals were sacrificed after 168 hours. Plasma levels of radioactivity were followed from each animal for 23 timepoints. Total radioactivity which included the test compound and metabolites was determined in excreta and in organs and tissues collected at sacrifice. Metabolism was investigated in urine and faeces.

Samples were analyzed by radio HPLC, radio TLC, LC-MS and NMR methods. Toxicokinetic parameters were calculated with the software TOPFIT version 2.0.

Table 21: Experimental groups for each dose level of [Furanone-4-^{14}C] flupyradifurone

Group no.	No. of animals	Route	Dose	Sacrifice time (hours after last dose)
1	4 M	Oral (gavage)	2 mg/kg bw	168
2	4 F	Oral (gavage)	2 mg/kg bw	168

Results

Recovery

The total recovery for the orally administered test was approximately between 96% and 102% of the administered dose in the excreta and the body of male and female rats.

Table 22: Recovery of radioactivity in urine, faeces, gastrointestinal tract and the body following a single oral dose of [furanone-4-^{14}C] flupyradifurone (2 mg/kg bw)

	% of administered dose	
	Male	**Female**
Faeces	16.59	10.38
Urine	78.96	91.37
Sum of excreta	**95.56**	**101.0**
Body without GIT	0.48	0.17
GIT	0.02	0.01
Total Body	**0.49**	**0.18**
Balance	**96.05**	**101.90**

Absorption

The total amount of absorbed [furanone-4-^{14}C] flupyradifurone is at least 79% for males and 91% for females since the sum of the dose in urine and body excluding GIT is 79% and 91%, respectively. The absorption started immediately after dosing as could be seen from the quick increase of radioactivity in plasma.

Table 23: Time course of radioactivity in the plasma following oral dosing of [furanone-4-^{14}C] flupyradifurone

	Concentration [mg a.s. equiv./kg]	
Time [h]	**Male, 2mg/kg**	**Female, 2mg/kg**
0.17	0.2883	0.4562
0.33	0.7373	1.1720
0.67	1.2720	1.7870
1	1.4180	1.8570
1.5	1.4570	1.9120
2	1.4460	1.8610
3	1.3570	1.7410
4	1.2550	1.5880
6	1.0220	1.2990
8	0.8145	1.0140
24	0.0621	0.0515
28	0.0462	0.0326
32	0.0375	0.0264

48	0.0168	0.0091
52	0.0162	0.0079
56	0.0149	0.0076
72	0.0113	0.0062
96	0.0087	n.d.
120	0.0066	n.d.
144	0.0048	n.d.
152	0.0048	n.d.
168	n.d.	n.d.

Distribution and plasma kinetics

[Furanone-4-^{14}C] flupyradifurone was quickly absorbed and distributed. The C_{max} was reached at approximately 1.5 hour after administration. The plasma concentrations declined to 50% of C_{max} within 8 hours and to 2 - 3% of C_{max} within 24 hours for both male and female rats. At time of sacrifice, 168 hours after administration, the plasma concentrations were below LOQ for both male and female rats. Male rats exhibited about two-fold higher concentrations than females in the time range ≥48 hours. This behavior is probably caused by higher amounts of [furanone-4-^{14}C] flupyradifurone metabolized to C1 and C2 fragments being incorporated into biomolecules or exhaled as $^{14}CO_2$ in males. At the time of sacrifice, 168 hours after administration, the plasma concentration was below LOQ for both male and female animals (Table 23).

The mean values of total radioactivity were used for a toxicokinetic modeling using the TOPFIT software. A good fit could only be achieved with a three compartment model due to the obvious biphasic nature of the plasma curves. The modeling was performed for the time range between 0 and 152 hours for males and 0 to 72 hours for females. The biokinetic parameters were very similar for both sexes but females exhibited a somewhat shorter mean residence time than males. The area under curve (AUC) was 16.0 mg/kg*h for male and 18.2 mg/kg*h for female rats. The model confirmed a biphasic decline of plasma radioactivity with a short first half life of elimination of approximately 3 hours which is probably mainly attributable to the elimination of the parent compound and a significantly longer second half-life of about 53 hours, probably related to the incorporation of radioactivity into biomolecules. The corresponding concentration-time curves of the modeled and the measured data are shown in Figure 2 below.

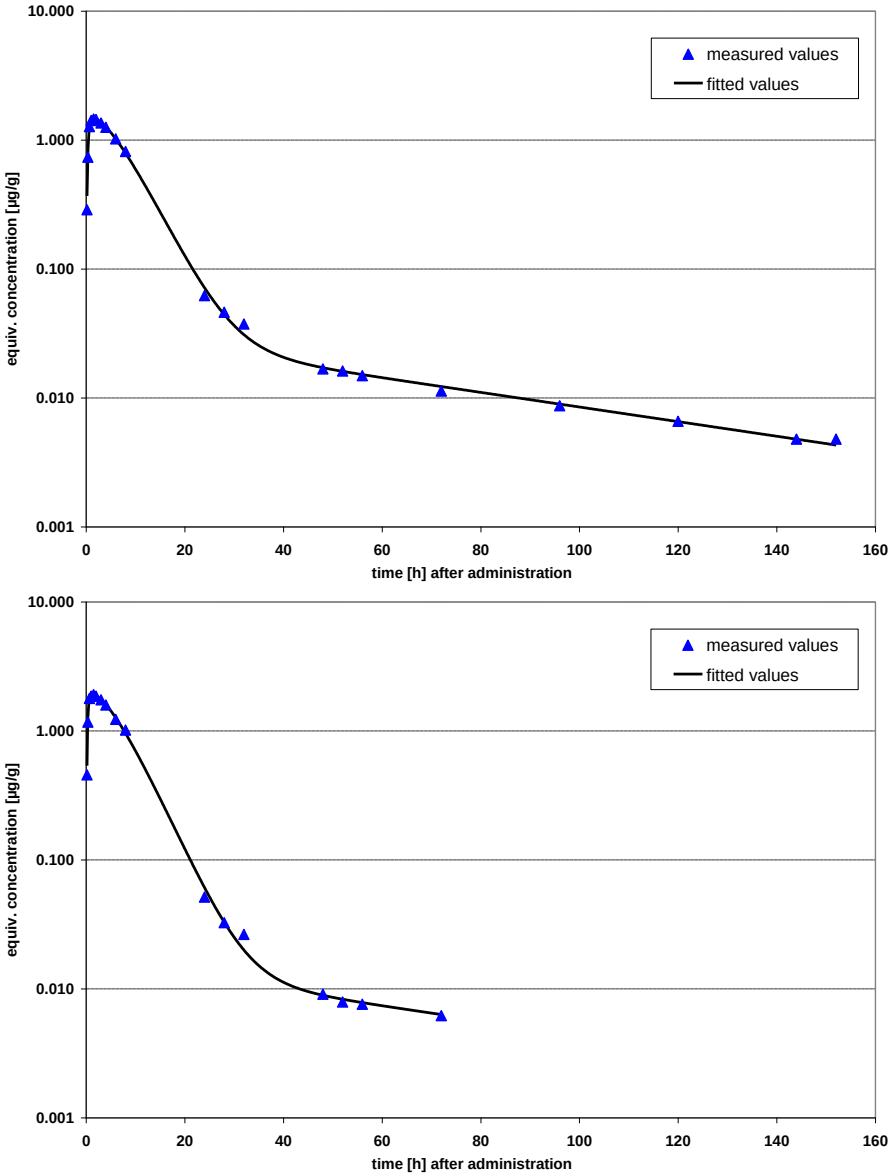


Figure 2: Timecourse of radioactivity in the plasma following a single oral dose of [furanone-4-^{14}C] flupyradifurone (2 mg/kg bw). (upper curve: male rats; lower curve: female rats)

Excretion

Approximately 79% and 91% of the administered dose was detected in the urine of male and female rats, respectively, and about 75% and 87% was excreted within 24 hours. The major route of excretion is therefore by urine, and is somewhat higher in female rats compared to male rats. Faecal excretion accounted for about 17% of the given dose in male rats, and the major part of faecal residues, approximately 15% of the dose, was excreted within the first 24 hours. In female rats approximately 10% of the dose is found in faeces where about 9% of the dose is excreted in the first 24 hours. The cumulative excretion results are summarized in Table 24.

Table 24: Cumulative excretion of radioactive residues via urine and faeces after oral administration of [furanone-4-^{14}C] flupyradifurone to male and female rats

Time [h]	**% of dose**	
Faeces	**Male, 2 mg/kg**	**Female, 2 mg/kg**
24	14.76	9.45
48	16.40	10.29
72	16.50	10.33
96	16.54	10.35
120	16.56	10.36
144	16.57	10.37
168	16.59	10.38
Urine		
4	8.74	6.52
8	33.86	18.32
12	39.55	---
24	75.04	87.31
48	78.40	90.44
72	78.75	90.89
96	78.86	91.14
120	78.91	91.24
144	78.94	91.33
168	78.96	91.37
Total	**95.56**	**101.70**

Radioactive residues in organs and tissues at sacrifice

Approx. 0.5% of the dose was detected in the body of male rats at sacrifice 168 hours after oral administration; only a very minor amount of 0.02% was found in the GIT. Residual concentrations of radioactivity were in the range of 0.0025 to 0.0336 mg/kg. The lowest concentration was detected in the plasma and the highest value in the thyroid. But basically, levels for most organs and tissues were very similar and in the range of approximately 0.005 to 0.01 mg/kg. For female rats, approx. 0.2% of the dose was still present in the body at sacrifice and only a negligible proportion of 0.01% in the GIT. Residual concentrations of radioactivity were in the range of 0.0012 to 0.0131 mg/kg. The lowest concentration was detected in the plasma and the highest value in the thyroid. As for males, levels for most organs and tissues were very similar and in the range of approximately 0.002 to 0.005 mg/kg. For most organs and tissues the residues in males were 2 - 3 times higher as compared to females. This difference is also very likely caused by the higher rate of incorporation of radioactivity into biomolecules

The equivalent concentrations of the residual radioactivity in organs and tissues at sacrifice are presented in Table 25.

Table 25: Total radioactive residues in organs and tissues at sacrifice after oral administration of [furanone-4-^{14}C] flupyradifurone to male and female rats.

Organ or tissue	**concentration [mg a.s. equiv./kg]**

	Male, 2 mg/kg bw	**Female, 2 mg/kg bw**
Blood cells	0.0083	0.0038
Plasma	0.0025	0.0012
Carcass	0.0079	0.0031
Heart	0.0065	0.0029
Brain	0.0072	0.0033
Kidneys	0.0104	0.0045
Liver	0.0128	0.0081
Testes	0.0059	---
Ovaries	---	0.0039
Uterus	---	0.0035
Adrenal gland	0.0200	0.0114
Harderian gland	0.0241	0.0091
Thyroid gland	0.0336	0.0131
Spleen	0.0081	0.0032
Lung	0.0075	0.0048
Eye	0.0053	0.0048
Skin	0.0111	0.0047
Bone femur	0.0085	0.0049
Fat perirenal	0.0118	0.0058
Muscle leg	0.0069	0.0023

Metabolites in urine and faeces

Flupyradifurone was metabolized to approximately 20 metabolites. The parent compound represented the predominant part of the radioactivity in urine while in faeces the amounts of the metabolite BYI 02960-OH were more or less similar. All other identified and characterised metabolites represented a minor part of the dose. The metabolic profiles in urines and faeces were very similar for both sexes but male rats exhibited a higher rate of metabolite formation as compared to female animals. A summary of the distribution of the parent compound and metabolites in the excreta is provided in Table 26.

Table 26: Amounts of metabolites in the excreta of rats after administration of [furanone-4-^{14}C] flupyradifurone

Compound	**% of the total dose administered**	
	Male: 2 mg/kg	**Female: 2 mg/kg**
Parent compound	54.68	75.96
difluoroethyl-amino-furanone	3.49	0.96
BYI 02960-OH-gluA (isomer 1)	1.13	0.49
BYI 02960-OH-gluA (isomer 3)	2.17	1.01
BYI 02960-des-difluoroethyl	2.12	3.35
BYI 02960-OH-SA	0.24	0.34

BYI 02960-OH	20.60	13.25
BYI 02960-iso-OH	0.26	0.07
Total identified	**84.70**	**95.42**
unknown 1*	5.30	2.45
unknown 2	0.46	0.19
unknown 3	0.57	0.05
unknown 4	0.03	---
unknown 5	0.40	0.12
unknown 6	0.05	0.16
unknown 7	0.02	0.09
unknown 8	0.03	0.11
unknown 9	0.24	0.44
unknown 10	0.02	0.02
unknown 11	0.25	0.23
unknown 12	---	0.14
unknown 13	0.53	0.02
unknown 14	0.10	0.06
unknown 15	0.02	---
Total characterized	**8.00**	**4.08**
Total	**95.55**	**101.75**

* unknown 1 from urine of male rats was further characterized by TLC analysis of the isolated metabolite fraction.

Acceptability
The study was considered acceptable.

Conclusions
Flupyradifurone was completely absorbed as demonstrated by the high amounts of radioactivity detected in urine and the body without GIT at sacrifice (>79% of the administered dose). Excretion of radioactivity was fast and mainly renal. Female rats showed a slightly higher renal excretion rate (91%) than male rats (78%). The major part of the dose (>89% for males and >96% for females) was excreted within 24 hours after treatment. The parent compound, one major and six minor metabolites were identified in all samples. Identification rates were high and amounted to >90% of the given dose. The metabolic transformation of flupyradifurone was principally oxidative in nature and took place at least at 3 different structural positions. Basically, male rats showed a higher rate of metabolism with only ca. 55% of unchanged parent compound found in excreta whereas 76% of unchanged BYI 02960 was found in the excreta of female rats. But the metabolic patterns were very similar in males and females.

Study 4

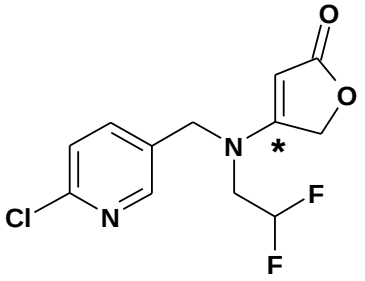
Characteristics

Reference	:	Koester, J. 2011	exposure	:	Single exposure by gavage
type of study	:	Toxicokinetic study (Quantitative	dose	:	5 mg/kg bw

		whole body autoradiography)			
year of execution	:	2008-2010	vehicle	:	0.5% aqueous Tragacanth® suspension
test substance	:	[furanone-4-^{14}C] flupyradifurone, batch no. KATH 6109 (test 1 and 2, males) and KATH 6252 (test 3, females), chemical purity >99%, radiochemical prurity >99%, 3.94 MBq/mg	GLP statement	:	yes
Route	:	oral	guideline	:	OECD 417
Species	:	Rat Wistar Hsd/Cpb: WU	acceptability	:	acceptable
group size	:	8 males + 1 control, 8 females + 1 control			

Study design

The study was performed according to OECD Guideline 417. Male and female rats in groups of eight rats were administered orally by gavage a single dose of [furanone-4-^{14}C] flupyradifurone at target dose levels of 5 mg/kg bw after ca. 16 hours of fasting. A control animal of each gender was treated with 5 mg/kg bw non-radiolabelled flupyradifurone (Table 27).



* denotes the ^{14}C-label position

After administration of [furanone-4-^{14}C] flupyradifurone, the rats were kept individually, which allowed for separate and quantitative collection of urine, faeces, and expired air. Urine was collected separately for each animal in intervals of 1, 4, 8, 24 hours, and every 24 hours until 168 hours. The radioactivity was determined by LSC. The faeces samples were collected every 24 hours separately for each animal. The radioactivity was determined by combustion/LSC. Carbon dioxide and other volatiles from expired air were collected from four male and four female animals for the time ranges 0 - 24 h and 24 - 48 h. At sampling, the exact volume was determined, from which an aliquot was taken for the determination of radioactivity by LSC.

One animal of each gender was sacrificed using carbon dioxide at 1, 4, 8, 24, 48, 72, 120, and 168 hours post administration, respectively. The control animals were sacrificed 4 hours after dosing. The distribution of total radioactivity to, and elimination from blood, organs and tissues was analyzed qualitatively and quantitatively by whole-body autoradioluminography.

Table 27: Experimental groups for each dose level of [furanone-4-^{14}C] flupyradifurone

Group no.	No. of animals	Route	Dose	Sacrifice time (hoiurs after last dose)
1	8 M	Oral (gavage)	5 mg/kg bw	1, 4, 8, 24, 48, 72, 120, 168

	1 M (control)	Oral (gavage)	5 mg/kg bw	4
2	8 F	Oral (gavage)	5 mg/kg bw	1, 4, 8, 24, 48, 72, 120, 168
	1 F (control)	Oral (gavage)	5 mg/kg bw	4

Results

Distribution

The distribution in male and female rats was fast; maximum concentrations were reached at one hour after administration. The maximum concentrations in several organs and organs, *i.e.* liver, kidney, brown fat, myocardium, almost all glandular organs and the olfactory bulb were higher than in blood. The administered dose of [furanone-4-^{14}C] flupyradifurone was rapidly absorbed and distributed to the entire body, preferably to those organs or tissues that are responsible for metabolism. The equivalent concentrations in blood, organs and tissues declined following a biphasic kinetics showing a fast phase from 1 to 24 hours (females: 1 to 48 hours) and a slower decline from 24 to 168 hours (female: 48 to 168 hours). After 168 hours the radioactivity concentrations in blood and all organs and tissues had declined significantly but were still higher in almost all of the organs than in blood. Slightly lower values were detected in the skeleton muscle, myocardium, lung, pancreas, pineal and vitreal body. Highest values were in perirenal fat (males), uterus and nasal mucosa. For all these organs, the concentration was lower than in the blood. The results are summarized in Table 28 (male rats) and Table 29 (female rats).

Table 28: Distribution of radioactivity in organs and tissues of male rats after a single oral dose of [furanone-4-^{14}C] flupyradifurone (5 mg/kg bw)

Organ or tissue	**Equivalent concentration CEQ [µg a.s. equiv./g]**							
	Time of sacrifice [hours after administration]							
	1 h	**4 h**	**8 h**	**24 h**	**48 h**	**72 h**	**120 h**	**168 h**
Blood	3.896	2.737	1.634	0.086	0.039	0.039	0.024	0.017
Liver	6.860	4.734	2.976	0.303	0.168	0.150	0.080	0.036
Renal cortex	5.743	4.346	2.247	0.146	0.056	0.059	0.035	0.021
Renal medulla	7.529	6.423	3.037	0.205	0.071	0.075	0.042	0.027
Kidney total	6.636	5.384	2.642	0.176	0.063	0.067	0.038	0.024
Brown fat	4.286	2.554	1.665	0.145	0.110	0.085	0.059	0.030
Perirenal fat	0.552	0.418	0.258	0.033	0.020	0.012	0.031	0.018
Skeleton muscle	3.733	2.896	1.426	0.073	0.026	0.029	0.018	0.014
Myocardium	5.274	3.544	2.131	0.097	0.037	0.035	0.026	0.016
Lung	2.794	2.555	1.250	0.077	0.030	0.028	0.017	0.013
Spleen	3.754	2.878	1.464	0.103	0.056	0.057	0.035	0.019
Pancreas	4.960	3.144	2.003	0.117	0.044	0.046	0.026	0.016
Bone marrow	3.320	2.395	1.309	0.182	0.104	0.081	0.041	0.019
Testis	2.493	2.066	1.229	0.080	0.044	0.042	0.030	0.021
Brain	1.372	1.055	0.715	0.081	0.066	0.060	0.052	0.033
Spinal cord	1.624	1.253	0.788	0.108	0.080	0.079	0.067	0.042
Pituitary gland	4.639	2.890	1.766	0.123	0.077	0.073	0.040	0.026
Pineal body	3.763	2.534	1.506	0.105	---	0.048	0.031	0.014

Adrenal gland	7.487	4.793	2.914	0.358	0.143	0.131	0.072	0.039
Thymus	3.869	2.635	1.587	0.140	0.106	0.097	0.045	0.022
Thyroid gland	5.391	3.848	2.280	0.304	0.253	0.174	0.080	0.036
Salivary gland	5.224	3.691	2.237	0.138	0.063	0.059	0.037	0.020
Nasal mucosa	1.943	1.880	1.727	0.592	0.419	0.401	0.179	0.164
Vitreal body	1.785	1.875	1.301	0.197	0.091	0.059	0.049	0.015
Harderian gland	5.384	4.421	---	0.443	0.367	0.313	0.090	0.050
Olfactory bulb	5.823	4.097	2.875	0.547	0.434	0.371	0.128	0.052

--- : Organ or tissue was visible in the rat sections but not discernible in the radioluminograms

Table 29: Distribution of radioactivity in organs and tissues of female rats after a single oral dose of [furanone-4-^{14}C] flupyradifurone (5 mg/kg bw)

Organ or tissue	**Equivalent concentration CEQ [µg a.s. equiv. /g]**							
	Time of sacrifice [hours after administration]							
	1 h	**4 h**	**8 h**	**24 h**	**48 h**	**72 h**	**120 h**	**168 h**
Blood	4.250	2.587	1.341	0.061	0.009	0.007	0.006	0.006
Liver	7.757	4.648	2.705	0.143	0.024	0.018	0.011	0.009
Renal cortex	5.937	3.373	2.027	0.094	0.015	0.010	0.008	0.008
Renal medulla	7.971	5.223	3.525	0.156	0.018	0.015	0.012	0.011
Kidney total	6.954	4.298	2.776	0.125	0.016	0.012	0.010	0.009
Brown fat	3.604	2.221	0.938	0.089	0.018	0.019	0.010	0.008
Perirenal fat	0.654	0.365	0.179	0.014	<LOQ	<LOQ	<LOQ	<LOQ
Skeleton muscle	4.498	2.441	1.398	0.058	0.006	<LOQ	<LOQ	<LOQ
Myocardium	5.606	3.174	1.826	0.075	0.009	0.006	0.006	0.006
Lung	3.714	1.997	1.036	0.050	0.006	0.005	0.004	<LOQ
Spleen	4.238	2.273	1.313	0.068	0.013	0.010	0.007	0.007
Pancreas	5.546	3.081	1.691	0.082	0.010	0.006	0.005	---
Bone marrow	3.187	2.295	1.002	0.075	0.019	0.013	0.008	0.006
Ovary	4.143	2.246	1.447	0.075	0.012	0.011	0.006	<LOQ
Uterus	4.305	2.670	1.414	0.068	0.018	0.013	0.010	0.008
Brain	1.376	0.811	0.462	0.023	0.007	0.007	0.006	0.009
Spinal cord	1.464	0.941	0.495	0.029	0.009	0.009	0.008	0.011
Pituitary gland	4.838	2.950	1.516	0.076	0.012	---	---	---
Pineal body	3.176	2.444	1.244	0.053	---	---	---	---
Adrenal gland	7.927	4.739	2.498	0.172	0.057	0.036	0.021	0.026
Thymus	4.287	2.577	1.319	0.072	0.021	0.019	0.010	0.007
Thyroid gland	6.024	3.585	1.806	0.127	0.050	0.032	0.026	0.026
Salivary gland	5.732	3.450	1.833	0.085	0.013	0.010	0.007	0.006
Nasal mucosa	1.689	1.622	0.751	0.390	0.250	0.203	0.160	0.117
Vitreal body	1.831	1.615	1.110	0.212	0.041	0.032	0.013	0.009
Harderian gland	6.238	3.491	2.034	0.120	0.021	0.011	0.014	---
Olfactory bulb	---	---	2.393	0.194	0.068	0.064	0.021	0.017

--- : Organ or tissue was visible in the rat sections but not discernible in the radioluminograms

<LOQ : below limit of quantitation

Excretion

[furanone-4-^{14}C] flupyradifurone was excreted rapidly and completely within ca. 48 hours. The major part of the radioactivity (up to 81% in male and up to 88% in female rats) was excreted with urine and the minor part (about 16% for male and about 7% for female rats) with faeces. The exhalation of ^{14}C-carbon dioxide was tested with male and female animals for a period of 48 hours. Between 2.02% and 3.25% (males) and 0.50% and 0.96% (females) of the administered radioactivity was exhaled during this period. This demonstrated that for a small portion of the dose (higher in males than in females) the furanone ring of the molecule obviously underwent biotransformation to C1- and C2-fragments and the terminal product $^{14}CO_2$.

Table 30: Excretion of radioactivity in urine, faeces and expired air of male rats after a single oral administration of [furanone-4-^{14}C] flupyradifurone (5 mg/kg bw)

	% of the given dose							
	Time of sacrifice [h after administration]							
	1	**4**	**8**	**24*)**	**48**	**72**	**120**	**168**
Exhaled air								
24 h					2.33	2.74	2.54	1.71
48 h					2.83	3.25	3.01	2.02
Urine								
1 h	4.30							
4 h		13.29	4.49	6.27	3.44	3.56	6.76	19.80
8 h			41.15	41.14	27.44	28.12	48.81	50.47
24 h				45.15	73.40	69.05	74.38	77.72
48 h					75.77	71.95	77.50	80.11
72 h						72.26	77.87	80.39
96 h							78.00	80.48
120 h							78.09	80.52
144 h								80.56
168 h								80.59
Faeces								
24 h	*	*	*	41.20	13.54	13.41	10.80	12.76
48 h					15.09	15.48	13.07	13.68
72 h						15.84	13.30	13.76
96 h							13.36	13.79
120 h							13.39	13.80
144 h								13.82
168 h								13.83
Sum total	**4.30**	**13.29**	**41.15**	**86.35**	**93.69**	**91.35**	**94.50**	**96.44**

*) : Because of the untypical excretion behavior (41.20% in faeces after 24 hours), this animal was not considered

* : faeces not collected

Table 31: Excretion of radioactivity in urine, faeces and expired air of female rats after a single oral administration of [furanone-4-^{14}C] flupyradifurone (5 mg/kg bw)

	Percent of radioactive dose administered							
	Time of sacrifice [h after administration]							
	1	**4**	**8**	**24**	**48 *)**	**72**	**120**	**168**
Exhaled air								
24 h					0.50	0.63	0.66	0.82
48 h					0.58	0.71	0.77	0.96
Urine								
1 h	3.82							
4 h		32.45	35.72	38.42	36.43	42.56	28.90	35.24
8 h			60.08	54.58	53.23	48.11	35.08	54.17
24 h				87.30	54.81	90.78	76.99	84.22
48 h					57.56	92.71	80.60	86.19
72 h						92.89	81.22	86.49
96 h							81.60	86.66
120 h							82.01	87.88
144 h								87.98
168 h								88.04
Faeces								
24 h	*	*	*	6.57	33.30	4.32	4.94	4.69
48 h					34.84	5.18	6.46	5.60
72 h						5.22	6.58	5.75
96 h							6.73	5.79
120 h							6.76	5.81
144 h								5.83
168 h								5.84
Sum total	**3.82**	**32.45**	**60.08**	**93.87**	**92.98**	**98.82**	**89.54**	**94.84**

*) : Because of the untypical excretion behavior (41.20% in faeces after 24 hours), this animal was not considered
* : faeces not collected

Acceptability
The study was considered acceptable.

Conclusions
[furanone-4-^{14}C] flupyradifurone was readily absorbed from the gastrointestinal tract of male and female rats and evenly distributed throughout the rat bodies immediately after the single oral administration of 5 mg/kg bw. [furanone-4-^{14}C] flupyradifurone related radioactivity was rapidly cleared from blood and distributed primarily to those organs or tissues that are responsible for metabolism, excretion and secretion. The excretion of radioactivity *via* urine and faeces was almost completed two days after administration with more than 80% of the dose detected in the urine of both sexes.
The detection of $^{14}CO_2$ in the exhaled air during a sampling period of 48 hours (up to 3.25% in male and 0.96% in female rats) demonstrated that for a small portion of the dose the furanone ring obviously underwent biotransformation to C1- and C2-fragments and the terminal product $^{14}CO_2$. Peak concentrations of radioactivity for almost all organs and tissues were reached already one hour

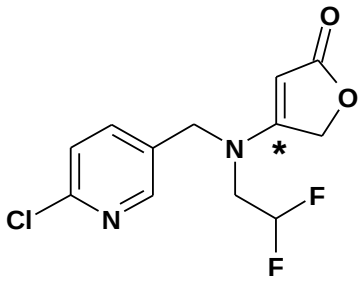
after dosing. From then onwards, the concentrations declined following a biphasic kinetics. The second slower decline phase, which started after 24 hours in males and after 48 hours in females, is probably due to the formation of small carbon units (C1- or C2-fragments) that entered the carbon pool used for the biosynthesis of endogenous compounds. This is presumably also the reason that at the end of the test (day 7) low radioactive residues were still measured in almost all organs and tissues.
The terminal residues were always by a factor of 1.4 to 4.7 higher in males than in females. A similar ratio of approx. 3 was also found for the formation of $^{14}CO_2$ in males as compared to females. The reason is probably a gender specific quantitative difference in metabolism leading to more C1- and C2-fragments and also a higher incorporation of these components into the endogenous carbon pool.
From the results of this study, any significant accumulation or retention of [furanone-4-^{14}C]BYI 02960 in male and female rats can be excluded.

Study 5

Characteristics

Reference	:	Koester, J. 2011	exposure	:	Single exposure by gavage
type of study	:	Toxicokinetic study (Metabolism in Organs and Tissues in rat)	dose	:	3 mg/kg bw
year of execution	:	2010-2011	vehicle	:	0.5% aqueous Tragacanth® suspension
test substance	:	[Furanone-4-^{14}C]-Flupyradifurone, batch no. KML 9029, chemical purity >99%, radiochemical purity >99%, 3.94 MBq/mg	GLP statement	:	yes
Route	:	oral	guideline	:	OECD 417
Species	:	Rat Wistar Hsd/Cpb: WU	acceptability	:	acceptable
group size	:	4/sex			

Study design
The study was performed according to OECD Guideline 417. Male and female rats in groups of four rats were administered orally by gavage a single dose of flupyradifurone at target dose levels of 3 mg/kg bw (Table 32). Flupyradifurone was radiolabelled with ^{14}C in the 4-position of the furanone ring of the molecule.



* denotes the ^{14}C-label position

Urine and faeces were collected for the time range between dosing and sacrifice; the animals were sacrificed after 6 hours. Total radioactivity which included the test compound and metabolites was determined at sacrifice in urine, plasma, liver, kidney, muscle (leg) and fat (perirenal). The metabolism was investigated in urine and plasma samples and in extracts of liver, kidney, muscle and fat. Samples were analyzed by radio HPLC, radio TLC, LC-MS and NMR methods.

Table 32: Experimental groups for each dose level of [furanone-4-^{14}C] flupyradifurone

Group no.	No. of animals	Route	Dose	Sacrifice time (hours after last dose)
1	4 M	Oral (gavage)	5 mg/kg bw	6
2	4 F	Oral (gavage)	5 mg/kg bw	6

Results

Recovery

The total recovery for the orally administered test was between approximately 98% and 101% of the administered dose in urine, organs and tissues, skin and the combined GIT and faeces samples of male and female rats (Table 33). In male and female rats, approx. 40% of the dose was detected in organs and tissues, the highest amount was found in the residual carcass (approx. 24 - 27%), followed by skin, liver, muscle, kidney, and fat for which the lowest values were measured.(approx. 0.06 - 0.09%). For the 0 - 6 h collection period, the renal excretion in female rats was by a factor of approx. 1.17 higher than in male rats.

Table 33: Recovery of radioactivity in urine, plasma and organs 6 h after a single oral dose of [furanone-4-^{14}C] flupyradifurone (3 mg/kg bw)

Percent of given dose (mean values)		
	Male	**Female**
Urine	36.64	42.82
Plasma	0.70	0.55
Carcass	24.00	26.73
Kidneys	0.73	1.04
Liver	3.56	3.61
GIT + faeces	23.63	12.57
Skin	9.18	9.42
Fat (perirenal)	0.06	0.09
Muscle (leg)	2.09	1.26
Balance	**100.60**	**98.09**

Metabolism:

Table 34 shows the amount of flupyradifurone and its metabolites after six hours in urine of male and female rats as percentage of the given dose. In urine, the parent compound was the dominating radioactive component (approximately 22% of the dose in males and 38% in females).
The concentrations of radioactivity in plasma can be found in Table 35, in liver in Table 36, in kidney in Table 37, in muscle in Table 38 and in fat tissue in Table 39. The highest radioactivity concentrations were detected in the liver (approx. 2.9 mg/kg for both sexes) and kidney (approx. 2.7 mg/kg for males and 4.3 mg/kg for females) as the main metabolic and excretory organs. The residue concentrations for plasma and the other tissues were comparable for both sexes and ranged from approx. 0.6 mg/kg for the perirenal fat to approx. 1.5 mg/kg for the leg muscle. Six hours after administration [furanone-4-^{14}C] flupyradifurone was only incompletely metabolized. Metabolic reactions took place at least at 3 different structural positions of the molecule. The majority of the radioactive residues were identified (approximately 89% - 100% in plasma and in extracts of organs and tissues as well as ≥ 88% of radioactivity in urine samples). In all samples of plasma, organs and tissues the parent compound was the by far largest component accounting for more than 72% of the total radioactivity. None of the identified metabolites accounted for more than 12% of the total radioactivity. The metabolism was qualitatively similar in male and female rats, but with quantitative differences. The degradation of the parent compound to the different metabolites was significantly higher in male as compared to female rats.

Table 34: Quantification of parent compound and metabolites in urine of male and female rats sacrificed 6 h after single oral administration of [furanone-4-^{14}C] flupyradifurone (3 mg/kg bw)

	% of the given dose	
	Male	**Female**
Sampling period [h]	**0 - 6 h**	**0 - 6 h**
Parent compound	22.1	37.6
unknown	1.4	0.4

unknown	1.5	0.4
unknown	0.2	---
unknown	0.4	---
unknown	0.3	---
BYI 02960-difluoroethyl-amino-furanone	1.5	0.2
BYI 02960-OH-gluA (isomer 1)	0.4	---
unknown	0.2	---
BYI 02960-OH-gluA (isomer 3)	0.9	---
BYI 02960-des-difluoroethyl	0.8	0.8
unknown	0.2	---
unknown	0.1	---
BYI 02960-OH	6.9	3.3
Total identified	32.5	42.0
Total characterized *	4.1	0.8
Sum total	**36.6**	**42.8**
Identification rate	**88.8%**	**98.1%**

* : Peaks were characterized based on their retention time in HPLC-analysis

Table 35: Concentration of parent compound and metabolites in plasma of male and female rats sacrificed 6 h after single oral administration of [furanone-4-^{14}C] flupyradifurone (3 mg/kg bw). Data are presented as mg/kg

	Male	**Female**
Sampling period [h]	**0 - 6 h**	**0 - 6 h**
Parent compound	1.087	1.385
unknown	0.056	0.007
unknown	---	0.010
BYI 02960-difluoroethyl-amino-furanone	0.101	0.012
BYI 02960-des-difluoroethyl	---	0.008
BYI 02960-OH	0.068	0.026
Total identified	1.257	1.369
Total characterized *	0.056	0.016
Sum total	**1.313**	**1.385**

* : Peaks were characterized based on their retention time in HPLC-analysis

Table 36: Concentration of parent compound and metabolites in the liver of male and female rats sacrificed 6 h after single oral administration of 3 mg/kg [furanone-4-^{14}C] flupyradifurone (3 mg/kg bw)

	Concentration [mg a.s. equiv./kg]	
	Male	**Female**
Sampling [h]	**6 h**	**6 h**
Parent compound	2.110	2.778
unknown	0.150	---
unknown	0.015	---
BYI 02960-difluoroethyl-amino-furanone	0.108	0.015
BYI 02960-OH-gluA (isomer 1)	0.026	---
BYI 02960-OH-gluA (isomer 3)	0.055	0.013
BYI 02960-des-difluoroethyl	0.036	0.024
unknown	0.012	---
unknown	0.007	---
unknown	0.016	---
BYI 02960-OH	0.242	0.077
Total identified	2.577	2.908
Total characterized *	0.200	---
Sum total	**2.928**	**2.937**

* : Peaks were characterized based on their retention time in HPLC-analysis

Table 37: Concentration of parent compound and metabolites in the kidney of male and female rats sacrificed 6 h after single oral administration of [furanone-4-^{14}C] flupyradifurone (3 mg/kg bw)

	Concentration [mg a.s. equiv./kg]	
	Male	**Female**
Sampling [h]	**6 h**	**6 h**
Parent compound	1.969	4.025
unknown	0.174	0.060
unknown	0.017	---
BYI 02960-difluoroethyl-amino-furanone	0.125	---
BYI 02960-OH-gluA (isomer 1)	0.010	---
BYI 02960-OH-gluA (isomer 3)	0.020	---
BYI 02960-des-difluoroethyl	0.036	0.047
BYI 02960-OH	0.319	0.197
Total identified	2.479	4.269
Total characterized *	0.191	0.060
Sum total	**2.732**	**4.346**

* : Peaks were characterized based on their retention time in HPLC-analysis

Table 38: Concentration of parent compound and metabolites in muscle of male and female rats sacrificed 6 h after single oral administration of [furanone-4-^{14}C] flupyradifurone (3 mg/kg bw)

	Concentration [mg a.s. equiv./kg]	
	Male	**Female**
Sampling [h]	**6 h**	**6 h**
Parent compound	1.160	1.435
unknown	0.026	---
BYI 02960-difluoroethyl-amino-furanone	0.090	0.009
BYI 02960-des-difluoroethyl	0.013	0.012
BYI 02960-OH	0.083	0.032
Total identified	1.346	1.487
Total characterized *	0.026	---
Sum total	**1.382**	**1.489**

* : Peaks were characterized based on their retention time in HPLC-analysis

Table 39: Concentration of parent compound and metabolites in the fat of male and female rats sacrificed 6 h after single oral administration of [furanone-4-^{14}C] flupyradifurone (3 mg/kg bw)

	Concentration [mg a.s. equiv./kg]	
	Male	**Female**
Sampling [h]	**6 h**	**6 h**
Parent compound	0.474	0.650
unknown		---
BYI 02960-difluoroethyl-amino-furanone	0.023	---
BYI 02960-OH	0.040	---
Total identified	0.537	0.650
Sum total	**0.558**	**0.651**

Acceptability

The study was considered acceptable.

Conclusions

The distribution of the radioactivity within the organs and tissues (*i.e.* blood, liver, kidney, muscle and fat) showed a distinctive preference for liver and kidney as the main metabolizing and excretory organs. The metabolic transformation of flupyradifurone was principally oxidative in nature and took place at least at 3 different structural positions of the molecule.

The metabolic pattern was in good accordance with that obtained from the corresponding ADME rat studies. With regard to the extent of metabolism, a clear sex difference was observed since it was higher in male than in female rats, *i.e.* the metabolic degradation of the parent compound was much less pronounced in females.

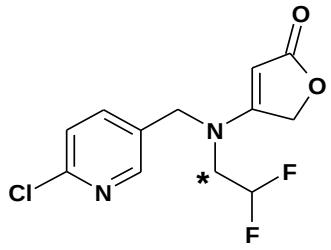
Study 6

Characteristics

Reference	:	Weber, E. 2011	exposure	:	single dose by gavage
type of study	:	Toxicokinetic study (ADME in male rat)	dose	:	2 mg/kg
year of execution	:	2010-2011	vehicle	:	0.5% aqueous Tragacanth® suspension
test substance	:	[Ethyl-1-^{14}C] flupyradifurone, batch no. KATH 6412, chemical purity >99%, radiochemical purity >99%, 3.93 MBq/mg	GLP statement	:	yes
Route	:	oral	guideline	:	OECD 417
Species	:	Rat Wistar Hsd/Cpb: WU	acceptability	:	acceptable
group size	:	4 male			

Study design

The study was performed according to OECD Guideline for testing chemicals 417. Four male rats were administered orally by gavage a single dose of [Ethyl-1-^{14}C] flupyradifurone suspended in 0.5% aqueous Tragacanth® (2 mL) at target dose level of 2 mg/kg bw. Flupyradifurone was radiolabelled with ^{14}C in the 1-position of the ethyl side chain:



* denotes the position of the ^{14}C-label

Urine and faeces were collected for the time range between dosing and sacrifice and the animals were sacrificed after 72 hours (Table 40). Plasma levels of radioactivity were followed from each animal for 12 time points. Total radioactivity which included the test compound and metabolites was determined in excreta and in organs and tissues collected at sacrifice. Metabolism was investigated in urine and faeces. Samples were analyzed by radio HPLC, radio TLC, LC-MS and NMR methods. Toxicokinetic parameters were calculated with the software TOPFIT version 2.0.

Table 40: Experimental groups for each dose level of [Ethyl-1-^{14}C] flupyradifurone

Group no.	No. of animals	Route	Dose	Sacrifice time (hours after last dose)
1	4 M	Oral (gavage)	2 mg/kg bw	72

Results

Recovery

The results in percent of the given dose in expired air, urine, faeces, organs and tissues at sacrifice are summarized in Table 41. The total recovery for the orally administered test was approximately 100%.

Table 41: Recovery of radioactivity in urine, faeces, gastrointestinal tract and the body following a single oral dose of [ethyl-1-^{14}C] flupyradifurone (2 mg/kg bw)

	% of given dose
Expired air	0.20
Faeces	13.51
Urine	82.24
Sum of excreta	**95.95**
Body excluding GIT	3.19
GIT	0.73
Total Body	3.92
Balance	**99.86**

Absorption

[Ethyl-1-^{14}C] flupyradifurone was almost completely absorbed in male rats. The absorption rate was at least 85% because >82% of the dose was detected in urine and >3% in the body without GIT. The absorption commenced immediately after dosing as can be seen from the quick increase of radioactivity in plasma micro samples (Table 42).

Table 42: Timecourse of radioactivity in the plasma following a single oral dose of [ethyl-1-^{14}C] flupyradifurone (2 mg/kg bw)

Time point	**Equivalent concentration (mg/kg)**
10 min	0.663
20 min	1.391
40 min	1.878
1 h	2.017
1.5 h	1.963
2 h	1.911
4 h	1.532
8 h	0.995
24 h	0.357
32 h	0.358
48 h	0.230
72 h	0.162

Distribution and plasma kinetics

Flupyradifurone was quickly distributed as can be seen form the analysis of plasma at different time points. The maximum concentration of radioactivity (C_{max}) was already reached one hour after administration. At this time, the radioactivity level in plasma corresponded approximately to the equidistribution concentration. The plasma concentration then declined to approx. 50% of C_{max}

within 8 hours and to approx. 10% of the maximum value within 48 hours. At the time of sacrifice, the plasma concentration amounted to ca. 8% of C_{max}.
The mean values of total radioactivity were used for a toxicokinetic modelling using the TOPFIT software. The fitting was conducted assuming a two-compartment model with no data weighting. A good fit was achieved for the entire time range (Figure 3). The AUC was 45.4 mg/L*h and the half life of elimination was 49.9 hours.

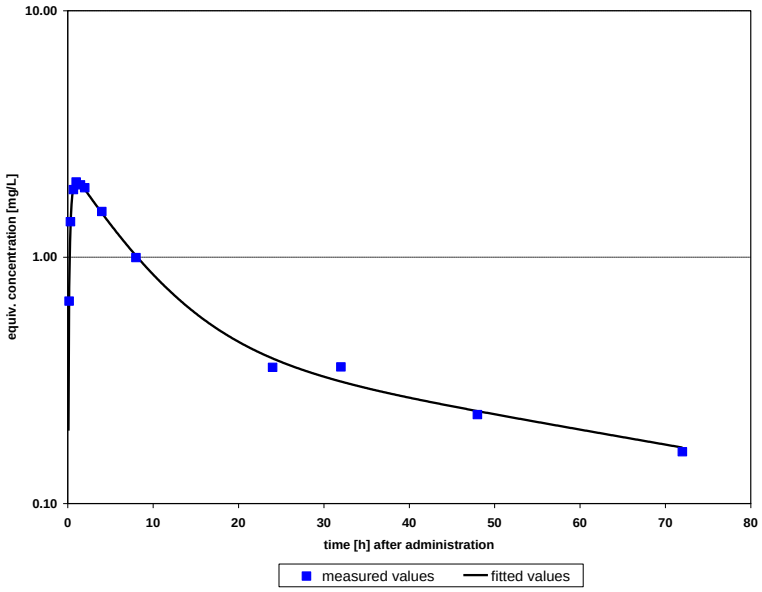


Figure 3: Timecourse of radioactivity in the plasma following a single oral dose of [ethyl-1-^{14}C] flupyradifurone (2 mg/kg bw)

Excretion
Only a very small proportion of the dose (0.2% in total) was detected in the expired air. This result confirms the stability of the radio label in the ethyl group with regard to extensive metabolic transformation. The major route of excretion was renal. In total, approximately 82% of the dose was detected in urine, the majority of which (about 76% of the dose) was excreted within 24 hours. Faecal excretion accounted for ca. 13.5% of the given dose. Also the major part of faecal residues (about 11% of the dose) was excreted within 24 hours after treatment. These results are in good accordance with the excretion behavior detected in all other rat studies with flupyradifurone. The cumulative excretion results in percent of the administered radioactivity are summarized in Table 43.

Table 43: Cumulative excretion of radioactive residues via urine, faeces and expired air after a single oral dose of [ethyl-1-^{14}C] flupyradifurone (2 mg/kg bw).

	Time [h]	**% of dose**
Expired Air	24	0.15
	48	0.19
	72	0.20
Faeces	24	11.08
	48	13.12
	72	13.51
Urine	4	14.95
	8	46.40
	12	60.48
	24	76.12
	48	80.60
	72	82.24
Total		**95.95**

Radioactive residues in organs and tissues at sacrifice

Approximately 4% of the dose was detected in the body at sacrifice 72 hours after oral administration; 0.73% was found in the GIT and 3.19% in the body without GIT. The residual concentration of radioactivity was in the range of 0.025 to 0.158 mg/kg. The lowest concentration was detected in the Harderian gland and the highest value was found in plasma. However, concentrations for most organs and tissues were very similar and in the range of approximately 0.05 to 0.1 mg/kg. The equivalent concentrations of the residual radioactivity in organs and tissues at sacrifice are presented in Table 44.

Table 44: Total radioactive residues in organs and tissues at sacrifice after a single oral dose of [ethyl-1-^{14}C] flupyradifurone (2 mg/kg bw)

	Equivalent concentration (mg/kg)
Blood cells	0.104
Plasma	0.158
Carcass	0.064
Heart	0.078
Brain	0.083
Kidneys	0.066
Liver	0.095
GIT	0.129
Testes	0.065
Adrenal gland	0.073
Harderian gland	0.025
Thyroid	0.088
Spleen	0.075
Lung	0.088
Eye	0.138
Skin	0.079
Bone (femur)	0.052
Perirenal fat	0.054
Muscle	0.055

Metabolites in urine and faeces

Flupyradifurone was metabolized into a number of metabolites. The unmetabolized parent compound represented the predominant part of the radioactivity in urine while in faeces the metabolite BYI 02960-OH was more prominent. BYI 02960-DFA was detected in both, urine and faeces. All other identified and characterized metabolites represented a minor part of the dose and were not common to either urine or faeces. A summary of the distribution of the parent compound and metabolites in urine and faeces is provided in Table 45.

Table 45: Amounts of metabolites in urine and faeces after a single oral dose of [ethyl-1-^{14}C] flupyradifurone (2 mg/kg bw)

Peak no.	**(BYI 02960-)**	**% of the dose**		
		Urine	**Faeces**	**Total**
1	DFA	5.28	0.49	5.77
6	difluoroethyl-amino-furanone	3.63	---	3.63
7	OH-gluA (isomer 1)	1.40	---	1.40
8	OH-gluA (isomer 3)	1.79	---	1.79
6	difluoroethyl-amino-furanone	3.63	---	3.63
7	OH-gluA (isomer 1)	1.40	---	1.40
8	OH-gluA (isomer 3)	1.79	---	1.79
11	OH	16.13	7.60	23.73

12	Parent compound	51.96	3.79	55.75
13	iso-OH	---	0.43	0.43
	Identified	80.19	12.31	92.50
2	unknown 1	0.64	---	0.64
3	unknown 2	0.68	---	0.68
4	unknown 3	0.36	---	0.36
5	unknown 4	0.37	---	0.37
9	unknown 5	---	0.10	0.10
10	unknown 6	---	0.17	0.17
	Characterized	2.04	0.27	2.31
	Total in extracts	82.23	12.58	94.81
Total in urine and faeces		**82.23**	**13.51**	**95.74**

Acceptability
The study was considered acceptable.

Conclusions
Flupyradifurone was almost completely absorbed; more than 85% of the administered dose was detected in the urine and the body without GIT at sacrifice. The excretion of radioactivity was fast and mainly renal. The major part of the dose (>87%) was excreted within 24 hours after treatment, but continued until sacrifice. At sacrifice, a small proportion of ca. 3% of the dose was detected in the body without GIT. The residue concentration in plasma was 0.158 mg/kg; for most other organs and tissues levels were in the range between 0.05 and 0.1 mg/kg. The parent compound, one major and five minor metabolites were identified in all samples. Identification rates were >95% of the total radioactivity in urine and >85% of the total radioactivity in faeces. The metabolic transformation of flupyradifurone was principally oxidative in nature and took place at 3 different positions of molecule.

Study 7

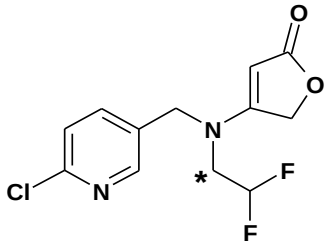
Characteristics

Reference	:	Koester, J. 2011	exposure	:	Single dose by gavage
type of study	:	Toxicokinetic study (Metabolism study in organs and tissues of male and female rats (3 time-points).	dose	:	3 mg/kg bw
year of execution	:	2010-2011	vehicle	:	0.5% aqueous Tragacanth® suspension
test substance	:	[Ethyl-1-^{14}C] flupyradifurone, batch no. KML 9028, chemical purity >98%, radiochemical purity >99% (HPLC) or >98% (TLC), 3.93 MBq/mg	GLP statement	:	yes
Route	:	oral	guideline	:	OECD 417
Species	:	Rat Wistar Hsd/Cpb: WU	acceptability	:	acceptable

group size : 4/time point/sex

Study design

The study was performed according to OECD Guideline 417. Male and female rats in groups of four rats were administered orally by gavage a single dose of flupyradifurone at target dose levels of 3 mg/kg bw (Table 46). Flupyradifurone was radiolabelled with ^{14}C in the 1-position of the ethyl side chain:



* denotes the position of the ^{14}C-label

Groups of male and female rats (4/time point/sex) were orally administered with a single dose of 3 mg/kg bw [ethyl-1-^{14}C] flupyradifurone by gavage in 0.5% aqueous Tragacanth®. The animals were sacrificed at 1, 6 or 24 hours after dosing. The total radioactivity was determined over different time periods (0 - 1 hour, 0 - 6 hours, or 0 – 24 hours) in urine, while in plasma, liver, kidney, muscle and fat tissue after sacrifice. The metabolism was investigated in urine, plasma, and in extracts of liver, kidney, muscle, and fat tissue. Samples were analyzed by radio HPLC, radio TLC, LC-MS and NMR methods.

Table 46: Experimental groups for each dose level of [furanone-4-^{14}C] flupyradifurone

Group no.	No. of animals	Route	Dose	Sacrifice time (hours after last dose)
1	4 M	Oral (gavage)	5 mg/kg bw	1
	4 F	Oral (gavage)	5 mg/kg bw	1
2	4 M	Oral (gavage)	5 mg/kg bw	6
	4 F	Oral (gavage)	5 mg/kg bw	6
3	4 M	Oral (gavage)	5 mg/kg bw	24
	4 F	Oral (gavage)	5 mg/kg bw	24

Results

Recovery

The detailed recovery rates of radioactivity in urine, organs and tissues and the combined GIT and faeces sample are shown in Table 47. The mean recoveries for male rats ranged from approximately 96% to 98% and for female rats from approximately 100% to 104% of the given dose.

In male rats, sacrificed at 1 hour after administration, approx. 64.6% of the dose was detected in organs and tissues and approx. 27.2% in the GIT and faeces sample (Table 47). After 24 hours, the value for organs and tissues declined to approx. 7.5%. These values indicated a fast distribution of the absorbed radioactivity within the body followed by a quick elimination finally leading to a significant increase of the urinary excretion from ca. 6.2% to ca. 71.8%. The situation was slightly different in female rats. After sacrifice at 1 hour h after administration, approx. 81% of the dose was

detected in organs and tissues and approx. 13.9% in the GIT and faeces sample. After 24 hours, the value of organs and tissues declined to approx. 5.3%. These values indicated again a fast distribution of the absorbed radioactivity within the body followed by a quick elimination finally leading to a significant increase of the urinary excretion from approx. 8.8% to approx. 85.9%.

Table 47: Recovery of radioactivity in urine, plasma and organs following a single oral dose of [ethyl-1-^{14}C] flupyradifurone (3 mg/kg bw)

	as % of the given dose					
	Male			**Female**		
Test period [hour]	**1**	**6**	**24**	**1**	**6**	**24**
Urine	6.22	36.53	71.80	8.76	39.70	85.88
Plasma	1.09	0.62	0.21	1.27	0.61	0.13
Carcass	38.32	24.47	4.25	51.19	29.57	3.08
Kidneys	1.30	0.73	0.08	1.17	0.63	0.06
Liver	6.80	3.44	0.60	7.30	3.62	0.39
GIT + faeces	27.24	21.18	16.61	13.94	14.26	9.44
Skin	14.64	9.19	2.13	17.54	10.48	1.45
Fat	0.10	0.04	0.01	0.15	0.09	0.01
Muscle	2.31	1.14	0.17	2.34	1.35	0.13
Balance	**98.03**	**97.33**	**95.85**	**103.70**	**100.30**	**100.60**

Metabolism

Table 48 shows the amount of flupyradifurone and its metabolites after 1, 6, and 24 hours in urine of male and female rats as percentage of the given dose. In urine, the parent compound was the dominating radioactive component. The concentrations of radioactivity in plasma can be found in Table 49, in liver in Table 50, in kidney in Table 51, in muscle in Table 52 and in fat tissue in Table 53. Flupyradifurone was intensively metabolized. Metabolic reactions took place at least at 3 different structural positions of the molecule. The majority of the radioactive residues were identified (approx. 97 - 100% in plasma and in extracts of organs and tissues as well as ≥ 95% of radioactivity in urine). In the 24 hours samples of plasma, and organs and tissues BYI 02960-DFA was by far the dominating metabolite accounting for more than 50% of the radioactivity. All other identified metabolites contributed to less than 10%. The contribution of the parent compound in these samples ranged from 6 to 38% of the radioactivity. In urine, the parent compound was the dominating radioactive component (approximately 48% of the dose in males and 77% in females).
The metabolism was qualitatively similar in male and female rats, but with quantitative differences. The degradation of the parent compound to the different metabolites in male rats was significantly higher than in female rats.

Table 48: Quantification of parent compound and metabolites in urine of male and female rats sacrificed 1 h, 6 h, and 24 h after a single oral dose of [ethyl-1-^{14}C] flupyradifurone (3 mg/kg bw)

	% of the given dose					
	Male			**Female**		
Sampling period	**0 - 1 h**	**0 - 6 h**	**0 - 24 h**	**0 - 1 h**	**0 - 6 h**	**0 - 24 h**

Parent compound	4.96	25.48	47.69	8.19	36.15	76.48
DFA	---	0.22	1.91	---	0.11	1.70
unknown	---	0.38	0.72	---	0.18	0.42
unknown	---	0.15	0.35	---	---	---
unknown	---	0.30	0.67	---	---	---
unknown	---	---	0.43	---	---	---
unknown	---	---	0.27	---	---	---
BYI 02960-difluoroethyl-amino-furanone	0.12	1.55	3.09	0.07	0.33	0.87
BYI 02960-OH-gluA (isomer 1)	0.12	0.66	1.55	---	0.10	---
unknown	---	---	0.46	---	0.14	---
unknown	---	---	---	---	0.38	---
BYI 02960-OH-gluA (isomer 3)	0.13	0.74	1.72	---	0.08	---
unknown	0.08	---	0.34	---	---	---
unknown	---	0.07	0.10	---	---	---
unknown	---	0.15	0.18	---	---	---
BYI 02960-OH	0.82	6.85	12.33	0.51	2.24	6.42
Total identified	6.15	35.49	68.28	8.76	39.00	85.46
Total characterized *	0.08	1.04	3.52	---	0.70	0.42
Sum total	**6.22**	**36.53**	**71.80**	**8.76**	**39.70**	**85.88**
Identification rate	**98.8%**	**97.2%**	**95.1%**	**100.0%**	**98.2%**	**99.5%**

* : Peaks were characterized based on their retention time in HPLC-analysis:

Table 49: Concentration of parent compound and metabolites in plasma of male and female rats sacrificed 1 h, 6 h, and 24 h after a single oral dose following a single oral dose of [ethyl-1-^{14}C] flupyradifurone (3 mg/kg bw)

	Concentration [mg a.s. equiv./kg]					
	Male			**Female**		
Sampling time	**1 h**	**6 h**	**24 h**	**1 h**	**6 h**	**24 h**
Parent compound	1.998	0.976	0.031	2.671	1.486	0.063
BYI 02960-DFA	0.058	0.348	0.448	0.057	0.175	0.280
Unknown	---	0.024	---	---	---	---
BYI 02960-difluoroethyl-amino-furanone	0.052	0.084	0.005	---	---	---
Unknown	---	0.018	---	---	---	---
BYI 02960-OH	0.058	0.045	0.007	---	---	---
Total identified	2.166	1.452	0.491	2.728	1.661	0.343
Total characterized *	---	0.042	---	---	---	---
Sum total**	**2.166**	**1.494**	**0.491**	**2.728**	**1.661**	**0.343**

* : Peaks were characterized based on their retention time in HPLC-analysis
** : Sum total = total identified or characterized + solids (unextractable) + samples not analyzed

Table 50: Concentration of parent compound and metabolites in the liver of male and female rats sacrificed 1 h, 6 h, and 24 h after a single oral dose following a single oral dose of [ethyl-1-^{14}C] flupyradifurone (3 mg/kg)

	Concentration [mg a.s. equiv./kg]					
	Male			**Female**		
Sampling time	**1 h**	**6 h**	**24 h**	**1 h**	**6 h**	**24 h**
Parent compound	3.900	1.903	0.084	5.519	2.977	0.084
BYI 02960-DFA	0.049	0.183	0.218	0.033	0.116	0.136
Unknown	---	0.012	---	0.019	---	---
Unknown	---	0.012	---	---	---	---
BYI 02960-difluoroethyl-amino-furanone	0.070	0.086	---	0.022	0.018	---
BYI 02960-OH-gluA (isomer 1)	---	0.021	---	---	---	---
unknown	---	0.013	---	---	---	---
unknown	---	0.013	---	0.017	0.016	---
unknown	---	---	---	---	---	0.002
unknown	---	---	---	---	---	0.002
unknown	---	---	---	0.014	---	---
unknown	0.042	0.031	---	0.021	0.010	---
BYI 02960-OH	0.187	0.150	0.014	0.106	0.063	0.005
Total identified	4.206	2.343	0.316	5.696	3.190	0.225
Total characterized *	0.042	0.081	---	0.055	0.010	0.004
Sum total **	**4.281**	**2.507**	**0.367**	**5.770**	**3.225**	**0.243**

* : Peaks were characterized based on their retention time in HPLC-analysis
** : Sum total = total identified or characterized + solids (unextractable) + samples not analyzed

Table 51: Concentration of parent compound and metabolites in the kidney of male and female rats sacrificed 1 h, 6 h, and 24 h after a single oral dose of [ethyl-1-^{14}C] flupyradifurone (3 mg/kg bw)

	Concentration [mg a.s. equiv./kg]					
	Male			**Female**		
Sampling time	**1 h**	**6 h**	**24 h**	**1 h**	**6 h**	**24 h**
Parent compound	4.268	2.108	0.082	4.695	2.530	0.102
BYI 02960-DFA	0.042	0.176	0.200	0.038	0.133	0.144
unknown	---	0.036	---	---	---	---
unknown	---	0.019	---	---	---	---
BYI 02960-difluoroethyl-amino-furanone	0.084	0.153	---	---	---	---
unknown	---	0.034	---	---	---	---
BYI 02960-OH	0.341	0.334	0.020	0.155	0.115	0.011
Total identified	4.735	2.772	0.303	4.888	2.778	0.257
Total characterized *	---	0.088	---	---	---	---
Sum total **	**4.747**	**2.896**	**0.317**	**4.901**	**2.795**	**0.267**

* : Peaks were characterized based on their retention time in HPLC-analysis
** : Sum total = total identified or characterized + solids (unextractable) + samples not analyzed

Table 52: Concentration of parent compound and metabolites in muscle of male and female rats sacrificed 1 h, 6 h, and 24 h after a single oral dose of [ethyl-1-^{14}C] flupyradifurone (3 mg/kg bw)

	Concentration [mg a.s. equiv./kg]					
	Male			**Female**		
Sampling time	**1 h**	**6 h**	**24 h**	**1 h**	**6 h**	**24 h**
Parent compound	2.201	1.097	0.075	2.827	1.568	0.055
BYI 02960-DFA	0.021	0.129	0.124	0.023	0.070	0.108
BYI 02960-difluoroethyl-amino-furanone	0.050	0.070	---	0.016	0.019	---
BYI 02960-OH	0.061	0.049	---	0.035	0.021	0.003
Total identified	2.332	1.346	0.198	2.901	1.678	0.166
Sum total **	**2.336**	**1.352**	**0.208**	**2.902**	**1.682**	**0.167**

* : Peaks were characterized based on their retention time in HPLC-analysis
** : Sum total = total identified or characterized + solids (unextractable) + samples not analyzed

Table 53: Concentration of parent compound and metabolites in fat of male and female rats sacrificed 1 h, 6 h, and 24 h after a single oral dose of [ethyl-1-^{14}C] flupyradifurone (3 mg/kg bw)

	Concentration [mg a.s. equiv./kg]					
	Male			**Female**		
Sampling time	**1 h**	**6 h**	**24 h**	**1 h**	**6 h**	**24 h**
Parent compound	0.911	0.389	0.030	1.079	0.624	0.013
BYI 02960-DFA	---	0.051	0.074	---	0.031	0.032
unknown	---	0.035	---	---	---	---
BYI 02960-OH	---	0.018	---	---	---	---
Total identified	0.911	0.458	0.104	1.079	0.654	0.045
Total characterized *	---	0.035	---	---	---	---
Sum total **	**0.911**	**0.493**	**0.108**	**1.079**	**0.655**	**0.047**

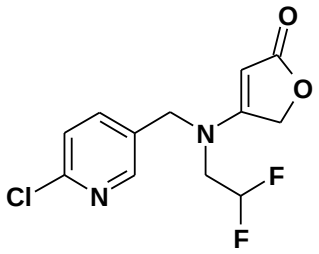
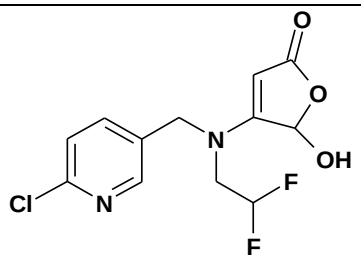
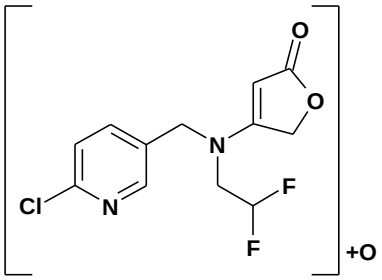
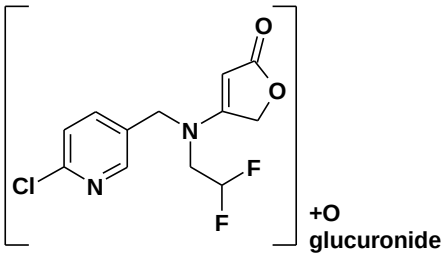
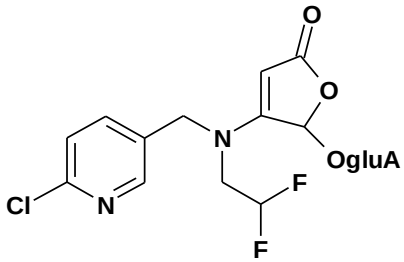
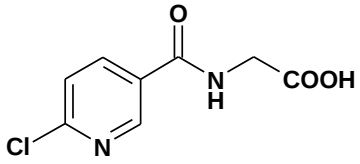
* Peaks were characterized based on their retention time in HPLC-analysis
** Sum total = Total identified or characterized + solids (unextractable) + samples not analyzed

Acceptability
The study was considered acceptable.

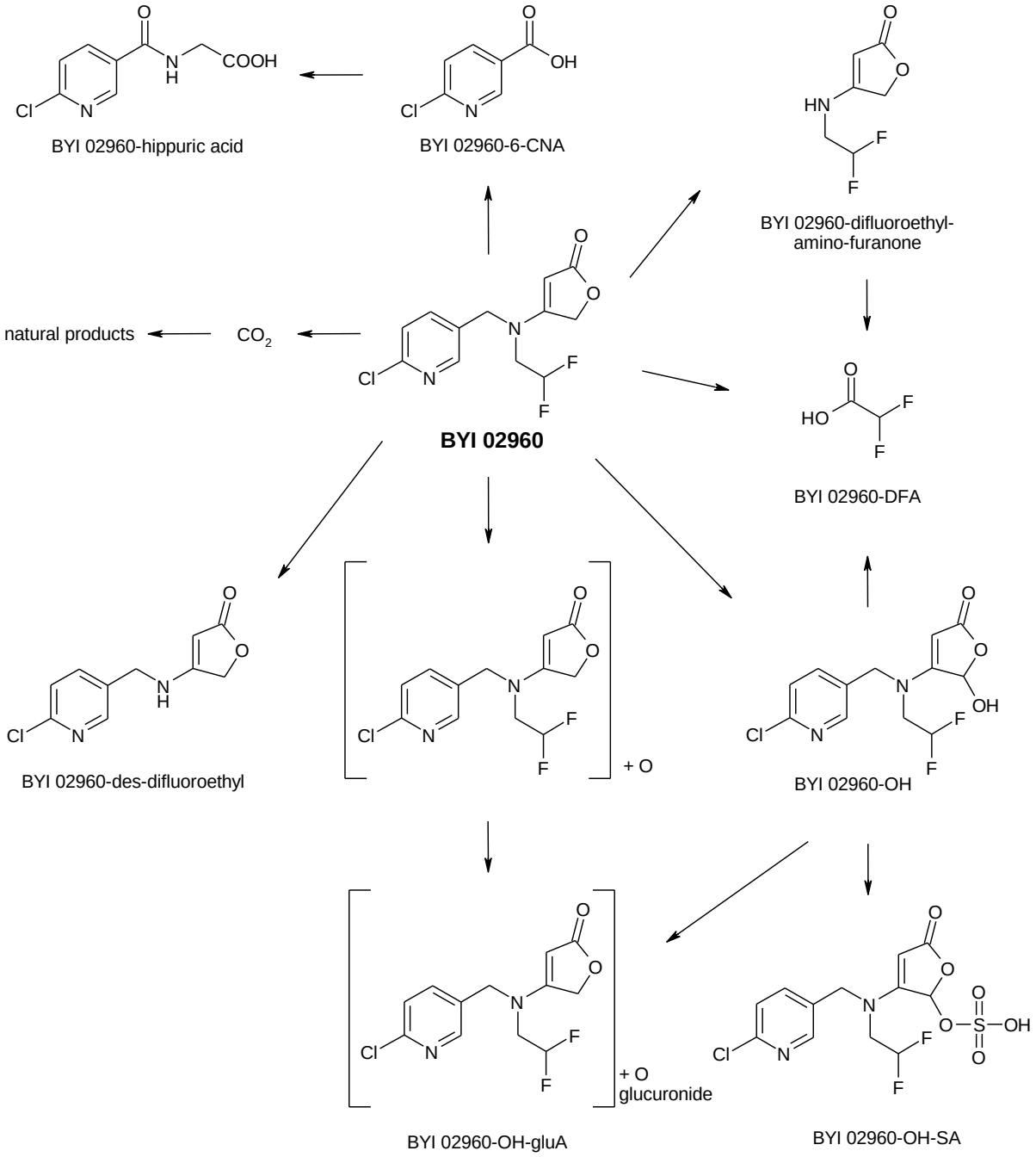
Conclusions
The distribution of the radioactivity within the organs and tissues (*i.e.* blood, liver, kidney, muscle and fat tissue) showed a distinctive preference for liver and kidney as the main metabolizing and excretory organs. The highest radioactivity concentrations were measured in plasma, and in organs and tissues at one hour after administration. They decreased significantly within the test period of 24 hours. There were no indications of irreversible binding or retention of radioactivity in organs and tissues. The parent compound, major and several minor metabolites were identified in all samples. Identification rates were high with ≥ 95% of radioactivity in urine and approx. 93 - 100% of the radioactivity in plasma, organs and tissues. The metabolic transformation of flupyradifurone was principally oxidative in nature and took place at least at 3 different structural positions of the molecule.
BYI 02960-DFA was by far the dominating metabolite in plasma, organs and tissues. On the other hand, the parent compound was the main constituent in urine. With regard to the extent of metabolism, a clear sex difference was observed since it was higher in male than in female rats. The metabolic degradation of the parent compound was much less pronounced in females as compared to males.
Toxicokinetic studies – Repeated dose, oral route
All single dose experiments revealed no indication of a potential for retention, accumulation and/or persistence of the administered radioactivity in organs or tissues. This observation is supported by the low log Kow of BYI 02960 of 1.2. Therefore, in line with paragraph 26 of the OECD Test Guideline 417 (July 22nd, 2010), a repeated dose study was not considered necessary.

List of identified metabolites

A comprehensive list of major metabolites detected in the rat is provided in the following table.

Report Name	Chemical Structure	IUPAC Name
active substance: BYI 02960		4-[(6-chloro-3-pyridylmethyl)(2,2-difluoroethyl)amino]furan-2(5H)-one
BYI 02960-OH		4-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}-5-hydroxyfuran-2(5H)-one
BYI 02960-iso-OH		
BYI 02960-OH-gluA (isomer 1)		
BYI 02960-OH-gluA (isomer 3)		3-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}-5-oxo-2,5-dihydrofuran-2-yl beta-D-glucopyranosiduronic acid
BYI 02960-hippuric acid		N-[(6-chloropyridin-3-yl)carbonyl]glycine

4.1.2 Proposed metabolic pathway



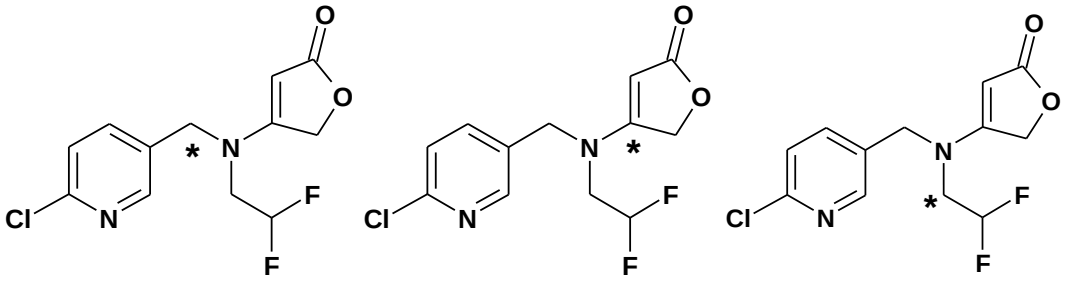
4.1.3 Human information

No information.

4.1.4 Summary and discussion on toxicokinetics

Summary

Absorption, distribution, excretion and metabolism of flupyradifurone were investigated using three different labelling positions. The active substance was labelled with ^{14}C in the pyridinylmethylene bridge, in the 4-position of the furanone ring and in the 1-position of the ethyl side chain:



Pyridinylmethyl-^{14}C flupyradifurone | Furanone-4-^{14}C flupyradifurone | Ethyl-1-^{14}C flupyradifurone

The pyridinylmethyl-labelled compound was used in an ADME-study in which male and female rats were orally administered with a single low dose of 2 mg/kg bw or a high dose of 200 mg/kg bw. Due to the high water solubility of flupyradifurone, male rats were also given an intravenous dose of 2 mg/kg bw. In this study, the excretion via urine and faeces was investigated as well as the distribution in the plasma and the radioactivity concentration in organs and tissues at sacrifice. The metabolism was investigated in urine and faeces.

A quantitative whole body autoradiography study was conducted also using the pyridinylmethyl-labelled compound following a single oral dose of 5 mg/kg bw to male and female rats. In this study the excretion of radioactivity was determined in urine, faeces and expired air, as well as the distribution in the plasma and the radioactivity concentration in the organs and tissues at various time points.

The furanone-4-labelled compound was used in an ADME-study in which male and female rats were orally administered with a single dose of 2 mg/kg bw. In this study, the excretion via urine and faeces was investigated as well as the distribution in the plasma and the radioactivity concentration in the organs and tissues at sacrifice. The metabolism was investigated in urine and faeces.

A quantitative whole body autoradiography study was also conducted using the furanone-4-labelled compound following a single oral dose of 5 mg/kg bw to male and female rats. In this study the excretion of radioactivity was determined in urine, faeces and the expired air as well as the distribution in the plasma and the radioactivity concentration in the organs and tissues at various time points.

In an organ metabolism study, male and female rats were orally administered with a single dose of 3 mg/kg bw [furanone-4-^{14}C] flupyradifurone. Animals were sacrificed 6 hours after dosage and the metabolism was investigated in urine, plasma, and in extracts of liver, kidney, muscle and fat tissues.

The ethyl-1-labelled compound was used in an ADME-study in which male rats were orally administered with 2 mg/kg bw. In this study, the excretion via urine, faeces and expired air was investigated as well as the distribution in the plasma and the radioactivity concentration in the organs and tissues at sacrifice. The metabolism was investigated in urine and faeces.

In an organ metabolism study, male and female rats were orally administered with a single dose of 3 mg/kg bw [ethyl-1-^{14}C] flupyradifurone. The animals were sacrificed at 1, 6 or 24 hours after dosing. The total radioactivity was determined over the different time periods in urine, while in plasma, liver, kidney, muscle and fat tissue at different time points after sacrifice. The metabolism was investigated in urine, plasma, and in extracts of liver, kidney, muscle, and fat tissue.

Conclusions

Following oral administration of a single low dose of flupyradifurone to male and female rats, the gastrointestinal absorption of radioactivity was high. It accounted for >80 % of the dose independent of the labelling position used. The value of >80% can be used for further toxicological risk assessment. Excretion was very fast, mainly renal and almost completed after 24 hours. No radioactivity was detected in the expired air after dosing of the pyridinylmethyl- and ethyl-1-labelled compounds, proving the stability of these labelling positions in the molecule. Only after administration of [furanone-4-^{14}C] flupyradifurone between 1% and 3% of the administered radioactivity was exhaled. This demonstrated that for a small portion of the dose (higher in male than in female rats) the furanone ring of the molecule obviously was opened and was subject of biotransformation to C-1 fragments.
The maximum plasma concentration was reached in most cases within 1 or 2 hours after administration of low doses. Only after administration of the high dose of 100 mg/kg bw the peak plasma concentration was observed between 2 and 4 hours after dosage. After reaching the peak concentration, the radioactivity levels in plasma declined steadily by several orders of magnitude in all studies independent of sex or labelling position of the test compound.

Quantitative whole body autoradiography revealed a fast absorption and distribution of the test compound with peak values observed already 1 hour after administration. At this time, the concentrations in liver and kidney were significantly higher than in blood, suggesting a preferred clearance from blood and distribution mainly to these organs which are mainly responsible for metabolism (liver) and excretion (kidney). Higher concentrations than in blood were also detected in the myocardium (heart) and in several glands. Very low levels were found in the brain, spinal cord and renal fat tissue. These results were similar in male and female rats, and independent of the labelling position of the test compound. A fast decline of radioactivity concentrations was observed for all organs and tissues in male and female rats during the entire test period. Concentrations fell for most organs and tissues below 5% of the maximum concentration after one day. After seven days, only very low concentrations were found in a few organs and tissues of rats dosed with the pyridinylmethyl-labelled test compound. In the study using the furanone-4-labelled compound, low radioactive residues were measured in almost all organs and tissues due to the incorporation of C1- or C2-fragments into the endogenous carbon pool. The residues in male rats were higher by a factor of 1.4 to 4.7 as compared to female rats. A similar ratio of approximately 3 (male/female) was also found for the formation of $^{14}CO_2$, and originates presumably from sex related differences in metabolism leading to more C1- and C2-fragments and also higher incorporation of these components into the endogenous carbon pool in male rats. In general males and female rats exhibited very similar absorption, distribution and excretion behavior. The results of these studies demonstrate that there is no indication of any accumulation or significant retention of radioactivity

in male and female rats. This observation is supported by the low KOW of 1.2. Concentrations of radioactivity detected in tissues and organs at sacrifice were either very low or below the limit of detection.

Flupyradifurone was intensively metabolised in the rat. Numerous metabolites were formed, most of them being minor ones. The parent compound represented the predominant part of the radioactivity in urine of male and female rats. In faeces of male rats, the metabolite BYI 02960-OH was more prominent than the parent compound. Two metabolites, BYI 02960-6-CNA and BYI 02960-hippuric acid were also prominent in male but not in females rats. The organ metabolism study using the ethyl-1-^{14}C label showed that in the 24 hours samples of plasma, and organs and tissues BYI 02960-DFA was by far the dominating metabolite accounting for more than 50% of the radioactivity. In general, the metabolic profiles in urine and faeces were very similar for both sexes but male rats showed a higher rate of metabolite formation as compared to female rats.

The principal metabolic reactions of flupyradifurone in rats were:
Hydroxylation followed by conjugation with glucuronic acid or sulfate,
cleavage of the difluoroethyl group forming BYI 02960-des-difluoroethyl, and difluoroacetic acid (BYI 02960-DFA),
cleavage of the molecule at the pyridinylmethylene bridge forming BYI 02960-6-CNA, which was further conjugated with glycine to BYI 02960-hippuric acid and BYI 02960-difluoroethyl-amino-furanone.
The figure below schematically shows the sites of the molecule, which are involved in the metabolic reactions:

Summarizing, the results of the metabolism studies conducted in the rat, a proposed metabolic pathway of flupyradifurone can be described as shown in this Figure 4:

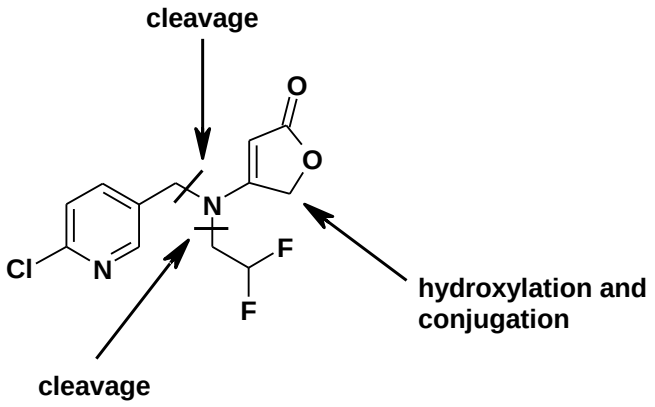


Figure 4: A proposed metabolic pathway of flupyradifurone in rat.

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Characteristics

reference	:	Gillissen, U. 2009	exposure	:	Once by gavage
type of study	:	Acute oral toxicity study	doses	:	300, 2000 mg/kg bw
year of execution	:	2009	vehicle	:	2% Cremophor EL in tap water
test substance	:	BYI 02960 (batch 2009-000239, purity 96.2%)	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 423
species	:	Rat, Wistar Hsd Cpb:Wu	acceptability	:	Acceptable
group size	:	3f (step 1 and step 2)/dose	**LD50-cut off**	:	>300-2000 mg/kg bw

Study design
The study was performed in accordance with OECD 423.

Four groups of three fasted female Wistar rats (HsdCpb: WU) were given a single oral dose (10 mg/kg) of BYI 02960 (batch 2009-000239, purity 96.2%) in 2% Cremophor EL. Clinical signs and mortality rates were determined several times on the day of administration and subsequently normally once daily for an observation period of at least 14 days. Weight gain of the animals was checked weekly until the end of the study. Animals which died or were killed in moribund state were weight (except on day of administration) and dissected as soon as possible and examined macroscopically. Surviving animals were sacrificed, dissected and examined macroscopically.

Results
Mortality:

Dose mg/kg bw	Mortality	Time of death
1st 2000	1/3	2h
2nd 2000	3/3	3h
1st 300	0/3	--
2nd 300	0/3	--

Symptoms of toxicity:
In animals dosed with 2000 mg/kg bw clinical signs included decreased motility, tremor, piloerection, labored breathing and clonical cramps. In animals treated with 300 mg/kg bw breathing sounds were noted.

Body weight:
The mean body weights increased throughout the study period.

Pathology:

In animals dosed with 2000 mg/kg that died during the observation period gross pathological findings included black or black spotted liver and hemorrhagic lung. No particular findings were observed in the surviving animals.

Acceptability
This study is considered acceptable.

Conclusions
The LD50-cut off of BYI 02960 was found to be >300-2000 mg/kg bw.

4.2.1.2 Acute toxicity: inhalation

Characteristics

reference	:	Folkerts, A. 2010	exposure	:	4 hours; nose-only
type of study	:	Acute inhalation toxicity study	doses	:	12.33 mg/L (nominal concentration), 4.7 mg/L (analytical concentration) MMAD 2.04 um with GSD 1.86
year of execution	:	2009	vehicle	:	50% (w/w) PEG 400 (Lutrol)
test substance	:	BYI 02960 (batch 2009-000239, purity 96.2%)	GLP statement	:	Yes
route	:	Inhalation	guideline	:	According to OECD 403
species	:	Rat, Wistar Hsd Cpb: WU (SPF)	acceptability	:	Acceptable
group size	:	5/sex	**LC_{50}**	:	> 4.7 mg/L

Study design
The study was performed in accordance with OECD 403 (1981).

Five male and five female Wistar Hsd Cpb:WU (SPF) rats were simultaneously exposed under nose only conditions for 4 h. The test article was aerosolized as 50% (w/w) solution in PEG 400 (Lutrol). Clinical signs and mortality rates were examined several times on the day of exposure and at least once daily for an observation period of 14 days. At the end of the study animals were sacrificed, dissected and gross pathological changes were recorded.

Results
Mortality: No mortality occurred.

Symptoms of toxicity:
Clinical signs after exposure to 4.7 mg/L BYI 02960 included increased breathing, labored breathing patters, irregular breathing, piloerection, motility reduced or increased, anxiety, tremor, limp, gait high-legged, exophthalmia, nose red encrustations, stridor, and abdominal position with uncoordinated movements. 48 hours after exposure all rats were without clinical signs.

Body weight: No treatment related effect on body weight was observed.

Pathology: No macroscopic pathologic abnormalities were observed.

Acceptability
The study is considered acceptable.

Conclusions
Acute LD_{50} for male and female rats was found to be >4.7 mg/L

4.2.1.3 Acute toxicity: dermal

Characteristics

reference	:	Gillissen U., 2009b	exposure	:	24 hours on a 10% skin area (semi-occlusive)
type of study	:	Acute dermal toxicity study	doses	:	2000 mg/kg bw
year of execution	:	2009	vehicle	:	Distilled water
test substance	:	BYI 02960 (batch 2009-000239, purity 96.2%)	GLP statement	:	Yes
route	:	Percutaneous	guideline	:	According to OECD 402
species	:	Rat, Wistar Hsd Cpb:Wu	acceptability	:	Acceptable
group size	:	5/sex	**LD_{50}**	:	>2000 mg/kg bw

Study design
The study was performed in accordance with OECD 402.

Groups of five animals/sex were exposed to BYI 02960 (batch 2009-000239, purity 96.2%). The test material was moistened and applied to a skin area of 30 cm^2 at a dose of 2000 mg/kg bw under semi-occlusive dressing. After 24 hours the dressings were removed and the area was rinsed. Clinical sign and mortality rates were determined several times on the day of application and subsequently at least once daily for an observation period of at least 14 days. The weight gain of the animals was checked weekly until the end of the study. At the end of the study animals were sacrificed, dissected and examined for gross pathological changes.

Results
Mortality: No mortality was observed
Symptoms of toxicity: No treatment related findings were observed
Body weight: The mean body weights increased throughout the study period.
Pathology: No macroscopic pathologic abnormalities were observed.

Acceptability
The study is considered acceptable.

Conclusions
The acute dermal LD50 of BYI 02960 in male and female rats was found to be greater than 2000 mg/kg bw.

4.2.1.4 Acute toxicity: other routes

4.2.2 Human information

4.2.3 Summary and discussion of acute toxicity

Table 54: Acute toxicity, LD_{50}/LC_{50} values

Test substance	LD_{50}/LC_{50}	Species	Route	Vehicle	Reference
BYI 02960	>300- 2000 mg/kg bw	Rat	Oral	2% Cremophor EL in tap water	Gillissen, U. 2009
BYI 02960	>2000 mg/kg bw	Rat	Dermal	Distilled water	Gillissen, U. 2009b
BYI09260	> 4.7 mg/L	Rat	Inhalation	50% PEG 400 (Lutrol)	Folkert, A. 2010

In addition there were 2 mortalities out of 12 rats in the acute neurotoxicity study at 800 mg/kg bw (see chapter 4.12.1.1).

4.2.4 Comparison with criteria

The acute oral LD50 value for flupyradifurone is between 300 and 2000 mg/kg bw in an OECD 423 test and a classification 'Acute Tox. 4, H302' is warranted according to CLP.
No mortality occurred at the highest dose level in an acute inhalation toxicity study, thus classification for inhalatory toxicity is not warranted.
The acute dermal LD50 value for flupyradifurone is >2000 mg/kg bw and a classification for dermal toxicity is not warranted.

4.2.5 Conclusions on classification and labelling

Flupyradifurone (BYI 02960) needs to be classified as Acute oral toxic Cat. 4 H302 according to Regulation (EC) 1272/2008. Flupyradifurone does not need to be classified on the basis of its acute dermal, and inhalation toxicity in rats.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS presented three studies performed with flupyradifurone (purity 96.2 %) in accordance with OECD Test Guidelines (TG) and GLP (good laboratory practice) for acute toxicity, one study for each route of exposure. Based on the outcome of these studies, the DS proposed to classify flupyradifurone as Acute Tox. 4 by the oral route (H302). The DS did not propose an Acute Toxicity Estimate (ATE, oral) for the purpose of classifying substances or mixtures containing flupyradifurone.

Comments received during public consultation

Three MSCAs agreed with the proposed classification. However, one of them pointed out that classification as Acute Tox. 4; H302 should be based on mortality at 2 000 mg/kg bw/day but not at 300 mg/kg bw/day.

Assessment and comparison with the classification criteria

Acute toxicity: oral

In the acute oral toxicity study, similar to OECD TG 423, two groups of three fasted female Wistar rats were given 300 mg/kg bw of flupyradifurone, two groups received a single dose of 2 000 mg/kg bw. In all rats given 300 mg/kg bw, breathing sounds from 50 minutes to 7 hours after exposure were noted. In the high-dose group, decreased motility, tremor, piloerection, laboured breathing and clonic cramps from 20 minutes to 5 days after dosing were observed. In the four animals that died after dosing with 2 000 mg/kg bw (one after 2 hours, the other three after 3 hours of exposure) gross pathological findings included black or black spotted liver and haemorrhagic lung. The dissection of the surviving animals did not show any particular findings.

Classification in Category 4 via the oral route, is required where the LD_{50} is > 300 and ≤ 2 000 mg/kg bw. Based on the rat acute oral toxicity study the LD_{50} for flupyradifurone is likely to be > 300 but lower than 2000 mg/kg bw. RAC agrees with the DS that flupyradifurone should be classified as Acute Tox. 4; H302 for the oral route. In addition, to facilitate consistent classification of mixtures containing flupyradifurone, a harmonised ATE value should also be proposed. According to the CLP regulation, the ATE value for a substance should be derived using the LD_{50} where available, which is not the case here. Therefore, the ATE is derived using the appropriate conversion value from Table 3.1.2 of CLP Regulation that relates to a classification category. The converted acute toxicity point estimate for a substance classified as Acute Tox. 4; H302 is 500 mg/kg bw. In conclusion, RAC is of the opinion that the converted ATE for flupyradifurone should be used, and proposes to assign an ATE of 500 mg/kg bw for acute oral toxicity.

Acute toxicity: inhalation

The acute inhalation study has been carried out according to OECD TG 403 (1981), in which rats have been exposed during a period of 4h (nose only). The test article was aerosolised as a 50% (w/w) solution in PEG 400 (Lutrol). Clinical signs of toxicity after exposure to 4.7 mg/L (analytical concentration) flupyradifurone included increased and irregular breathing, laboured breathing patterns, stridor, piloerection, anxiety, tremor, exophthalmia, increased or reduced motility, high-legged gait, abdominal position with uncoordinated movements and disappeared in all animals 48 hours after exposure. Neither treatment related effects on body weight nor macroscopic pathologic abnormalities were observed.

Classification for acute toxicity via the inhalation route is required where the LC_{50} value is ≤ 5 mg/L (dusts and mists). The rat 4 h-LC_{50} for flupyradifurone is > 4.7 mg/L. RAC agrees with the DS that **no classification is warranted for acute inhalation toxicity**.

Acute toxicity: dermal

In the acute dermal toxicity study in rats, carried out according to OECD TG 402, a dose of 2 000 mg/kg bw moistened test material (flupyradifurone) was applied to 30 cm^2 of the rat skin under a semi-occlusive dressing. No signs of toxicity/treatment related findings were observed.

Classification for acute toxicity via the dermal route is required where the LD_{50} is ≤ 2 000 mg/kg bw. The LD_{50} was > 2 000 mg/kg bw. RAC agrees with the DS that **no classification is warranted for acute dermal toxicity**.

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

The clinical signs that were apparent after single oral and inhalation exposures to flupyradifurone in acute toxicity studies were indicative of non-specific, general acute toxicity.

Additionally, a short-term oral neurotoxicity study was performed, which is reported under 'Others' (see chapter 4.12.1.1). This study gave a LOAEL of 50 mg/kg and a NOAEL of 35 mg/kg bw in rats based on piloerection and dilated pupils. In the mid-dose group (200 mg/kg bw), at the time-of-peak effect after dosing (2-hours after dosing), treatment-related observations included piloerection, rapid respiration, gait incoordination and flattened body posture in both sexes, with a higher incidence of tremors in both sexes. In addition, automated measures of motor activity were reduced during the first 10-min interval of the session, while activity for the entire test session was comparable to controls. At the low-dose level, the only treatment-related effects were limited to higher incidences of piloerection (both sexes) and dilated pupils (females only) at the time-of-peak effect after dosing. All effects were reversible, with none observed at later time points of the study. There were no macroscopic or microscopic treatment-related observations in either sex at any dose level.

4.3.2 Comparison with criteria

Since the effects induced by flupyradifurone were reversible and no morphological changes or organ damage was reported, classification in Category 1 or 2 is not appropriate. Category 3 classification should be considered for narcotic effects in animal studies, which may include lethargy, lack of coordination, loss of righting reflex, and ataxia. However, the neurotoxic effects reported were not narcotic in nature and thus do not support classification in Category 3.

4.3.3 Conclusions on classification and labelling

No classification is proposed for STOT SE under the CLP Regulation.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The DS did not propose any classification for STOT SE 1 or 2 as no toxicity to a specific organ were observed, neither in the acute toxicity studies in rats nor in a short-term oral neurotoxicity rat study. In each of these GLP-compliant studies conforming to OECD TG flupyradifurone (purity 96.2 %) was administered. In addition, the DS did not propose to classify flupyradifurone as STOT SE 3 for narcotic effects or respiratory tract irritation considering that no such effects were observed.

Comments received during public consultation

There were no comments provided.

Assessment and comparison with the classification criteria

In the acute oral and in the acute inhalation toxicity study in rats, the clinical signs were indicative of non-specific, general acute toxicity. Additionally, no acute organ toxicity was observed in a short-term oral neurotoxicity study, giving a LOAEL of 50 mg/kg bw and a NOAEL of 35 mg/kg bw based on piloerection and dilated pupils (females only).

All effects (e.g. rapid respiration, gait incoordination and flattened body posture at the time-of-peak effect after the dose of 200 mg/kg bw) were reversible. There were no macroscopic or microscopic treatment related observations in either sex at any dose level.

Overall, no specific target-organ toxicity was identified at doses equal to the top of the guidance value range listed in the CLP Regulation (Annex I: 3.8.2.1.9.3, Table 10.3.8.2). Accordingly, flupyradifurone does not meet the criteria for classification for STOT SE Categories 1 or 2 under the CLP Regulation.

STOT SE 3 is assigned for respiratory tract irritation (RTI) and/or narcotic effects. Flupyradifurone administrated by inhalation at a concentration of 4.7 mg/L for a period of 4 h induced general clinical signs of toxicity for a limited period of time (48 h) with no specific narcotic effects. Some findings are only suggestive of possible respiratory irritation (laboured and irregular breathing). Since flupyradifurone is not an irritant to the skin or the eyes, RAC agrees with the DS that there is no evidence to justify a classification for STOT SE 3. Overall, RAC agrees with the DS´s proposal that **no classification for STOT SE** is warranted.

4.4 Irritation

4.4.1 Skin irritation

4.4.1.1 Non-human information

Characteristics

reference	:	Gmelin, C. 2009	exposure	:	4-hours semi-occlusive
type of study	:	Skin irritation study	doses	:	0.5 g
year of execution	:	2009	vehicle	:	Distilled water
test substance	:	BYI 02960 (Batch 2009-000239, purity 96.2%)	GLP statement	:	Yes
route	:	Dermal	guideline	:	According to OECD 404
species	:	Rabbit, New Zealand White Crl:KBL(NZW)BR	acceptability	:	Acceptable
group size	:	3 females	**Effect**	:	Not skin irritating

Study design
The study was performed in accordance with OECD 404 (2002).

Three New Zealand female rabbits (Crl:KBL(NZW)BR) were exposed to 0.5 g of pulverized BYI 02960 (purity 96.2%) moistened with water on a skin area of 2.5 x 2.5 cm^2 under semi-occlusive dressing. After 4 hours of exposure the patch was removed and the exposed area was washed with water. Dermal irritation was scored at approximately 1, 24, 48 and 72 hours after patch removal.

Results
The results are summarised in the tables below.

Individual irritation scores

Scores observed after	**1 hour**	**24 hours**	**48 hours**	**72 hours**
Erythema	0/0/0	0/0/0	0/0/0	0/0/0
Oedema	0/0/0	0/0/0	0/0/0	0/0/0

Mean value irritation scores

Animal	mean 24-72 hrs	
	erythema	oedema
1	0	0
2	0	0
3	0	0
mean	0	0

Acceptability

The study is considered acceptable.

4.4.1.2 Human information

No data available

4.4.1.3 Summary and discussion of skin irritation

Flupyradifurone was found to be non-irritating to rabbit skin.

4.4.1.4 Comparison with criteria

Flupyradifurone does not meet the criteria for classification as a skin irritant.

4.4.1.5 Conclusions on classification and labelling

No classification is proposed for skin irritation under the CLP Regulation.

RAC evaluation of skin corrosion/irritation
Summary of the Dossier Submitter's proposal According to the DS, flupyradifurone does not meet the criteria for classification as a skin irritant. **Comments received during public consultation** There were no comments provided. **Assessment and comparison with the classification criteria** In a standard GLP-compliant study similar to OECD TG 404 in rabbits using 0.5 g of pulverized flupyradifurone (purity 96.2 %) moistened with water, no irritative effects were seen at any time during the study. RAC agrees with the DS that **no classification for skin irritation** is warranted.

4.4.2 Eye irritation

4.4.2.1 Non-human information

Characteristics

reference	:	Gmelin, C. 2009	exposure	:	single instillation in conjunctival sac
type of study	:	Acute eye irritation study	doses	:	0.1 g
year of execution	:	2009	vehicle	:	none
test substance	:	BYI 02960 (Batch 2009-000239, purity 96.2%)	GLP statement	:	Yes
route	:	Ocular	guideline	:	According to OECD 405
species	:	Rabbit, New Zealand White Crl:KBL(NZW)BR	acceptability	:	Acceptable
group size	:	Three females	**Effect**	:	Not eye irritating

Study design
The study was performed in accordance with OECD 405 (2002).

0.1 g of the pulverized substance was placed into the conjunctival sac of one eye of the first animal. The other eye, which remained untreated, served as control. The eye was rinsed approximately 24 hours following instillation. Eye irritations were scored at 1, 24, 48 and 72 hours post application.

Results
The results are summarised in the tables below.

Individual irritation scores

Scores observed after	**1 hour**	**24 hours**	**48 hours**	**72 hours**
Cornea/opacity	0/0/0	0/0/0	0/0/0	0/0/0
Iris	0/0/0	0/0/0	0/0/0	0/0/0
Conjunctiva redness	2/2/2	2/1/1	0/0/0	0/0/0
Conjunctiva chemosis	0/0/1	1/0/0	0/0/0	0/0/0

Mean value irritation scores

Animal	mean 24-72 hrs			
	Corneal opacity	Iris	Conjunctiva redness	Conjunctiva chemosis
1	0	0	0.7	0.3
2	0	0	0.3	0
3	0	0	0.3	0
mean	0	0	0.4	0.1

Acceptability
The study is considered acceptable.

4.4.2.2 Human information

No data available.

4.4.2.3 Summary and discussion of eye irritation

Flupyradifurone is considered not irritating to eyes.

4.4.2.4 Comparison with criteria

Flupyradifurone does not meet the criteria for classification as an eye irritant.

4.4.2.5 Conclusions on classification and labelling

No classification is proposed for eye irritation under the CLP Regulation.

RAC evaluation of serious eye damage/irritation
Summary of the Dossier Submitter's proposal The DS proposed no classification for eye damage. **Comments received during public consultation** There were no comments provided. **Assessment and comparison with the classification criteria** The eye damage/irritation potential of flupyradifurone was assessed in a GLP-compliant study, similar to OECD TG 405 (2002) with 0.1 g pulverized flupyradifurone (purity 96.2 %). In all three animals conjunctival redness was observed at 1 and 24 h after dosing whereas chemosis was found only in one animal. The effects, however, were fully reversed after 48 h. The mean scores (24 to 72 h) were 0.7, 0.3 and 0.3, respectively, for conjunctival redness and 0.3, 0 and 0, respectively, for conjunctival chemosis, i.e. below the classification criteria. RAC agrees with the DS that there is **no evidence to justify a classification for eye irritation**.

4.4.3 Respiratory tract irritation

No data available

4.4.3.1 Non-human information

4.4.3.2 Human information

4.4.3.3 Summary and discussion of respiratory tract irritation

4.4.3.4 Comparison with criteria

4.4.3.5 Conclusions on classification and labelling

4.5 Corrosivity

4.5.1 Non-human information

4.5.2 Human information

4.5.3 Summary and discussion of corrosivity

4.5.4 Comparison with criteria

Flupyradifurone was not corrosive when tested for skin irritation.

4.5.5 Conclusions on classification and labelling

No classification is proposed for corrosivity under the CLP Regulation.

4.6 Sensitisation

4.6.1 Skin sensitisation

4.6.1.1 Non-human information

Characteristics

reference	:	Vohr, H.-W. 2009	exposure	:	Epicutaneous application dorsal part of both ears; 3 consecutive days
type of study	:	Murine Local Lymph Node Assay (LLNA)	doses	:	0, 2, 10, 50%
year of execution	:	2009	vehicle	:	Dimethylformamide
test substance	:	BYI 02960 (Batch 2009-000239, purity 96.2%)	GLP statement	:	Yes
route	:	Dermal	guideline	:	Not according to OECD 429 (2002)
species	:	Mouse; Hsd Win: NMRI	acceptability	:	Acceptable
group size	:	6 female/group	**Effect**	:	Not skin sensitising

Study design
The study performed was similar to OECD 429 (2002), but instead of ^{3}H-Thymidin or ^{125}I-iododeoxyuridine and 10^{-5}M fluorodeoxyuridine incorporation to measure cell proliferation, cell counts were used as described below.

Six female NMRI mice (Hsd Win:NMRI) per group received 25 µl BYI 02960 (batch 2009-000239, purity 96.2%) at 0%, 2%, 10% and 50% epicutaneously onto the dorsal part of both ears. The treatment was repeated three times. The animals were sacrificed one day after the last application. After sacrifice the weight of the lymph nodes was determined and after crushing the lymph nodes through a sieve into a 12-well plate, the cell counts per ml were determined. Before the first treatment and before sacrifice the thickness of both auricles was measured and on day 4 of the study the ear weight of sacrificed animals was measured.

A concurrent positive control was not included. In the study it is indicated that the last reliability test (November 2008) using Alpha Hexyl Cinnamic Aldehyde formulated in acetone/olive oil (4:1) at concentration of 3%, 10% and 30% showed the sensitizing potential of the test item. However, the data was not included in the study.

Results
No signs of systemic toxicity were noticed. No effect on body weight was observed.

Treatment	Cell count Stimulation Index	Lymph node weight Stimulation Index	Ear weight Stimulation Index	Ear thickness Stimulation Index
Vehicle	1.00	1.00	1.00	1.00
2%	1.21	1.01	1.00	1.00
10%	0.97	0.92	1.00	1.01
50%	0.87	0.93	0.97	1.00

Acceptability
The study is considered acceptable although not according to OECD 429. However, the method was validated versus the normal LLNA and shown to have an acceptable predictive value (Kolle et al., 2012).

Conclusions

BYI 02960 has no sensitising potential in the LLNA test in concentrations up to and including 50% using an EC1.5 value as cutoff between sensitisers and non-sensitisers.

4.6.1.2 Human information

4.6.1.3 Summary and discussion of skin sensitisation

Table 55: Sensitisation studies

Test substance	Effect/Classification	Species	Route	Vehicle	Reference
BYI 02960	Not skin sensitising (LLNA)	Mouse	Dermal	Dimethylformamide	Vohr, H.-W. 2009

BYI 02960 has no sensitising potential in the LLNA test in concentrations up to and including 50%.

Flupyradifurone does not have sensitising properties in a LLNA.

4.6.1.4 Comparison with criteria

Flupyradifurone does not meet the criteria for classification as a skin sensitiser.

4.6.1.5 Conclusions on classification and labelling

No classification is proposed for skin sensitisation under the CLP Regulation.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

According to the DS, flupyradifurone does not meet the criteria for classification as a skin sensitiser based on a GLP-compliant but non-guideline Murine Local Lymph Node Assay (LLNA) in mice.

Comments received during public consultation

There were no comments provided.

Assessment and comparison with the classification criteria

The DS summarised in the CLH report a GLP-compliant skin sensitisation study similar to the LLNA based on lymph node cell counts (LNCC).

In that study, six female NMRI mice per group received 25 µL flupyradifurone (purity 96.2 %) at 0 %, 2 %, 10 % and 50 % epicutaneously onto the dorsal part of the ears. The treatment was repeated three times. One day after the last application, the animals were sacrificed. There were no signs of skin irritation. A positive control was not included.

The performed study is a variant of the LLNA (LLNA/IMDS (Integrated Model for the Differentiation of Skin reactions) which is based on LNCC, including the measurement of ear swelling after treatment): After weighing the removed auricular lymph nodes and crushing them through a sieve, the cell counts were determined. Additionally, an 8 mm diameter section of ear was punched out and weighed.

In the LNCC assay, the stimulus index is calculated by dividing the mean node and ear

weights, change in ear thickness (indicating the extent of ear swelling after treatment) and nodal cell counts by the mean values of these parameters in the vehicle control. There was no treatment related increase in any of the parameters which were evaluated up to and including a concentration of 50 % of the tested substance using an EC 1.4 value as the cut-off point between sensitisers and non-sensitisers.

Although the sensitising potential of the positive control (alpha hexyl cinnamic aldehyde) was demonstrated for the test system on a separate occasion in November 2008 (3 %, 10 %, 30 % - but not 50 % - in acetone/olive oil 4:1) and although Kolle *et al*. (2012) published an acceptable predictive value, RAC considers that the following uncertainties remain:

- publications on the LNCC indicate limitations and low predictability of the LNCC compared to the LLNA and other non-radioactive methods,
- the study does not include a positive control,
- historical control data from the performing laboratory were not provided,
- there is no information why the study was not performed with female mice of the usual CBA/Ca or CBA/J strains,
- there are no validated OECD TG for the LNCC study,
- there is no additional supporting information (e.g. human data, *in vitro*/*in chemico*/*in silico* tests).

However, after the public consultation, an OECD- and CLP-compliant LLNA in CBA/J mice (conducted in 2012 to satisfy non-EU requirements) was submitted by industry (Anon., 2012). After the applicability and biocompatibility test with concentrations of 25 % and 50 % (w/v) in DMF, the main assay was performed with 25 µL flupyradifurone (purity 96.2 %) at 10 %, 25 %, 50 % in dimethylformamide (DMF) applied epicutaneously onto the dorsal part of the ears on Days 1, 2 and 3. The study included a negative control group with DMF and a positive control group with 25 % alpha-hexyl cinnamaldehyde (HCA) in DMF. On Day 6, the cell proliferation in the local lymph nodes was measured by incorporation of tritiated methyl thymidine and the values obtained were used to calculate the stimulation indices (SI).

There were no signs of irritation seen at any dose level. The SI values at concentrations of 0 %, 10 %, 25 % and 50 % (w/v) were 1.0, 2.3, 2.5 and 1.6 whereas the positive control showed an SI value of 13.5.

Therefore, based on the LLNA study that was not included in the original CLH proposal from the DS, RAC is of the opinion that flupyradifurone does not warrant a classification for skin sensitisation.

Supplemental information - In depth analyses by RAC

Industry provided a study report on a GLP- and OECD-compliant LLNA/IMDS performed in 2002 on 144 female NMRI mice (6 animal/group and 6 animals/control group) instead of CBA mice to confirm the reliability of the LLNA method with alpha hexyl cinnamic aldehyde (purity 85 %) at concentrations of 0 %, 3 %, 10 %, 30 % using six different vehicles (polyethylene glycol 400; DAE 433; dimethylformamide; methyl ethyl ketone; acetone/olive

oil 4:1 and cremophor EL/physiological saline solution 2 % v/v). The sacrifice was done on day 3. According to the study director, the test could confirm the sensitivity and reliability of the LLNA. However, there were some discrepancies in the outcome with regard to the potency between the vehicles used.

Besides, inclusion of a concurrent positive control is recommended by the OECD TG because it demonstrates competency of the laboratory to successfully conduct each assay. It also enables an assessment of intra-, and inter-laboratory reproducibility and comparability. Some regulatory authorities also require a positive control for each study and therefore users are encouraged to consult the relevant authorities prior to conducting the LLNA. Accordingly, the routine use of a concurrent positive control is encouraged to avoid the need for additional animal testing to meet such requirements that might arise from the use of a periodic positive control. Because the LNCC is not a common and highly reproducible test, RAC considers that the use of a concurrent positive control was needed.

4.6.2 Respiratory sensitisation

4.6.2.1 Non-human information

No information available.

4.6.2.2 Human information

No information available.

4.6.2.3 Summary and discussion of respiratory sensitisation

4.6.2.4 Comparison with criteria

4.6.2.5 Conclusions on classification and labelling

No classification based on absence of data.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for respiratory sensitisation in the absence of data on the potential of flupyradifurone to cause respiratory sensitisation.

Comments received during public consultation

No comments were provided.

Assessment and comparison with the classification criteria

A respiratory sensitiser is described as a substance that will lead to hypersensitivity of the airways. In the absence of any data, flupyradifurone **does not require classification for respiratory sensitisation**.

4.7 Repeated dose toxicity

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

4.7.1.1.1 28-day oral studies

Study 1

Characteristics

Reference	:	Capt, A. 2007	exposure	:	28 days
type of study	:	Exploratory 28-day rat oral toxicity study	dose	:	0, 75, 200, 350 mg/kg bw/day
year of execution	:	2006	vehicle	:	Corn oil supplemented with 10% ethanol and 10% water
test substance	:	BYI 02960 (Batch NLL 7780-11-5, purity 98.3%)	GLP statement	:	No
Route	:	gavage	guideline	:	-
Species	:	Rat, Wistar RJ: WI (IOPS HAN)	acceptability	:	Acceptable as range-finding study
group size	:	5/sex/dose	**NOAEL**	:	75 mg/kg bw/day

Study design

Groups of five animals per sex were orally dosed BYI 02960 (batch NLL 7780-16-5, purity 98.3%) for at least 28 days at dose levels of 75, 200 and 350 mg/kg bw/day. Animals were observed daily for mortality and clinical signs. Body weight and food consumption were recorded once weekly. Selected haematology and clinical chemistry parameters were assayed at the end of the study. At the end of the study, all animals were necropsied, selected organs were weighed and a range of tissues were examined microscopically. A portion of the liver was homogenized for microsomal preparations to determine cytochrome P-450 isoenzyme profile.

Results

Dose (mg/kg bw/day)	**0**	**75**	**200**	**350**
	m f	**m f**	**m f**	**m f**
Mortality	Two females at 350 mg/kg bw/day, one female at 200 mg/kg bw			

Dose (mg/kg bw/day)	**0**		**75**		**200**		**350**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Clinical signs	Increased salivation in all exposed animals							
Body weight	Slight not statistically significant lower mean body weight (max 8%) in males at 350 mg/kg/day throughout the study and in females in the first week.							
Body weight gain (g)	180	71	170	65	176	76	159	88
Food consumption	Reduced at 350 mg/kg/day in males (17%) and females (29%) between study day 1 and 8, and by 8% in males between day 8 and 15. At 200 mg/kg/day reduced in females by 16% between day 1 and 8.							
Ophthalmology	Not determined							
Urinalysis	Not determined							
FOB	Not determined							
Haematology	No treatment related changes.							
Clin. Chemistry								
-total bilirubin (µmol/L)	1.2	1.6	**0.7* (-42%)**	0.9 (-44%)	**0.6* (-50%)**	**0.7* (-56%)**	**0.4** (-67%)**	**0.5** (-69%)**
- Glucose concentration	5.42	4.80	4.60 (-15%)	5.07 (+6%)	**3.92** (-28%)**	5.12 (+7%)	**2.87** (-47%)**	3.57 (-26%)
- Triglyceride concentration	1.14	0.45	1.50 (+32%)	0.46 (+2%)	1.32 (+16%)	0.87 (+93%)	1.89 (+66)	**1.51* (+236 %)**
- Creatinine concentration	50	51	51 (+2%)	56 (+10%)	**54* (+8%)**	**63* (+24%)**	53 (+6%)	**63* (+24%)**
- Alanine aminotransferase	39	33	43 (+10%)	35 (+6%)	38 (-3%)	**43** (+30%)**	46 (+18%)	**51** (+55%)**
- Alanine phosphatase	165	95	163 (-1%)	110 (+16%)	166 (+1%)	96 (+1%)	165 (0%)	**126* (+36%)**

Dose (mg/kg bw/day)	**0**		**75**		**200**		**350**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Organ weights								
-liver weight								
-absolute (g)	10.92	5.86	9.97 (-9%)	6.37 (+9%)	12.01 (+10%)	7.32 (+25%)	12.43 (+14%)	**8.74** (+49%)**
- relative to body weight (%)	3.095	2.552	2.913 (-6%)	2.850 (+12%)	3.442 (+11%)	**3.157* (+24%)**	**3.814* (+23%)**	**3.704** (+45%)**
- relative to brain weight	542.7	315.1	482.5 (-11%)	328.8 (+4%)	602.6 (+11%)	382.3 (+21%)	625.6 (+15%)	**460.6** (+46%)**
Pathology								
Macroscopy (liver)								
- enlarged	0/5	0/5	0/5	0/5	1/5	0/4	3/5	3/3
- prominent lobulation	0/5	0/5	1/5	0/5	2/5	0/4	5/5	2/3
Microscopy								
Centrilobular hepatocellular hypertrophy: diffuse								
- minimal	0	0	0	0	0	2	0	0
- slight	0	0	0	0	4	1	1	2
- moderate	0	0	0	0	1	0	3	1
- marked	0	0	0	0	0	0	1	0
- total	0	0	0	0	5	3	5	3
Follicular cell thyroid hypertrophy: diffuse								
- minimal	0	0	0	0	4	0	4	2
- slight	0	0	0	0	0	0	1	1
- total	0	0	0	0	4	0	5	3
Hepatotoxicity testing								
- P-450			X 1.2	NC	X 1.3	X 1.2	NC	X 1.2
- BROD			X 8	NC	X 25	X 8	X 25	X 28
- EROD			NC	NC	NC	**X 4***	**X 3***	**X 4***
- PROD			NC	NC	X 5	NC	X 5	X 4
- Lauric acid			NC	NC	NC	NC	NC	NC

*a high inter-individual variability was observed in EROD activities, X = increase compared to controls

Two females were found dead on Study Day 6 at 350 mg/kg bw/day and one female died at 200 mg/kg bw/day. The only clinical sign found was increased salivation in all animals at all dose levels. Mean body weight was slightly reduced in males by between 5% to 8% (not statistically significant) and in females by 4% on Study Day 8 (not statistically significant). Mean food consumption was reduced by maximally 17% in males and by 29% in females.

Clinical chemistry evaluations showed a decrease in total bilirubin in both sexes at the highest two dose levels. Glucose concentration was only significantly reduced in males at 200 and 350 mg/kg bw/day. Triglyceride concentration and alaline phosphatase were significantly increased in high dose females. Both creatinine concentration and alanine aminotransferase were significantly increased in mid and high dose females. Creatine concentration was also significantly increased in males at the mid dose only. The statistically significant difference observed in total bilirubin at 75 mg/kg in males was due to only one animal with a total bilirubin concentration of 0.0 µmol/l, while the mean of the remaining animals was 0.85 µmol/l. Therefore, this effect was considered to not be treatment-related.

Mean absolute and relative liver weights were 14 to 23% higher in males and 45 to 49% higher in females, compared to controls at 500 mg/kg bw/day. Upon macroscopic examination, enlargement and prominent lobulation of the liver were observed in both sexes. The liver effects observed at 200 and 350 mg/kg/day were correlated with microscopic centrilobular hepatocellular hypertrophy. The one incidence of prominent lobulation at 75 mg/kg/day in males were without associated microscopic findings and were considered not to be adverse.

Hepatocellular enzymatic activity assays showed that EROD activity was not found to be clearly affected by treatment. As BROD activity was induced at 350 mg/kg/day in both sexes and at 200 mg/kg/day in males, with no clear induction of PROD activity, BYI 02960 appears to be an inducer of the Cytochrome P-450 3A family.

Acceptability
The study was not performed under GLP, but it was performed in a GLP-certified laboratory. The study is acceptable as a dose range finding study.

Conclusions
Based on the clinical chemical finding, the increased organ weights and pathological findings the NOAEL is set at 75 mg/kg bw/day.

Study 2

Reference	:	Blanck, M. 2008	exposure	:	28 days
type of study	:	Exploratory 28-day rat oral study	dose	:	0, 500, 5000 ppm M: 0, 33.6, 385 mg/kg bw/day)
year of execution	:	2007	vehicle	:	none
test substance	:	BYI 02960 (batch NLL 7780-27-1, purity 99.7%)	GLP statement	:	No
Route	:	Oral (Dietary)	guideline	:	-
Species	:	Rat, Wistar Rj:WI (IOPS HAN)	acceptability	:	Acceptable as range-finding study
group size	:	5 males/dose	**NOAEL**	:	33.6 mg/kg bw/d

Study design

Groups of five males were treated with BYI 02960 (batch NLL 7780-27-1, purity 99.7%) via the diet for at least 28 days at concentrations of 500 ppm (actual analyzed dose level of 410 ppm, which equates to 33.6 mg/kg/day BYI 02960) and 5000 ppm (equivalent to 385 mg/kg bw/day). All animals were observed for mortality and clinical signs daily, body weight and food consumption were measured weekly. A detailed physical examination was performed weekly throughout the study. Before necropsy a blood sample was collected from the retro-orbital venous plexus of each surviving animal for selected clinical chemistry determinations and hormone analysis. All animals were necropsied, selected organs weighed and a range of tissues were taken, fixed and examined microscopically. The remaining portions of the liver were homogenized for microsomal preparations in order to determine cytochrome P-450 isoenzyme profile.

Results

Dose (ppm)	**0**	**500**	**5000**
Mortality	No mortalities occurred		
Clinical signs	No clinical signs were observed		
Body weight	At 5000 ppm, mean body weight was reduced by between 17 and 19% throughout the study period (statistically significant for the first 3 weeks)		
Body weight gain (g)	186	194	**116***
Food consumption	At 5000 ppm food consumption was consistently reduced by between 12 and 39% throughout the study period, most pronounced between study day 1 and 8.		
Ophthalmology	Not performed		

Dose (ppm)	**0**	**500**	**5000**
Urinalysis	Not performed		
FOB	Not performed		
Haematology	Not performed		
Clin. Chemistry			
- total bilirubin (µmol/L)	1.1	0.7 (-36%)	**0.3*** **(-73%)**
- Glucose concentration	6.91	6.60 (-4%)	**3.76**** **(-46%)**
- Urea	4.98	4.82 (-3%)	**6.83**** **(+37%)**
- Total cholesterol	1.43	1.29 (-10%)	**2.01*** **(+41%)**
- T4	48.5	58.7 (+21%)	39.5 (-19%)
- TSH	9.64	11.23 (+17%)	17.46 (+81%)
Organ weights			
-liver weight			
-absolute (g)	9.79	10.38 (+6%)	11.25 (+15%)
- relative to body weight (%)	2.751	2.873 (+4%)	**3.906**** **(+42%)**
- Thyroid gland			
- absolute (g)	0.0171	0.0165 (-4%)	0.0182 (+6%)
- relative to body weight (%)	0.0048	0.00457 (-5%)	**0.00632**** **(+32%)**
Pathology			
Macroscopy (liver)			
- prominent lobulation	0/5	2/5	4/5
Microscopy			

Dose (ppm)	**0**	**500**	**5000**
Centrilobular hepatocellular hypertrophy: diffuse			
- slight	0	0	2
- moderate	0	0	3
- total	0	0	5
Follicular cell thyroid hypertrophy: diffuse			
- minimal	0	0	2
- slight	0	0	3
- total	0	0	5
Hepatotoxicity testing			
- P-450		NC	NC
- BROD		NC	X 2.1
- EROD		NC	NC
- PROD		NC	NC
- UDPGT		NC	X 1.11

*p≤0.05 **p≤0.01 NC = no significant change

At 5000 ppm mean body weight was reduced with a maximum of 19% during the first three weeks of the study. Overall mean cumulative body weight was significantly reduced by 38% compared to controls. Mean food consumption was consistently reduced by maximally 39% with the effect being more pronounced between study day 1 and 8. Total bilirubin and glucose concentration were significantly decreased and urea and total cholesterol were significantly increased at 5000 ppm. Hormone analysis showed a non-significant increase in TSH (+81%) and a slight decrease in T4 (-19%) in the plasma which was considered treatment related. At the high dose relative liver and thyroid weight were significantly increased. These were associated microscopic findings including slight and moderate centrilobular hepatocellular hypertrophy and minimal and slight follicular cell hypertrophy.

Acceptability

The study was not performed under GLP, but it was performed in a GLP-certified laboratory. The study is acceptable as a dose range finding study.

Conclusion

Based on the clinical chemical findings, the increased relative liver and thyroids weights and the associated pathological findings, the dose level of 500 ppm (33.6 mg/kg/day) is considered to be the NOAEL in this study.

Study 3

Reference	:	Blanck, O. 2007	exposure	:	28-days
type of study	:	Preliminary 28-day mouse oral toxicity study	Dose	:	0, 300, 600, 1200 ppm M: 0, 50, 98, 207 mg/kg bw/day F: 0, 59, 122, 240 mg/kg bw/day
year of execution	:	2007	Vehicle	:	none
test substance	:	BYI 02960 (NLL 7780-27-1, purity 99.7%)	GLP statement	:	No
Route	:	(Oral) dietary	guideline	:	-
Species	:	Mouse C57BL/6J	acceptability	:	Acceptable as range-finding study
group size	:	5 sex/dose	**NOAEL**	:	98 mg/kg bw/day

Study design

BYI 02960 was administered continuously via the diet to groups of C57BL/6J mice (five/sex) for at least 28 days at concentrations of 300, 600 and 1200 ppm (equating approximately to 50, 98 and 207 mg/kg bw/day in males and 59, 122, 240 mg/kg bw/day in females). No stability study was performed on the submitted feed. Taking into consideration the available stability data (study SA 07131), the actual concentration received by the treated animals is assumed to be between 80% and 90% of nominal concentrations. Animals were observed daily for mortality and clinical signs. Body weight and food consumption were recorded weekly. Selected clinical chemistry parameters were determined at the end of the study. All animals were subjected to necropsy, selected organs were weighed and a range of tissues were fixed and examined microscopically.

Results

Dose (ppm)	**0**	**300**	**600**	**1200**
	m f	**m F**	**m f**	**m f**
Mortality	No mortalities occurred during the study			
Clinical signs	No clinical signs were observed			
Body weight	In males at 1200 ppm, mean body weight was slightly reduced by 6% on study day 8 only.			
Body weight gain (g)	3.3 3.8	3.2 3.9	3.7 3.3	2.8 4.0
Food consumption	No effect on mean food consumption was noted			
Ophthalmology	Not performed			
Urinalysis	Not performed			

Dose (ppm)	**0**		**300**		**600**		**1200**	
	m	**f**	**m**	**F**	**m**	**f**	**m**	**f**
FOB	Not performed							
Haematology	Not performed							
Clin. Chemistry								
- Alanine aminotransferase	39	35	39 (0%)	40 (+14%)	30 (-23%)	36 (+3%)	29 (+26%)	**50** (+43%)**
- Alanine phosphatase	112	151	110 (-2%)	174 (+15%)	113 (+1%)	137 (-9%)	118 (+5%)	**183* (+21%)**
Organ weights								
-epididymis								
-absolute (g)	0.10		**0.08* (-20%)**		**0.08* (-20%)**		**0.07** (-30%)**	
- relative to body weight (%)	0.470		**0.384* (-18%)**		0.401 (-15%)		**0.364** (-23%)**	
- Spleen								
-absolute (g)	0.040	0.061	**0.049* (+23%)**	0.053 (-13%)	0.044 (+10%)	0.054 (-11%)	0.044 (+10%)	0.051 (-16%)
- relative to body weight (%)	0.1956	0.3534	**0.2394** (+22%)**	0.3094 (-12%)	0.2148 (+9.8%)	0.3153 (-11%)	0.2214 (+13%)	0.3052 (-14%
Pathology Macroscopy	No treatment related findings							
Microscopy	No treatment related findings							

*p≤0.05 **p≤0.01

There were no mortalities or clinical signs during the course of the study in any treatment group. Mean body weight was slightly reduced by 6% in males at 1200 ppm at study day 8 only. At 1200 ppm mean body weight gain/day was -0.03 g (p≤0.05) compared with 0.14 g/day in the control group between Study Days 1 and 8. Overall cumulative mean body weight gain was reduced by 15% between Study Days 1 and 29. As the effect on body weight was slight and transient and in the absence of other findings, it was considered to be a non-adverse effect of treatment. In historical

control data from 28-day mouse study performed in the same laboratory with animals from the same strain and the same age, it can be seen that the range for mean cumulative body weight gain in males is between 2.2 to 3.9 grams. The cumulative body weight gain of 2.8 g for males administered at 1200 ppm is therefore within this range.
When compared to the controls, a higher alanine aminotransferase (+43%, p <0.01) and alkaline phosphatase (+21%, p <0.05) activities were observed in females at 1200 ppm. However in view of the variation of the individual values and the lack of a clear dose response these changes were considered not to be treatment-related.

Lower epididymis weights were found in treated animals when compared to controls but this change was considered not to be relevant since it was not dose-related and not associated with relevant histological findings. Furthermore the epididymis weights were within historical control data as can be seen in the following table. These historical control data were taken from two 28-day mouse toxicity studies performed in the same laboratory using mice from the same supplier administered with the same diet, A04Cp1-10 from S.A.F.E (SA 00226 performed in 2001 and SA 08036 performed in 2008; more 28-day mouse studies were performed in the laboratory during this period but epididymis weights were not collected).
Mean absolute and relative spleen weights were statistically significantly higher in males at 300 ppm when compared to controls, but this change was considered not to be relevant since it was not dose-related.

Table 56: Epididymis weight changes

Dose levels in ppm	Animal Numbers	Absolute organ weights (g)	Organ to body weight ratios (%)	Organ to brain weight ratios (%)
0	RT1M1406	0.10	0.51	23.26
	RT1M1407	0.08	0.39	18.18
	RT1M1408	0.09	0.45	19.57
	RT1M1409	0.11	0.53	25.00
	RT1M1410	0.10	0.47	22.22
	Mean	**0.10**	**0.47**	**21.645**
	SD	**0.01**	**0.054**	**2.761**
300	RT2M1416	0.09	0.45	21.43
	RT2M1417	0.08	0.40	18.60
	RT2M1418	0.08	0.37	17.78
	RT2M1419	0.07	0.36	16.67
	RT2M1420	0.07	0.34	15.91
	Mean	**0.08***	**0.384***	**18.077***
	SD	**0.01**	**0.044**	**2.138**
600	RT3M1426	0.07	0.33	15.91
	RT3M1427	0.09	0.46	20.00
	RT3M1428	0.08	0.38	17.78
	RT3M1429	0.09	0.47	20.45
	RT3M1430	0.08	0.36	17.39
	Mean	**0.08***	**0.401**	**18.307**
	SD	**0.01**	**0.059**	**1.894**
1200	RT4M1436	0.07	0.37	15.56
	RT4M1437	0.07	0.35	15.91
	RT4M1438	0.08	0.41	18.18
	RT4M1439	0.07	0.34	15.56
	RT4M1440	0.07	0.34	15.22
	Mean	0.07**	0.364**	16.084**
	SD	0.00	0.029	1.198

Historical control data			
	Absolute organ weights	Organ to body weight ratio (%)	Organ to brain weight ratio (%)

Mean	0.08	0.376	18.120
Standard deviation	0.01	0.060	3.409
Number of animals	10	10	10
Minimum value	0.07	0.315	14.894
Maximum value	0.10	0.500	23.810
Percentile 5%	0.07	0.321	15.039
Percentile 95%	0.10	0.479	23.560

Acceptability

The study was not performed under GLP, but it was performed in a GLP-certified laboratory. The study is acceptable as a dose range finding study.

Conclusion

The decreased epididymis weight was within background historical control data. Due to the low number of animals by dose group, the study has to be considered as supplementary.

Continuous dietary administration of BYI 02960 to the C57BL/6J mouse for at least 28 days resulted in a No Observed Adverse Effect Level (NOAEL) in males and a No Observed Effect Level (NOEL) in females at a nominal concentration of 600 ppm (98 mg/kg bw/d). Considering the reduction of body weight gain at 1200 ppm, this might be considered as a LOAEL. Additionally ALT was also increased at the high dose.

The dose was adjusted for the stability of the diet, and therefore calculated to a NOAEL of 166 mg/kg bw/d. Since the study is a preliminary study, and not critical for risk assessment. The RMS can agree with a worst-case NOAEL of 98 mg/kg bw/d.

Note: The storage stability in the other studies presented in the DAR gave no indication of concern.

Study 4

Reference	:	Odin-Feurtet, M. 2008	exposure	:	Repeated by diet, 28 days
type of study	:	Preliminary 28 day dog oral toxicity study	dose	:	0, 500, 2000, 4000 ppm M: 0, 16, 62, 118 mg/kg bw/day F: 0, 18, 77, 131 mg/kg bw/day
year of execution	:	2008	vehicle	:	none
test substance	:	BYI 02960 (batch NLL 7780-44-6, purity 99.5%)	GLP statement	:	No
Route	:	Oral (dietary)	guideline	:	-
Species	:	Beagles	acceptability	:	Acceptable as range-finding study
group size	:	2/sex/dose	**NOAEL**	:	62 mg/kg bw/day

Study design

Groups of two males and two females received BYI 02960 mixed in their diet at concentrations of 500, 2000 or 4000 ppm (equating approximately to 16, 62, 118 mg/kg body weight/day in males and 18, 77, 131 mg/kg body weight/day in females) for at least 28 days. Mortality and clinical signs were checked at least once daily. Food consumption was recorded daily and body weight was measured weekly. Once during the acclimatization phase and at the end of the treatment, Ophthalmological examination, detailed physical examination, blood analysis (haematology, clinical chemistry) and urinalysis were performed. All animals were subjected to a detailed necropsy. Selected organs were weighed and a range of tissues were taken and processed for histopathological examination.

Results

Dose (ppm)	**0**	**500**	**2000**	**4000**
	m f	**m f**	**m f**	**m f**
Mortality	No mortalities occurred			
Clinical signs	At 4000 ppm genital discharge between day 15 to 22 in one female (attributed to oestrus)			
Body weight	At 4000 ppm overall body weight loss of 0.2 kg in one male.(-2%)			
Body weight gain (g)	No body weight gain in one male and one female at 4000 ppm (0.5-1.1 kg weight gain in controls)			
Food consumption	At 4000 ppm lower food consumption in all animals.			
Ophthalmology	No treatment-related findings			

Dose (ppm)	**0**		**500**		**2000**		**4000**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Urinalysis	No treatment-related finding							
FOB	Not performed							
Haematology - platelet counts	Increased platelet counts in both females (+43 and +36%, relative to their own pre-study value) and in one of the two males at 4000 ppm (+30%) and in one of the two females at 2000 ppm (+28%).							
Clin. Chemistry	At 4000 ppm, an increase was observed in creatinine concentration in one female (+31%, relative to its own pre-study value).							
Organ weights	No treatment-related findings							
Pathology Macroscopy - enlarged thyroid	0	0	0	0	0	0	0	2
Microscopy **Centrilobular glycogen accumulation** - minimal - slight - total	 0 2 2	 2 0 2	 0 2 2	 2 0 2	 1 0 1	 2 0 2	 0 0 0	 0 0 0

*p≤0.05 **p≤0.01, NC = no significant change

There was an overall body weight loss in one male and an absence of overall weight gain in one female at 4000 ppm. Lower food consumption was observed in both male and female animals compared to the controls.

Haematology assessment revealed an increased platelet counts in both females (+43 and +36%, relative to their own pre-study value) and in one of the two males (+30%, relative to its own pre-study value) at 4000 ppm. At 2000 ppm, an increase was noted in one of the two females (+28%, relative to its own pre-study value). In isolation, these treatment-related changes were not considered to be adverse.

Enlarged thyroid glands were noted in 2/2 females at 4000 ppm but this change was not considered to be treatment-related as there was no effect on thyroid weight and there were no microscopic findings for the thyroid. In the liver, centrilobular glycogen accumulation was decreased in incidence and/or severity at 4000 and 2000 ppm in males and at 4000 ppm in females. This finding was considered to be treatment-related, but not adverse.

Acceptability
The study was not performed under GLP, but it was performed in a GLP-certified laboratory. The study is acceptable as a dose range finding study.

Conclusion
Based on the body weight loss and reduced body weight gain a NOAEL of 2000 ppm is set (equating to 62 mg/kg/day in males and to 77 mg/kg/day in females).

4.7.1.1.2 Semichronic oral studies

Study 1

Reference	:	Odin-Feurtet, M. 2009	exposure	:	Repeated diet, 90 days
type of study	:	90-day rat oral toxicity study	dose	:	0, 100, 500, 2500 ppm M: 0, 6.0, 30.2, 156 mg/kg bw/day F: 0, 7.6, 38.3, 186 mg/kg bw/day
year of execution	:	2008	vehicle	:	none
test substance	:	BYI 02960 (batch NLL 7780-44-6, purity 99.5%)	GLP statement	:	yes
Route	:	diet	guideline	:	OECD 408
Species	:	Rats, Wistar Rj:WI (IOPS HAN)	acceptability	:	Acceptable
group size	:	10/sex/dose	**NOAEL**	:	6.0 mg/kg bw/day

Study design

Groups of 10 males and 10 females Wistar rats were fed diets containing BYI 02960 (batch NLL 7780-44-6, purity 99.5%) at concentrations of 100, 500, 2500 ppm (equating to approximately 6.0, 30.2, 156 mg/kg bw/day in males and 7.6, 38.3, 186 mg/kg bw/day in females) for at least 90 days. An additional 10 animals per sex were fed control or high dose test diet for at least 90 days and subsequently fed control diet and observed for reversibility or persistence of toxic effects after a post-treatment recovery period of at least 28 days. The stability of the test substance in the diet (concentrations of 20, 100 and 2500 ppm) was checked over a period of at least 92 or 81 days of frozen storage followed by 10 days at room temperature or for at least 102 or 91 days of storage at room temperature. Clinical signs were recorded daily, body weight was measured weekly. Food consumption was measured twice weekly during the first 6 weeks of treatment and weekly thereafter. All surviving animals from the main study groups were subjected to a neurotoxicity assessment (exploratory locomotor activity, open field observations, sensory reactivity and grip strength) during weeks 12 to 13 of the study. Ophthalmological examinations were performed on all animals during the acclimatization phase and on all animals of the control and high dose groups during week 13. Blood samples were collected from the sublingual vein of the first five suitable animals of each group for further determination of the test substance and its metabolites. Urine samples were collected overnight on the week before scheduled necropsies from all animals. Before scheduled necropsies a blood sample was collected from the retro-orbital venous plexus of each

animal for haematology and clinical chemistry determinations. All animals were necropsied, selected organs weighed and a range of tissues were taken, fixed and examined microscopically.

Results

Dose (ppm)	**0**		**100**		**500**		**2500**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality	No mortalities occurred							
Clinical signs	No clinical signs were observed							
Body weight	At 2500 ppm, a lower body weight (6-10%) was observed in both sexes throughout the study (significant in females only).							
Body weight gain (g)	293	110	308	110	287	**97***	**259***	**94****
Food consumption	Slight reduction in both males and females at 2500 ppm							
Ophthalmology	No treatment-related changes							
Urinalysis	No treatment-related changes							
FOB	No treatment-related changes							
Haematology -platelet count	1160	1233	1121 (-3%)	1324 (+7%)	1224 (+6%)	1187 (-4%)	1235 (+6%)	**1415*** **(+15%)**
Clin. Chemistry -total bilirubin (µmol/L)	1.33	2.05	1.1 (-15%)	1.9 (-5%)	1.1 (-15%)	1.8 (-10%)	**0.8**** **(-38%)**	**1.1**** **(-45%)**
- Glucose (mmol/l)	6.59	6.03	6.56 (0%)	5.82 (-3%)	6.19 (-6%)	5.58 (-7%)	**5.23**** **(-21%)**	**4.69**** **(-22%)**
- Total cholesterol (mmol/l)	1.54	1.55	1.78 (+16%)	1.68 (+8%)	1.70 (+10%)	1.86 (+20%)	1.97 (+28%)	**2.26**** **(+46%)**
- Triglyceride	0.85	0.44	1.03	0.44	0.85	0.48	1.15	0.73

Dose (ppm)	**0**		**100**		**500**		**2500**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
concentration			(+21%)	(0%)	(0%)	(+9%)	(+35%)	(+66%)
Organ weights								
-liver weight								
-absolute (g)	10.92	6.18	11.37 (+4%)	5.91 (-4%)	10.92 (0%)	5.93 (-4%)	11.78 (+8%)	6.65 (+8%)
- relative to body weight (%)	2.194	2.290	2.231 (+2%)	2.170 (-5%)	2.255 (+3%)	2.283 (0%)	**2.546** (+16%)**	**2.624** (+15%)**
- thyroid gland								
-absolute (g)	0.0195	0.0162	0.0222 (+14%)	0.0162 (0%)	0.0228 (+17%)	0.0165 (+2%)	0.0234 (+20%)	0.0171 (+6%)
- relative to body weight (%)	0.00393	0.00602	0.00439 (+12%)	0.00599 (0%)	**0.00473* (+20%)**	0.00633 (+5%)	**0.00494** (+26%)**	0.00679 (+13%)
- Heart								
-absolute (g)	1.48	1.05	1.53 (+3%)	1.02 (-3%)	1.51 (+2%)	0.99 (-6%)	1.43 (-4%)	**0.94** (-10%)**
- relative to body weight (%)	0.298	0.388	0.302 (+1)	0.378 (-3%)	0.312 (+5%)	0.380 (2%)	0.305 (+2%)	0.374 (-4%)
- Kidney								
-absolute (g)	2.90	1.88	2.93 (+1%	1.77 (-6%)	2.92 (+1%)	1.75 (-7%)	2.79 (-4%)	**1.71* (-9%)**
- relative to body weight (%)	0.585	0.697	0.581 (-1%)	0.650 (-7%)	0.602 (+3%)	0.674 (-5%)	0.596 (+2%)	0.678 (-3%)
Pathology								
Macroscopy								
- enlarged liver	0/10	0/10	0/10	0/10	0/10	0/10	4/10	1/10
- dark thyroid gland	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10
Microscopy								
Centrilobular								

Dose (ppm)	**0**		**100**		**500**		**2500**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
hepatocellular hypertrophy: diffuse								
- minimal	0	0	0	0	0	0	6	3
- slight	0	0	0	0	0	0	4	0
- total	0	0	0	0	0	0	10	3
Follicular cell thyroid hypertrophy: diffuse	0	0	0	0	0	0	3	0
- minimal	0	0	0	0	0	0	3	0
- total								

*p≤0.05 **p≤0.01

At 2500 ppm, a lower body weight was observed in both sexes throughout the study (6-10%). A reduced mean body weight gain/day was observed in high dose males and in mid and high dose females. At 500 ppm the overall mean body weight gain was 12% lower than the controls in females at the end of the treatment period. In the absence of any other changes in the parameters assessed, this finding was considered not to be adverse.

Ophthalmological examination revealed haemorrhage of the iris and a damaged eye in one male and one female, respectively, exposed to 2500 ppm. In view of the single occurrence, these findings were considered incidental.

Haematological evaluation revealed a higher mean platelet count in high dose females when compared to the control group (+15%). In addition, mean total bilirubin and glucose concentrations were slightly lower in both sexes and mean total cholesterol and triglycerides concentrations were slightly higher when compared to the controls. At the end of the recovery phase, the mean concentration remained slightly lower. The other treatment-related changes were considered to be reversible as no relevant difference was noted after the recovery period.

At 2500 ppm, mean liver to body weight ratio was statistically significantly higher in both sexes when compared to controls. This change, even if partly due to lower terminal body weight, was associated with relevant histopathological findings and was considered to be treatment-related. Mean thyroid gland to body weight ratio was statistically significantly higher in males when compared to controls and was associated with minimal follicular cell hypertrophy. At the end of the recovery phase, no treatment-related findings were observed in the liver and the thyroid gland. All the treatment-related findings observed at the end of the treatment period were thus considered to be reversible.

Acceptability
The study is considered acceptable

Conclusion
Based on the increased relative and absolute thyroid weights the NOAEL in this study is set at 100 ppm (corresponding to 6 mg/kg bw per day).

Study 2

Reference	:	Odin-Feurtet, M. 2009	exposure	:	Repeated diet, 90 days
type of study	:	90-day mouse oral toxicity	dose	:	0, 100, 500, 2500 ppm M: 0, 16, 81, 407 mg/kg bw/day F: 0, 19, 98, 473 mg/kg bw/day
year of execution	:	2008	vehicle	:	none
test substance	:	BYI (batch NLL 7780-44-6, purity 99.5%)	GLP statement	:	yes
Route	:	diet	guideline	:	OECD 408
Species	:	Mice C57BL/6J	acceptability	:	Acceptable
group size	:	10/sex/dose	**NOAEL**	:	81 mg/kg bw/day

Study design

The study follows OECD guideline 408 except for that FOB and ophthalmology were not performed.

Mice (10/sex/dose) received BYI 02960 (batch NLL 7780-44-6, purity 99.5%) at dietary concentrations of 100, 500 and 2500 ppm (equating approximately to 16, 81, 407 mg/kg bw/day in males and 19, 98, 473 mg/kg bw/day in females) for at least 90 days. Clinical signs were recorded daily, body weights and food consumption were measured weekly. On the day of necropsy, a blood sample was collected from the retro-orbital venous plexus of each surviving animal for selected clinical chemistry determinations. All animals were necropsied, selected organs weighed and a range of tissues were taken, fixed and examined microscopically.

Results

Dose (ppm)	**0**		**100**		**500**		**2500**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality	No treatment related mortalities occurred							
Clinical signs	No clinical signs were observed							
Body weight	A lower body weight was observed at 2500 ppm in both sexes throughout the study (max 11%).							
Body weight gain (g)	7.2	5.4	6.9	5.7	6.5	5.2	**4.1****	5.0
Food consumption	At 2500 ppm a slight reduction in food consumption (max 11%) between study days 1 and 22 in females only.							

Dose (ppm)	**0**		**100**		**500**		**2500**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Ophthalmology	Not performed							
Urinalysis	Not performed							
FOB	Not performed							
Haematology	Not performed							
Clin. Chemistry								
-total cholesterol (mmol/l)	1.91	1.52	1.87 (-2%)	1.50 (-1%)	1.77 (-7%)	1.42 (-5%)	**1.34** (-30%)**	**1.16** (-24%)**
- Alkaline phosphatise (IU/I)	76	131	74 (-3%)	129 (-2%)	81 (+7%)	134 (+2%)	**105** (+38%)**	144 (+10%)
- Alanine aminotransferase(IU/I)	27	36	32 (+19%)	38 (+6%)	32 (19%)	38 (+6%)	35 (+30%)	**74* (+106%)**
- Aspartate aminotransferase (IU/I)	88	130	91 (+3%)	129 (-1%)	95 (+8%)	132 (2%)	105 (+19%)	177 (+36%)
- Urea (mmol/l)	11.14	12.76	14.04 (+26%)	13.31 (+4%)	13.98 (+25%)	14.79 (+16%)	**16.78** (+51%)**	**15.23* (+19%)**
- Total protein (g/l)	59	58	57 (-3%)	58 (0%)	59 (0%)	57 (-2%)	**56* (-5%)**	**55** (-5%)**
- Albumin (g/l)	34	36	34 (0%)	35 (-3%)	35 (+3%)	35 (-3%)	33 (-3%)	**33** (-8%)**
Organ weights -liver weight								
-absolute (g)	0.96	0.82	1.02 (+6%)	0.82 (0%)	0.95 (-1%)	0.84 (+2%)	1.02 (+6%)	**0.92** (+12%)**
- relative to body weight (%)	4.170	4.474	4.422 (+6%)	4.498 (+1%)	4.207 (+1%)	4.609 (+3%)	**4.989** (+20%)**	**5.264** (+18%)**

Dose (ppm)	**0**		**100**		**500**		**2500**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
- kidney weight								
-absolute (g)	0.35	0.26	0.35 (0%)	0.27 (+4%)	0.32 (-9%)	0.26 (0%)	**0.31** (-11%)**	0.25 (-4%)
- relative to body weight (%)	1.541	1.439	1.531 (+1%)	1.474 (+2%)	1.437 (-5%)	1.417 (-2%)	1.515 (0%)	1.425 (-1%)
- brain weight								
-absolute (g)	0.45	0.45	0.46 (+2%)	0.46 (+2%)	0.44 (-2%)	0.44 (-2%)	0.44 (-2%)	0.45 (0%)
- relative to body weight (%)	1.943	2.468	1.985 (+2%)	2.513 (+2%)	1.960 (+1%)	2.415 (-2%)	**2.172** (+12%)**	2.516 (+2%)
- thymus								
-absolute (g)	0.027	0.034	0.028 (+4%)	0.032 (-6%)	0.029 (+7%)	0.032 (-6%)	0.028 (+4%)	0.035 (+3%)
- relative to body weight (%)	0.1156	0.1848	0.1234 (+7%)	0.1740 (-6%)	0.1274 (+10%)	0.1773 (-4%)	**0.1391** (+20%)**	0.1975 (+7%)
Pathology								
Macroscopy								
- pale liver	0/10	0/10	0/10	0/10	0/10	1/10	1/10	6/10
Microscopy								
Hepatocellular vacuolation: diffuse								
- minimal	6	7	3	7	8	6	0	2
- slight	4	3	7	3	2	3	6	3
- moderate	0	0	0	0	0	0	4	5
- total	10	10	10	10	10	9	10	10
Corticoepithelial vacuolation: multifocal/diffuse								
- minimal	7	0	4	0	5	0	0	0
- slight	3	0	2	0	3	0	0	0
- moderate	0	0	2	0	0	0	0	0

Dose (ppm)	**0**		**100**		**500**		**2500**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
- total	10	0	8	0	8	0	0	0

*p≤0.05, **p≤0.01

There were no treatment-related mortalities during the study. One female of the mid dose group was killed for humane reasons on study day 61. Macroscopic examination revealed dilated and tear in esophagus, food content in thoracic cavity, enlarged adrenal glands, dark liver, white foci on stomach, atrophic/small thymus and enlarged bronchial lymph node. The cause of death was due to initial esophageal impaction followed by tearing and a subsequent local inflammation reaction and can therefore be considered incidental.

At 2500 ppm body weight was reduced in both sexes compared to control (max 11%). Mean body weight gain was reduced from study days 1 to 22 in males and between study days 1 and 8 in females. The overall cumulative mean body weight was reduced by 43% in males and 7% in females.

In the high dose animals clinical chemistry evaluation revealed a lower mean total cholesterol and total protein concentration in both sexes, and a higher urea and alkaline concentration in both sexes. Alanine aminotransferase activity was induced in females whereas albumin concentrations were reduced.

Mean absolute and relative liver weights were statistically significantly higher in females. Mean absolute kidney weight were statistically significantly lower in males when compared to controls. At macroscopic examination pale liver was noted in 6/10 females. At microscopic examination, a slight increase in severity of diffuse hepatocellular vacuolation was noted in the liver in both sexes. In the kidney, a loss of the normal multifocal/diffuse cortical epithelial vacuolation was noted in males. Relative brain and thymus weight were significantly increased in high dose males, without corroborating pathological finding these effects are likely due to the decreased body weight and are considered not treatment related.

Acceptability
The study is considered acceptable

Conclusion
Based on the clinical chemical findings, the increased liver and decreased kidney weights and the associated pathological findings the NOAEL in this study is set at 500 ppm (equating to 80.6 mg/kg bw/day in males and 98.1 mg/kg bw/day in females).

Study 3

Reference	:	Eigenberg, D.A. 2010	exposure	:	Repeated diet, 90 days
type of study	:	90-day dog oral toxicity study	Dose	:	0, 400, 1200, 3600/2400* ppm M: 0, 12, 33, 102/85* mg/kg bw/day F: 0, 12, 41, 107/78* mg/kg bw/day
year of execution	:	2009	vehicle	:	Corn oil and acetone
test substance	:	BYI 02960 (batch 2009-000239, purity 96.2%)	GLP statement	:	yes
Route	:	diet	guideline	:	OECD 409
Species	:	Dog, Beagle	acceptability	:	Acceptable
group size	:	4/sex/dose	**NOAEL**	:	12 mg/kg bw/day

**The highest dose (3600 ppm) was reduced to 2400 ppm from study week 9 onward due to clinical signs seen in two dogs on day 44 and continual weight loss in the high-dose group.*

Study design

The study was carried out according to OECD guideline 409.

BYI 02960 was administered via the diet to groups of 4 male and 4 female Beagle dogs at dose levels of 400, 1200 or 3600/2400 ppm for at least 90 days (12, 33 and 102/85 mg/kg bw/ day for males and 12, 41 and 107/78 mg/kg bw/day for females). The 3600 ppm dose group was reduced to 2400 ppm beginning study week 9 due to clinical signs seen in two of the dogs on Day 44 and continual weight loss in the high-dose group. Cage side observations and food consumption were recorded daily and detailed clinical observations and body weights were recorded weekly. Haematology, clinical chemistry, and urinalysis evaluations were performed on all animals once prior to administration of the test substance and from all animals during study weeks 5, 9, and 13. Ophthalmic examinations were performed pre-exposure and pre-sacrifice. A gross necropsy was performed, organ weights were taken, and micropathology was performed.

Results

Dose (ppm)	**0**		**400**		**1200**		**3600/2400**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality	No mortalities occurred							
Clinical signs	In the high dose group: unsteady and stiff back legs and lower back in one male and one female.							
Body weight	-	-	+5%	-5%	-9%	-7%	-11%	-13%
Body weight gain	777	1325	1282	775	**60***	671	**-421***	**1***

Dose (ppm)	**0**		**400**		**1200**		**3600/2400**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
(g)								
Food consumption	Statistically significantly reduced in mid and high dose males during the first ten days and in high dose females during the first seven day.							
Ophthalmology	No treatment-related effects							
Urinalysis	No treatment-related effects							
Haematology								
-RBC								
- day -7	6.99	6.81	7.28	7.10	6.88	6.65	7.50	7.03
- day 28	7.02	6.91	7.13	6.99	6.85	6.41	6.64	6.42
- day 56	6.92	6.95	7.18	7.25	6.67	**6.44***	6.29	**5.48***
- day 84	7.07	6.80	7.36	7.31	6.97	6.53	6.75	6.25
-Hgb								
- day -7	16.3	15.7	17.1	15.9	15.7	15.7	16.8	16.5
- day 28	16.4	16.0	16.5	15.8	15.6	14.9	**14.9***	14.7
- day 56	16.1	16.4	16.8	16.6	15.1	15.3	**14.0***	**13.0***
- day 84	16.3	15.9	17.0	16.9	15.5	15.2	14.9	14.1
- Hct								
- day -7	47.5	45.5	50.0	46.0	45.5	44.6	49.6	47.6
- day 28	47.2	45.8	47.7	45.5	44.3	42.7	**42.3***	42.3
- day 56	46.4	46.2	48.0	47.6	43.2	43.0	**39.9***	**37.7***
- day 84	47.6	45.3	49.5	48.3	45.4	43.6	**43.2***	40.7
- MCV								
- day -7	67.8	66.8	68.7	64.9	66.2	67.2	66.2	67.8
- day 28	67.2	66.3	67.1	65.3	**64.6***	66.7	**63.7***	65.9
- day 56	67.0	66.5	67.0	65.7	64.7	66.7	**63.6***	64.5
- day 84	67.3	66.6	67.3	66.1	65.1	66.7	**64.1***	65.0
Clin. Chemistry study day 56								
-Creatin phosphokinase	242	282	248 (+2%)	**175* (-38%)**	2834[#] (+1071%)	1538[#] (+445%)	2944[#] (+1117%)	**4836* (+1615%)**
- Aspartate aminotransferase								

Dose (ppm)	**0**		**400**		**1200**		**3600/2400**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
(IU/I)	38	42	36 (-5%)	**31* (-26%)**	241 (+534%)	**130* (+210%)**	266 (+600%)	**370* (+780%)**
- Alanine aminotransferase (IU/I)	30	28	36 (+20%)	27 (-4%)	221 (+637%)	**151* (+439%)**	311 (+937%)	**552* (+1871%)**
- Glucose (mg/dl)	89	83	86 (-3%)	87 (+5%)	92 (+3%)	92 (+11%)	88 (-1%)	**103* (24%)**
- Creatin (mg/dl)	1.0	0.9	0.9 (-10%)	0.9 (-10%)	0.9 (-10%)	0.8 (-20%)	**0.8* (-20%)**	**0.7* (-22%)**
Organ weights								
-liver weight								
-absolute (g)	269	226	260 (-3%)	201 (-11%)	261 (-3%)	225 (0%)	**307* (+14%)**	252 (+12%)
- relative to body weight (%)	2.757	2.892	2.533 (-8%)	2.621 (-9%)	2.911 (+6%)	3.035 (+5%)	**3.525* (+29%)**	**3.596* (+24%)**
- kidney weight								
-absolute (g)	47	38	55 (+17%)	34 (-11%)	51 (+9%)	36 (-5%)	55 (+17%)	38 (0%)
- relative to body weight (%)	0.485	0.469	0.539 (+11%)	0.442 (-6%)	**0.570* (+18%)**	0.488 (+4%)	**0.633* (+31%)**	0.545 (+16%)
- prostate								
-absolute (g)	7.375		9.847 (+34%)		10.410 (+41%)		**12.322* (+67%)**	
- relative to body weight (%)	0.075		0.096 (+28%)		**0.116* (+55%)**		**0.141* (+88%)**	

Dose (ppm)	**0**		**400**		**1200**		**3600/2400**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
- lung								
-absolute (g)	90.448	82.549	86.208 (-5%)	68.825 (-17%)	94.794 (+5%)	72.206 (-13%)	91.983 (+2%)	72.190 (-13%
- relative to body weight (%)	0.924	1.025	**0.838* (-9%)**	0.899 (-12%)	**1.039* (+12%)**	0.977 (-5%)	1.052 (+14%)	1.030 (0%)
Pathology	No treatment-related effects							
Macroscopy								
Microscopy								
liverAccumulation of Brown pigment in Kupffer cells								
- minimal	0	0	0	0	0	0	0	2
- total	0	0	0	0	0	0	0	2
Skeletal muscle Myofiber atrophy/degeneration: focal								
- minimal	0	0	0	0	2	3	1	4
- slight	0	0	0	0	0	1	1	0
- total	0	0	0	0	2	4	2	4

*p≤0.05, # Value not statistically significant due to a large SD from dogs that had normal values.

Clinical signs consisted of unsteady and stiff back legs and lower back in one high dose male and one high dose female. Body weight and body weight gain were reduced at 1200 and 3600/2400 ppm.

There was a compound-related reduction in RBC, Hgb, and Hct during the 1, 2, and 3 month haematology evaluations in both sexes at the high dose. In the 1200 and 3600/2400 ppm dose groups, there was a compound-related increase in creatine phosphokinase, aspartate aminotransferase and alanine aminotransferase at the 2-month test interval. The values for CK, AST, and ALT were normal at the 3 month evaluation.

Relative and absolute liver weights were increased in the high dose groups. There was a statistical significant compound-related increase in relative kidney weight for males in the mid and high dose group. The statistical increase in the prostate weight for the 3600/2400 ppm dose group males is not considered compound-related as these were young dogs and variations in prostate weight due to

maturation can be expected. The statistical significant values for relative lung weight in the 400 and 1200 ppm male groups are not considered to be compound-related as there is no clear dose-response relationship.
Microscopic pathology revealed minimal brown pigments in Kupffers in the liver of high dose females and myofiber atrophy/degeneration in skeletal muscle in both sexes at the mid and high dose.

Historical control data from studies performed in 2010 and 2011:

Sex	Mean ±SD	range
Liver absolute weight (g)		
Males	259.6 ± 37.4	213.2 to 337.1
Females	233.3±45.7	185.4 to 336.5
Liver relative weight		
Males	2.93 ± 0.39	2.47 to 3..78
Females	3.07 ± .0.48	2.34 to 3.99
Prostate absolute weight:		
Males	7.02 ± 3.10	2.30 to 13.31
Prostate relative weight:		
Males	0.08 ± 0.039	0.26 ot 0.171
Females		
Lung absolute weight:		
Males	85.5 ± 7.2	74.6 to 96.2
Females	75.7 ± 12.5	66.9 to 109.6
Lung relative weight:		
Males	0.97 ± 0.09	0.81 to 1.12
Females	1.01 ± 0.25	0.84 to 1.71

Acceptability
The study is considered acceptable.

Conclusion
Based on reduced body weight, reduced body weight gain, clinical chemistry, significant increase in kidney weight and focal myofiber atrophy/degeneration the No Observed Adverse Effect Level is 400 ppm (12 mg/kg bw/day for males and females).

4.7.1.1.3 1-year toxicity study

Study 1

Reference	:	Cady, A. 2012	exposure	:	Repeated diet, 1-year
type of study	:	1-year dog oral toxicity study	Dose	:	0, 150, 300, 1000 ppm M: 0, 4.6, 7.8, 28.1 mg/kg bw/day F: 0, 4.1, 7.8, 28.2 mg/kg bw/day
year of execution	:	2010-2011	vehicle	:	Corn oil and acetone
test substance	:	BYI 02960 (batch 2009-000239, purity 96.2%)	GLP statement	:	yes
Route	:	diet	guideline	:	OECD 452
Species	:	Dog, Beagle	acceptability	:	Acceptable
group size	:	4/sex/dose	**NOAEL**	:	7.8 mg/kg bw/day

Study design

The study was carried out according to OECD guideline 452.

Male and female Beagle dog (4/sex/dose) were fed diet containing BYI 02960 (batch 2009-000239, purity 96.2%) for at least one year at dietary level of 150, 300 and 1000 ppm, equivalent to 4.6, 7.8 and 28.1 mg/kg bwd/day for males and 4.1, 7.8 and 28.2 mg/kg bw/day for females). Body weights were measured weekly and food consumption was measured daily. All animals were generally observed at least daily for clinical signs of toxicity. Detailed clinical observations were performed on a weekly basis. Ophthalmic exams were conducted prior to initiation of dosing and prior to termination of the study. Clinical chemistry, haematology and urinalysis evaluation were performed once prior to administration and at approximately 3, 6, 9 and 12 months. At the end of the study, a gross necropsy was conducted, organs were weighed and tissues were collected for microscopic examination.

Results

Dose (ppm)	**0**		**150**		**300**		**1000**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality	No mortalities occurred							
Clinical signs	No treatment-related effect							
Body weight	No treatment-related effect							

Dose (ppm)	**0**		**150**		**300**		**1000**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Body weight gain (g)	Body weight gain reduced in females at 1000 ppm in the first week only (-88%). No overall effect on BWG found.							
Food consumption	No treatment-related effect							
Ophthalmology	No treatment-related effect							
Urinalysis	No treatment-related effect							
Haematology	No treatment-related effect							
Clin. Chemistry	No treatment-related effect							
Organ weights	No treatment-related effect							
Pathology <u>Macroscopy</u>	No treatment-related effect							
<u>Microscopy</u> -skeletal muscle, protocol (gastrocnemius) – degeneration myofiber	0	0	0	0	0	0	2 (1.5)	2 (1.0)
- Muscle, other (biceps femoris) – degeneration, myofiber	0	0	0	0	0	0	3 (1.3)	3 (1.0)

*p≤0.05, **p≤0.01, () = Average severity of dogs with lesion: 1 (minimal) to 5 (severe).

Body weight or overall body weight gain were not affected by treatment. Body weight gain was reduced at 1000 ppm in females in the first week only (-88%). Food consumption, ophthalmology, urinalysis, haematology, clinical chemistry, organ weights and macroscopic pathology evaluations revealed no treatment-related effects. Test substance-related micropathological changes was limited to degeneration noted in skeletal muscle, protocol (gastrocnemius); and muscle, other (biceps femoris). Degeneration of the myofiber comprised of one or more of the following changes: atrophy, necrosis, and/or presence of inflammatory cells around the affected myofiber.

Acceptability
The study is considered acceptable

Conclusion
Based on micropathology findings in males and females, the LOAEL in this study was 1000 ppm, equivalent to 28.1 and 28.2 mg/kg bw/day for male and female dogs, respectively. The NOAEL in this study was 300 ppm, equivalent to 7.8 mg/kg bw/day for both male and female dogs.

4.7.1.2 Repeated dose toxicity: inhalation

4.7.1.2.1 28-day inhalation studies

Based on the results of the acute inhalation toxicity study and the physical properties of BYI 02960 (compound not volatile with an extrapolated vapour pressure of 9.1×10^{-7} Pa at 20 °C), no repeat inhalation study is triggered.

4.7.1.3 Repeated dose toxicity: dermal

4.7.1.3.1 28-day dermal studies

Characteristics

reference	:	Cada A.; 2012	exposure	:	28 days, 6 h/d, semi-occlusive (10% of the total body surface area)
type of study	:	28-day dermal toxicity study	dose	:	50, 150 or 500 mg/kg bw/d
year of execution	:	2012	vehicle	:	Deionized water
test substance	:	BYI 02960, lot/batch no. 2009-000239, purity 96.2 %, beige powder	GLP statement	:	yes
route	:	Dermal	guideline	:	Partly in accordance with OECD 410
species	:	Rat, Wistar Han CRL: WI (HAN)	acceptability	:	acceptable
group size	:	10 /sex/dose	NOAEL	:	500 mg/kg bw/d

Study design
The study was performed partly in accordance with OECD 410: the highest dose should have resulted in toxic effects, or the limit dose of 1000 m/kg bw/d should have been used as the highest dose level. According to the study report, the high dose of 500 mg/kg bw/d was selected after consideration of the amount of test material that can be reliably applied to the skin. See acceptability. Male and female rats, 10 rats/sex/group (one control and three test substance-dosed groups) were administered vehicle or BYI 02960 (50, 150, or 500 mg/kg/day) daily by dermal application for at least 28 consecutive days (minimum of 6 h/day) and euthanized one day following the last dose. An additional 6 rats were used to test compound wetting suitability. Two rats/group

were treated with 200 mg BYI 02960 using 2 mL deionized water, 0.5% methylcellulose, or corn oil. After at least 2 h of exposure, water was found to be an appropriate wetting agent and was chosen to be used to dose in this study.

Results

Table 57

Dose (mg/kg bw/d)	**0**		**50**		**150**		**500**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	No mortalities occurred								
Clinical signs	Clinical signs of toxicity attributable to the test substance were not observed								
Body weight gain	Body weights and body weight gain were not affected by test substance administration.								
Food consumption (g/day)									
- Week 1								dc	
- Week 2				dc				dc	
Haematology	Hematology or coagulation parameter changes attributable to the test substance were not observed								
Clinical chemistry	Clinical chemistry parameter changes attributable to the test substance were not observed								
Organ weights									
- Liver (absolute)								d (6%)	
- Liver (relative)								d (9%)	
Pathology									
macroscopy	There were no test substance-related gross lesions observed								
microscopy	There was no test substance-related micropathology change in males or females at any dose level								

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls

Table 58: Food consumption (g/animal/day)

Dosage Level of BYI 02960	**0 mg/kg**	**50 mg/kg**	**150 mg/kg**	**500 mg/kg**
Males				
Week 1 (Days 0–7) (% Control)	20.87	21.14 (101%)	20.77 (100%)	20.91 (100%)
Week 2 (7-14) (%Control)	22.02	23.25 (106%)	22.27 (101%)	23.07 (105%)
Females				
Week 1 (Days 0–7) (% Control)	17.11	15.78 (92%)	16.64 (97%)	15.12* (88%)
Week 2 (7-14) (%Control)	19.33	17.34* (90%)	18.17 (94%)	17.51* (89%)

* P<=5%

At 500 mg/kg/day
No adverse effects attributable to exposure to the test substance were observed.

In the first and second week of daily dermal administration, females had statistical significantly lower food consumption (g/animal/day) as compared to controls. In addition, food efficiency (g/kg bw/day) was significantly lower in the first week. These findings were not considered to be biologically significant as changes were not consistently affected in a dose-related fashion, were not found in males, and did not impact clinical observations or body weights at any point.
The absolute and relative liver weights at 500 mg/kg/day males were non-statistically decreased by 6% and 9%, respectively, compared to control rats. There were no test substance-related clinical or gross or microscopic pathology changes to correlate this finding.

At 150 mg/kg/day
No adverse effects attributable to exposure to the test substance were observed.

At 50 mg/kg/day
No effects attributable to exposure to the test substance were observed.
Females administered with 50 mg/kg/day had significantly lower food consumption in the second week as compared to controls. This finding was not considered biologically significant as changes were not consistently affected in a dose-related fashion, were not found in males, and did not impact clinical observations or body weights at any point.

Acceptability
The study is considered acceptable despite the fact that not the limit dose of 1000 mg/kg bw/d is used. It is not fully explained in the report why 500 mg/kg bw/d is the amount of test material that can be reliably applied to the skin. However, the study did not indicate any route-specific effects and NOAEL in this dermal study is higher than the oral NOAEL, that is used for the derivation of the reference values.

Conclusions
Based on the lack of adverse findings observed in this study, the 28-day dermal exposure NOAEL in male and female rats dosed with BYI 02960 was 500 mg/kg bw/day.

4.7.1.4 Repeated dose toxicity: other routes

4.7.1.5 Human information

4.7.1.6 Other relevant information

4.7.1.7 Summary and discussion of repeated dose toxicity

The results of the subacute and semichronic toxicity studies are summarised in Table 59 and Table 60, respectively.

Table 59: Subacute studies

Duration	Species	Route	NOAEL mg/kg bw/day	LOAEL mg/kg bw/day	Critical effects	Reference

28-day	rat	oral, gavage	75[1]	200[1]	Changes in biochemical parameters, increased liver weight, enlarged liver, prominent lobulation, hepatocellular hypertrophy, follicular cell hypertrophy	Capt, A. 2007
28-day	rat	oral, diet	33.6[1]	385[1]	Changes in biochemical parameters, increased relative liver and thyroid weight, prominent lobulation of the liver, hepatocellular hypertrophy, follicular cell hypertrophy	Blanck, M. 2008
28-day	mouse	oral, diet	98[1]	-		Blanck, O. 2007
28-day	dog	oral, diet	62[1]	118[1]	Overall body weight loss and reduced body weight gain	Odin-Feurtet, M. 2008
28-day	Rat	Dermal	500	-		Cada A.; 2012

1. Dose range finding studies.

Table 60: Semichronic studies

Duration	Species	Route	NOAEL mg/kg bw/day	LOAEL mg/kg bw/day	Critical effects	Reference
90-day study	rat	oral, diet	6.0	30.2	Changes in biochemical parameters, increased liver and thyroid weight, enlarged liver, dark thyroid gland, hepatocellular hypertrophy, follicular cell hypertrophy	Odin-Feurtet, M. 2009
90-day study	mouse	oral, diet	81	407	Reduced body weight, changes in biochemical parameters, increased liver weight, decreased kidney weight, pale liver, hepatocellular vacuolation, loss of normal multifocal/diffuse cortical epithelial vacuolation.	Odin-Feurtet, M. 2009
90-day study	dog	oral, diet	12	33	Reduced body weight gain, changes in biochemical parameters, significant increased kidney weight, myofiber atrophy/degeneration.	Odin-Feurtet, M. 2008
1-year study	dog	oral, diet	7.8	28.1	Degeneration of skeletal muscle and other mucle (biceps femoris) degeneration	Cady, A. 2012

Oral

Four subacute oral range-finding studies were performed, with rat, mouse and dog.

In a 28-day study with rats exposed to 0, 75, 200 or 350 mg/kg bw/d via gavage, toxicologically relevant effects were clinical chemical findings, increased organ weights and pathological findings in the 200 mg/kg bw/d group. The NOAEL in this study is set at 75 mg/kg bw/d.

In a 28-day study with rats exposed to 0, 33.6 or 385 mg/kg bw/d via diet, toxicologically relevant effects were clinical chemical findings, increased liver and thyroid weights and associated pathological findings in the 385 mg/kg bw/d group. The NOAEL in this study is set at 33.6 mg/kg bw/d.

In a 28-day study with mouse exposed to 300, 600 and 1200 ppm (equating approximately to 50, 98 and 207 mg/kg bw/day in males and 59, 122, 240 mg/kg bw/day in females) via diet, no toxicologically relevant effects were observed. The NOAEL in this study is set at 33.6 mg/kg bw/d.

In a 28-day study with dogs exposed to 0, 500, 2000 or 4000 ppm (equating approximately to 0, 16, 62, 118 mg/kg body weight/day in males and 0, 18, 77, 131 mg/kg body weight/day in females) via diet, toxicologically relevant effects were body weight loss and reduced body weight gain at the 4000 ppm group. The NOAEL in this study is set at 2000 ppm (62 mg/kg bw/d).

Four semichronic oral studies were performed, with rat, mouse and dog.
In a 90-day toxicity study with rat exposed to 0, 100, 500, 2500 ppm (equating to approximately 0, 6.0, 30.2, 156 mg/kg bw/day in males and 0, 7.6, 38.3, 186 mg/kg bw/day in females) via diet, toxicologically relevant effects were clinical chemical findings, increased relative liver and thyroid weight and associated pathological findings in the 2500 ppm group and thyroid weight effects in the 500 ppm males. The NOAEL in this study is 100 ppm (6.0 mg/kg bw/d).
In a 90-day toxicity study with mouse exposed to 0, 100, 500, 2500 ppm (equating approximately to 16, 81, 407 mg/kg bw/day in males and 19, 98, 473 mg/kg bw/day in females) via diet, toxicologically relevant effects were clinical chemical findings, increased relative liver and decreased kidney weights and associated pathological findings in the 2500 ppm group. The NOAEL in this study is 500 ppm (81 mg/kg bw/d).
In a 90-day toxicity study with dog exposed to 0, 400, 1200 or 3600/2400 ppm (equating approximately to 0, 12, 33 and 102/85 mg/kg bw/ day for males and 0, 12, 41 and 107/78 mg/kg bw/day for females) via diet, toxicologically relevant effects were reduced body weight, reduced body weight gain, clinical chemistry, significant increase in kidney weight and focal myofiber atrophy/degeneration in the 1200 ppm group. The NOAEL in this study is 400 ppm (12 mg/kg bw/d).
In a 1-year toxicity study with dog exposed to 0, 150, 300 and 1000 ppm (equating approximately to 0, 4.6, 7.8 and 28.1 mg/kg bwd/day for males and 0, 4.1, 7.8 and 28.2 mg/kg bw/day for females) via diet, toxicologically relevant effects were micropathology (degeneration of the skeletal muscle (gastrocnemius and biceps femoris (atrophy, necrosis and/or presence of inflammatory cells)) findings in the 1000 ppm group. The NOAEL in this study is 300 ppm (7.8 mg/kg bw/d).
In a 90-day neurotoxicity study, through approximately 13 weeks of continuous dietary exposure to BYI 02960 at 0, 100, 500 or 2500 ppm, there were no neurotoxic treatment-related findings apparent at any dietary level in either sex. Based on these findings, a NOAEL of 2500 ppm was established for the rat (equating to 143 mg/kg bw/day for males).

Dermal
Male and female rats, 10 rats/sex/group (one control and three test substance-dosed groups) were administered BYI 02960 (50, 150, or 500 mg/kg/day) daily by dermal application for at least 28 consecutive days (minimum of 6 h/day) and euthanized one day following the last dose. Based on the lack of adverse findings observed in the study, the 28-day dermal exposure NOAEL in male and female rats dosed with BYI 02960 was 500 mg/kg bw/day.

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

The most sensitive outcome were micropathological findings in skeletal muscle at the LOAEL (28.2 mg/kg bw/d) in a one year dog study, with a NOAEL of 7.8 mg/kg bw/d. Test substance-related micropathological changes were limited to degeneration noted in skeletal muscle, protocol (gastrocnemius); and muscle, other (biceps femoris). Degeneration of the myofiber comprised of one or more of the following changes: atrophy, necrosis, and/or presence of inflammatory cells around the affected myofiber. The level of severity was minimal to slight.

In a 90-day dog study, the NOAEL was 12 mg/kg bw/day for males and females, and the LOAEL was 33/41 mg/kg bw/day (males/females). Findings at the LOAEL were reduced body weight, reduced body weight gain, clinical chemistry, significant increase in kidney weight and focal myofiber atrophy/degeneration. The changes in clinical chemistry consisted mainly of strong increases in liver enzymes (alanine aminotransferase, creatin phosphokinase, aspartate aminotransferase). However, the relative liver weight increase was only significant at the high dose and no histopathological changes of the liver were observed at the mid dose, which indicates that the changes in enzyme levels at the mid dose were probably adaptive. Also of significance was that the 102/107 mg/kg bw/day dose was reduced to 85/78 mg/kg bw/day from study week 9 onward due to clinical signs seen in two of the dogs on Day 44 and continual weight loss seen in this group. This group also showed the same effects as the 33/41 mg/kg bw/day dose, with in addition a compound-related reduction in RBC, Hgb, and Hct during the 1, 2, and 3 month haematology evaluations in both sexes. The reduction in Hb was observed in both high dose males and females and most severe at day 56 (the reduction was respectively 13% and 20.7%). After the dose reduction, the RBC and Hgb levels recovered to levels that were no longer significantly different from the controls.

A decrease in Hgb and Hct values was also observed in female rats during a carcinogenicity study at a dose of 120 mg/kg bw/day. The decrease in Hgb was at most 6.3% at 6 months, which was significant, but below the guideline limits for classification. In the same study, slightly lower total bilirubin concentrations in both sexes and slightly higher mean total cholesterol in females were observed at 120 mg/kg bw/day.

The LOAEL in a 28-day dog study was 118/131 mg/kg bw/day, based on body weight loss and reduced body weight gain. Also enlargement of the thyroid gland was observed in 2/2 females, but this was judged as not adverse, as there was no effect on thyroid weight and there were no microscopic findings for the thyroid. The NOAEL in this study was 62/77 mg/kg bw/day. It should be noticed that the highest dose level of 118/131 mg/kg bw/day was clearly below the STOT RE guidance value in a 28-day study (300 mg/kg bw/day).

A 90-day rat study showed effects on the mean body weight gain in females and reduced thyroid weight in males at 30.2 mg/kg bw/day, which were considered adverse. At the highest dose of 156/186 mg/kg bw/day more severe effect were observed including reduced body weight gain in females and changes in clinical chemistry in both sexes. The changes in clinical chemistry consisted of increased platelet counts and cholesterol and decreases in bilirubin and glucose. There was an increase in liver and thyroid weight and histopathological effects. In the 28-day rat study using gavage application, mortality was observed in females at 200 and 350 mg/kg bw/day but not at a comparable high dose in the diet study.

The effects in the 90-day mouse study were limited to effects above the guidance value for STOT RE 2 (100 mg/kg bw) as the NOAEL was determined at 81/98 mg/kg bw/day. In the 28-day study, the effects at the highest dose of 200 mg/kg bw/day were limited.

The NOAELs in the available rat and mouse chronic toxicity and carcinogenicity studies were 15.8 and 43 mg/kg bw/day, respectively. This is above the guidance value for STOT RE in a 2-year study (12.5 mg/kg bw/day).

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

Classification in Category 1 is applicable, when significant toxic effects observed in a 90-day repeated-dose study conducted in experimental animals are seen to occur at or below the guidance values (C) of ≤ 10 mg/kg bw/day. Considering the severity and concentration at which adverse effects occurred, classification in category 1 is not warranted.

Classification in Category 2 is applicable, when significant toxic effects observed in a 90-day repeated-dose study conducted in experimental animals are seen to occur within the guidance value range of $10 < C \leq 100$. The equivalents of this range are:
$30 < C \leq 300$ mg/kg bw/day for 28-days
$2.5 < C \leq 25$ mg/kg bw/day for one year
$1.25 < C \leq 12.5$ mg/kg bw/day for two years

Minimal to slight myofiber atrophy/degeneration and weight loss were observed in a 28-day dog study at 118/131 mg/kg bw/day, in a 90-day dog study at 33/41 and 102/107 mg/kg bw/day, as well as in a one year dog study at 28 mg/kg bw/day. The effects at the high dose (102/107 mg/kg bw/day) in the 90-day study were considered so severe that the dose level for this group was reduced. Changes in haematology (> 20% Hb reduction) were observed at the high dose group of the 90-day study, but were less than 20% after correction from 107 mg/kg bw/day to 100 mg/kg bw/day (upper limited STOT RE 2 for a 90-day study). Therefore, blood was not considered a specific target organ. Increases in liver enzymes where observed in the mid- and high dose groups of the 90-day study. However, as there were no histopathological changes in the liver at the mid dose, this effect was not considered an indication of specific liver toxicity. In addition, no effects on clinical chemistry were reported in the one year dog study at 28 mg/kg bw/day, which is only slightly lower than the mid dose of the 90-day study (33/41 mg/kg bw/day).

Significant effects in rats occurred at 30.2 mg/kg bw/day in a 90-day study. However, these effects were not considered sufficiently severe. The effects at the highest dose level of 171 mg/kg bw/day would warrant classification with STOT RE 2. However, interpolation between 30 and 171 mg/kg bw to estimate the level of effects at 100 mg/kg bw/day is difficult. There were incidences of

mortality in a 28-day gavage study at 200 mg/kg bw/day (1/5 female) and 350 mg/kg bw/day (2/5 females). No such effects were observed in a comparable diet study.

The effects observed in the studies with mice at the relevant dose level for STOT RE 2 are not considered severe enough for classification.

Considering the observed effects in dogs were consistent, severe, and occurred below or around the guideline values, classification as STOT RE Category 2 is proposed. The muscles are identified as the target organ. However, it is unclear whether the effects on the muscles are a direct effect of the substance or whether the effect is secondary to the weight loss. In that case, no target organ can be identified as this is probably a general effect.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Flupyradifurone (BYI 02960) needs to be classified as STOT RE Cat. 2 H373 (muscle) according to Regulation (EC) 1272/2008.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS evaluated eight studies conducted by the oral route: four subacute (28-day) range-finding (non-GLP, non-guideline) studies in rats, mice and dogs performed in GLP-certified laboratories, three subchronic (90-day) in rats, mice and dogs (GLP- and OECD-compliant) and a 1-year chronic repeated toxicity study in dogs. In addition, the DS reported one subacute (28-day) dermal rat study, GLP-compliant and partly similar to OECD TG 410.

In the 90-day dog study, haemoglobin was not reduced up to 20 % in the high-dose group after dose reduction from 107 to 100 mg/kg bw/day. The DS therefore considered that this effect does not warrant classification for STOT RE 2. Also the increased liver enzymes which were found in the mid- and high-dose groups of this study were not considered to indicate specific liver toxicity, as there were no associated histopathological changes at the mid-dose group. This is supported by the fact that no effects on clinical chemistry were reported in the 1-year dog study at 28 mg/kg bw/day as this dose level is only slightly lower than the 90-day mid-dose level (33/41 mg/kg bw/day). The DS also highlighted that a decrease in Hb and haematocrit (Hct) values was also observed in female rats during a carcinogenicity study at 120 mg/kg bw/day. The decrease in Hb was at most 6.3 % at 6 months, which was significant, but below the guideline limits for classification. In the same study, slightly lower total bilirubin concentrations in both sexes and slightly higher mean total cholesterol in females were observed at 120 mg/kg bw/day. The DS reported that the most relevant toxicological effects were reduced body weight, reduced body weight gain, clinical chemistry abnormalities, statistically significantly increased kidney weight and focal myofiber atrophy/degeneration from the mid-dose level (33 mg/kg bw/day). The muscular effects were also observed in the 1-year dog study from a dose level of 28.1 mg/kg bw/day for males and 28.2 mg/kg bw/day for females. They

are described as degeneration of the skeletal muscle (gastrocnemius) and of the biceps femoris (atrophy, necrosis and/or presence of inflammatory cells).

In the 90-day rat study, significant effects (clinical chemical findings, increased relative thyroid and liver weights) were observed at 30.2 mg/kg bw/day. However, these effects were not severe enough but at the highest dose (171 mg/kg bw/day) classification for STOT RE 2 would be warranted. An extrapolation to estimate these effects at 100 mg/kg bw/day is no possible. No target organ was proposed from the findings in rats.

Effects observed in mice studies do not warrant classification following repeated dose toxicity to flupyradifurone according to the DS. Information on repeated exposure in humans is not available.

Overall, the DS reported that flupyradifurone induced consistent, severe effects in dogs in repeated dose studies, below or around the guideline values for STOT RE 2. These effects included weight loss, minimal myofiber atrophy/degeneration, changes in haematology (> 20 % Hb reduction) and clinical chemistry parameters. The DS concluded that flupyradifurone warrants classification as STOT RE 2; H373 with "muscle" as a target organ.

Comments received during public consultation

Five MSCAs supported the proposal to classify flupyradifurone for STOT RE 2; H373 (muscle). One of them suggested to include also the liver, another to include the thyroid as a target organ whereas another considered the blood to be of specific concern. Concerning the **thyroid**, the DS responded that effects were observed in males only and were reversible without a clear relationship to the treatment. In the absence of clear signs of thyroid toxicity within the range for classification in other repeated dose toxicity studies, the DS decided not to include the thyroid as a specific target organ. Regarding the **blood**, the MSCA reported that the decrease in Hb was associated with brown pigmentation (minimal) in liver Kupffer cells of females (which may be hemosiderin deposition) and that other haematology effects were noted (decreased MCV, RBC and Hct). The DS responded that these effects were only observed in the 90-day dog study and the decrease in Hb was only (marginally) over 20 % at day 56 (20.7 % decrease). Other blood parameters were not significantly changed or were also reversible. The DS further clarified that the pigmentation in liver Kupffer cells was observed in high dose females only and it is not clear if this effect (as well as the Hb level) is specific to blood toxicity. For the **liver**, one MSCA highlighted that hepatotoxicity of flupyradifurone has been observed in three different species (rat, mouse and dog) and submitted an attachment during the public consultation. According to the MSCA, effects included absolute and relative liver weight gain, clinical chemistry findings (decrease of total bilirubin and glucose concentration and increase of triglycerides, urea, creatinine, alanine aminotransferase and alanine phosphatase) and observations of enlarged liver, centrilobular hepatocellular hypertrophy and prominent lobulation. The DS responded that the effects observed seem reversible and more consistent with an adaptive response and not sufficiently severe, rather than signs of marked organ dysfunction.

Assessment and comparison with the classification criteria

There are no human data available with respect to repeated dose toxicity. However, the specific organ toxicity of flupyradifurone has been well tested in animals (rats, mice and dogs) via the

oral route. There is also one 28-day dermal rat study. Due to the outcome of the acute inhalation toxicity study and the low vapour pressure of flupyradifurone (9.1×10^{-7} Pa at 20 °C) an inhalation study was not performed.

In addition to the studies considered by the DS for the evaluation of this endpoint, RAC also assessed a 28-day immunotoxicity and a 90-day neurotoxicity study (both performed in rats) as well as two long-term studies performed in rats and mice, two reproductive rat studies, two developmental toxicity studies performed in rats and rabbits and a developmental neurotoxicity rat study.

Repeated-dose toxicity studies

Based on the available repeated-dose toxicity studies, the main effects of concern were effects in the muscles (e.g. dogs). In addition, in different species effects on body weight were observed. A table which includes all relevant studies considered for this endpoint is presented below. Supplemental information (in depth analyses by RAC) is presented in the background document.

Oral studies

Rats, 28-day (two studies)

In a Wistar rat study (5/sex/dose), flupyradifurone (purity 98.3%) in corn oil supplemented with 10 % ethanol and 10 % water was administered at concentrations of 0, 75, 200 and 350 mg/kg bw/day by gavage.

On day 2, one female was found dead at 200 mg/kg bw/day and on day 6, two females died at 350 mg/kg bw/day. All animals showed increased salivation at all dose levels but no other clinical signs were observed. At the highest dose, the mean body weight was slightly, but not statistically significantly reduced in males throughout the study and in females in the first week. The mean food consumption at 350 mg/kg bw/day was reduced by 17% in males and by 29 % in females between study day 1 and 8 compared to the control group and by 8% in males between day 8 and 15. At 200 mg/kg bw/day, only females showed a reduction in food consumption (16 %) between day 1 and 8. Changes in clinical chemistry parameters occurred consistently at 200 and 350 mg/kg bw/day, e.g. significant reduction in total bilirubin (in both sexes) and in glucose levels (in males), increase of triglyceride levels (in both sexes) and creatinine levels as well as increases in ALAT and ALP activities (in females).

Mean relative and absolute liver weights were increased compared to the controls at the higher dose levels, particularly in females (+45 % to +49 %) at the highest dose. Besides, liver enlargement and prominent lobulation was observed as well as centrilobular or diffuse hypertrophy of hepatocytes at 200 and 350 mg/kg bw/day. In the thyroid, minimal follicular cell hypertrophy was found at 200 mg/kg bw/day (males) and 350 mg/kg bw/day (both sexes).

Hepatocellular enzymatic activity assays showed that BROD activity was affected at 200 mg/kg bw/day in males (25-fold) and at 350 mg/kg bw/day in both sexes (25- and 28-fold) where no clear induction of neither PROD nor EROD activity was observed.

In the second 28-day rat study, groups of five male Wistar rats were fed diets containing flupyradifurone (purity 99.7 %) at concentrations of 0, 500 and 5 000 ppm (equivalent to doses of 0, 33.6 and 385 mg/kg bw/day).

Deaths did not occur, but treatment-related effects were the same in the high dose group as in the study summarised above (e.g. 17-19 % lower mean body weight and 38 % lower mean

body weight gain from days 1 to 29 and up to 39 % lower food consumption).

The clinical parameters showed a similar profile at 385 mg/kg bw/day as in the study mentioned above (e.g. significant reduction in total bilirubin and glucose levels).

At the high dose (385 mg/kg bw/day), relative liver and relative thyroid weights were significantly increased, and were associated with prominent lobulation of the liver and microscopic findings such as slight (2/5 rats) and moderate (3/5 rats) centrilobular hepatocellular hypertrophy and minimal (2/5 rats) and slight (3/5 rats) follicular cell hypertrophy.

Rats, 28-day immunotoxicity study (one study)

In a study conducted according to U.S. E.P.A., OPPTS Series 870 (Health Effects TG), flupyradifurone (purity 96.2 %) was administered via the diet to female Wistar rats (10/dose) at concentrations of 0, 125, 600 and 3 000 ppm (0, 10, 50 and 230 mg/kg bw/day). Two additional groups of rats were included in the study design i.e. one group was fed with untreated diet whereas the other was given 3.5 mg/kg bw/day of the immunosuppressive agent cyclophosphamide.

No treatment-related deaths were reported. However, one animal had to be humanely sacrificed due to automutilation of both forelimbs. In the high-dose group, mean body weight was reduced from study day 8 (-11 %) to study day 29 when compared to the controls. The body weight reduction was statistically significant in the first two weeks of treatment. Between study day 1 and 8, the high-dosed animals did not gain any body weight compared to controls. Afterwards there was no difference between treated animals and controls. Food consumption was reduced by approximately 34 % on study day 8 and by 9 to 13 % later on but compared to the control group this effect was not statistically significant.

The effects in the spleen and thymus weight did not show any dose response relationship. With regard to the immunological effects, a 55 % increase of SRBC-specific IgM was observed in the low-dose group whereas a decrease of mean specific IgM was reported in the mid- (-15 %) and high-dosed (-18 %) animals compared to controls. Rats treated with cyclophosphamide showed a 93 % reduction of SRBC-specific IgM.

Mice, 28-day (one study)

Flupyradifurone (purity 99.7 %) at concentrations of 0, 300, 600, 1 200 ppm (0, 50/59, 98/122, 207/240 (M/F) mg/kg bw/day) was administered via the food to C57BL/6J mice (5/sex/dose). No stability study was performed on the submitted feed but taking into account the available stability data, the actual concentration in feed received by the animals is assumed to be between 80 % and 90 % of nominal concentrations.

No mortalities or clinical signs were observed. The cumulative mean body weight gain was reduced in the high dosed males by 15 % between study days 1 and 29. However, in the absence of other findings and based on available historical control data from the same laboratory with animals from the same strain and the same age, this result was not considered to be adverse.

Although alanine aminotransferase and alkaline phosphatase levels were higher in the high dosed females, the absence of a clear dose response relationship and the inter-individual variation do not clearly indicate a treatment-related effect.

Other effects (e.g. lower absolute and relative epididymis weights and higher absolute and

relative spleen weights in males at 50 mg/kg bw/day) were not considered to be treatment-related.

Dogs, 28-day (one study)

Beagle dogs (2/sex/dose) received flupyradifurone (purity 99.5 %) at 0, 500, 2 000 or 4 000 ppm (0, 16/18, 62/77 or 118/131 (M/F) mg/kg bw/day, respectively) mixed in their diet.

Overall body weight loss in one male and absence of overall weight gain in one female, lower food consumption in males and females, enlarged thyroid glands in both females without any microscopic findings (therefore not considered treatment-related) were observed at the high dose groups (118/131 M/F mg/kg bw/day). Centrilobular glycogen accumulation was decreased in incidence and/or severity at 4 000 and 2 000 ppm (62 mg/kg bw/day) in males and at 4 000 ppm in females. Increased platelet counts in both females and in one male were found in the high dose group and in one female at 2 000 ppm (77 mg/kg bw/day) as well as increased creatinine levels in one female at the high dose group.

Rats, 90-day (one study)

Wistar rats (10/sex/dose) were fed diets containing flupyradifurone (purity 99.5 %) at 100, 500 and 2 500 ppm (6.0/7.6, 30.2/38.3 and 156/186 M/F mg/kg bw/day, respectively). To investigate the reversibility of effects, an additional 10 rats of each sex were fed control or high-dose diet for at least 90 days and subsequently fed control diet for at least 28 days.

Neither mortality nor treatment-related clinical signs or evidence of neurotoxicity were observed. In the high dose group, a lower body weight of 6 to 10 % was observed, non-significant in males but statistically significant in females as well as a significant reduction of body weight gain in both males and females (-12/-15 % (m/f)). Food consumption at this dose was also slightly reduced (percentage of the reduction was not provided) in tated females compared to the control group.In females dosed with concentration of 2 500 ppm (186 mg/kg bw/day), the mean platelet count was 6/15 % (m/f) higher, the mean total bilirubin was 38/45 % (m/f) and, the glucose concentrations were 21/22 % (m/f) lower, the mean total cholesterol was 38/46 % higher and triglyceride concentrations were 35/66 % higher compared to controls. Clinical chemistry parameters were generally dose-dependently affected.

Statistically significant effects at the high dose groups when compared to controls consisted in higher relative liver weights in both sexes associated with relevant minimal to slight histopathological findings in males (10/10) and females (3/10). Statistically significant increased relative thyroid weights in males at the mid- and high-doses (20 and 26 %) were associated with minimal follicular cell hypertrophy in a limited number of male rats (3/10) at the high dose only. After the recovery phase, none of these treatment-related findings were observed and they were therefore considered to be reversible.

In the high-dose females, the absolute heart weight was significantly reduced (-10 %) whereas the reduction of the relative heart weight compared to controls was 4 % lower. No information was provided whether these effects were also reversible after the recovery period.

Rats, 90-day neurotoxicity (one study)

The study was performed according to OECD TG 424 with dose levels of 0, 5.7/6.9, 29.4/34.8, 143/173 (M/F) mg/kg bw/day with male and female rats (12/sex/dose). Up to and including the highest dose, there was no treatment-related mortality, clinical signs or ophthalmological

changes during the study and no treatment-related effects were observed in any of the neurotoxicology endpoints, including neuropathological examinations, in either sex. No quantitative data are available. Overall at the end of the study, mean body weight gain at 143/173 (M/F) mg/kg bw/day level represented 85% and 79% of the control values in males and females, respectively. Mean food consumption was significantly lower in both sexes during the first week of treatment (-18/-29 % (m/f)) and remained slightly lower in both sexes throughout the study (up to -14/-13 % (m/f), the effect being statistically significant on several occasions). Enlarged liver (without histopathological changes) was noted in females only (4/6). There was no effect at the mid dose in either males or females.

Mice, 90-day (one study)

Flupyradifurone (purity 99.5%) at concentrations of 0, 100, 500, 2 500 ppm (0, 16/19, 81/98, 407/473 (M/F) mg/kg bw/day) was administered to C57BL/6J mice (10/sex/dose). The study is similar to OECD TG 408 except for the performance of functional observational battery (FOB) and the ophthalmological examination.

There were no treatment-related death or clinical signs. One female of the mid-dose group (98 mg/kg bw/day) had to be humanely sacrificed on study day 61 for reasons unrelated to treatment. In the high dose group, the overall cumulative mean body weight was reduced by 43 % in males but only by 7 % in females. Body weight gain was not related to decreased food consumption, which was only slightly decreased in females at the top dose.

Haematology was not performed in this study. The mean total cholesterol and total protein concentration in the high dose animals was statistically significantly decreased compared to untreated animals whereas urea (statistically significantly in both males and females) and alkaline phosphatase (statistically significantly in males) as well as the aspartate aminotransferase (non-statistically significantly in both males and females) levels were increased in both sexes. In addition, alanine aminotransferase activity was statistically significantly induced in females and albumin concentrations were statistically significantly reduced in females. The above changes started to be observed at the mid-dose where they were slight and none of them were statistically significant.

At the high-dose group, absolute (+12 %) and relative (+18 %) liver weights were statistically significantly higher in females and pale liver was observed in 6/10 females. Males also had an increase in relative liver weights (statistically significantly, +20 %) but not in absolute liver weight, pale liver was observed in 1/10 males. At microscopic examination, a slight increase in severity of diffuse hepatocellular vacuolation (observed also in controls and low- and mid-dose mice) was found in both sexes (controls: minimal (6/7 m/f), slight (4/3), moderate (0/0); high-dose: minimal (0/2 m/f), slight (6/3), moderate (4/5)). In the kidney, a loss of the normal multifocal/diffuse cortical epithelial vacuolation was observed and the absolute kidney weight was statistically significant lower in males (-11 %) compared to controls.

Dogs, 90-day (one study)

Flupyradifurone (purity 96.2 %) in corn oil and acetone was administered to beagles (4/sex/dose) at concentrations of 0, 400, 1 200, 3 600/2 400 ppm (0, 12/12, 33/41, 102/107 F/M mg/kg bw/day). At the beginning of study week 9, the 3600 ppm dose group had to be reduced (from 102 to 85 (M) and from 107 to 78 (F) mg/kg bw/day) due to clinical signs observed in two dogs on day 44 and continual weight loss in the high dose group. Haematology was provided at day 7 and at 1, 2, 3 months.

There were no treatment-related death. Unsteady and stiff back legs and lower back in one male and one female were observed in the high dose group. Body weight and body weight gain were statistically significantly reduced at 1 200 and 3 600/2 400 ppm. Food consumption was reduced in mid- and high-dose males and high-dose females during the first week of the study. The changes were statistically significant only for body weight gains of males and females at the highest dose and for males at the mid dose.

A treatment-related reduction in RBC (red blood cells), Hb and Hct was found in the 1, 2 and 3 month haematology evaluations in both sexes at the high dose. Other clinical chemistry parameters, which were severely increased at the 2-month test interval in the mid- and high-dose groups (e.g. creatine phosphokinase, 10 to 16 fold, aspartate aminotransferase, 5 to 8 fold and alanine aminotransferase, 4 to 18 fold), returned to normal at the 3-month evaluation.

In the high dose group, relative increase in liver weights were statistically significantly increased in both sexes (+29/24 %, m/f), whereas a significant increase in the absolute liver weight was only noted in males (+14 %). A significant increase in relative kidney weight was observed for males in the mid- and high-dose groups (+18 and 31 %, respectively). Interestingly, no microscopic changes were noted in the liver. In contrast, both sexes at the mid- and high-dose groups showed minimal to slight myofiber atrophy/degeneration in skeletal muscles (2/4 males and 4/4 females for both doses, with a slight increase in severity of the effects).

Dogs, 1-year (one study)

Beagles (4/sex/dose) were fed diets containing flupyradifurone (purity 96.2 %) in corn oil and acetone at concentrations of 0, 150, 300 and 1 000 ppm (0, 4.6/4.1, 7.8/7.8, 28.1/28.2 (M/F) mg/kg bw/day).

There were no treatment-related death or clinical signs. Body weight gain was only transiently reduced in females (-88 %) in the high dose group. Treatment-related histopathological changes were limited to degeneration in skeletal muscle (e.g. gastrocnemius, 2/4 males and 2/4 females; biceps femoris, 3/4 males and 3/4 females) comprising one or more of the following changes: atrophy, necrosis and/or presence of inflammatory cells around the affected myofiber.

Dermal study

Rats, 28-day (one study)

The 28-day dermal study in rats performed with flupyradifurone (purity 96.2 %) showed statistically non-significant decreases in absolute and relative liver weights at 500 mg/kg bw/day in males compared to the control group. 500 mg/kg bw/day was the highest dose tested although the test should have been performed also with 1 000 mg/kg bw/day. It is not completely clear from the study report why this dose was not considered to be reliably applied to the skin. However, as there was no indication of severe effects at 500 mg/kg bw/day, classification might not be warranted based on this study although some minor uncertainty remain as the extrapolated value according to Regulation (EC) No 1272/2008 for STOT RE 2 is 600 mg/kg bw/day.

Combined chronic toxicity and/or carcinogenicity studies in rats and mice

Two long-term studies have been performed in rats and mice. For neoplastic effects, see the carcinogenicity section. Occurrence of non-neoplastic effects in these studies can be relevant for classification for STOT RE. The dose levels relevant for classification with STOT RE are as

follows: 2-year study: STOT RE 1: C ≤ 2.5 mg/kg bw/d and STOT RE 2: 2.5 < C ≤ 25 mg/kg bw/day.

In the combined chronic toxicity and carcinogenicity study in **rats** conducted according to OECD TG 453 (GLP), rats were dosed via the diet at 0, 80, 400 and 2 000 ppm. The average daily intake over the whole study was 0, 3.17, 15.8 and 80.9 mg/kg bw/day (males) and 0, 4.48, 22.5 and 120 mg/kg bw/day (females). Females at the top dose had a statistically significant loss of weight (-13 %) and loss of body weight gain (-22 %) compared to controls at termination, which was associated with a slight decreased food consumption throughout the study. At the interim sacrifice and at the end of the study, effects related to the treatment were noted in the liver of both males and females compared to controls at the high dose (80.9/120 mg/kg bw/day) in both sexes. They consisted in centrilobular hypertrophy, centrilobular/periportal hepatocellular macrovacuolation, hepatocellular brown pigments and brown pigments in Kupffer cells and higher incidences of interstitial mononuclear cell infiltrate. No adverse effect was noted in both sexes at 400 ppm (15.8/22.5 mg/kg bw/day) which is well above the guidance value for STOT RE 2 (12.5 mg/kg bw/day). White foci in the lung (associated with foamy macrophages, chronic interstitial inflammation and perivascular inflammation) were noted in 21/46 of high-dose females, which were considered to be associated with the treatment but above the guidance value. Ovarian cysts were noted in 18/46 females (compared to 8/29 in the controls) but they were not considered related to flupyradifurone administration. Some females presented iris mydriasis and abnormal pale colour of fundus of the retina (4/48 and 3/48 respectively) compared to 0/30 in controls. In addition, higher incidences of lens opacity was noted in high dose females (46/48, 96 %) compared to controls. In the thyroid, increased incidences of colloid alteration were noted in males at the mid- and top-doses. The effect at the mid-dose (15.8 mg/kg bw/day) was not considered to be adverse by the DS, as increased incidence of colloid alteration occurs naturally in aging rats and was not associated with relevant follicular hypertrophy at this dose level. Other effects were within historical control data, not treatment related or of doubtful biological significance. Therefore, RAC agrees with the DS that the findings in rats are not severe enough and/or are above guidance values for STOT RE 2 to trigger a classification.

In the carcinogenicity study in **mice** conducted according to OECD TG 451 (GLP), mice were dosed via the diet for 78 weeks at 0, 70, 300 and 1 500 ppm. The average daily intake over the whole study was 0, 10.0, 43, 224 mg/kg bw/day (males) and 0, 12.2, 53, 263 mg/kg bw/day (females). Body weights and body weight gains were affected in both males and females (-19 % and -13 %, respectively) compared to control mice. The liver (males and females) and the kidneys (males only) appeared to be the target organs but the effects were considered not adverse by the DS. Atrophic/small kidneys were noted in 5/42 high-dose males (in comparison to 0/38 in controls) as well as reduced absolute and relative kidney weights. Interestingly, decreased incidence and severity of bilateral basophilic tubules, focal cortical mineralization and corticoepithelial vacuolation were noted in high-dose males (all statistically significant). RAC notes that these changes in the kidney occurred with lower incidence and severity than in control animals and are therefore not relevant for classification.

RAC agrees therefore that kidney effects in males do not fulfil the classification criteria for STOT RE 2. Regarding the liver, histopathological examination showed increased incidence of centrilobular hepatocellular vacuolation (high-dose males, statistically significant), whilst a decreased incidence of periportal hepatocellular macrovacuolation was noted in high-dose females (statistically significant). No statistically significant increase in the incidence of centrilobular hepatocellular vacuolation was also noted in mid-dose males (43 mg/kg bw/day).

These changes in the liver were considered to be treatment-related but not adverse due to the lack of associated degenerative changes. Therefore, RAC agrees with the DS that the findings in mice are not severe enough to trigger classification and/or are above guidance values for STOT RE 2.

Reproductive and developmental toxicity studies

Findings from reproductive toxicity studies can also be of relevance for classification for STOT RE according to the CLP regulation (CLP Annex I section 3.9.2.5).

The one generation study with rats showed treatment related findings at high dose levels (147.5 to 182.3 mg/kg bw/day), with reported NOAELs in the range of 17.5 mg/kg bw/day (parental and offspring). In parental males, equivocal increases in liver weights were observed in both the 700 ppm (50.1 mg/kg bw/day) and 2 000 ppm (147.5 mg/kg bw/day) dose groups. In parental females, test substance-related decreases in absolute and relative spleen weight were observed at 2 000 ppm (~170 mg/kg bw/day). The decrease in body weight and body weight gain throughout pre-mating and gestation constitute the most severe effects (without decreased food consumption) and are shown in the Table below.

Table: *Parental males and females changes in mean body weight and mean body weight gain at different phases of the one-generation study (% difference compared to controls)*

	Pre-mating		**Gestation**		**Lactation**	
	BW	BW gain	BW	BW gain	BW	BW gain
Males	147.5 mg/kg bw/day: ↓9.3 %	No effect	na	na	na	na
Females	168.9 mg/kg bw/day: ↓9.4 %	60 mg/kg bw/day: ↓12.3 % 168.9 mg/kg bw/day: ↓33.4 %**	48.8 mg/kg bw/day: ↓4.5 % 164.4 mg/kg bw/day: ↓11.3 %**	No effect	60.9 mg/kg bw/day: ↓10.6 %* 182.3 mg/kg bw/day: ↓10.2 %**	No effect

*, statistically significant ($p < 0.05$); **, statistically significant ($p < 0.01$); na: not applicable

In females, declines in absolute body weight and body weight gain were observed mainly during premating, gestation and during lactation in the high-dose group. Effects observed are consistent and statistically significant from a dose level of 164.4 mg/kg bw/day. The decreased body weight was statistically significant in parental females at 60.9 mg/kg bw/day during lactation.

In the two-generation reproductive study, parental (P) males were exposed to dose levels of 6.5, 32.3 and 119.8 mg/kg bw/day (100, 500 and 1 800 ppm, respectively). Females (P) received 7.8, 39.2 and 140.2 mg/kg bw/day (100, 500 and 1 800 ppm, respectively). The F1 generation males and females were exposed to similar dose levels. The decrease in body weight and body weight gain associated with decreased food consumption throughout pre-mating and gestation (of the P and F1 generations) constitute the most severe effects and are summarised in the Table below.

Table: *Parental and F1 males and females changes in mean body weight and mean body weight gain at different phases of the two-generation study (% difference compared to controls).*

	Pre-mating		**Gestation**		**Lactation**	
	BW⁺	**BW gain**	**BW⁺**	**BW gain**	**BW⁺**	**BW gain**

Males (P)	No effect	No effect	na	na	na	na
Males (F1)	119.8 mg/kg bw/day: ↓10 %** (with 10 %** **increased** fc)	No ss effect but dr decrease	na	na	na	na
Females (P)	140.2 mg/kg bw/day: ↓11 %** (Day 20) (with 10 % **decreased** fc)	No ss effect but dr decrease (**21 % at 39.2 mg/kg bw/day** and 43 % at 140.2 mg/kg bw/day)	151.4 mg/kg bw/day: ↓10 %** (Days 0-20) (slight **increased** fc)	151.4 mg/kg bw/day: ↓10 %** (Days 0-20) (slight **increased** fc)	150.4 mg/kg bw/day: ↓8 %** (Day 21) (slight **increased** fc)	No ss effect (Days 0-21) (slight **increased** fc)
Females (F1)	39.2 mg/kg bw/day: ↓8 %** (Day 20) 140.2 mg/kg bw/day: ↓16 %** (with 12 % **decreased** fc)	No ss effect but dr decrease (**16 %** at 39.2 and 22 % at 140.2 mg/kg bw/day) (with 12 % **decreased** fc)	35.5 mg/kg bw/day: ↓6 %** (Days 20) 140.2 mg/kg bw/day: ↓13 %** (Days 0-20) (with 27 % **increased** fc)	151.4 mg/kg bw/day: ↓19 %** (Days 0-20) (with 27 % **increased** fc)	39.8 mg/kg bw/day: ↓8 %** (Days 21) 150.4 mg/kg bw/day: ↓13 %** (Day 21) (with 7 % **increased** fc)	No ss effect (Days 0-21) (slight **increased** fc)

*, statistically significant ($p < 0.05$); **, statistically significant ($p < 0.01$); +, directly compared to control values; dr, dose-related; fc, food consumption; ss, statistically significant.

The DS reported that at the highest dose level (140.2 mg/kg bw/day), statistically significant decreases in body weight were observed in parental P and F1 females during premating, gestation and lactation. In F1 males, at the highest dose level (119.8 mg/kg bw/day) significant decreases in body weight were observed beginning on Day 0 and continued throughout exposure (pre-mating period of 10 weeks). The DS also reported that statistically significant decreases in body weight and body weight gain during premating and gestation were also observed in F1 females at the mid-dose level (39.2 and 35.5 mg/kg bw/day for pre-mating and gestation, respectively) but the decreased body weight was less than 10 %. Body weight gains were decreased in P and F1 females during premating and gestation in the high-dose groups, and non significantly during pre-mating in the mid-dose group (-21 % for P generation females and -16 % for F1 generation females). In a comment received during public consultation in relation to individual estrous cycles, industry analysed individual mean body weights in the F1 animals and noted that there was a 15.9 % decrease in mean body weight compared to controls at the end of the pre-mating period. Further, at the beginning of gestation (GD0), P0 body weight was reduced by 9.7 % in high-dose animals compared to controls while in the F1 generation, GD0 body weight was reduced by 17 % compared to controls.

RAC notes that individual animal data were not available but also observes that the food consumption was slightly to moderately increased at the mid- and high-dose levels.

Other effects in P and F1 adults included liver hypertrophy associated with increased liver weights. They were not correlated with histopathological findings. In offspring (F1 and F2 pups), organ weight changes observed in high-dose F1 pups are considered to be secondary to the decreased body weights and not a direct test substance effect.

In an oral developmental neurotoxicity study with female Wistar rats, the average mean daily intake of the test substance based on the average dietary consumption for the last two weeks of gestation and three weeks of lactation at 120, 500, or 1 200 ppm, respectively, was 0, 10.3, 42.4, and 102 mg/kg bw/day. In maternal animals decreased body weight gain was found. Body weight was non-statistically significantly decreased in high-dose males and females on PND 17 and 21 (-4 % to -5 %). Although these differences from control are small and not statistically significant, they are attributed to the test substance because of the relationship with dose (both genders and at multiple time points). These lower body weights were comparable to the 4–6 % lower body weight for the high-dose dams throughout lactation. The test substance in either sex at any other dietary levels did not affect body weight. Body weight gain for PND 4–17 was statistically significantly decreased in high-dose males (-7 %). Body weight gain for the shorter period of PND 11–17 was statistically significantly decreased in high-dose males (-10 %) and females (-7 %). There were similar non-statistically significant trends in body weight gain for PND 4–17 in high-dose females (-6 %), for PND 4–21 and PND 11–21 in both sexes. These differences from control are attributed to the test substance. Body weight gain was not affected by the test substance in either sex at the 10.3 and 42.4 ppm dietary levels. There was no difference in body weight after weaning for males and females at any dietary level. In the offspring, slightly increased motor and locomotor activity in males and slightly increased auditory startle habituation in females was observed.

The developmental study in rats showed similar effects on body weights and body weight gains of dams. In the high-dose females (150 mg/kg bw/day) a mean maternal body weight gain and the maternal corrected body weight change were decreased between GD 6-21 (not statistically significant). The mean body weight changes were significantly lower than controls by 14 to 91 % at all measurement intervals between GD 6 and GD 18 ($p \leq 0.01$). The food consumption was significantly decreased between GD 6 and 12. At 50 mg/kg bw/day the mean maternal body weight gain was reduced by 49 % between GD 6-8, and the mean maternal body weight change was significantly lower than controls by 23 % between GD 6 and GD 10. The mean food consumption was reduced by 8 % between GD 6-8 ($p \leq 0.05$). No treatment-related body weight changes were observed in the low-dose group.

In rabbits, the high-dose group (40 mg/kg bw/day) mean maternal body weight between GD 6 and 10 was lower than in controls. The mean maternal corrected body weight changes were reduced by 12 % compared to the controls (not statistically significant) and were slightly outside the range of in-house historical control data. Food consumption was transiently reduced by 20 % between GD 6 and 8 ($p \leq 0.01$) and by 11 % between GD 8 and 10 (not statistically significant). The DS considered these effects on (corrected) body weight gain and food consumption were slight, and mainly transient.

Summary of effects in repeated dose toxicity studies relevant for STOT RE

Table: *Summary of the described study reports including NOAEL and LOAEL*

Duration	Species	Route	NOAEL mg/kg bw/day	LOAEL mg/kg bw/day#	Reported effects relevant for STOT RE 2
28-day	rat	oral, gavage	75[1]	**200**	Changes in biochemical parameters, increased liver weight, enlarged liver, prominent lobulation, hepatocellular hypertrophy, follicular cell hypertrophy
28-day	rat	oral, diet	33.6[1]	385	Changes in biochemical parameters,

					increased relative liver and thyroid weight, prominent lobulation of the liver, hepatocellular hypertrophy, follicular cell hypertrophy
28-day	mouse	oral, diet	98[1]	-	
28-day	dog	oral, diet	M:62; F: 77[1]	**118**	Overall body weight loss and reduced body weight gain
28-day	rat	dermal	500	-	
28-day immunotoxicity study	rat	oral, diet	50	**230**	Body weight loss and reduced body weight gain, decreased food consumption
90-day	rat	oral, diet	6.0	**30.2**	Changes in biochemical parameters, increased liver and thyroid weight, enlarged liver, dark thyroid gland, hepatocellular hypertrophy, follicular cell hypertrophy
90-day	mouse	oral, diet	81	407	Reduced body weight, changes in biochemical parameters, increased liver weight, decreased kidney weight, pale liver, hepatocellular vacuolation, loss of normal multifocal/diffuse cortical epithelial vacuolation
90-day	dog	oral, diet	12	**33**	Reduced body weight gain, changes in biochemical parameters, significant increased kidney weight, myofiber atrophy/degeneration
90-day neurotoxicity	rat	oral, diet	M: 29.4 F: 34.8	M: 143 F: 173	Reduced body weight gain and food consumption. Enlarged liver (F: 4/6)
1-year	dog	oral, diet	7.8	**28.1**	Degeneration of skeletal muscle and other muscle (biceps femoris) degeneration
2-year	rat	oral, diet	15.8/18.5 (104/52 weeks)	-	Effects in the liver and to a less extend effects in the thyroid and lung
2-year	mouse	oral, diet	M: 45; F: 53	-	Effects in the liver and the kidney
One-generation	Rat	oral, diet	F and pups: 17.5	**F: 60.0** **Pups: 60.9**	Reduced body weight, reduced body weight gain, alterations in food consumption during premating and decreased spleen weights in females. Pups: Maternal effects leading to secondarily-mediated effects on body weight, body weight gain and changes in brain weight
Two-generation	rat	oral, diet	P: 6.4	**P: 32**	Reduced body weight, reduced body weight gain
Dev. toxicity	rat	oral, gavage	F and pubs: 50	-	F: Reduced body weight gain and reduced food consumption, increased absolute liver weight
Dev. toxicity	rabbit	oral, gavage	F: 15 Pups: 40	-	Reduced body weight gain, reduced food consumption
Dev. neurotoxicity	rat	oral, diet	F: 42.4 Offspring: 42.4	F: 102 Offspring: 102	F: decreased body weight gain. increased auditory startle habituation Offspring: decreased body weight gain, increased startle amplitude in females only on PND 60 and increased motor and locomotor activity on PND 13 in males only

[1] Dose range studies; # values in bold are within guidance values and therefore relevant for classification

According to the CLP criteria, substances are classified in STOT RE 2 based on evidence from studies in experimental animals that can be presumed to have the potential to be harmful to human health following repeated exposure. For classification in Category 2, animal data in which significant toxic effects of relevance to human health were observed at generally moderate exposure concentrations. The CLP Regulation provides a 'guidance value' of 10-100 mg/kg bw/day from a 90-day study to assist classification in Category 2. For a 28-day study this guidance value is ≤ 300 mg/kg bw/day.

Summing up on the myofiber atrophy/degeneration, RAC considers that the **effects in muscles** observed in the long-term dog studies are rather severe since atrophy and necrosis were observed in the 1-year dog study. In addition, these effects do not seem to be a secondary effect due to weight loss as in the 1-year dog study weight loss did not occur. RAC concludes that classification for **STOT RE 2 is warranted** as myofiber atrophy/degeneration (irreversible effect) was observed in both sexes at dose levels much lower than 100 mg/kg bw/day in a 90-day dog study, supported by the same findings in a 1-year dog study at 28.1/28.2 mg/kg bw/day (at slightly higher doses than the extrapolated CLP-Regulation value for STOT RE 2 which is ≤ 25 mg/kg bw/day). RAC points out that the values and ranges stated in the legislation are not intended as strict demarcation values. Besides, in the 90-day study in dog, clear changes in clinical chemistry (at the 2-month evaluation) indicative of muscle toxicity (creatinine phosphokinase (CK) and aspartate aminotransferase) support the conclusion that the muscle effects are sufficiently severe for classification although no CK isoenzyme analysis was performed. In addition, as no underlying mode of action is demonstrated, the relevance for humans cannot be excluded.

Regarding the liver, although increased absolute and relative liver weight, clinical chemistry findings (e.g. decrease of total bilirubin and glucose concentration and increase of triglycerides, alanine aminotransferase and alanine phosphatase) as well as centrilobular or diffuse hepatocellular hypertrophy and prominent lobulation were observed in three different species (in rats and dogs at dose levels relevant for classification as STOT RE 2) and in both males and females, RAC considers these effects to be non-specific (adaptive) effects and not as indications of a marked organ dysfunction.

The results of the two chronic toxicity/carcinogenicity studies in rats and mice provide further support that the effects in the liver might not be severe enough to consider the liver as a target organ. Indeed, in the carcinogenicity study in rats, 1/60 males had marked eosinophilic foci at the top dose, 1/60 male rats had a marked periportal diffuse macrovacuolation at the top dose and 1/60 male rats had a marked periportal diffuse macrovacuolation at the mid dose. In the lung, 1/60 females had a marked focal foamy macrophage. All the other findings (in the liver, the lung and the thyroid) were minimal to moderate with a slight treatment-related increase in severity in both males and females. In mice, all findings were minimal to moderate with the exception of 5/50 male mice with a marked centrilobular diffuse vacuolation. All the other findings (in the liver and the kidney) were minimal to moderate with a slight treatment-related increase in severity in both males and females.

Effects on the **thyroid** which were clearly within the values according to Regulation (EC) No 1272/200 for STOT RE consisted in minimal follicular cell hypertrophy observed at 200 mg/kg bw/day in males in a 28-day rat study and in enlarged thyroid glands without any histopathological changes in the 28-day dog study (in males at 118 mg/kg bw/day and in females at 131 mg/kg bw/day). However, RAC does not consider these effects significant enough to warrant classification as there is no evidence of organ dysfunction.

Based on several independent studies in rats and rabbits, RAC concludes that flupyradifurone significantly affects the **body weight and (corrected) body weight gain** of parental animals and offspring that could not be counterbalanced by a significant increase in food consumption in the one- and two-generation studies. Significant body weight changes were observed well within the guidance value for STOT RE 2 (equivalent to a 90-day rat study: 10 < C ≤ 100 mg/kg bw/day). However, these effects are not considered severe enough to justify a classification for specific organ toxicity.

Other effects like the effects on the **blood system** (e.g. decreased Hb, Hct, MCV in the high dose group in the 90-day-study in dogs) are not severe enough to justify a classification with the blood as a target organ.

Overall, RAC concludes that, based on irreversible myofiber atrophy/degeneration in both sexes in dogs at doses within the relevant guidance values in the CLP Regulation, flupyradifurone should be classified as **STOT RE 2; H373: "May cause damage to organs (muscle) through prolonged or repeated exposure"**. As there is only one repeated dermal dose toxicity study in rats with some uncertainty with regard to the highest dose tested and as there are no studies performed via the inhalation route, RAC cannot exclude the possibility that the substance can exert toxicity by these routes. RAC therefore considers that the route should not be included in the hazard statement.

4.9 Germ cell mutagenicity (Mutagenicity)

4.9.1 Non-human information

4.9.1.1 In vitro data

Study 1

Study design and results

Type of study: Ames test, plate incorporation and preincubation method

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
S. typh. TA 98 TA 100 TA 102 TA 1535 TA 1537	point mut. point mut. point mut. point mut. point mut.	- - - - -	- - - - -	rat liver	Aroclor 1254	16-5000 µg/plate	Herbold, B. (2009)

Test substance: BYI 02960 (batch number 2009-000239; purity 96.2%); tested in DMSO
Precipitation: No precipitation of the test substance was reported.
Toxicity: at 5000 µg/plate, the substance had only a weak, strain-specific bacteriotoxic effect
GLP statement: yes
According to OECD 471: yes
Adequate positive (+S9: 2-AA; -S9: NaN_3, NF, 4-NPDA, MMC and Cumene) and negative (sterility) controls were included.

Table 61: Plate incorporation assay with BYI 02960 - Mean number of revertants

Strain	**TA 98**		**TA 100**		**TA 102**		**TA 1535**		**TA 1537**	
Metabolic activation	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**
Neg. control 0 µg/plate	25	31	124	198	212	294	8	12	7	10
BYI 02960										
16 µg/plate	26	38	128	182	216	297	8	13	6	8
50 µg/plate	27	25	121	190	218	314	7	10	6	10
158 µg/plate	24	35	145	177	207	335	7	10	5	8
500 µg/plate	24	29	135	213	212	314	7	11	6	8
1581 µg/plate	28	26	131	202	220	288	8	8	5	7
5000 µg/plate	24	35	116	188	223	214	7	7	9	7
Pos. control	68 117	2031 2221	341 406	1719 1881	657 742	613 1183	805 835	90 59	35 52	304 135

Table 62: Pre-incubation assay with BYI 02960 - Mean number of revertants

Strain	**TA 98**		**TA 100**		**TA 102**		**TA 1535**		**TA 1537**	
Metabolic activation	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**
Neg. control 0 µg/plate	17	35	128	176	220	263	8	10	7	10
BYI 02960										
16 µg/plate	19	31	113	164	197	224	9	11	7	8
50 µg/plate	17	33	112	150	215	248	8	9	6	10
158 µg/plate	19	32	126	190	227	268	8	9	7	7
500 µg/plate	19	33	131	183	199	311	7	10	7	9
1581 µg/plate	18	22	116	165	197	245	9	9	6	8
5000 µg/plate	18	28	107	116	173	244	8	8	7	9
Pos. control	73 154	917 1065	412 638	1405 1914	418 448	553 1191	629 746	82 60	32 71	309 239

BYI 02960 did not cause any significant increase in the number of revertant colonies in either the presence or absence of metabolic activation. Therefore, BYI 02960 was non-mutagenic with or without S9 mix in the plate incorporation as well as in the pre-incubation modification of the Ames test.

Acceptability
The study is considered acceptable.

Conclusions
BYI 02960 is not mutagenic in the Ames test.

Study 2

Study design and results

Type of study: Ames test, plate incorporation and preincubation method

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
S. typh. TA 98 TA 100 TA 102 TA 1535 TA 1537	 point mut. point mut. point mut. point mut. point mut.	 - - - - -	 - - - - -	rat liver	Phenobarbital/ß-naphthoflavone	3-5000 µg/plate	Sokolowski, A. (2011)

Test substance: BYI 02960 (batch number PFV107N005; batch; purity 97.2%); tested in DMSO
Precipitation: No precipitation of the test substance was reported.
Toxicity: No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation. Only in experiment I in strain TA 1537 a minor reduction in the number of revertants (below the indication factor of 0.5), was observed with and without S9 mix at 5000 pg/plate.
GLP statement: yes
According to OECD 471: yes
Adequate positive (+S9: 2-AA; -S9: NaN_3, 4-NOPD, MMS) and negative (sterility and vehicle) controls were included.

Table 63: Plate incorporation assay with BYI 02960 - Mean number of revertants

Strain	TA 98		TA 100		TA 102		TA 1535		TA 1537	
Metabolic activation	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**
Neg. control (DMSO)	29	39	139	174	491	633	16	20	13	13
Neg. control (untreated)	48	37	173	162	485	540	20	17	17	24
BYI 02960										

Strain	**TA 98**		**TA 100**		**TA 102**		**TA 1535**		**TA 1537**	
Metabolic activation	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**
3 µg/plate	30	40	136	175	493	610	13	19	12	16
10 µg/plate	30	36	141	152	488	611	17	19	14	13
33 µg/plate	31	40	134	159	434	612	18	21	11	14
100 µg/plate	32	39	147	163	452	649	16	21	12	15
333 µg/plate	32	38	156	164	462	622	16	20	12	15
1000 µg/plate	28	39	140	172	455	629	18	20	8	15
2500 µg/plate	31	44	149	153	450	636	13	19	11	14
5000 µg/plate	35	36	132	160	390	543	14	18	4 *	5 *
Pos. control	275	2932	2135	2911	4489	2782	2100	407	63	344

* air bubbles and manual count

Table 64: Pre-incubation assay with BYI 02960 - Mean number of revertants

Strain	**TA 98**		**TA 100**		**TA 102**		**TA 1535**		**TA 1537**	
Metabolic activation	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**	**-S-9**	**+S-9**
Neg. control (DMSO)	29	40	120	122	383	366	12	20	18	20
Neg. control (untreated)	47	42	136	157	338	356	12	15	16	18
BYI 02960										
33 µg/plate	29	42	132	115	354	433	13	24	15	18
100 µg/plate	30	37	106	122	373	385	12	20	19	20
333 µg/plate	27	47	138	122	380	449	13	20	16	22
1000 µg/plate	24	44	96	109	330	487	15	20	18	17
2500 µg/plate	31	34	96	118	321	411	14	18	17	15
5000 µg/plate	26	37	89	106	283	278	15	17	19	19
Pos. control	393	1612	1793	2218	1084	1897	1837	264	93	246

BYI 02960 did not cause any significant increase in the number of revertant colonies in either the presence or absence of metabolic activation. Therefore, BYI 02960 was non-mutagenic with or without S9 mix in the Ames test.

Acceptability
The study is considered acceptable.

Conclusions
BYI 02960 is not mutagenic in the Ames test.

Study 3

Study design and results

Type of study: mammalian cells *in vitro*, cytogenetic assay

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose range[a]	Reference
				Tissue	Inducer		
Chinese hamster V79 cells	Chromosome aberration	-	-	rat liver	Aroclor 1254	-S9: 0, 500, 1000, 2000, 2500 and 3000 µg/mL (4 hours treatment, harvest 18 hours after the beginning of treatment) 0, 2000, 2500 and 3000 µg/mL (4 hours treatment, harvest 30 hours after the beginning of treatment) 0, 100, 200, 400, 600 and 800 µg/mL (18 hours treatment, harvest at the same time) +S9: 0, 500, 1000, 2000, 2500 and 3000 µg/mL (4 hours treatment, harvest 18 hours after the beginning of treatment) 0, 2000, 2500 and 3000 µg/mL (4 hours treatment, harvest 30 hours after the beginning of treatment)	Thum, M. (2009)

[a] Based on the results of the survival index and of the mitotic index, the following concentrations were selected for reading:
-S9:
500, 1000 and 2500 µg/mL (4 hours treatment, harvest 18 hours after the beginning of treatment);
2500 µg/mL (4 hours treatment, harvest 30 hours after the beginning of treatment);
200, 400 and 800 µg/mL (18 hours treatment, harvest at the same time).

+S9:
500, 1000 and 3000 µg/mL (4 hours treatment, harvest 18 hours after the beginning of treatment)
3000 µg/mL (4 hours treatment, harvest 30 hours after the beginning of treatment)

Test substance: BYI 02960 (batch number 2009-000239; purity 96.2%); tested in DMSO
Precipitation: No precipitation of the test substance was observed.
Toxicity: Without S9 mix cytotoxic effects were observed at 1000 µg/mL and above after 4 hours treatment and at 400

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose range[a]	Reference
				Tissue	Inducer		
µg/mL and above after 18 hours treatment. With S9 mix cytotoxic effects were observed at 2000 µg/mL and above. GLP statement: yes According to OECD 473: yes Adequate positive and negative controls were included.							

Table 65: Summary of results (metaphases with aberrations, polyploidy metaphases, survival and mitotic index, 4 hours treatment)

Treatment group	Harvest time (hours)	Metaphases with aberrations (%)		Polyploid metaphases[a]	Survival index in %	Mitotic nuclei in 2000 cells[b]	
		incl. gaps	excl. gaps			absolute	in %
without metabolic activation							
DMSO	18	1.0	1.0	13.5	100.0	134	100.0
BYI 02960							
500 µg/mL	18	4.0	3.5	15.5	88.9	148	110.4
1000 µg/mL	18	1.5	1.5	11.0	77.8*	129	96.3
2500 µg/mL	18	4.0	4.0	14.5	77.0*	72	53.7***
Mitomycin C (0.1 µg/mL)	18	56.5	55.5***	8.0	57.1*	100	74.6**
DMSO	30	1.5	1.0	9.0	100.0	123	100.0
BYI 02960							
2500 µg/mL	30	2.0	2.0	12.5	60.6*	86	69.6***
with metabolic activation							
DMSO	18	3.5	2.0	36.0	100.0	128	100.0
BYI 02960							
500 µg/mL	18	2.5	2.0	35.0	81.1	123	96.1
1000 µg/mL	18	3.0	1.5	37.0	81.8	139	108.6
3000 µg/mL	18	3.5	3.5	26.5	65.0*	132	103.1
Cyclophosphamide (2 µg/mL)	18	74.0	71.5***	17.0	49.0*	59	46.1***
DMSO	30	3.0	1.5	19.0	100.0	156	100.0
BYI 02960							
3000 µg/mL	30	3.0	3.0	17.5	46.1*	86	55.1***

[a] The number of polyploid metaphases observed in a culture in addition to 100 metaphases analysed for chromosome aberrations were recorded (2 cultures);

[b] The mitotic index was determined by counting a total of 1000 cells per culture (2 cultures);

* relevant reduction of survival index;

** $p < 0.05$;

***$p < 0.01$.

Table 66: Summary of results (metaphases with aberrations, polyploidy metaphases, survival and mitotic index, 18 hours treatment)

Treatment group	**Harvest time (hours)**	**Metaphases with aberrations (%)**		**Polyploid metaphases[a]**	**Survival index in %**	**Mitotic nuclei in 2000 cells[b]**	
		incl. gaps	**excl. gaps**			**absolute**	**in %**
without metabolic activation							
DMSO	18	3.0	3.0	5.5	100.0	91	100.0
BYI 02960	18						
200 µg/mL	18	4.0	4.0	6.0	80.7	80	87.9
400 µg/mL	18	2.5	2.0	5.0	86.9	80	87.9
800 µg/mL	18	3.5	3.5	8.5	68.2*	63	69.2**
Mitomycin C (0.03 µg/mL)	18	52.0	51.5***	4.5	71.0*	103	113.2

[a] The number of polyploid metaphases observed in a culture in addition to 100 metaphases analysed for chromosome aberrations were recorded (2 cultures);
[b] The mitotic index was determined by counting a total of 1000 cells per culture (2 cultures);
* relevant reduction of survival index;
** $p < 0.05$;
*** $p < 0.01$.

None of the cultures treated with BYI 02960 in the absence or presence of S9 mix showed statistically significant or biologically relevant increases of numbers of metaphases with aberrations. Based on the results of this test, BYI 02960 is considered not to be clastogenic for mammalian cells in vitro.

Acceptability
The study is considered acceptable.

Conclusions
BYI 02960 did not induce structural chromosome aberrations in V79 cells when tested up to and including cytotoxic concentrations.

Study 4

Study design and results

Type of study: mammalian cells *in vitro*, gene mutation test

Indicator cells	**Endpoint**	**Res. –act.**	**Res. +act.**	**Activation**		**Dose range**	**Reference**
				Tissue	**Inducer**		
Chinese hamster V79 cells	Gene mutations (HGPRT)	-	-	rat liver	Aroclor 1254	-S9: 0, 46, 92, 184, 368, 736, 1472, 2944 µg/mL; +S9: 0, 46, 92, 184, 368, 736, 1472, 2944 µg/mL	Entian, G. (2009)
Test substance: BYI 02960 (batch number: 2009-000239; purity 96.2%); tested in DMSO Precipitation: precipitation of the test substance was observed in the culture medium at 2944 µg/mL Toxicity: Without and with S9 mix, the test substance induced no decreases in survival to treatment or in relative population growth. The test substance was tested up to 2944 µg/mL, a concentration which slightly exceeded the limit concentration of 10 mM. GLP statement: yes According to OECD 476: yes Adequate positive and negative controls were included.							

Table 67: Gene mutation in mammalian cells, without metabolic activation

Treatment group	Survival to treatment (colony mean)	Population growth (% of NC)	Cloning efficiency (%)	Mutant frequency ($x10^{-6}$)
experiment 1 (5-hour exposure period)				
Untreated control	136	129.7	78.7	1.1
	131	97.9	80.2	9.4
Negative control	121	100	87	5.3
DMSO	128.7	100	91.3	10.0
BYI 02960 46 µg/mL	144	128.4	75.3	0.0
	121.7	106	81.8	5.1
BYI 02960 92 µg/mL	152.3	112.2	73.3	14.2
	128	107.8	81.7	4.6
BYI 02960 184 µg/mL	135.7	120.8	74.3	6.2
	155	120.6	78.7	10.6
BYI 02960 368 µg/mL	114.3	80.6	82.5	1.0
	142.7	135.7	74.3	0.0
BYI 02960 736 µg/mL	148	119.8	87.2	5.7
	154	122.9	83.7	4.5
BYI 02960 1472 µg/mL	130	114.5	79.2	3.7
	165	144.4	80	3.6
BYI 02960 2944 µg/mL [p]	129.3	101.8	73.2	5.1
	63.3	68.8	79	7.4
Positive control (EMS, 900 µg/mL)	126.7	46.8	65.5	628.5*
	102.3	33.9	71.7	751.7*
experiment 2 (5-hour exposure period)				
Untreated control	176.3	62.2	83.2	2.0
	170.7	103.3	78.7	6.4
Negative control	178	100	75.7	2.2
DMSO	206.7	100	76	4.9
BYI 02960 46 µg/mL	163.3	58.7	76.7	6.0
	186.3	91	74.5	5.6
BYI 02960 92 µg/mL	160.7	55.3	74.3	1.7
	194	97.4	71.8	4.6
BYI 02960 184 µg/mL	167	54.5	81.2	8.7
	174.3	94.9	74.3	9.5
BYI 02960 368 µg/mL	147	54.9	74.5	4.5
	147.3	84.9	77.8	1.6
BYI 02960 736 µg/mL	171	56.2	82.5	1.5

	149.7	75.8	75.7	4.4
BYI 02960 1472 µg/mL	162.3	63.3	72.8	4.6
	160.3	87.8	75.2	3.9
BYI 02960 2944 µg/mL [P]	161.3	75	64.3	1.9
	160	60.1	80	6.8
Positive control (EMS, 900 µg/mL)	71.3	19.9	75.2	680.7*
	90.7	37	79.2	495.8*

[P] precipitation of the test substance

* significant (α = 0.05) increase in the one-sided Dunnett Test.

Table 68: Gene mutation in mammalian cells, with metabolic activation

Treatment group	Survival to treatment (colony mean)	Population growth (% of NC)	Cloning efficiency (%)	Mutant frequency (x10-6)
experiment 1 (5-hour exposure period)				
Untreated control	182.7	128.3	64.3	9.7
	144.7	130	69.7	0.6
Negative control	173.3	100	68.8	1.2
DMSO	179	100	77.3	1.4
BYI 02960 46 µg/mL	169.7	106.1	70.3	1.8
	172	127	76.7	4.9
BYI 02960 92 µg/mL	143	89.4	78.3	1.6
	164.7	124.1	76.7	[c]
BYI 02960 184 µg/mL	155.3	103.4	72.5	2.3
	145	137	73	9.7
BYI 02960 368 µg/mL	181.7	127.7	81	3.1
	160.7	134.1	73.3	5.7
BYI 02960 736 µg/mL	185.3	111.7	71.2	9.4
	166.7	102.2	83.8	6.5
BYI 02960 1472 µg/mL	167	90.4	80.7	8.3
	177.3	137.5	80.8	8.2
BYI 02960 2944 µg/mL [p]	157.3	98.3	76.7	2.7
Positive control (DMBA, 20 µg/mL)	175	57.1	79.7	161.6*
	100	22.4	84.5	150.4*
experiment 2 (5-hour exposure period)				
Untreated control	149.3	105.4	68.2	0.6
	161	78.1	83.5	0.5
Negative control	171.7	100	93.8	0.9
DMSO	181.3	100	78	2.1
BYI 02960 46 µg/mL	162.3	114.3	83.3	2.0
	139.3	49.1	82.5	0.5
BYI 02960 92 µg/mL	170.7	94.4	82	2.0
	156.3	55.7	67	0.0
BYI 02960 184 µg/mL	158.7	114	80.8	2.6
	194.3	45.8	74.7	1.1
BYI 02960 368 µg/mL	170.3	99.3	78.5	0.5
	192.3	67.4	79.8	1.6
BYI 02960 736 µg/mL	180.3	85.8		[c]
	189.3	38.3	80.7	1.0

BYI 02960 1472 µg/mL	187.7	116.6	82	0.0
	181.3	33.6	93.5	0.9
BYI 02960 2944 µg/mL [P]	161.3	82.4	76	2.7
	162.3	48.8	85.7	0.5
Positive control (DMBA, 20 µg/mL)	63	22.8	76	121.7*
	33.7	12.6	79.5	139.9*

[c] Due to contamination in one cell culture, for only one culture the mutant frequency was determined.
[P] precipitation of the test substance
* significant (α = 0.05) increase in the one-sided Dunnett Test.

BYI 02960 was tested up to 2944 µg/mL, a concentration which exceeded the requested limit concentration of 10 mM. Under both activation conditions, no cytotoxic effects were induced. There were no indications of mutagenic effects of BYI 02960 in the V79/HPRT forward mutation assay either without or with S9 mix.

Acceptability
The study is considered acceptable.

Conclusions
In this study, BYI 02960 has no mutagenic potency in vitro in the CHO/HPRT assay.

4.9.1.2 In vivo data

Study 1

Study design and results

Type of study: mouse micronucleus test

Indicator cells	Endpoint	Result	Dose range	Reference
Mouse, Crl:NMRI Two intraperitoneal injections separated by 24 hours: 5/male/dose (vehicle and positive control, low, mid and high dose)	Micronuclei (bone marrow)	-	Pretest dose selection: 10, 40, 100 and 1000 mg/kg bw (m/f) Main test: 10, 20 and 40 mg/kg bw Dosing route: intraperitoneal Dosing volume: 10 mL/kg bw Vehicle (corn oil) and an adequate positive control was included	Herbold, B. (2009)
Test substance: BYI 02960 (batch number: 2009-000239; purity 96.2%); tested in corn oil Toxicity: at all tested dose levels, apathy, roughened fur, loss of weight, sternal recumbency, spasm, periodically stretching of body and difficulty in breathing GLP statement: yes According to OECD 474: yes				

Table 69: Results of the micronucleus test with BYI 02960

Treatment group	Number of evaluated PCE	Number of NCE per 2000 PCE	MNNCE per 2000 NCE	MNPCE per 2000 PCE
Negative control (corn oil)	10,000	3800	1.5	3.4
BYI 02960				
10 mg/kg bw	10,000	3134	1.0	1.4
20 mg/kg bw	10,000	3139	1.9	3.0
40 mg/kg bw	10,000	3008	1.0	3.8
Positive control				
Cyclophosphamide (20 mg/kg bw)	10,000	3308	1.9	20.6*

* $P < 0.01$ in non-parametric Wilcoxon ranking test

There was no indication of a clastogenic effect of intraperitoneally administered BYI 02960 in the micronucleus test on the male mouse, i.e. in a somatic test system in vivo.

Acceptability
The study is considered acceptable.

Conclusions
BYI 02960 has no potential to induce micronuclei in mouse bone marrow cells.

Study 2

Study design and results

Type of study: mouse micronucleus test

Indicator cells	Endpoint	Result	Dose range	Reference
Mouse, Crl:NMRI Two intraperitoneal injections separated by 24 hours: 7/female/dose (vehicle and positive control, low, mid and high dose)	Micronuclei (bone marrow)	-	Pretest dose selection: 10, 50, 75 and 100 mg/kg bw (f) Main test: 12.5, 25 and 50 mg/kg bw Dosing route: intraperitoneal Dosing volume: 10 mL/kg bw Vehicle (10% DMSO / 90% corn oil) and an adequate positive control was included	Wieland, J. (2011)
Test substance: BYI 02960 (batch number: PVF107N005; purity 97.2%); tested in 10% DMSO / 90% corn oil Toxicity: at 25 mg/kg bw, reduction of spontaneous activity, eyelid closure and ruffled fur were observed; at 50 mg/kg bw reduction of spontaneous activity, eyelid closure, ruffled fur and abdominal position were observed GLP statement: yes According to OECD 474: yes				

Table 70: Results of the micronucleus test with BYI 02960

Treatment group	**Number of evaluated PCE**	**PCE per 2000 erythrocytes**	**PCEs with micronuclei (%)**
Negative control (corn oil)	14,000	1229	0.121
BYI 02960			
12.5 mg/kg bw	14,000	1250	0.114
25 mg/kg bw	14,000	1234	0.143
50 mg/kg bw	14,000	1177	0.093
Positive control			
Cyclophosphamide (40 mg/kg bw)	14,000	1125	2.264*

* P < 0.05 in non-parametric Mann-Whitney test

There was no indication of a clastogenic effect of intraperitoneally administered BYI 02960 in the micronucleus test on the female mouse.

Acceptability
The study is considered acceptable.

Conclusions
BYI 02960 has no potential to induce micronuclei in mouse bone marrow cells.

4.9.2 Human information

4.9.3 Other relevant information

4.9.4 Summary and discussion of mutagenicity

The results from the *in vitro* and *in vivo* genotoxicity studies are summarized in Table 71 and Table 72, respectively.

Table 71: In vitro genotoxicity studies

Test substance	**Type of study**		**Result**		**Reference**
	Indicator cells	**Endpoint**	**without activation**	**with activation**	
BYI 02960	S. typh. TA 98 TA 100	Point mutation	negative	negative	Herbold, B. (2009)

Test substance	Type of study		Result		Reference
	Indicator cells	Endpoint	without activation	with activation	
	TA 102 TA 1535 TA 1537				
BYI 02960	S. typh. TA 98 TA 100 TA 102 TA 1535 TA 1537	Point mutation	negative	negative	Sokolowski, A. (2011)
BYI 02960	Chinese hamster V79 cells	Chromosome aberration	negative	negative	Thum, M. (2009)
BYI 02960	Chinese hamster V79 cells	Gene mutations (HGPRT)	negative	negative	Entian, G. (2009)

Table 72: In vivo genotoxicity studies

Test substance	Type of study		Result	Reference
	Species	Endpoint		
BYI 02960	Mouse, Crl:NMRI	Micronuclei (bone marrow)	negative	Herbold, B. (2009)
BYI 02960	Mouse, Crl:NMRI	Micronuclei (bone marrow)	negative	Wieland, J. (2011)

BYI 02960 was not mutagenic in two Ames tests.
BYI 02960 did not induce structural chromosome aberrations in V79 cells when tested up to and including cytotoxic concentrations and showed no mutagenic potency in vitro in the CHO/HPRT assay.
BYI 02960 did not induce micronuclei in mouse bone marrow cells in two separate in vivo studies.
Overall it can be concluded that BYI 02960 has no mutagenic or clastogenic potential.

4.9.5 Comparison with criteria

Annex VI of CLP states for the hazard class germ cell mutagenicity that "the classification in **Category 2** is based on positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from:
- Somatic cell mutagenicity tests *in vivo*, in mammals; or
- Other *in vivo* somatic genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assay".
No such effects were observed in the available studies.
Flupyradifurone does not meet the criteria for classification as germ cell mutagen.

4.9.6 Conclusions on classification and labelling

No classification is proposed for germ cell mutagenicity under the CLP Regulation.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The Dossier Submitter did not propose a classification for germ cell mutagenicity as neither in the *in vitro* studies using bacteria and mammalian cells nor in the *in vivo* studies was any mutagenic potency of flupyradifurone observed.

Comments received during public consultation

No specific comments were received for this endpoint.

Assessment and comparison with the classification criteria

The mutagenic and DNA damaging potential of flupyradifurone was studied ***in vitro*** in two GLP-compliant Ames tests, similar to OECD guideline 471, conducted with flupyradifurone (purity 96.2 %/97.2 %), in a mammalian cell cytogenetic assay test and in a mammalian cell gene mutation test, both OECD- and CLP-compliant, conducted with flupyradifurone (purity 96.2 %) and ***in vivo*** in two mouse OECD- and CLP-compliant micronucleus tests, conducted with flupyradifurone (purity 96.2 %/97.2 %).

There was neither an increase in the number of revertant colonies in the Ames tests, nor any clastogenicity or mutagenic effects *in vitro* nor in the two *in vivo* micronucleus assays in mice.

RAC therefore agrees with the conclusion of the DS that classification of flupyradifurone for germ cell mutagenicity is not warranted.

4.10 Carcinogenicity

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

4.10.1.1.1 Chronic toxicity and carcinogenicity

Study 1

Characteristics

reference	:	Garcin, J.C. 2012	exposure	:	Main groups: 104 weeks Satellite groups: 52 weeks
type of study	:	Combined chronic toxicity and carcinogenicity study	doses	:	0, 80, 400 and 2000 ppm **Average daily intake:** 0-104 weeks: Males: 0, 3.17, 15.8, 80.9 mg/kg bw/day Females: 0, 4.48, 22.5, 120 mg/kg bw/day 0-52 weeks: Males: 3.57, 18.5 and 95.1 mg/kg bw/day Females: 5.97, 25.3, 136 mg/kg bw/day
year of execution	:	2009-2012	vehicle	:	None
test substance	:	BYI 02960 (batch number 2009-000239, purity 96.2%)	GLP statement	:	Yes
route	:	Oral (diet)	guideline	:	According to OECD 453
species	:	Rat, Wistar Ri:WI (IOPS HAN)	acceptability	:	Acceptable
group size	:	Main group: 60/sex/dose Satellite group (interim sacrifice 52 weeks): 10/sex/dose	**NOAEL**toxicity (104 weeks)	:	15.8 mg/kg bw/day
			NOAELtoxicity (52 weeks)		18.5 mg/kg bw/day
			NOAELcarcinogenicity (104 weeks)		80.9 mg/kg bw/day

Study design

The study is performed according to OECD 453.

Results

The results are summarised in Table 73. Additional information on the observed effects is provided in Table 74-78.

Table 73

Dose (ppm in diet)	**0**		**80**		**400**		**2000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	35	31	39	30	30	28	33	14	
Clinical signs - hair loss - soiled fur							 i i		
Body weight								ds (-13%)	
Body weight gain								ds (-22%)	

Dose (ppm in diet)	**0**	**80**	**400**	**2000**	**dr**
	m f	**m f**	**m f**	**m f**	
Food consumption				d	
Ophthalmoscopy - iris mydriasis - retina, fundus abnormal colour, pale - lens opacity				i (8.3%) i (6.3% i (96%)	
Haematology	No substance-related findings				
Urinalysis	No substance-related findings				
Clinical chemistry - cholesterol - bilirubin				i d ds	
Organ weights *Satellite group*					
- terminal BW (g)		d	d	d ds (87%)	
- liver - relative				is is	
Main group					
- terminal BW (g)				d ds	
- liver					
- relative				is is (12%) (10%)	
Pathology					
Macroscopy *Satellite group* - liver: enlarged, pale				i	
Macroscopy *Main group* - lung: white foci - ovarian cyst				i i	
Microscopy	See table below				
Neoplastic lesions	No substance-related findings				

dr dose related
ds/is statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls

Table 74: Treatment-related ophthalmological findings at the second year examination (main groups).

Sex	Males				Females			
Dose level of BYI 02960 (ppm)	0	80	400	2000	0	80	400	2000
Group size	26	24	31	31	30	32	33	48
Iris mydriasis	3 (11.5%)	2 (8.3%)	3 (9.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (8.3%)
Lens opacity	23 (88%)	22 (92%)	29 (94%)	29 (94%)	15 (50%)	22 (69%)	27 (82%)	46 (96%)
HCD – Mean % (Min-Max)					92.0% (81.4 – 100.0)			
Retina, fundus abnormal color, pale	0 (0%)	1 (4.2%)	1 (3.2%)	1 (3.2%)	0 (0%)	0 (0%)	0 (0%)	3 (6.3%)

HCD= Historical Control Data

Table 75: Mean liver weight ± SD at the 24-month scheduled sacrifice (% change when compared to the controls).

MEAN LIVER WEIGHT ±SD AT 24-MONTH SCHEDULED SACRIFICE (% CHANGE WHEN COMPARED TO CONTROLS)								
Sex	Males				Females			
BYI 02960 Dose-level (ppm)	0	80	400	2000	0	80	400	2000
Absolute liver weight (g)	12.401 ±1.9940	12.065 ±1.8556 (-3%)	11.971 ±1.8261 (-3%)	12.915 ±1.4947 (+4%)	8.878 ±1.7052	9.246 ±1.6570 (+4%)	9.406 ±2.1925 (+6%)	8.308 ±1.3457 (-6%)
Liver to body weight ratio (%)	1.971 ±0.2142	2.008 ±0.2758 (+2%)	1.941 ±0.1859 (-2%)	2.215** ±0.3085 (+12%)	2.242 ±0.2658	2.291 ±0.3077 (+2%)	2.278 ±0.3116 (+2%)	2.458** ±0.2783 (+10%)
Liver to brain weight ratio (%)	530.945 ±90.8128	518.885 ±81.6223 (-2%)	514.364 ±79.8667 (-3%)	546.361 ±64.5187 (+3%)	418.821 ±79.1927	440.047 ±79.7220 (+5%)	449.204 ±105.7920 (+7%)	392.895 ±67.0007 (-6%)

**: $p \leq 0.01$

Table 76: Incidence and severity of microscopic changes in the liver at 24-month scheduled sacrifice

INCIDENCE AND SEVERITY OF MICROSCOPIC CHANGES IN THE LIVER, ALL ANIMALS OF THE CARCINOGENICITY PHASE								
Sex	Male				Female			
BYI 02960 Dose-level (ppm)	0	80	400	2000	0	80	400	2000
Number of examined animals	60	60	60	60	60	60	60	60
Eosinophilic focus(i) of hepatocellular alteration								
Minimal	22	21	19	28	14	9	8	16
Slight	4	6	9	9	2	3	1	6
Moderate	1	0	2	7	0	0	1	1
Marked	0	0	0	1	0	0	0	0
Total	**27****	**27**	**30**	**45****	**16**	**12**	**10**	**23**
Tigroid focus(i) of hepatocellular alteration								
Minimal	29	28	32	42	30	30	24	36
Slight	5	8	7	3	5	7	5	5
Moderate	0	0	0	0	1	0	1	0
Total	**34**	**36**	**39**	**45**	**36**	**37**	**30**	**41**
Mixed focus(i) of hepatocellular alteration								
Minimal	0	0	0	3	0	0	0	0
Total	**0***	**0**	**0**	**3**	**0**	**0**	**0**	**0**
Hepatocellular hypertrophy: centrilobular								
Minimal	0	0	6	23	0	0	0	27
Slight	0	0	0	2	0	0	0	1
Total	**0****	**0**	**6***	**25****	**0****	**0**	**0**	**28****
Hepatocellular macrovacuolation: centrilobular: diffuse								
Minimal	3	4	3	18	1	0	0	19
Slight	0	0	1	6	0	1	0	5
Moderate	0	1	0	0	1	0	0	0
Total	**3****	**5**	**4**	**24****	**2****	**1**	**0**	**24****
Hepatocellular macrovacuolation: periportal: diffuse								
Minimal	5	4	2	2	18	22	12	11
Slight	2	3	1	4	8	11	13	2
Moderate	2	1	1	0	2	3	1	0
Marked	0	0	1	1	0	0	0	0
Total	**9**	**8**	**5**	**7**	**28****	**36**	**26**	**13****
Hepatocellular brown pigment : focal								
Minimal	0	0	0	0	1	0	1	13
Slight	0	0	0	0	0	0	0	3
Total	**0**	**0**	**0**	**0**	**1****	**0**	**1**	**16****

*: Significant ($p \leq 0.05$) **: Significant ($p \leq 0.01$)

Statistical significances in the control findings are considered an error.

(to be continued)

INCIDENCE AND SEVERITY OF MICROSCOPIC CHANGES IN THE LIVER, ALL ANIMALS OF THE CARCINOGENICITY PHASE								
Sex	Male				Female			
BYI 02960 Dose-level (ppm)	0	80	400	2000	0	80	400	2000
Number of examined animals	60	60	60	60	60	60	60	60
Accumulation of brown pigment in Kupffer cells								
Minimal	8	11	10	9	10	9	14	18
Slight	2	5	8	4	4	4	1	8
Moderate	0	0	0	1	1	1	1	1
Total	**10**	**16**	**18**	**14**	**15***	**14**	**16**	**27***
Interstitial mononuclear cell infiltrate: focal								
Minimal	20	19	21	24	22	28	29	35
Slight	1	0	1	2	1	4	0	1
Moderate	0	0	0	0	1	0	0	0
Total	**21**	**19**	**22**	**26**	**24**	**32**	**29**	**36***

*: Significant (p≤0.05) **: Significant (p≤0.01)

Statistical significances in the control findings are considered an error.

Table 77: Incidence and severity of microscopic changes in the lung at the 24-month scheduled sacrifice

INCIDENCE AND SEVERITY OF MICROSCOPIC CHANGES IN THE LUNG, ALL ANIMALS OF THE CARCINOGENICITY PHASE								
Sex	Male				Female			
BYI 02960 Dose-level (ppm)	0	80	400	2000	0	80	400	2000
Number of examined animals	60	60	60	60	60	60	60	60
Alveolar foamy macrophages: focal								
Minimal	18	24	22	25	27	18	11	25
Slight	10	12	8	7	2	11	16	18
Moderate	2	4	4	3	1	0	2	6
Marked	0	0	0	0	0	0	0	1
Total	**30**	**40***	**34**	**35**	**30****	**29**	**19***	**50****
Chronic interstitial inflammation: focal								
Minimal	1	4	4	3	2	3	5	10
Slight	0	1	0	0	0	0	0	0
Moderate	0	0	1	0	0	0	0	0
Total	**1**	**5**	**5**	**3**	**2***	**3**	**5**	**10***
Perivascular inflammation: focal								
Minimal	23	20	30	20	17	22	17	35
Slight	1	3	2	1	0	1	0	1
Total	**24**	**23**	**32**	**21**	**17****	**23**	**17**	**36****

*: Significant (p≤0.05) **: Significant (p≤0.01)

Statistical significances in the control findings are considered an error.

Table 78: Incidence and severity of microscopic changes in the thyroid gland at the 24-month scheduled sacrifice

INCIDENCE AND SEVERITY OF MICROSCOPIC CHANGES IN THE THYROID GLAND, ALL ANIMALS OF THE CARCINOGENICITY PHASE								
Sex	Male				Female			
BYI 02960 Dose-level (ppm)	0	80	400	2000	0	80	400	2000
Number of examined animals	60	60	60	60	60	60	60	60
Colloid alteration								
Minimal	14	12	28	18	11	10	10	11
Slight	7	9	9	18	1	3	0	4
Moderate	0	1	1	4	0	1	0	0
Marked	0	0	0	0	1	0	0	0
Total	**21****	**22**	**38****	**40****	**13**	**14**	**10**	**15**
Follicular cell hypertrophy: diffuse								
Minimal	1	0	1	3	0	1	0	3
Total	**1**	**0**	**1**	**3**	**0**	**1**	**0**	**3**
Brown pigments: follicular cells								
Minimal	16	10	18	22	8	7	5	17
Slight	1	0	1	1	0	0	0	0
Moderate	0	0	1	0	0	0	0	0
Total	**17**	**10**	**20**	**23**	**8**	**7**	**5**	**17**

** : Significant ($p \leq 0.01$)

Statistical significances in the control findings are considered an error.

Body weight and body weight gain changes

The body weight and body weight gains have been reported as follows in the study report.

At 2000 ppm in males, the mean cumulative body weight gain was significantly lower than controls during the first week of treatment (- 27%, $p \leq 0.01$) and over the first three months of the study (-13%, $p \leq 0.01$). Thereafter, mean cumulative body weight gain was comparable to controls. The mean body weight was lower compared to controls from Week 1 to Week 54 (statistically significant in most occasions). Thereafter the mean body weight was comparable to controls. In females, mean body weight and cumulative body weight gains were lower compared to controls throughout the study (-5% to -17%; $p \leq 0.01$ or 0.001 for body weight and -18% to -62%; $p \leq 0.001$, 0.01 or 0.05 for cumulative body weight gains.

Overall, the mean cumulative body weight gain was decreased by 23% when compared to controls at the end of the study; the mean body weight was 13% lower.

At 400 ppm, mean cumulative body weight gain was statistically significantly lower than in controls during the first week of treatment for males (-6%, $p \leq 0.05$) and females (-12%, $p \leq 0.05$) and was comparable to control thereafter. Mean body weight was not affected throughout the study.

At 80 ppm, there was no treatment-related effect on body weight parameters in either sex.

Table 79: Mean body weights (BW and cumulative body weight gains (BWG))

Group mean body weights (BW) and cumulative body weight gains (BWG) (g)				
BYI 02960 dosage level (ppm)	0	80	400	2000
Males				
Initial BW (Day 1) (%C)	**237**	**237 (100)**	**237 (100)**	**237 (100)**
BW Week 2 (Day 8) (%C)	296	295 (100)	293 (99)	280 ** (95)
BW Week 14 (Day 92) (%C)	544	537 (99)	540 (99)	506 ** (93)
BW Week 26 (Day 176) (%C)	623	612 (98)	615 (99)	585 ** (94)
BW Week 54 (Day 372) (%C)	714	699 (98)	712 (100)	680 * (95)
BW Week 78 (Day 540) (%C)	749	730 (97)	752 (100)	721 (96)
BW Week 106 (Day 736) (%C)	**653**	**623 (95)**	**655 (100)**	**616 (94)**
BWG Weeks 1-2 (Days 1 to 8) (%C)	59	58 (98)	56 * (94)	43 ** (73)
BWG Weeks 1-14 (Days 1 to 92) (%C)	308	301 (98)	303 (98)	269 ** (87)
BWG Weeks 14-26 (Days 92 to 176) (%C)	78	75 (96)	75 (96)	80 (103)
BWG Weeks 26-54 (Days 176 to 372) (%C)	89	90 (101)	95 (107)	93 (104)
BWG Weeks 54-78 (Days 372 to 540) (%C)	31	42 (135)	39 (126)	39 (126)
BWG Weeks 78-106 (Days 540 to 736) (%C)	-91	-84 (nc)	-82 (nc)	-113 (nc)
Overall BWG Weeks 1-106 (Days 1 to 736) (%C)	**416**	**392 (94)**	**420 (101)**	**379 (91)**
Females				
Initial BW (Day 1) (%C)	**167**	**168 (100)**	**168 (100)**	**167 (100)**
BW Week 2 (Day 8) (%C)	192	191 (100)	190 (99)	181 ** (95)
BW Week 14 (Day 92) (%C)	290	288 (99)	289 (100)	267 ** (92)
BW Week 26 (Day 176) (%C)	319	313 (98)	316 (99)	289 *** (91)
BW Week 54 (Day 372) (%C)	364	354 (97)	355 (97)	317 ** (87)
BW Week 78 (Day 540) (%C)	421	430 (102)	417 (99)	349 ** (83)
BW Week 106 (Day 736) (%C)	**421**	**437 (104)**	**444 (105)**	**364 ** (87)**
BWG Weeks 1-2 (Days 1 to 8) (%C)	25	23 (92)	22 * (88)	15 ** (60)
BWG Weeks 1-14 (Days 1 to 92) (%C)	122	120 (98)	122 (100)	100 ** (82)
BWG Weeks 14-26 (Days 92 to 176) (%C)	29	26 (90)	27 (93)	22 ** (76)
BWG Weeks 26-54 (Days 176 to 372) (%C)	48	39 (81)	37 (77)	28 *** (58)
BWG Weeks 54-78 (Days 372 to 540) (%C)	59	76 (129)	62 (105)	34 ** (58)
BWG Weeks 78-106 (Days 540 to 736) (%C)	39	21 (54)	35 (90)	15 * (38)
Overall BWG Weeks 1-106 (Days 1 to 736) (%C)	**256**	**271 (106)**	**275 (107)**	**198 *** (77)**

%C: % *vs* control C = control nc = not calculated
* Statistically different (p□0.05) from the control
** Statistically different (p□0.01) from the control
*** Statistically different (p□0.001) from the control

These data can be summarized as follows in Table 80.

Table 80: Body weight and body weight gain additional data

Dose (ppm)	0		80		400		2000		dr
	m	f	m	f	m	f	m	f	
BW W106	653	421	623 (-5%)	437 (+4%)	655	444 (+5%)	616 (-6%)	364 ds (-13%)	
BWG W1-106	416	256	392 (-6%)	271 (+6%)	420 (+1%)	275 (+7%)	379 (-9%)	198 ds (-23%)	

The historical control body weight data have been collected from carcinogenicity studies performed at Bayer CropScience (Centre of Toxicology, Sophia-Antipolis, France), initiated between February 2005 and January 2010. Only the studies in which the age of animals was identical at the start of the study were considered. This represents a group of 5 studies. For technical reasons, some body weight sessions were not recorded on the exact same day for all studies; therefore when possible the most approaching data were taken instead.

Table 81: Historical body weight control data from carcinogenicity studies performed on Wistar rats initiated from February 2005 to January 2010

Sex	Ref Session (Days)	MEAN	NUMBER	STANDARD DEVIATION	MINIMUM	MAXIMUM
F	1 (Day 1)	162	290	11.1	132	193
F	2 (Day 8)	188	290	13.5	150	224
F	3 (Day 92)	287	289	23.1	227	346
F	4 (Day 176)	313	285	26.9	251	386
F	5 (Day 372)	357	250	44.9	269	509
F	6 (Day 540)	415	221	66.9	280	610
F	7 (Day 736)	437	154	77.5	292	676
M	1 (Day 1)	221	360	14.0	178	254
M	2 (Day 8)	278	360	17.1	222	325
M	3 (Day 92)	535	358	44.2	424	672
M	4 (Day 176)	613	355	53.1	499	767
M	5 (Day 372)	708	314	69.7	549	888
M	6 (Day 540)	739	254	82.3	546	982
M	7 (Day 736)	672	137	83.2	485	892

Table 82: Historical body weight gain control data from carcinogenicity studies performed on Wistar rats initiated from February 2005 to January 2010

Sex	SESSION	MEAN	NUMBER	STANDARD DEVIATION	MINIMUM	MAXIMUM
F	S 1-2	26	290	7	8	50
F	S 1-3	125	289	18	80	174
F	S 3-4	27	285	11	-1	74
F	S 4-5	42	250	27	-9	145
F	S 5-6	58	221	38	-39	199
F	S 6-7	32	154	42	-91	186
F	S 1-7	275	154	74	135	505
M	S 1-2	57	360	7	18	76

M	S 1-3	314	358	41	191	451
M	S 3-4	78	355	20	28	146
M	S 4-5	92	314	34	-83	179
M	S 5-6	27	254	36	-116	126
M	S 6-7	-53	137	60	-263	114
M	S 1-7	453	137	83	266	659

Haematological findings

The haematological changes were reported as follows in the report M-428257-01-1:

Effects were observed at 2000 ppm only. At 2000 ppm in males, relative to the controls, mean leucocyte counts were higher at Month 12 (+22%, $p \leq 0.01$), 18 (+31%, $p \leq 0.01$) and 24 (+63%, $p \leq 0.05$). This variation was associated with higher mean absolute lymphocyte count at Month 12 (+19%, $p \leq 0.01$) and 18 (+32%, $p \leq 0.01$) and with higher mean absolute neutrophil count at Month 12 (+36%, $p \leq 0.05$) and 24 (+143%, $p \leq 0.05$).

At 2000 ppm in females, throughout the first year of treatment, statistically significant differences were noted in some erythrocyte parameters. Mean hemoglobin concentration and/or mean corpuscular volume were lower; as a consequence mean hematocrit and/or mean corpuscular hemoglobin were lower too. These variations were very slight (≤ -4%, relative to the controls).

In this group, mean platelet counts were slightly higher (< +20%, relative to the controls) during the first year and mean total leucocyte count and mean absolute lymphocyte count were slightly higher at Month 4 (+31% and +34% respectively, relative to the controls) and at Month 6 (+34% and +42% respectively, relative to the controls).

Altogether these changes were considered not to be toxicologically relevant in view of their low magnitude and their transient occurrence. The few other differences observed, even if statistically significant, were considered to be incidental and not treatment-related. Haematological findings in males are tabulated in Table 83.

Table 83: Haematological changes in the males during the rat carcinogenicity study with flupyradifurone

Parameters	Dose levels in ppm				Historical control data
	0	80	400	2000	
Month 4					
White blood cell count	14.79	14.66	14.58	15.67	13.15 ±2.89 [5.2-24.5]
Neutrophil count	2.68	2.17	2.14	2.38	1.96 ±0.653 [0.8-7.5]
Lymphocyte count	11.30	11.75	11.62	12.51	10.42 ± 2.5 [3.9-20.1]
Month 6					
White blood cell count	12.13	12.39	12.91	14.07	12.43 ± 2.433 [7.5- 23.0]
Neutrophil count	1.85	2.00	1.94	2.28	1.98 ± 0.558 [1.0-4.2]
Lymphocyte count	9.56	9.60	10.17	10.93	9.73 ± 2.108 [5.6-19.4]
Month 12					
White blood cell count	10.55	11.27	11.73	12.91 ** (+ 22.4%)	10.95 ± 2.262 [6.5- 17.3]
Neutrophil count	2.05	2.18	2.19	2.79* (+36.1%)	2.16 ± 0.670 [4.3-13.4]
Lymphocyte count	7.8	8.44	8.86	9.31++ (+19.4%)	8.07 ± 1.915 [4.3-13.4]
Month 18					
White blood cell count	8.92	10.01	9.08	11.68 ** (+30.9)	10.60 ± 2.404 [5.8-17.5]
Neutrophil count	2.07	2.16	1.87	2.63	2.67 ± 0.763 [1.2 – 4.6]
Lymphocyte count	6.32	7.25	6.70	8.37 ** (+32.4%)	7.24 ± 1.828 [3.9 – 13.0]
Month 24					
White blood cell count	11.10	11.10 11.68	11.68	18.06 ++ (+62.7%)	11.57 ± 4.645 [5.1 – 29.3]
Neutrophil count	3.58	3.60	4.11	8.69 + (143%)	4.04 ± 2.343 [1.4 – 13.7]
Lymphocyte count	6.81	6.72	6.80	8.38	6.70 ± 2.822 [2.2- 19.9]

Counts expressed in 10^9/L

**: Dunnett LSD Test significant at 0.01 level

*: Dunnett LSD Test significant at 0.05 level

++: Dunn Rank Sum Test significant at 0.01 level

+: Dunn Rank Sum Test significant at 0.05 level

All the data are within historical control data except for the white blood cell and neutrophil counts after 24 months, but the mean values are within the range of historical control data.

Haematological findings in females are tabulated in Table 84:

Table 84: Haematological changes in the females during the rat carcinogenicity study with flupyradifurone

Parameters	Dose levels in ppm				Historical control data
	0	80	400	2000	
Month 4					
HGB (g/dL)	15.55	15.77	15.55	15.46	15.68± 0.546 [14.2-17.4]
HCT (L/L)	0.4518	0.4555	0.4493	0.4518	0.4672 ±0.0219 [0.408-0.545]
MCV (fL)	52.4	52.2	52.2	51.3* (-2%)	53.5± 1.77 [49-58]
MCH (pg)	18.03	18.06	18.11	17.57 ++ (-2.6%)	17.94± 0.591 [16.3-19.4]
Platelet (10^9/L)	1192.8	1190.8	1182.6	1390.1 * (+16.5%)	1204.6± 158.01 [732-1603]
White blood cell count (10^9/L)	8.12	8.43	8.84	10.64 ** (+31%)	8.33± 2.289 [3.7-19.8]
Lymphocytes (10^9/L)	6.65	6.69	7.19	8.90 ** (+33.8%)	6.63± 1.965 [2.6-17.3]
Month 6					
HGB (g/dL)	15.14	15.36	15.33	14.48 ** (-6.3%)	15.33 ± 0.655 [13.8-16.9]
HCT (L/L)	0.4453	0.4537	0.4535	0.4323 + (-2.9%)	0.4555 ± 0.0201 [0.408-0.512]
MCV (fL)	53.4	53.2	53.5	52.3* (-2.07%)	53.3 ± 1.69 [49 – 59]
MCH (pg)	18.16	18.04	18.08	17.49 ** (-3.7%)	17.92 ± 0.576 [16.6 – 19.5]
Platelet (10^9/L)	1238.8	1214.9	1199.9	1451.8 + (+17.2%)	1209.1 ± 181.33 [111-1663]
White blood cell count (10^9/L)	6.33	7.17	7.02	8.51 ** (+34.44%)	7.12 ± 1.855 [3.4-13.1]
Lymphocytes (10^9/L)	4.84	5.64	5.40	6.85 ** (+41.53%)	5.57 ± 1.587 [2.5-11.2]
Month 12					
HGB (g/dL)	14.59	14.86	15.01	14.04 * (-3.8%)	15.72± 0.597 [13.2-17.3]
HCT (L/L)	0.4296	0.4380	0.4389	0.4144* (-3.5%)	0.4730 ±0.0189 [0.418-0.515]
MCV (fL)	52.7	52.5	52.4	51.1 * (-3.04%)	52.0 ± 1.55 [48-55]
MCH (pg)	17.88	17.83	17.93	17.32++ (-3.13%)	17.26 ± 0.669 [15.6-19.3]
Platelet (10^9/L)	1101.9	1127.2	1171.7	1306.4** (+18.6%)	1168.2 ± 193.78 [597-1693]
White blood cell count (10^9/L)	5.77	6.09	6.33	6.44	10.95 ± 2.262 [6.5-17.3]
Lymphocytes (10^9/L)	4.08	4.27	4.56	4.64	8.07 ± 1.915 [4.3-13.4]

*: Dunnett LSD Test significant at 0.05 level
++: Dunn Rank Sum Test significant at 0.01 level
+: Dunn Rank Sum Test significant at 0.05 level

All data are within the historical control range except the lymphocyte counts after 4 months and haemoglobin concentration and haematocrit value after 12 months. The mean values are within the historical control range.

No effects of the treatment on the mortality were observed. Clinical signs were limited to soiled fur, hyper-reactivity to external stimuli and resistance to handling, observed during the first year of treatment, and soiled fur and hair loss observed during the second year of treatment in high-dose males.

In high-dose females the mean body weight and cumulative body weight gain were lower compared to controls throughout the study (statistically significant). Overall the mean cumulative body weight gain was decreased by 23% when compared to the controls at the end of the study; the mean body weight was 13% lower. In high-dose males, mean body weight was lower compared to controls from week 1 to week 54 (statistically significant on most occasions); thereafter the mean body weight was comparable to the controls.

In high-dose females, food consumption was marginally lower than control in many occasions throughout the study. In all other groups mean food consumption was considered to be comparable to the controls throughout the study.

Iris mydriatis and an abnormal pale colour of fundus of the retina was noted in 4/48 and 3/48 high-dose females, respectively, compared to 0/30 in controls. In addition, higher incidences of lens opacity were observed at this dose level in comparison to the controls. However, lens opacity is a common finding in rats of this age and strain, and the values were well within the historical control data, while the value in the control group was particularly low and outside the historical control data of the test lab. Therefore this change was considered to be not treatment-related.

The transient haematological changes observed were considered to be not treatment-related in view of their low magnitude and transient occurrence.

Slightly lower total bilirubin concentrations were observed at 2000 ppm in both sexes. Slightly higher mean total cholesterol was observed throughout the study in high-dose females. No relevant changes were noted in other groups and at other dose levels.

No treatment-related findings were observed at urinalysis.

At the interim sacrifice and at the end of the study duration, mean liver to body weight ratios were statistically significantly higher when compared to controls at 2000 ppm in both sexes. Enlarged and pale liver was noted in 3/10 high-dose males at interim sacrifice and was considered to be associated with the treatment. At the completion of the 2-year study period white foci in the lung were noted in 21/46 of high-dose females, which were considered to be associated with the treatment. Also higher incidence of ovarian cysts was noted in 18/46 females, when compared to 8/29 in the controls. However, as microscopically there were no differences in terms of percentages in ovarian cysts between the control group (18/29, 62%) and high-dose group (31/46, 67%), this finding was considered to be not treatment-related.

At the interim sacrifice, higher incidences of eosinophilic and tigroid foci of altered hepatocytes were observed in high-dose males. Centrilobular hypertrophy was observed in both sexes at 2000 ppm and also at 400 ppm in 2/10 males (dose-related effects). However, in the absence of any

increase of incidences of foci of alterations or hepatocellular macrovacuolation, this change at 400 ppm was considered to be not an adverse effect. Centrilobular hepatocellular macrovacuolation and lower incidences of periportal hepatocellular vacuolation were noted in both sexes at 2000 ppm. In the thyroid gland, colloid alteration was found to be increased in incidence and severity in both sexes at 2000 pm and in males at 400 ppm. At the terminal sacrifice, higher incidences of eosinophilic, mixed and tigroid foci of altered hepatocytes were observed in males at 2000 ppm. Centrilobular hypertrophy and higher incidences of centrilobular hepatocellular macrovacuolation were observed in both sexes of high-dose group. Higher incidences of hepatocellular brown pigments and brown pigments in Kupffer cells, higher incidence of interstitial mononuclear cell infiltrate and lower incidences of periportal hepatocellular macrovacuolation were noted in high-dose females. No adverse effect was noted in both sexes at 400 ppm; there was only minimal centrilobular hypertrophy in 6 male animals without any increase of foci of alteration or hepatocellular macrovacuolation.

In the lung higher incidences of foamy macrophages, chronic interstitial inflammation and perivascular inflammation were observed in high-dose females at the terminal sacrifice.

In the thyroid, increased incidences of colloid alteration were noted in males at 2000 and 400 ppm. The effect at 400 ppm was not considered to be adverse, as increased incidence of colloid alteration occurs naturally in aging rats and was not associated with relevant follicular hypertrophy at this dose level.

No neoplastic lesions were observed at any dose levels.

Historical control data are provided only for ophthalmologic findings and are based on selected 2-year studies performed in-house between February 2005 and February 2008.

Acceptability
The study is considered acceptable.

Conclusions
No neoplastic lesions were observed in the study at any dose level. The NOAEL for carcinogenicity was set at the highest dose level of 2000 ppm, equal to the average daily intake of 80.9 and 120 mg/kg bw/day over 104 weeks for male and female rats, respectively.

Based on effects in the liver and in a less extend in the thyroid and lung (females), the NOAEL for general toxicity was set at 400 ppm in diet, equal to the average daily intake of 15.8 and 22.5 mg/kg bw/day over 104 weeks for male and female rats, respectively (18.5 and 25.3 mg/kg bw/day, respectively, over 52 weeks).

Study 2

Characteristics

reference	:	Kennel, P. 2012	exposure	:	Main groups: at least 78 weeks Satellite groups: 52 weeks
type of study	:	Carcinogenicity study	doses	:	0, 70, 300 and 1500 ppm **Average daily intake:** 0-78 weeks:

					Males: 0, 10.0, 43 and 224 mg/kg bw/day Females: 0, 12.2, 53 and 263 mg/kg bw/day 0-52 weeks: Males: 10.5, 45 and 232 mg/kg bw/day Females: 12.9, 56 and 263 mg/kg bw/day
year of execution	:	2009-2012	vehicle	:	None
test substance	:	BYI 02960 (batch number 2009-000239, purity 96.2%)	GLP statement	:	Yes
route	:	Oral (diet)	guideline	:	According to OECD 451
species	:	Mouse, C57BL/6J	acceptability	:	Acceptable
group size	:	Main group: 50/sex/dose Satellite group (interim sacrifice 52 weeks): 10/sex/dose	**NOAEL**toxicity **NOAEL**carcinogenicity	:	43 mg/kg bw/day 224 mg/kg bw/day

Study design

The study is performed according to OECD 451

Results

The results are summarised in Table 85. Additional information on the observed effects is provided in Table 86-84.

Table 85

Dose (ppm in diet)	**0**		**70**		**300**		**1500**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	11	8	5	10	9	5	8	7	
Clinical signs	No treatment-related findings								
Body weight							ds (-6%)	d (-7%)	
Body weight gain					d	d	ds (-19%)	ds (-13%)	
Food consumption								d	
Haematology	No substance-related findings								
Urinalysis	Not evaluated in the study								
Clinical chemistry	Not evaluated in the study								
Organ weights *Satellite group*									
- terminal BW (g)							d		
kidney - absolute - relative to brain weight							ds (-13%) ds (-14%)		
brain - relative to body weight							is		
Main group									
terminal BW (g)							ds	d	

Dose (ppm in diet)	**0**	**70**	**300**	**1500**		**dr**
	m f	**m f**	**m f**	**m**	**f**	
liver - absolute - relative to body weight				is (9%) is (14%)	is (8%)	
kidney - absolute - relative				d d		
brain - relative to body weight				is	is	
Pathology						
Macroscopy *Satellite group*	No treatment-related changes					
Macroscopy *Main group* **kidney**: - atrophic/small				is		
Microscopy	See table below.					
Neoplastic lesions	No substance-related findings					

dr dose related
ds/is statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls

Appendix Table: Body weight and body weight gain additional data

Dose (ppm)	0		70		300		1500		dr
	m	f	m	f	m	f	m	f	
BW W78	31.2	28.5	30.9 (-1%)	28.4	30.7 (-1%)	27.1 (-5%)	29.4ds (-6%)	26.6d (-7%)	
BWG W1-78	10.8	11.2	10.4 (-4%)	11.5 (+3%)	10.2d (-6%)	10.1d (-10%)	8.7ds (-19%)	9.7ds (-13%)	

Table 86: Mean liver weight ± SD at the 18-month scheduled sacrifice

MEAN LIVER WEIGHT ± SD AT SCHEDULED SACRIFICE (% CHANGE WHEN COMPARED TO CONTROLS)								
Sex	Males				Females			
Dose-level of BYI 02960 (ppm)	0	70	300	1500	0	70	300	1500
Absolute liver weight (g)	1.128 ± 0.0784	1.128 ± 0.0846 (0%)	1.137 ± 0.0727 (+1%)	1.233 ** ± 0.0861 (+9%)	1.285 ± 0.1749	1.287 ± 0.1751 (0%)	1.220 ± 0.2035 (-5%)	1.294 ± 0.1899 (+1%)
Liver to body weight ratio (%)	4.166 ± 0.2381	4.171 ± 0.2616 (0%)	4.172 ± 0.2388 (0%)	4.759 ** ± 0.2743 (+14%)	5.110 ± 0.3489	5.167 ± 0.4445 (+1%)	4.986 ± 0.6718 (-2%)	5.509 ** ± 0.8036 (+8%)
Liver to brain weight ratio (%)	249.155 ± 16.3057	248.368 ± 19.3286 (0%)	251.190 ± 15.1611 (+1%)	270.814 ** ± 16.0455 (+9%)	272.958 ± 34.0733	270.002 ± 33.9804 (-1%)	257.339 ± 44.1777 (-6%)	273.926 ± 39.0896 (0%)

**:p≤0.01

Table 87: Mean kidney weight ± SD at the 18-month scheduled sacrifice

MEAN KIDNEY WEIGHT ± SD AT SCHEDULED SACRIFICE (% CHANGE WHEN COMPARED TO CONTROLS)								
Sex	Males				Females			
Dose-level of BYI 02960 (ppm)	0	70	300	1500	0	70	300	1500
Absolute kidney weight (g)	0.4959 ± 0.0482	0.5148 ± 0.0489 (+4%)	0.4932 ± 0.0505 (-1%)	0.4290 ** ± 0.0384 (-13%)	0.4424 ± 0.0497	0.4533 ± 0.0832 (+2%)	0.4381 ± 0.0455 (-1%)	0.4187 ± 0.0420 (-5%)
Kidney to body weight ratio (%)	1.8295 ± 0.1406	1.9004 ± 0.1321 (+4%)	1.8073 ± 0.1583 (-1%)	1.6538 *** ± 0.0966 (-10%)	1.7650 ± 0.1179	1.8233 ± 0.3017 (+3%)	1.7957 ± 0.1407 (+2%)	1.7800 ± 0.1246 (+1%)
Kidney to brain weight ratio (%)	109.5244 ± 10.1927	113.2376 ± 10.4639 (+3%)	108.8135 ± 9.7886 (-1%)	94.2427 ** ± 7.5710 (-14%)	93.9341 ± 8.6909	95.1333 ± 17.5359 (+1%)	92.2409 ± 8.8349 (-2%)	88.5742 ± 7.4842 (-6%)

**:p≤0.01; ***:p≤0.001

Table 88: Incidence of macroscopic changes in the kidney at the 18-month scheduled sacrifice

INCIDENCE OF MACROSCOPIC CHANGES IN THE KIDNEY, SCHEDULED SACRIFICE, CARCINOGENICITY PHASE								
Sex	Males				Females			
BYI 02960 Dose level (ppm)	0	70	300	1500	0	70	300	1500
Atrophic/small	0/38	0/45	0/41	5/42 *	0/42	0/39	0/45	0/42

*:p≤0.05; **:p≤0.01 (Appendix M)

Table 89: Incidence and severity of microscopic changes in the liver at the 18-month scheduled sacrifice

Incidence and severity of microscopic changes in the liver (all animals) 18-month carcinogenicity phase								
Sex	Males				Females			
Dose level of BYI 02960 (ppm)	0	70	300	1500	0	70	300	1500
Number of animals examined	50	50	50	50	50	50	50	50
Hepatocellular macrovacuolation : mainly periportal: diffuse								
Minimal	0	0	0	0	32	28	28	13
Slight	0	0	0	0	2	1	1	0
Total	**0**	**0**	**0**	**0**	**34**	**29**	**29**	**13****
Hepatocellular vacuolation : mainly centrilobular: diffuse								
Minimal	10	16	2	0	0	0	0	0
Slight	16	16	22	5	0	1	0	1
Moderate	2	2	12	31	0	1	0	3
Marked	0	0	0	5	1	0	0	0
Total	**28**	**34**	**36**	**41****	**1**	**2**	**0**	**4**

**: p≤0.01

Table 90: Incidence and severity of microscopic changes in the kidney at the 18-month scheduled sacrifice

Incidence and severity of microscopic changes in the kidney (all animals) 18-month carcinogenicity phase								
Sex	Males				Females			
BYI 02960 Dose level (ppm)	0	70	300	1500	0	70	300	1500
Number of animals examined	50	50	50	50	50	50	50	50
Basophilic tubules : bilateral								
Minimal	23	23	18	3	3	2	3	1
Slight	4	2	3	0	0	0	0	0
Total	**27**	**25**	**21**	**3****	**3**	**2**	**3**	**1**
Cortical mineralization: focal								
Minimal	12	19	13	1	1	0	0	0
Moderate	0	0	1	0	0	0	0	0
Total	**12**	**19**	**14**	**1****	**1**	**0**	**0**	**0**
Corticoepithelial vacuolation								
Minimal	3	2	5	19	0	0	0	0
Slight	4	3	7	9	0	0	0	0
Moderate	29	26	31	4	0	0	0	0
Marked	13	14	4	0	0	0	0	0
Total	**49**	**45**	**47**	**32****	**0**	**0**	**0**	**0**

**: p≤0.01

No test-substance related mortality or clinical signs were observed. Reduced body weight was observed in both sexes at the highest dose level (statistically significant in males). Mean cumulative body weight gain over the whole study period was also significantly reduced in males and females of the high-dose group and was 19% lower than in controls in males and 13% lower than in controls

for females. Mean body weight gain over the whole study period was also slightly reduced in mid-dose animals of both sexes (not statistically significant). Food consumption was decreased on several occasions throughout the study in high-dose females (average 3% below controls).

At the interim sacrifice, absolute kidney weight and kidney to brain weight ratio were 13 to 15% lower than controls in high-dose males (statistically significant). Mean brain to body weight ratio was 9% higher than controls in high-dose males ($p \leq 0.05$), but this effect was considered to be related to the decreased body weight. At the macroscopic observation, no treatment-related changes were noted in either sex at any dose level. Histopathology was not performed at the interim sacrifice.

At the terminal sacrifice, absolute and relative liver weights were 9 to 14% higher than controls in high-dose males ($p \leq 0.01$). Relative liver weight was also increased in high-dose females ($p \leq 0.01$), but this effect was considered to be related to the reduced body weight. Statistically significantly reduced absolute and relative kidney weights were observed in high-dose males. Relative brain weight was slightly higher than controls in both sexes of the high-dose group (+5% in males and +7% in females, $p \leq 0.01$), but this effect was considered to be related to the reduced body weight.

At the macroscopic observation, atrophic/small kidneys were noted in 5/42 high-dose males (in comparison to 0/38 in controls). At histopathological examination increased incidence of centrilobular hepatocellular vacuolation was noted in high-dose males (statistically significant), whilst a decreased incidence of periportal hepatocellular macrovacuolation was noted in high-dose females (statistically significant). Not statistically significant increase in the incidence of centrilobular hepatocellular vacuolation was also noted in mid-dose males. These changes in the liver were considered to be treatment-related but not adverse due to the lack of associated degenerative changes. In the kidney, decreased incidence and severity of bilateral basophilic tubules, focal cortical mineralization and corticoepithelial vacuolation were noted in high-dose males (all statistically significant). These changes in the kidney were considered to be treatment-related but not adverse, as they occurred with lower incidence and severity than in control animals).

No neoplastic changes were evidenced at any dose level in either sex.

Historical control data for histopathological findings were provided from 11 in-house studies with C57BL/6J mice performed in the period October 2000 – March 2007.

Acceptability
The study is considered acceptable.

Conclusions
No neoplastic changes were evidenced in any group at any dose level. The NOAEL for carcinogenicity was set at the highest dose level of 1500 ppm, equal to the average daily intake of 224 and 263 mg/kg bw/day over 18 months for male and female mice, respectively.

Based on effects in the liver and in the kidney (male) the NOAEL for general toxicity was set at 300 ppm in diet, equal to the average daily intake of 45 and 53 mg/kg bw/day over 18 months for male and female mice, respectively.

4.10.1.2 Carcinogenicity: inhalation

4.10.1.3 Carcinogenicity: dermal

4.10.2 Human information

4.10.3 Other relevant information

4.10.4 Summary and discussion of carcinogenicity

In Table 91 the results of the chronic toxicity and carcinogenicity studies with the test substance are summarised.

Table 91: Oral chronic toxicity and carcinogenicity studies

Duration (weeks)	Species	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Critical effects	Reference
104	rat	15.8	80.9	Effects on liver, thyroid and lung	J.C. Garcin, 2012
78	mouse	43	224	Effects on liver and kidney	P. Kennel, 2012

In a two-year toxicity/carcinogenicity study with rats, effects in the liver and in a less extend in the thyroid and lung (females) were observed in the 2000 ppm dose group. The NOAEL for general toxicity was set at 400 ppm in diet, equal to the average daily intake of 15.8 mg/kg bw/d. No neoplastic lesions were observed in the study at any dose level.
In an 18-months carcinogenicity study with mice, effects in the liver and in the kidney (male) were observed in the 1500 ppm dose group. The NOAEL for general toxicity was set at 300 ppm equal to the average daily intake of 45 mg/kg bw/day.
No increase in tumor incidence was reported in the two available studies.

4.10.5 Comparison with criteria

No increase in tumor incidence was reported in the two studies. Thus, flupyradifurone does not meet the criteria for classification as a carcinogen.

4.10.6 Conclusions on classification and labelling

No classification is proposed for carcinogenicity under the CLP Regulation.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The carcinogenic potential of flupyradifurone has been investigated in two dietary GLP-compliant carcinogenicity studies, one in rats (similar to OECD TG 453) and one in mice (similar to OECD TG 451), conducted with flupyradifurone (purity 96.2 %).

There were no human data available.

None of the studies conducted in rats and mice revealed any evidence of carcinogenic effect. Therefore the DS concluded that no classification for carcinogenicity is warranted.

Comments received during public consultation

There were no specific comments for this endpoint.

Assessment and comparison with the classification criteria

The both long-term studies in rats and mice which did not show any neoplastic leasions at any dose level. Non-neoplastic effects are described in detail in the section *"RAC evaluation of specific target organ toxicity - repeated exposure"* and in the background document.

Therefore, RAC agrees with the DS that **no classification for carcinogenicity is warranted**.

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

4.11.1.1 Non-human information

STUDY 1

Characteristics

reference	:	Milius, A.D. 2010	exposure	:	Throughout the entire in-life phase of the study P: 10 weeks before mating, 7 days mating, 22 days gestation, 21 days lactation
type of study	:	One generation reproductive toxicity study (dose range-finding)	doses	:	Nominal: 0, 200, 700 and 2000 ppm in diet Actual daily intake: See study design
year of execution	:	2009/2010	vehicle	:	none
test substance	:	BYI 02960 (batch number 2009-000239; purity 96.2%)	GLP statement	:	Yes
route	:	Oral (diet)	guideline	:	None – pilot study
species	:	Rat, Wistar Crl:WI (Han)	acceptability	:	Acceptable
group size	:	10/sex/dose	**NOAEL**par toxicity	:	17.5 mg/kg bw/day (f).
			NOAELpar reproductive effects	:	147.5 mg/kg bw/day (m, highest dose tested)
			NOAELoffspring	:	17.5 mg/kg bw/day (f)

Study design

The study was performed in order to determine appropriate dietary levels for a definitive (guideline) two-generation reproductive toxicity study with BYI 02960. In this study, BYI 02960 was administered continuously in the feed to the Wistar rat (10 animals/dose/sex) at nominal dietary concentrations of 0, 200, 700, and 2000 ppm. All test diets (including control) were available for *ad libitum* consumption; the homogeneity and stability of BYI 02960 as a dietary admixture was confirmed. Body weight and food consumption determinations and detailed clinical examinations of each animal were conducted weekly throughout the study, as well as, an evaluation of multiple reproductive parameters. All animals placed on study were subject to a post-mortem examination, which included documenting and saving all gross lesions, weighing designated organs and, collecting representative tissue specimens. Micropathology was not performed on any tissue collected in this study.

The mean values of the actual substance intake from the intended test material dose levels for the parental generation are shown in*Table 92*.

Table 92: Mean values of the actual substance intake by parental animals during premating, gestation and lactation

Phase of Study	200 ppm in mg/kg/day[a]	700 ppm in mg/kg/day[a]	2000 ppm in mg/kg/day[a]
Premating (P-gen) - Male	14.5	50.1	147.5
Premating (P-gen) - Female	17.5	60.0	168.9
Gestation (P-gen) - Female	15.8	48.8	164.4
Lactation (P-gen) - Female	17.5	60.9	182.3

[a] Individual values were based on means for each particular phase.

Results

The results of the study are summarised in *Table 93*. Additionally, information on parental body weights, body weight gains, food consumption, reproductive performance, litter parameters and pup weights is presented in *Table 94-93*.

Table 93

Dose (ppm in diet)	**0**	**200**	**700**	**2000**	**dr**
	m f	**m f**	**m f**	**m f**	
F0 animals					
Mortality	No substance-related mortality				
Clinical signs	No substance-related findings				
Body weight			d (g) ds (l)	ds (pm, g, l)	
Body weight gain			d (pm)	d (pm) d (pm)	
Food consumption				d (pm)	
Mating/fertility/gestation	No substance-related findings				
Oestrus cycle	Not investigated				
Sperm evaluation	Not investigated				
Organ weight -liver -spleen			i	i i(a/r) ds (a/r)	
Pathology					
Macroscopy	No substance-related findings				
Microscopy	Not investigated				
F1 pups					

Dose (ppm in diet)	**0**	**200**	**700**	**2000**	**dr**
	m f	**m f**	**m f**	**m f**	
Litter size	No substance-related findings				
Viability index	No substance-related findings				
Lactation index	No substance-related findings				
Sex ratio	No substance-related findings				
Clinical signs	No substance-related findings				
Body weight - day 14 - day 21			 d ds d d	 d ds d ds	
Physical development	No substance-related findings				
Organ weight - brain			 d (a) i (r)	 ds (a) is (r)	
Pathology					
Macroscopy	No substance-related findings				

dr dose related
ds/is statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative organ weight
pm premating
l lactation
g gestation

Table 94: Mean (S.E.) body weight and food consumption during premating.

Observations/study week	Dose Group			
	Control 0 ppm	LDT 200 ppm	MDT 700 ppm	HDT 2000 ppm
P Generation Males				
Mean body weight (g) - Week 15 S.E.	452.9 13.42	463.2 14.30	454.6 11.51	432.6 14.36
Mean weight gain (g) Weeks 1-15	232.2	242.3	235.3	210.7
Mean food consumption (g/animal/day) Weeks 1-10	23.9	24.3	23.8	23.6
Mean food consumption (g/kg/day) Weeks 1-10	72.6	73.2	73.5	75.4
P Generation Females - Pre-mating				
Mean body weight (g) - Week 10 S.E.	231.0 3.87	230.3 5.22	221.7 6.00	207.0** 3.27
Mean weight gain (g) Weeks 1-10	79.0	79.5	69.3	52.6
Mean food consumption (g/animal/day) Weeks 1-10	17.1	17.2	16.8	15.6
Mean food consumption (g/kg/day) Weeks 1-10	86.8	88.4	88.4	85.7

[a]Data obtained from Tables 2, 3, and 4 in the study report

** Statistically different from control, $p \leq 0.01$

Table 95: Mean (S.E.) body weight and food consumption during gestation

P Generation Females - Gestation				
Observations/study week	Dose Group			
	Control 0 ppm	LDT 200 ppm	MDT 700 ppm	HDT 2000 ppm
Mean body weight (g) - Day 0 S.E.	233.5 4.33	231.3 6.22	225.5 4.69	206.4** 4.47
Mean body weight (g) - Day 6 S.E.	249.4 5.26	251.8 6.06	241.2 4.09	225.0** 3.85
Mean body weight (g) - Day 13 S.E.	274.7 5.15	274.8 7.39	261.4 4.26	241.6** 4.38
Mean body weight (g) - Day 20 S.E.	334.8 6.45	337.8 10.78	315.5 6.10	295.9** 4.42
Mean weight gain (g) - Days 0-20 S.E.	101.4 5.30	106.5 6.14	90.0 3.98	89.5 3.15
Mean food consumption (g/animal/day) Days 0–20	18.6	20.1	17.4	18.6
Mean food consumption (g/kg/day) Days 0–20	73.8	80.1	71.8	83.4

**: Statistically different from control, p≤0.01

Table 96: Mean (S.E.) body weight and food consumption during lactation

P Generation Females - Lactation				
Observations/study week	**Dose Group**			
	Control 0 ppm	**LDT 200 ppm**	**MDT 700 ppm**	**HDT 2000 ppm**
Mean body weight (g) - Day 0 S.E.	255.2 5.94	253.2 7.97	242.1 2.99	223.9** 5.85
Mean body weight (g) - Day 4 S.E.	266.1 4.96	270.0 7.13	253.6 4.16	241.6* 5.23
Mean body weight (g) - Day 7 S.E.	274.9 4.22	275.9 5.84	266.5 3.43	252.3* 5.39
Mean body weight (g) - Day 14 S.E.	296.6 4.78	296.4 6.25	276.8* 3.77	262.5** 6.28
Mean body weight (g) - Day 21 S.E.	290.1 4.80	287.1 10.04	272.8* 2.87	261.8** 3.95
Mean food consumption (g/animal/day) Days 0–21	49.3	47.3	45.0	45.3
Mean food consumption (g/kg/day) Days 0–21	179.3	171.3	171.5	182.7

[1] Data obtained from Tables 13, 14 and 15 in the study report

[2] * Statistically different from control, $p \leq 0.05$ ** Statistically different from control, $p \leq 0.01$

Table 97: Reproductive performance

Observation	**Dose Group (ppm)**			
	Control 0 ppm	**LDT 200 ppm**	**MDT 700 ppm**	**HDT 2000 ppm**
P Generation – F_1 Offspring				
Number Cohoused	10	10	10	10
Number Mated	10	9	9	9
Number of Animals Delivered	8	8	9	9
Number of Animals with Implants	9	8	9	9
Mating Index	100.0	90.0	90.0	90.0
Fertility Index	90.0	88.9	100.0	100.0
Gestation Index	88.9	100.0	100.0	100.0
Mean Number Days to Insemination (S.E.)	2.8 (0.32)	1.9 (0.26)	2.3 (0.33)	2.2 (0.28)
Mean Gestation Length (days) (S.E.) Median Gestation Length (days)	21.9 (0.23) 22.0	22.3 (0.16) 22.0	22.0 (0.24) 22.0	22.2 (0.28) 22.0

[1] Data obtained from Table 6 in the study report

Table 98: Litter parameters

Observation	Dose Group (ppm)			
	Control 0 ppm	LDT 200 ppm	MDT 700 ppm	HDT 2000 ppm
P Generation				
Total number of implantation sites	96	99	101	94
Total number of pups born	91	97	100	92
Number stillborn	0	4	0	0
Sex Ratio Day 0 (% male)	55.3	45.5	48.0	54.2
Mean litter size Day 0	11.4	12.1	11.1	10.2
Birth index	87.0	98.1	99.0	98.0
Live birth index	100.0	96.2	100.0	100.0
Viability index	100.0	98.2	99.1	100.0
Lactation index	100.0	100.0	100.0	100.0

[a] Data obtained from Table 6 and 20 in the study report

Table 99: Mean (SE) combined male and female pup weights (g).

F_1 Generation				
Lactation Day	Control 0 ppm	LDT 200 ppm	MDT 700 ppm	HDT 2000 ppm
0	6.1	6.0	5.7	6.0
S.E	0.18	0.18	0.11	0.23
4[b]	10.2	10.0	9.4	10.0
S.E.	0.48	0.39	0.22	0.43
4[c]	10.1	10.0	9.4	10.0
S.E.	0.51	0.39	0.22	0.41
7	16.2	16.2	15.0	15.3
S.E.	0.71	0.51	0.25	0.45
14	33.2	32.1	29.9*	29.9*
S.E.	0.86	0.83	0.57	0.71
21	50.6	48.4	45.9	45.7*
S.E.	1.42	1.05	1.11	1.11
GAIN	44.5	42.4	40.2*	39.7*

[a] Data obtained from Table 18 and 20 in the study report
[b] Before standardization (culling)
[c] After standardization (culling)
* Statistically different from control, $p \leq 0.05$

There were no test substance-related mortalities or clinical observations observed during the course of the study at any dietary level tested.

In males, no effects on body weight were observed at any dose level. In females, declines in absolute body weight were observed during premating in 2000 ppm group (9.4% decline when compared to controls), during gestation in 700 and 2000 ppm groups (4.5% and 11.3% respectively, value in 2000 ppm group reached statistical significance) and during lactation in 700 and 2000 ppm groups (10.6% during days 14-21 in 700 ppm group, 10.2% during days 0-21 in 2000 ppm groups, both statistically significant).

In males a very slight decrease in body weight gain during premating was observed in the 2000 ppm dose group (9.3% decline when compared to controls). In females, declines in body weight gain were noted during premating in 700 and 2000 ppm group (12.3% and 33.4%, respectively). Food consumption was decreased 8.8% in females at 2000 ppm during premating. No further changes on body weight gain and food consumption were noted.

There were no test substance-related effects on any reproductive parameter (e.g. mating, fertility, or gestation indices, days to insemination, gestation length, or the median number of implants) at any dietary level tested.

In males, equivocal increases in liver weights were observed in both the 700 and 2000 ppm dose groups. In females, test substance-related decreases in absolute and relative spleen weight were observed in the 2000 ppm dose level.

There was no test substance-related effects observed on the viability of the pups after delivery at any dietary level tested. No test substance-related clinical observations were observed at any dietary level tested. Declines in absolute pup weight were observed beginning post-natal day (PND) 14 and continuing to PND 21 in the 700 and 2000 ppm dietary groups with significance only observed for the females. Body weight gain for the males and females was declined in both the 700 and 2000 ppm dose group (9.7% and 10.8%, respectively).
Significant changes in brain weight in the 2000 ppm dose group and slight changes in the 700 ppm dose group were observed (decreased absolute and increased relative) and are considered to be secondary to body weight declines observed in the pups at these same dose groups. There were no test substance-related gross necropsy findings observed at any dietary level tested.

Historical control data were obtained from reproduction studies performed in the same test laboratory in the period of 1998-2008 in the Wistar rat.

Acceptability
The study is considered acceptable.

Conclusion
The parental systemic LOAEL is 700 ppm (60.0 mg /kg bw/day females), based on decreased body weight, decreased body weight gain, alterations in food consumption during premating, and decreased spleen weights in females. The parental systemic NOAEL is 200 ppm (17.5 mg /kg bw/day).
The reproductive NOAEL is 147.5 mg /kg bw/day based on no reproductive findings observed at the highest dose tested.
The offspring LOAEL is 700 ppm (60.9 mg /kg bw/day). The LOAEL is based on maternal effects leading to secondarily-mediated effects on pup weight, pup weight gain, and organ weight changes (brain). The offspring NOAEL is 200 ppm (17.5 mg /kg bw/day).

Study 2

Characteristics

reference	:	Milius, A.D. 2011	exposure	:	Throughout the entire in-life phase of the study P: 10 weeks before mating, 14 days mating, 22 days gestation, 21 days lactation F1: 3-4 weeks after weaning before mating, 14 days mating, 22 days gestation, 21 days lactation
type of study	:	Two-Generation Reproductive Toxicity Study	doses	:	Nominal: 0, 100, 500 and 1800 ppm Actual daily intake: See study design.
year of execution	:	2009/2011	vehicle	:	acetone
test substance	:	BYI 02960 (batch number 2009-000239; purity 96.2%)	GLP statement	:	Yes
route	:	Oral (diet)	guideline	:	OECD guideline 416
species	:	Rat, Wistar Crl:WI (Han)	acceptability	:	Acceptable
group size	:	30/sex/dose	**NOAEL**par toxicity	:	6.4 mg/kg bw/day.
			NOAELpar reproductive effects	:	39.6 mg/kg bw/day
			NOAELoffspring	:	6.4 mg/kg bw/day

Study design

The study was performed in accordance with OECD guideline 416.

The mean values of the actual substance intake from the intended test material dose levels for the P and the F1 generation are shown in *Table 100*.

Table 100: Mean values of the actual substance intake by parental animals during premating, gestation and lactation

Phase of Study	100 ppm in mg/kg/day[a]	500 ppm in mg/kg/day[a]	1800 ppm in mg/kg/day[a]
Premating (P-gen) - Male	6.6	32.5	117.4
Premating (F_1-gen) - Male	6.4	32.0	122.1
Mean P- and F_1-gen - Males	**6.5**	**32.3**	**119.8**
Premating (P-gen) - Female	7.7	38.7	137.0
Premating (F_1-gen) - Female	7.8	39.6	143.4
Mean P- and F_1-gen - Females	**7.8**	**39.2**	**140.2**
Gestation (P-gen) – Female	6.9	34.3	134.0
Gestation (F_1-gen) – Female	7.0	36.6	168.8
Mean Gestation P and F_1	**7.0**	**35.5**	**151.4**
Lactation (P-gen) - Female	7.8	37.4	140.4
Lactation (F_1-gen) - Female	7.7	42.2	160.3
Mean Lactation P and F_1	**7.8**	**39.8**	**150.4**

[a] Individual values were based on the means for each particular phase

Results

The results of the study are summarised in *Table 101*. Additionally, data on the mean body weight and food consumption during premating, gestation and lactation, estrous cycle, sperm measures, reproductive performance, litter parameters and pup weights are presented in *Table 102-104*.

Table 101

Dose (mg/kg bw/day)	**0**	**100**	**500**	**1800**	**dr**
	m f	**m f**	**m f**	**m f**	
F0 animals					
Mortality	No substance-related mortality				
Clinical signs	No substance-related findings				
Body weight				ds (pm, g, l)	
Body weight gain			d (-21%) (pm)	d (pm, g)	
Food consumption				ds (pm), i (g)	
Mating/fertility/gestation	No substance-related findings				
Oestrus cycle	No substance-related findings				

Dose (mg/kg bw/day)	**0**	**100**	**500**	**1800**	**dr**
	m f	**m f**	**m f**	**m f**	
Sperm evaluation	No substance-related findings				
Organ weight -liver -thyroid				i (a/r) i (a)	
Pathology Macroscopy	No substance-related findings				
Microscopy - centrilobular hypertrophy				i	
F1 pups					
Litter size	No substance-related findings				
Viability index	No substance-related findings				
Lactation index	No substance-related findings				
Sex ratio	No substance-related findings				
Clinical signs	No substance-related findings				
Body weight - birth - day 7 - day 14 - day 21				ds ds ds ds ds ds ds ds	
Physical development - delay in prepuital separation - delay in vaginal patency				i i	
Organ weight	No substance-related findings				
Pathology Macroscopy	No substance-related findings				
F1 animals **Mortality**	No substance-related mortality				
Clinical signs	No substance-related findings				
Body weight **- premating** **- gestation** **- lactation**			ds ds ds	ds ds ds ds	

Dose (mg/kg bw/day)	**0**	**100**	**500**	**1800**	**dr**
	m f	**m f**	**m f**	**m f**	
Body weight gain **- premating** **- gestation** **- lactation**			ds (- 21%) d (-16%)	ds (- 43%) d (-21%) ds (-19%)	
Food consumption				is (11%) ds (pm) is (g)	
Mating/fertility/gestation	No substance-related findings				
Number of implantation sites				ds (-17%)	
Oestrus cycle - number				ds (-17%)	
Sperm evaluation	No substance-related findings				
Organ weights	No substance-related findings				
Pathology Macroscopy	No substance-related findings				
Microscopy	No substance-related findings				
F2 pups **Litter size**				ds ds (-9%)	
Viability index	No substance-related findings				
Sex ratio	No substance-related findings				
Clinical signs	No substance-related findings				
Body weight - Birth - day 7 - day 14 - day 21			ds -6% ds -7% ds -7% ds -7%	d -8% ds -8% ds -12% ds -14% ds -12% ds -13%	
Organ weight	No substance-related findings				
Pathology Macroscopy	No substance-related findings				

dr dose related
ds/is statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative organ weight

Table 102: Mean (S.E.) body weights and food consumption during premating (P and F1 animals)

Observations/Study Week	Dose Group			
	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
P-Generation Males				
Mean body weight (g) - Week 14 S.E.	436.0 7.81	436.0 5.96	441.3 7.77	438.4 7.54
Mean weight gain (g) Weeks 1–14	200.8	201.9	200.0	198.0
Mean food consumption (g/animal/day) Weeks 1–10	21.4	21.9	21.9	21.7
Mean food consumption (g/kg/day) Weeks 1–10	65.4	67.2	65.6	65.5
P-Generation Females - Pre-mating				
Mean body weight (g) - Week 10 S.E.	237.4 3.33	235.5 3.09	229.0 2.92	213.3** 2.73
Mean weight gain (g) Weeks 1–10	63.4	61.8	50.4	36.3
Mean food consumption (g/animal/day) Weeks 1–10	16.3	16.0	16.0	14.7**
Mean food consumption (g/kg/day) Weeks 1-10	78.5	77.6	78.1	76.4
F_1-Generation Males				
Mean body weight (g) – Week 14 S.E.	454.5 8.27	456.0 7.51	452.3 8.28	410.8** 7.53
Mean weight gain (g) Weeks 1–14	152.3	152.0	166.2	145.1
Mean food consumption (g/animal/day) Weeks 1–10	23.4	23.8	23.4	22.8
Mean food consumption (g/kg/day) Weeks 1–10	62.2	63.7	64.5	68.8**
F_1-Generation Females - Pre-mating				
Mean body weight (g) - Week 10 S.E.	242.1 3.04	237.3 4.06	224.2** 2.90	203.5** 2.49
Mean weight gain (g) Weeks 1–10	52.2	51.5	43.7	41.2
Mean food consumption (g/animal/day) Weeks 1–10	16.8	16.3	16.3	14.8**
Mean food consumption (g/kg/day) Weeks 1–10	77.7	76.8	79.8	80.7

[a]Data obtained from Tables 2, 3, and 4 in the study report. Food consumption data represents grand means.
**Statistically different from control, $p \leq 0.01$

Table 103: Mean (S.E.) body weights and food consumption during gestation (P animals)

Observations/Study Week	Dose Group			
	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
P- Generation Females - Gestation				
Mean body weight (g) - Day 0 S.E.	238.1 4.27	235.2 3.25	232.2 3.10	214.8** 3.06
Mean body weight (g) - Day 6 S.E.	255.0 3.65	250.2 3.07	248.6 3.22	228.1** 3.25
Mean body weight (g) - Day 13 S.E.	276.0 3.54	272.3 3.57	267.9 3.63	247.1** 3.06
Mean body weight (g) - Day 20 S.E.	333.6 5.68	326.5 4.28	322.1 5.41	301.1** 4.43
Mean weight gain (g) - Days 0–20 S.E.	95.6 3.63	91.3 2.59	89.9 3.74	86.4 2.87
Mean food consumption (g/animal/day) Days 0–20	18.1	17.9	17.9	17.5
Mean food consumption (g/kg/day) Days 0–20	70.6	70.9	71.6	76.2

[a]Data obtained from Tables 8, 9, and 10 in the study report. Food consumption data represents grand means.
** Statistically different from control, $p \leq 0.01$

Table 104: Mean (S.E.) body weights and food consumption during gestation (F1 animals)

Observations/Study Week	Dose Group			
	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
F_1-Generation Females - Gestation				
Mean body weight (g) - Day 0 S.E.	243.4 3.27	238.3 4.82	224.4** 3.01	202.6** 2.85
Mean body weight (g) - Day 6 S.E.	257.6 3.09	251.0 4.57	238.7** 2.86	214.0** 3.23
Mean body weight (g) - Day 13 S.E.	277.8 3.33	270.0 4.54	258.3** 2.80	230.8** 2.98
Mean body weight (g) - Day 20 S.E.	335.1 4.52	327.4 5.28	313.8** 4.01	277.2** 4.28
Mean weight gain (g) - Days 0–20 S.E.	91.8 2.59	89.1 3.50	89.4 2.86	74.7** 2.11
Mean food consumption (g/animal/day) Days 0–20	18.2	17.6	17.8	19.0
Mean food consumption (g/kg/day) Days 0–20	70.2	69.7	74.3	89.3**

[a]Data obtained from Tables 8, 9, and 10 in the study report.. Food consumption data represents grand means.
* Statistically different from control, $p \leq 0.05$; ** Statistically different from control, $p \leq 0.01$

Table 105: Mean (S.E.) body weights and food consumption during lactation (P and F1 animals)

Observations/Study Week	Dose Group			
	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
P-Generation Females - Lactation				
Mean body weight (g) - Day 0 S.E.	262.8 3.22	256.1 2.95	250.8 4.30	233.3** 3.24
Mean body weight (g) - Day 4 S.E.	271.3 3.12	271.6 3.36	265.8 3.91	245.7** 3.66
Mean body weight (g) - Day 7 S.E.	277.4 2.81	276.7 3.05	271.2 3.90	251.6** 3.20
Mean body weight (g) - Day 14 S.E.	292.6 3.81	292.3 2.77	287.2 4.22	267.1** 3.17
Mean body weight (g) - Day 21 S.E.	285.8 3.67	281.4 2.80	280.1 3.59	264.3** 3.15
Mean food consumption (g/animal/day) Days 0–21	41.5	43.1	41.6	40.2
Mean food consumption (g/kg/day) Days 0–21	149.2	155.9	153.3	159.8
F_1-Generation Females - Lactation				
Mean body weight (g) - Day 0 S.E.	263.0 3.38	254.5 4.53	242.5** 3.27	218.4** 2.84
Mean body weight (g) - Day 4 S.E.	277.3 3.24	265.9 4.13	256.0** 3.51	225.1** 3.69
Mean body weight (g) - Day 7 S.E.	278.7 3.23	268.5 4.02	260.9** 3.43	231.3** 3.02
Mean body weight (g) - Day 14 S.E.	294.8 3.54	282.8 3.64	272.2** 3.72	244.3** 3.53
Mean body weight (g) - Day 21 S.E.	283.8 3.45	278.5 3.91	261.6** 4.17	247.1** 3.22
Mean food consumption (g/animal/day) Days 0–21	45.3	42.5	44.0	40.1
Mean food consumption (g/kg/day) Days 0–21	162.0	158.5	169.4	173.2

[a]Data obtained from Tables 13, 14, and 15 in the study report. Food consumption data represents grand means.
* Statistically different from control, $p \leq 0.05$; ** Statistically different from control, $p \leq 0.01$

Table 106: Estrous Cycle Length and Periodicity

Observation	Dose Group (ppm)			
	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
P-Generation				
Number of Estrous Cycles (S.E.)	3.5 (0.1)	3.7 (0.1)	3.4 (0.2)	3.4 (0.1)
Estrous Cycle Length (S.E.)	4.4 (0.3)	4.3 (0.1)	4.3 (0.2)	4.3 (0.1)
F_1-Generation				
Number of Estrous Cycles (S.E.)	3.5 (0.2)	3.3 (0.2)	3.3 (0.2)	2.9* (0.2)
Estrous Cycle Length (S.E.)	4.0 (0.2)	4.1 (0.1)	4.4 (0.2)	4.4 (0.1)

* Statistically different from control, $p \leq 0.05$ Data taken from Table 6 in the study report.

Table 107: Sperm measures

Sperm Analysis		Dose Group (ppm)			
		Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
P Generation Males					
Sperm Motility	% Motile	92.4	90.9	93.3	90.7
	% Progressive	62.1	60.7	63.3	59.2
Sperm Counts (sperm/gram)	Testis	26.9	N/A	N/A	23.7
	Epididymis	158.8	N/A	N/A	138.7
Sperm Morphology (mean total number)	Normal	193.2	N/A	N/A	197.0
	Abnormal	2.7	N/A	N/A	2.2
	Detached Head	4.1	N/A	N/A	0.8
F_1-Generation Males					
Sperm Motility	% Motile	90.7	94.5	92.0	92.7
	% Progressive	64.0	66.4	64.8	63.3
Sperm Counts (sperm/gram)	Testis	29.4	N/A	N/A	30.9
	Epididymis	158.8	N/A	N/A	141.8
Sperm Morphology (mean total number)	Normal	189.1	N/A	N/A	195.2
	Abnormal	3.8	N/A	N/A	4.2
	Detached Head	0.4	N/A	N/A	0.6

a Data obtained from Table 24 in the study report N/A = Not Applicable

Table 108: Reproductive performance

Observation	Dose Group (ppm)			
	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
P-Generation – F_1-Offspring				
Number Cohoused	30	30	30	30
Number Mated	30	29	29	30
Number of Animals Delivered	29	27	28	28
Number of Animals with Implants	29	27	28	28
Mating Index	100.0	96.7	96.7	100.0
Fertility Index	96.7	93.1	96.6	93.3
Gestation Index	100.0	100.0	100.0	100.0
Mean Number Days to Insemination (S.E.) Median	3.4 (0.67) 3.0	3.3 (0.59) 3.0	3.2 (0.52) 3.0	3.1 (0.59) 3.0
Mean Gestation Length (days) (S.E.) Median Gestation Length (days)	22.1 (0.11) 22.0	22.1 (0.12) 22.0	22.1 (0.12) 22.0	22.0 (0.12) 22.0
Total number of implantation sites (Median)	311 (11.0)	285 (11.0)	298 (10.5)	289 (10.0)
F_1-Generation – F_2-Offspring				
Number Cohoused	30	30	30	30
Number Mated	29	30	29	30
Number of Animals Delivered	27	28	28	29
Number of Animals with Implants	27	28	28	29
Mating Index	96.7	100.0	96.7	100.0
Fertility Index	93.1	93.3	96.6	96.7
Gestation Index	100.0	100.0	100.0	100.0
Mean Number Days to Insemination (S.E.) Median	2.3 (0.23) 2.0	3.7 (0.45) 4.0**	2.6 (0.24) 2.5	2.1 (0.18) 2.0
Mean Gestation Length (days) (S.E.) Median Gestation Length (days)	22.0 (0.09) 22.0	22.1 (0.15) 22.0	21.9 (0.09) 22.0	22.0 (0.08) 22.0
Total number of implantation sites (Median)	305 (12.0)	314 (11.0)	323 (11.0)	281 (10.0**)

[a]Data obtained from Table 6 in the study report ** Statistically different from control, $p \leq 0.01$

Table 109: Litter parameters

Observation	Dose Group (ppm)			
	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
P Generation				
Total number of pups born	297	277	282	281
Number stillborn	0	2	0	0
Sex Ratio Day 0 (% male)	55.1	53.0	54.7	49.6
Mean litter size Day 0 Median	10.2 11.0	10.3 11.0	10.1 10.0	10.0 10.0
Birth index	95.0	97.1	93.3	97.4
Live birth index	100.0	99.0	100.0	100.0
Viability index	98.0	99.3	99.7	98.1
Lactation index	99.1	99.5	100.0	99.1
F_1-Generation				
Total number of pups born	291	300	311	266
Number stillborn	1	0	1	4
Sex Ratio Day 0 (% male)	51.6	50.6	50.6	47.7
Mean litter size Day 0 Median	10.8 11.0	10.7 11.0	11.1 10.5	9.2 **10.0***
Birth index	95.6	92.7	96.4	94.8
Live birth index	99.7	99.7	99.2	98.2
Viability index	99.3	99.7	99.4	97.2
Lactation index	99.1	100.0	100.0	98.7

[a] Data obtained from Table 6 and 20 in the study report * Statistically different from control, $p \leq 0.05$

Table 110: Mean (S.E.) combined male and female pup weights (g)

F_1-Generation					F_2-Generation				
Lactation Day	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm	Lactation Day	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
0 S.E	6.1 0.11	6.0 0.11	5.9 0.11	5.6** 0.11	0 S.E	6.1 0.10	6.0 0.10	5.9 0.10	5.8 0.08
4[b] S.E.	10.2 0.26	10.3 0.25	9.8 0.28	9.3* 0.27	4[b] S.E.	10.1 0.23	9.8 0.21	9.6 0.25	9.6 0.22
4[c] S.E.	10.3 0.26	10.3 0.25	9.8 0.28	9.3* 0.26	4[c] S.E.	10.1 0.23	9.7 0.21	9.7 0.25	9.7 0.22
7 S.E.	16.1 0.32	16.1 0.35	15.3 0.40	14.2** 0.36	7 S.E.	16.0 0.32	15.6 0.23	15.3 0.36	14.7* 0.38
14 S.E.	32.3 0.46	31.9 0.61	30.6 0.70	28.0** 0.49	14 S.E.	32.1 0.60	31.2 0.41	29.9* 0.51	27.9** 0.75
21 S.E.	49.3 0.63	48.9 0.88	47.3 1.00	42.8** 0.69	21 S.E.	48.8 0.77	47.4 0.72	45.2** 0.72	42.7** 1.10
GAIN[d]	43.2	42.9	41.4	37.3**	GAIN[d]	42.7	41.5	39.4**	36.8**

[a] Data obtained from Table 18 in the study report [b] Before standardization (culling) [c] After standardization (culling)
[d] GAIN taken from Table 20 in the study report * Statistically different from control, $p \leq 0.05$ ** Statistically different from control, $p \leq 0.01$

Parental animals (P and F1 adults)

At the highest dose level, statistically significant decreases in body weight were observed in parental P and F1 females during premating, gestation and lactation. In F1 males, at the highest dose level significant decreases in body weight were observed beginning on Day 0 and continued throughout exposure. Statistically significant decreases in body weight during premating, gestation and lactation were observed also in F1 females at the mid-dose level however the weight loss was less than 10%.

Body weight gains were decreased in P and F1 females during premating and gestation in the high-dose groups, and non significant during pre-mating in the mid-dose group (21% for P-generation females and 16% for F1 generation females). Also increased food consumption was observed in the high-dose F1 females during gestation.

In the high-dose P males, increased absolute and relative liver weight (9% relative to controls) and increased absolute thyroid weights (13-21% relative to controls) were observed. Minimal centrilobular hypertrophy of the liver was observed in the high-dose P males, correlated with the increased liver weights. No other organ weight changes and microscopic changes were noted at any dose levels in both P and F1 generations.

No adverse effects on reproductive performance, sperm parameters or oestrous cycle were noted in P generation at any dose level. In the F1 generation, a significant decrease in the number of oestrous cycles was observed in the high-dose females (see *Table 106*). This finding paralleled the significant weight loss observed in these females. A statistically significant decline in the total number of implantation sites was also observed in the high-dose F1 females (see *Table 108*). No clear dose-response was obvious. These findings are considered to be secondary to the maternal toxicity, as evidenced from the significant weight loss, rather than true adverse reprotoxic effects.

Offspring (F1and F2 pups)

No effects on the litter size, pup viability, sex ratio and lactation index were observed in F1 pups at any dose level. A slight decrease in the litter size was noted in the high-dose F2 pups (see *Table 109*). This decrease paralleled the reduced body weight gain during gestation and a decreased number of implantation sites in the high-dose F1 females. There were no substance-related effects on the pup viability, sex ratio and lactation index.

A significant decrease in birth weight was observed (8.2% compared to the controls) in the F1 pups of the highest dose level. This decrease continued and increased by magnitude by post-natal day (PND) 21. A significant delay in preputial separation and a slight nonstatistical delay in vaginal patency were observed in parallel with the decreased pup weight exhibited during lactation in the same dose group.

Reduced absolute and relative brain (statistically significant), thymus (statistically significant only in males) and spleen weights (statistically significant) were observed in high-dose F1 pups, but were considered to be secondary to the decreased body weights at the same dose level and not a direct test substance effect.

The body weights at birth were comparable to controls in the F2 pups at all dose levels. Pup weight declines were observed beginning on PND 7 (declined 8.1%) and continued to Day 21 (declined 12.5%) at the high-dose level and on PND 14 to Day 21 at the mid-dose level. No effect on anogenital distance was observed in the F2-generation pups at any dose level. Reduced absolute and relative brain (statistically significant only in females), thymus and spleen weights (statistically significant) were observed in high-dose F2 pups, but were considered to be secondary to the decreased body weights at the same dose level and not a direct test substance effect. Significantly reduced thymus weight was also observed in mid-dose F2 female pups.

It is the position of Bayer CropScience (BCS) and the performing laboratory that the effects in the F1 and F2 generation are likely a result of treatment-induced reductions in BW and BWG rather than a direct, endocrine-mediated MOA. The defense of the applicant of its position is given in Annex 8.1 Clarification by the applicant on the possible endocrine mediated mode of action of flupyradifurone.

Acceptability
The study is considered acceptable.

Conclusions
A significant decrease in body weight, but <10% was observed in the mid-dose of the F1 females and a non-significant decrease in body weight gain was found in the parental animals (21 % in females) and the F1 animals (16 % in females). Therefore the parental systemic LOAEL is considered 500 ppm (32 mg/kg bw/d) based on decreased body weight gain in females. The parental systemic NOAEL is 100 ppm (6.4 mg/kg bw/d).

The reproductive NOAEL was 500 ppm (39.6 mg BYI 02960/kg bw/day females) based on decreased estrous cycle number, litter size, and the number of implants observed in the F_1 generation at the highest dietary level tested (LOAEL = 1800 ppm (143.4 mg BYI 02960/kg bw/day in females).

The offspring NOAEL was 100 ppm (6.4 mg/kg bw/d) based on the effect in the F_2 pups on body weight.

4.11.1.2 Human information

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Study 1

Characteristics

reference	:	Langrand-Lerche, A. D. 2010	exposure	:	Once daily, days 6-20 of gestation
type of study	:	Developmental toxicity study	doses	:	0, 15, 50 and 150 mg/kg bw/day
year of execution	:	2009/2010	vehicle	:	0.5% aqueous methylcellulose 400
test substance	:	BYI 02960 (batch number 2009-000239; purity 96.2%)	GLP statement	:	Yes
route	:	Oral (gavage)	guideline	:	OECD guideline 414
species	:	Rat, Sprague-Dawley Crl:CD (SD)	acceptability	:	Acceptable
group size	:	23 females/dose	**NOAEL**maternal toxicity	:	50 mg/kg bw/day
			NOAELdevelopmental toxicity	:	50 mg/kg bw/day

Study design

The study was performed in accordance with OECD guideline 414.

Results

The results of the study are summarised in *Table 111*. Additionally, information on maternal body weights, body weight gains, food consumption, reproductive performance, litter parameters and pup weights is presented in *Table 112-108*.

Table 111

Dose (mg/kg bw/day)	**0**	**15**	**50**	**150**	**dr**
Maternal effects					
Mortality	No substance-related mortality				
Clinical signs - salivation				i	
Pregnant animals	22	22	22	23	
Body weight (gain)				d	
Food consumption			ds	ds	
Uterus weight	No substance-related findings				
Pathology - liver weight				is	
Macroscopy					

Dose (mg/kg bw/day)	**0**	**15**	**50**	**150**	**dr**
Litter response					
Number of dams examined	22	22	22	23	
Pregnancy rate	96%	96%	96%	100%	
Resorptions					
- early	21	28	21	30	
- late	4	2	0	1	
- litters with total resorption	0	0	0	0	
Corpora lutea/dam	No substance-related findings				
Dams with live foetuses	22	22	22	23	
Live foetuses/dam	13.8 ± 3.0	14.5 ± 2.2	14.2 ± 2.3	13.7 ± 2.5	
Foetal weight	No substance-related findings				
Post implantation loss (mean %)	7.9 ± 10.3	8.6 ± 8.4	6.5 ± 9.3	9.0 ± 8.3	
Sex ratio, % male	51.3 ± 12.3	52.9 ± 14.1	46.7 ± 15.4	51.7 ± 10.3	
Examination of the foetuses					
External observations	No substance-related findings				
Skeletal findings (incidence)					
-evaluated foetuses/litter	158/22	165/22	161/22	162/23	
-# parietal (uni/bi): incomplete ossification (number litter/fetuses affected)	0/0	2/6*	0/0	4/9**	
- # hyoid centrum: incomplete ossification (number litter/fetuses affected)	0/0	2/2	1/1	4/9**	
- # 7th cervical centrum; unossified (number litter/fetuses affected)	2/6	2/7	4/4	7/11	
- # 5th sternebra: uncomplete ossification	10/17	11/21	7/8*	12/24	
- 13th costal cartilage (uni): short	0/0	0/0	0/0	2/2	
Visceral findings	No substance-related findings				

dr dose related
ds/is statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative organ weight
* statistically significant ($p \leq 0.05$)
** statistically significant ($p \leq 0.01$)

Table 112: Mean (±SD) maternal body weight gain (g)

Interval	Dose level of BYI 02960 in mg/kg/day				HCD#
	0	15	50	150	
Number of dams (pregnant)	22	22	22	23	
Pretreatment, GD 0-6:	29.4 ± 8.08	25.3 ± 7.50	27.6 ± 5.53	28.5 ± 5.44	28.05 - 39.25
Treatment, GD 6-8:	5.9 ± 3.82	5.9 ± 2.78	3.0 ± 3.75	-5.7 ± 5.18**	4.05- 8.48
Treatment, GD 8-10:	9.1 ± 1.96	7.4 ± 2.28*	8.5 ± 2.56	6.9 ± 3.48*	7.78 - 11.30
Treatment, GD 10-14:	16.8 ± 3.84	17.1 ± 7.81	16.1 ± 5.22	19.5 ± 4.09	17.17 - 22.33
Treatment, GD 14-18:	41.0 ± 8.88	43.8 ± 7.06	44.9 ± 6.48	41.7 ± 7.64	41.48 - 48.21
Treatment, GD 18-21:	43.6 ± 9.61	44.9 ± 17.09	47.5 ± 7.59	45.7 ± 8.89	47.09 - 58.21
Treatment, GD 6-21	116.4 ± 18.77	119.1 ± 25.52	120.1 ± 16.94	108.2 ± 16.75	122.55 - 143.29
Corrected BW gain	44.6 ± 16.80	40.9 ± 18.89	44.0 ± 12.81	37.3 ± 8.89	49.56 – 75.43

a Data obtained from **Table 4 in the Tables and Appendices section** of the study report.
HCD: Historical control range (lowest – highest) from Att.3 in the Tables and Appendices section of the study report.
**: p≤0.01 *: p≤0.05

Table 113: Caesarean section observations

Observation	Dose Level of BYI 02960 (mg/kg/day)				HCD
	0	15	50	150	
Maternal data:[a]					
No. Animals assigned	23	23	23	23	NA
No. Animals pregnant	22	22	22	23	NA
Pregnancy rate, %	96	96	96	100	NA
No. Animals non-pregnant	1	1	1	0	NA
Maternal wastage					
No. died (total)	0	0	0	0	NA
No. died pregnant	0	0	0	0	NA
No. died non-pregnant	0	0	0	0	NA
No. premature delivery	0	0	0	0	NA
Uterine data:[b]					
Total No. corpora lutea[c]	370	380	361	377	NA
Corpora lutea / dam	16.8±2.0	17.3±2.4	16.4±1.7	16.4±2.2	15.74-17.83
Total No. implantations[c]	329	349	334	345	NA
Implantations / dam	15.0±2.8	15.9±1.8	15.2±1.8	15.0±2.4	14.38-16.04
Total No. litters[c]	22	22	22	23	
Total No. live fetuses[c]	304	319	313	314	NA
Live fetuses / dam[c]	13.8±3.0	14.5±2.2	14.2±2.3	13.7±2.5	13.52-15.04
Total No. dead fetuses	0	0	0	0	NA
Dead fetuses / dam[c], %	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0-0.30
Total No. early resorptions[c]	21	28	21	30	NA
Total No. late resorptions[c]	4	2	0	1	NA
Early resorptions / dam	1.0±1.1	1.3±1.3	1.0±1.3	1.3±1.3	0.474-2.042
Late resorptions / dam	0.2±0.4	0.1±0.3	0.0±0.0	0.0±0.2	0.0-0.217
Litters with total resorptions[c]	0	0	0	0	NA
Mean fetal weight, combined sexes, g	5.41±0.28	5.27±0.60	5.35±0.24	5.27±0.25	5.30-5.57
Mean fetal weight, males, g	5.54±0.28	5.36±0.60	5.46±0.29	5.40±0.29	5.45-5.70
Mean fetal weight, females, g	5.26±0.29	5.18±0.59	5.23±0.24	5.14±0.25	5.16-5.42
Sex ratio, % male	51.3±12.3	52.9±14.1	46.7±15.4	51.7±10.3	46.2-53.5
Preimplantation loss per litter, %	10.5±16.2	7.4±9.2	7.2±9.3	8.3±10.6	3.32-13.21
Postimplantation loss per litter, %	7.9±10.3	8.6±8.4	6.5±9.3	9.0±8.3	3.95-13.15

a Data obtained from Table 1 in the Tables and Appendices section of the study report.
b Data obtained from Table 8 and Appendix F in the Tables and Appendices section of the study report.
* Statistically different ($p \leq 0.05$) from the control.
** Statistically different ($p \leq 0.01$) from the control.
c Statistical analysis was not conducted on this endpoint
HCD Historical control range (lowest – highest) of main uterine parameters from Att 3 in the Tables and Appendices section of the study report
NA not applicable

Table 114: Skeletal examinations

Dose level of BYI 02960 (mg/kg/day)	0	15	50	150	Historical Control Range	0	15	50	150	Historical Control Range
	Number of litters examined					Number of fetuses examined				
	22	22	22	23		158	165	161	162	
OBSERVATIONS	Number of litters affected (% of litters affected)					Number of fetuses affected (% of fetuses affected)				
Variations # Parietal (uni/bi) : incomplete ossification.	0 (0.0)	2 (9.1)	0 (0.0)	4 (17.4)	(0.0 – 9.1)	0 (0.0)	6 (3.6)*	0 (0.0)	9 (5.6)**	(0.0 – 1.3)
# Hyoid centrum : incomplete ossification.	0 (0.0)	2 (9.1)	1 (4.5)	4 (17.4)	(0.0 – 12.5)	0 (0.0)	2 (1.2)	1 (0.6)	9 (5.6)**	(0.0 – 1.9)
# 7th cervical centrum : unossified.	2 (9.1)	2 (9.1)	4 (18.2)	7 (30.4)	(0.0 – 41.7)	6 (3.8)	7 (4.2)	4 (2.5)	11 (6.8)	(0.0 – 12.2)
# 5th sternebra: incomplete ossification.	10 (45.5)	11 (50.0)	7 (31.8)	12 (52.2)	(19.0 – 70.8)	17 (10.8)	21 (12.7)	8 (5.0)*	24 (14.8)	(3.1 – 19.9)
13th costal cartilage (uni) : short.	0 (0.0)	0 (0.0)	0 (0.0)	2 (8.7)	(0.0 – 0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.2)	(0.0 – 0.0)

a Data obtained from Table 12 in the Tables and Appendices section of the study report.
 # : Statistical analysis was conducted on this observation (App M)
*** Statistically different (p≤ 0.05) from the control.**
**** Statistically different (p≤ 0.01) from the control.**

There were no mortalities during the study. At 150 mg/kg bw/day, increased salivation was noted in 20/23 animals on one or several occasions between gestation days (GD) 11 and 21 and soiling around the mouth was observed in 1/23 females on GD 21.

In the high-dose females a mean maternal body weight gain and the maternal corrected body weight change were decreased between GD 6-21 (not statistically significant). The mean body weight changes were significantly lower than controls by 14 to 91% on all intervals between GD 6 and GD 18 ($p \leq 0.01$). The food consumption was significantly decreased between GD 6 and 12. At 50 mg/kg bw/day the mean maternal body weight gain was reduced by 49% between GD 6-8, and the mean maternal body weight change was significantly lower than controls by 23% between GD 6 and GD 10. The mean food consumption was reduced by 8% between GD 6-8 ($p \leq 0.05$). No treatment-related body weight changes were observed in the low-dose group.

At 150 mg/kg bw/day the mean absolute liver weight was 13% higher than in the controls ($p \leq 0.01$). No other treatment-related findings were observed at the macroscopic examination at any dose level.

The pregnancy rate was at least 96% in all dose groups and was unaffected by treatment. The number of live fetuses, the number of implant sites per dam, the percentages of pre and post implantation losses, the number of early and late resorptions, the fetal death status and the percentage of male fetuses were unaffected by treatment in any dose group.

At 150 mg/kg/day, mean fetal body weights for combined sexes and females were reduced by 2 to 3%, compared to the controls (not statistically significant). No other treatment-related changes on fetal body weights were noted.

There were no treatment-related malformations or variations noted at the fetal external examination and fetal visceral examination. At 150 mg/kg bw/day, the incidence of parietal and hyoid centrum incomplete ossification were statistically significantly higher than in the control group ($p \leq 0.01$). No clear dose-response was obvious. The incidence of unossified 7th cervical centrum and 5th sternebra incomplete ossification were also higher in 150 mg/kg bw/day group than in the controls, but the incidence was not statistically significant and the observed values within the in-house historical control data. The incidence of the short 13th costal cartilage was also higher in the 150 mg/kg bw/day group than in the controls and was outside the in-house historical control data, but as the observation was noted unilaterally only and the incidence was low, the finding was considered to be not treatment-related.

The historical control data were generated from fifteen in-house Sprague-Dawley Crl:CD (SD) rat studies conducted between 2001-2008.

Conclusions
Minimal maternal toxicity was observed at the high-dose level, manifested as decreased body weight gain and reduced food consumption. The mean absolute liver weight was increased in the high dose group. Based on these findings the NOAEL for maternal toxicity was set at 50 mg/kg bw/day.

The increased incidence of parietal incomplete ossification showed no dose response, the unossified cervical centrum and the incomplete ossification of the sternebra was within the historical background. An increase of a short costal cartilage was seen at the high-dose. Based on these findings, the NOAEL for developmental toxicity was set at 50 mg/kg bw/day.

Study 2

Characteristics

reference	:	Langrand-Lerche, C. 2012	exposure	:	Gestation days (GD) 6-20
type of study	:	Complementary maternal tolerability study	doses	:	0, 20 and 30 mg/kg bw/day
year of execution	:	2011/2012	vehicle	:	0.5% aqueous methylcellulose 400
test substance	:	BYI 02960 (batch number 2009-000239; purity 96.2%)	GLP statement	:	Yes
route	:	Oral (gavage)	guideline	:	None (complementary tolerability study); however, the in-life portion of the study was conducted according to OECD guideline 414.
species	:	Rat, Sprague-Dawley Crl:CD (SD)	acceptability	:	Acceptable
group size	:	23/sex/dose	**NOAEL**maternal toxicity	:	30 mg/kg bw/day

Characteristics
The study was performed to provide additional information on the maternal tolerability of BYI 02960 and to determine more precisely the NOAEL for maternal toxicity. Groups of 23 sperm-positive female Sprague-Dawey rats were exposed to BYI 02960 by oral gavage from gestation day

(GD) 6 to 20, the sperm-positive day being GD 0. The doses given were 0, 20 and 30 mg/kg bw/day in aqueous solution of 0.5% methylcellulose 400. Clinical observations were recorded daily. Maternal body weights were recorded for all females on GD 0, 6, 8, 10, 12, 14, 16, 18 and 21. Food consumption was also measured for all the females during the intervals GD 1-6, 6-8, 8-10, 10-12, 12-14, 14-16, 16-18 and 18-21. At scheduled sacrifice, on GD 21, a macroscopic examination of the visceral organs was performed, the gravid uterine weight was recorded and the live fetuses were euthanized. In addition, the liver was weighed at scheduled sacrifice for all pregnant females. A portion of liver was retained in 10% neutral buffered formalin from all females on study for possible histological examination. Specimens were not examined and are retained in the archives.

Results

The results of the study are summarized in *Table 115*. Additional information on maternal body weight gain and caesarean section observations is provided in *Table 116-Table 117*.

Table 115

Dose (mg/kg bw/day)	**0**	**20**	**30**	**dr**
Maternal effects				
Mortality	No substance-related mortality			
Clinical signs	No substance-related clinical signs			
Pregnant animals	21	23	23	
Body weight (gain)	No treatment-related effects			
Food consumption	No treatment-related effects			
Pathology	No treatment-related findings			
Pregnancy rate	91%	100%	100%	
Number of animals died pregnant	0	1	0	
Number of animals died non-pregnant	0	0	0	
Number of animals with premature delivery	0	0	0	

dr dose related

Table 116: Mean (± SD) maternal body weight gain (g)

Interval	Dose level of BYI 02960 in mg/kg/day		
	0	20	30
Number of dams (pregnant)	21	23	23
Pretreatment, GD 0-6:	29 ± 5	28 ± 6	30 ± 8
Treatment, GD 6-8:	4 ± 4	4 ± 4	3 ± 4
Treatment, GD 8-10:	10 ± 4	10 ± 4	9 ± 4
Treatment, GD 10-14:	20 ± 5	19 ± 5	21 ± 5
Treatment, GD 14-18:	46 ± 9	46 ± 8	47 ± 8
Treatment, GD 18-21:	53 ± 9	52 ± 9	53 ± 10
Treatment, GD 6-21	133 ± 19	131 ± 18	133 ± 20
Corrected BW gain	55.6 ± 12.0	53.9 ± 8.5	58.0 ± 13.9

a Data obtained from Table 4 and 8 in the Tables and Appendices section of the study report.

Table 117: Caesarean section observations

Observation	Dose Level of BYI 02960 (mg/kg/day)		
	0	20	30
Maternal data:			
No. Animals assigned	23	23	23
No. Animals pregnant	21	23	23
Pregnancy rate, %	91	100	100
No. Animals non-pregnant	2	0	0
Maternal wastage			
No. died (total)	0	1	0
No. died pregnant	0	1	0
No. died non-pregnant	0	0	0
No. premature delivery	0	0	0

Data obtained from Table 1 and Appendix G in the Tables and Appendices section of the study report.

No treatment-related mortality or clinical signs were observed in the study. One animal of the mid-dose group died on GD 20 after having been dosed. Necropsy revealed red dark lungs and presence of foam in lungs and trachea, indicating of a gavage error. Body weight parameters and food consumption were unaffected by treatment. There were no treatment-related macroscopic findings at necropsy and no changes in liver weights at any dose level.

Historical control data on the maternal parameters were obtained from the in-house developmental toxicity studies with Sprague-Dawley Crl:CD (SD) rats performed in the period June 2001-April 2009.

Acceptability
The study is considered acceptable.

Conclusions
Based on the lack of adverse effects on mortality, clinical signs, body weight changes, food consumption, gross pathology and caesarean section data the highest dose level of 30 mg/kg bw/day was considered a NOAEL for maternal toxicity in this study.

STUDY 3

Characteristics

reference	:	P. Kennel, 2012	exposure	:	Gestation days (GD) 6-28
type of study	:	Developmental toxicity study	doses	:	0, 7.5, 15 and 40 mg/kg bw/day
year of execution	:	2011/2012	vehicle	:	0.5% aqueous methylcellulose 400
test substance	:	BYI 02960 (batch number 2009-000239; purity 96.2%)	GLP statement	:	Yes
route	:	Oral (gavage)	guideline	:	OECD guideline 414.
species	:	Rabbit, New Zealand White Crl: KBL (NZW)	acceptability	:	Acceptable
group size	:	23 females/dose	**NOAEL**maternal toxicity	:	15 mg/kg bw/day
			NOAELdevelopmental toxicity	:	40 mg/kg bw/day

Study design

The study was performed in accordance with OECD guideline 414.

Results

The results of the study are summarised in *Table 118*. Additionally, information on maternal body weights, body weight gains, food consumption, reproductive performance, litter parameters and pup weights is presented in *Table 119-115*.

Table 118

Dose (mg/kg bw/day)	**0**	**7.5**	**15**	**40**	**dr**
Maternal effects					
Mortality	No substance-related mortality				
Clinical signs	No treatment-related clinical signs				
Pregnant animals	23	22	23	23	
Body weight (gain)				d	
Food consumption				d	
Uterus weight	No substance-related findings				
Pathology	No substance-related findings				
Litter response					
Number of dams examined	23	22	23	22	
Pregnancy rate	100%	96%	100%	96%	
Resorptions					
- early	7	9	9	13	
- late	3	2	3	3	
- litters with total resorption	0	0	0	0	
Corpora lutea/dam	No substance-related findings				
Dams with live foetuses	22	22	23	22	
Number of implantations/dam	10.5 ± 2.1	10.9 ± 2.3	10.2 ± 2.0	11.5 ± 2.9	
Live foetuses/dam	9.7 ± 2.5	10.1 ± 2.1	9.4 ± 1.8	10.4 ± 2.4	
Foetal weight	No substance-related findings				
Post implantation loss (mean %)	8.7 ± 13.0	6.1 ± 9.2	7.4 ± 9.2	9.7 ± 9.5	
Sex ratio, % male	48.4 ± 18.6	52.4 ± 15.2	50.0 ± 16.4	47.4 ± 20.7	
Examination of the foetuses					

Dose (mg/kg bw/day)	**0**	**7.5**	**15**	**40**	**dr**
External observations	No substance-related findings				
Skeletal findings (incidence)					
-evaluated	213/22	223/22	216/23	228/22	
foetuses/litter	9/20	10/13	11/16	12/23	
- # hyoid centrum: incomplete ossification (number litter/fetuses affected)	8/18	8/11	7/10	10/19	
- # 5th and 6th sternebra: uncomplete ossification	1/2	2/2	0/0	3/5	
- pubis (uni/bi): incomplete ossification	21/85	22/87	19/85	17/95	
- insertion point (uni/bi) of pelvic girdle on 2nd sacral vertebra					
Visceral findings	No substance-related findings				

dr dose related

d/i decreased/increased, but not statistically significantly compared to the controls

Table 119: Mean maternal body weight gain (kg)

Interval	**Dose level of BYI 02960 (mg/kg/day)**				**Historical control range**
	0	**7.5**	**15**	**40**	
Number of dams (pregnant)	23	22	23	22	
Pretreatment, GD 3-6:	0.03 (±0.076)	0.03 (±0.073)	0.01 (±0.075)	0.02 (±0.070)	-0.067 to 0.046
Treatment, GD 6-8:	0.02 (±0.042)	0.02 (±0.035)	0.00 (±0.031)	-0.01 (±0.060)	-0.026 to 0.025
Treatment, GD 8-10:	0.04 (±0.046)	0.04 (±0.033)	0.03 (±0.025)	0.03 (±0.039)	-0.003 to 0.048
Treatment, GD 10-14:	0.03 (±0.081)	0.09 (±0.056) **	0.07 (±0.049) *	0.06 (±0.054)	0.023 to 0.088
Treatment, GD 14-18:	0.03 (±0.075)	0.05 (±0.054)	0.07 (±0.045)	0.04 (±0.049)	0.046 to 0.101
Treatment, GD 18-22:	0.04 (±0.046)	0.05 (±0.046)	0.03 (±0.042)	0.06 (±0.061)	0.031 to 0.083
Treatment, GD 22-26:	0.06 (±0.080)	0.04 (±0.067)	0.04 (±0.050)	0.07 (±0.058)	0.000 to 0.087
Treatment, GD 26-29:	0.06 (±0.069)	0.04 (±0.059)	0.03 (±0.049)	0.04 (±0.076)	-0.002 to 0.065
Treatment, GD 6-29	0.28 (±0.228)	0.31 (±0.111)	0.28 (±0.160)	0.28 (±0.116)	0.221 to 0.439
Corrected BW change	-0.25 (±0.177)	-0.24 (±0.110)	-0.25 (±0.157)	-0.28 (±0.126)	-0.25 to -0.08

a Data obtained from Table 3 in the study report.
* Statistically different ($p \leq 0.05$) from the control.
** Statistically different ($p \leq 0.01$) from the control.

Table 120: Ceasarean section observations

Observation	Dose Level of BYI 02960 (mg/kg/day)				Historical control range
	0	7.5	15	40	
Maternal data:[a]					
No. Animals assigned	23	23	23	23	NA
No. Animals pregnant	23	22	23	22	NA
Pregnancy rate, %	100	96	100	96	NA
No. Animals non-pregnant	0	1	0	1	NA
Maternal wastage					
Total No. intercurrent death or sacrifice (pregnant & non pregnant)	0	0	0	0	NA
Total No. intercurrent death or sacrifice (pregnant)	0	0	0	0	NA
Total No. intercurrent death or sacrifice (non pregnant)	0	0	0	0	NA
No. premature delivery	0	0	0	0	NA
No. abortion	1	0	0	0	NA
Uterine data at scheduled sacrifice:[b]					
Total No. corpora lutea[c]	253	280	265	283	NA
Corpora lutea / dam	11.5 ± 1.8	12.7 ± 1.9	11.5 ± 1.6	12.9 ± 2.6	10.79 - 13.21
Total No. implantations[c]	231	239	235	254	NA
Implantations / dam	10.5 ± 2.1	10.9 ± 2.3	10.2 ± 2.0	11.5 ± 2.9	9.10 - 10.95
Total No. litters[c]	22	22	23	22	NA
Total No. live fetuses[c]	213	223	216	228	NA
Live fetuses / dam[c]	9.7 ± 2.5	10.1 ± 2.1	9.4 ± 1.8	10.4 ± 2.4	8.23 - 9.90
Total No. dead fetuses	8	5	7	10	NA
Dead fetuses / dam[c], %	3.2 ± 6.8	2.0 ± 4.3	2.9 ± 5.4	3.8 ± 6.0	0.74 - 6.58
Total No. early resorptions[c]	7	9	9	13	NA
Total No. late resorptions[c]	3	2	3	3	NA
Early resorptions / dam	0.3 ± 0.8	0.4 ± 0.7	0.4 ± 0.5	0.6 ± 0.6	0.167 - 1.000
Late resorptions / dam	0.1 ± 0.5	0.1 ± 0.3	0.1 ± 0.5	0.1 ± 0.5	0.000 - 0.333
Litters with total resorptions[c, d]	0	0	0	0	NA
Pre-implantation loss per litter, %	9.2 ± 8.5	14.5 ± 13.6	11.0 ± 14.1	10.9 ± 11.0	9.08 - 26.73
Post-implantation loss per litter, %	8.7 ± 13.0	6.1 ± 9.2	7.4 ± 9.2	9.7 ± 9.5	4.07 - 19.25
Mean fetal weight, combined sexes, g	37.23 ± 6.20	38.07 ± 3.44	39.14 ± 4.26	37.89 ± 4.69	34.96 - 41.37
Mean fetal weight, males, g	37.31 ± 6.49	38.66 ± 4.05	39.48 ± 4.50	38.04 ± 5.77	34.92 - 42.33
Mean fetal weight, females, g	36.52 ± 6.86	37.60 ± 3.54	38.81 ± 4.41	37.75 ± 4.57	34.57 - 40.85
Sex ratio, % male	48.4 ± 18.6	52.4 ± 15.2	50.0 ± 16.4	47.4 ± 20.7	44.0 - 53.8

a Data obtained from Table 1 in the Tables and Appendices section of the study report.
b Data obtained from Tables 8 and 9 and Appendix F in the Tables and Appendices section of the study report.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.
c Statistical analysis was not conducted on this endpoint.
d Also includes litters with dead fetuses only or dead fetuses and resorptions.
HCD Historical control range (lowest – highest) of main uterine parameters from Attachment 3 in the Tables and Appendices section of the study report.
NA Not applicable.

Table 121: Skeletal examinations

Dose level of BYI 02960 (mg/kg/day)	0	7.5	15	40	Historical Control Range	0	7.5	15	40	Historical Control Range
	Number of litters examined					Number of fetuses examined				
	22	22	23	22		213	223	216	228	
	Number of litters examined					Number of heads examined				
	22	22	23	22		112	117	115	119	
Observations #	Number of litters affected (% of litters affected)					Number of fetuses affected (% of fetuses affected)				
Variations Hyoid centrum: incomplete ossification or unossified.	9 (40.9)	10 (45.5)	11 (47.8)	12 (54.5)	(4.8 – 63.6)	20 (17.9)	13 (11.1)	16 (13.9)	23 (19.3)	(1.1 – 28.6)
5th and 6th sternebrae: incomplete ossification.	8 (36.4)	8 (36.4)	7 (30.4)	10 (45.5)	(14.3 – 45.8)	18 (8.5)	11 (4.9)	10 (4.6)	19 (8.3)	(2.1 – 12.6)
Pubis (uni/bi): incomplete ossification.	1 (4.5)	2 (9.1)	0 (0.0)	3 (13.6)	(0.0 – 27.3)	2 (0.9)	2 (0.9)	0 (0.0)	5 (2.2)	(0.0 – 4.3)
Insertion point (uni/bi) of pelvic girdle on 2nd sacral vertebra.	21 (95.5)	22 (100)	19 (82.6)	17 (77.3)	(66.7 – 95.8)	85 (39.9)	87 (39.0)	85 (39.4)	95 (41.7)	(28.2 – 56.7)

a Study data obtained from Table 13 and historical control range from Attachment 4 in the Tables and Appendices section of the study report. Statistical analysis was conducted on all these observations.
\# Statistical analysis was conducted on the observations (Appendix M).
* Statistically different ($p \leq 0.05$) from the control.
** Statistically different ($p \leq 0.01$) from the control.

There were no treatment-related mortalities or clinical signs at any dose level tested.

In the high-dose group mean maternal body weight between GD 6 and 10 was lower than in controls ($p \leq 0.05$). Thereafter mean body weight gain was comparable to the controls throughout all intervals. Mean maternal corrected body weight change was reduced by 12% compared to the controls (not statistically significant) and slightly outside the range of in-house historical control data. Food consumption was transiently reduced by 20% between GD 6 and 8 ($p \leq 0.01$) and by 11% between GD 8 and 10 (not statistically significant). These effects on (corrected) body weight gain and food consumption were slight, and mainly transient.

At 15 and 7.5 mg/kg bw/day no treatment-related effects were observed on mean maternal body weights, body weight gains and maternal corrected body weight change. Mean maternal food consumption was similar to the controls.

There were no treatment-related macroscopic changes in the dams of any dose levels observed at necropsy. Mean liver weight was similar to the controls at all dose levels.

Pregnancy rate was similar in all groups. No treatment-related changes were noted on litter parameters, including the number of live fetuses, the number of implant sites per dam, the percentages of pre- and post-implantation losses, the number of early and late resorptions, the fetal death status, the fetal body weight and sex ratio.

There were no treatment-related malformations or variations noted at the fetal external examination. At 40 mg/kg/day, the incidence of the variation "eye bulge (uni/bi): protruding" was higher than in the control group and was outside the range of in-house HCD at the fetal level. However, as the observation was noted in only one litter and as the finding was not corroborated at the internal and skeletal observations, this increased incidence was considered to have occurred by chance.

There were no treatment-related malformations or variations noted at the fetal visceral and skeletal examinations. The observed skeletal variations (see

Table 121) were not statistically different from the controls and were well within the in-house historical control data. Therefore they were not considered to be treatment-related.

Historical control data were generated from 14 developmental toxicity studies with New Zealand White Crl: KBL (NZW) rabbits performed by the testing laboratory in the period of March 2000 – October 2009.

Acceptability
The study is considered acceptable.

Conclusions
The effects on (corrected) body weight gain and food consumption were slight and mainly transient, however considered adverse (>10%).The NOAEL for maternal toxicity and developmental toxicity was set at 15 mg/kg bw/day.

Developmental neurotoxicity

Study 1

Characteristics

reference	:	Gilmore R.; 2012	exposure	:	Dietary, GD 6 -LD 21
type of study	:	A Developmental Neurotoxicity Study	doses	:	0, 120, 500 and 1200 ppm
year of execution	:	2012	vehicle	:	None
test substance	:	BYI 02960 (batch number 2009-000239; purity 96.2%)	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 426
species	:	Rat, Wistar Rj:WI (IOPS HAN)	acceptability	:	Acceptable
group size	:	30 females/dose	NOAEL maternal	:	500 ppm (42.4 mg/kg bw/d)
			NOAEL developmental		500 ppm (42.4 mg/kg bw/d)
			NOAEL developmental neurotoxicity		500 ppm (42.4 mg/kg bw/d)

Study design

The study was performed according to OECD 426. BYI 02960 was administered via the diet from gestation Day (GD) 6 through lactation Day (LD) 21 to mated female Wistar rats at nominal concentrations of 0, 120, 500, or 1200 ppm with adjustment during lactation to maintain a more consistent dosage throughout the period of exposure. The average mean daily intake of the test substance (mg BYI 02960/kg bw/day) based on the average dietary consumption for the last two weeks of gestation and three weeks of lactation at 120, 500, or 1200 ppm, respectively, was 0, 10.3, 42.4, and 102 mg/kg bw/day. All test diets (including control) were provided for ad libitum consumption throughout the study, except during neurobehavioral testing. The Parental (P)-generation females were evaluated by cage-side and detailed clinical observations, body weight, food consumption, and reproductive endpoints. On postnatal Day (PND) 4, litters with a minimum of seven pups, including at least three per sex, were culled to yield, as closely as possible, four males and four females. Subsets of surviving offspring, representing 19–20 litters per dietary level, were subjected to evaluation using the following observations and measurements: detailed clinical observations and a detailed observational battery, pupil response, surface righting, balanopreputial separation or vaginal patency, body weight, food consumption, automated measures of activity (figure-eight maze), auditory startle habituation, learning and memory (passive avoidance after weaning and a water maze task beginning on PND 60 ± 2 days), and an ophthalmic examination. Neural tissues were collected from 10 rats/sex/dietary level (representing 20 litters) on PND 21 (brain only) and at study termination (approximately 75 days of age) for microscopic examination and morphometry.

Results

The results are summarised in *Table 122* (parental animals) and *Table 125* (offspring).

Parental animals

Table 122: The results of the parental females

Dose (ppm)	**0**	**120**	**500**	**1200**
	f	**f**	**F**	**f**
Mortality	No P-generation females were found dead during gestation or lactation.			
Clinical signs				
Gestation (Days 6–24)				
- Red Vaginal Discharge	0/30	0/30	1/30	0/30
- Hair loss	1/30	1/30	2/30	3/30
- Fused Digits	1/30	0/30	0/30	0/30
Lactation (Days 0 – 21)	1/27	1/29	3/29	4/30
- Hair loss	1/27	0/29	0/29	0/30
- Fused Digits				
Detailed observational battery	No test substance-related findings			
Body weight (g) (Gestation)				
GD 20				ds (-7%)
GD 0-20				ds (-21%)
Body weight (g) (Lactation)				
LD 0				ds (-4%)
LD 4				ds (-6%)

Dose (ppm)	**0**	**120**	**500**	**1200**
	f	**f**	**F**	**f**
Reproductive performance -	No test substance-related findings			

i/d: increased/decreased
is/ds: statistically significantly increased/decreased

Table 123: Mean (±S.E.) Maternal Body Weight and Food Consumption

Observations/Study Week	**Dietary Level of BYI 02960**			
	Control	**120 ppm**	**500 ppm**	**1200 ppm**
Gestation				
Mean Body Weight (g) GD 0	206.4±2.18 (27)	208.0±2.54 (29)	209.6±2.36 (29)	209.8±2.59 (30)
Mean Body Weight (g) GD 6	229.1±2.71 (27)	230.8±2.96 (29)	231.2±3.46 (29)	233.1±2.72 (30)
Mean Body Weight (g) GD 13	254.5±3.74 (27)	256.0±3.58 (29)	256.0±3.27 (29)	245.3±2.92 (30)
Mean Body Weight (g) GD 20	320.9±4.09 (27)	314.7±5.14 (28)	318.7±4.26 (29)	299.9**±4.05 (29)4%)
Mean Weight Gain (g) GD 0–20	114.5±2.80 (27)	107.5±3.38 (28)	109.1±2.59 (29)	90.3**±2.57 (29)
Mean Food Consumption (g/animal/day) GD 6–13	20.1±0.73 (27)	19.1±0.47 (28)	18.9±0.47 (29)	18.4±0.59 (30)
Mean Food Consumption (g/animal/day) GD 13–20	20.3±0.37 (27)	20.1±0.43 (27)	20.2±0.40 (29)	19.2±0.48 (29)
Lactation				
Mean body weight (g) LD 0	244.0±3.30 (27)	245.2±3.85 (29)	242.9±3.43 (29)	233.3*±2.76 (30)
Mean body weight (g) LD 4	262.0±3.19 (25)	256.7±4.48 (23)	256.1±3.16 (26)	247.2**±3.62 (25)
Mean body weight (g) LD 7	265.4±2.98 (23)	263.2±3.89 (23)	263.4±3.36 (23)	255.7±3.46 (23)
Mean body weight (g) LD 14	279.6±3.17 (23)	274.6±4.05 (23)	275.2±3.30 (23)	266.6±2.86 (23)
Mean body weight (g) LD 21	261.1±5.87 (23)	264.1±4.50 (23)	254.2±3.88 (23)	246.5±6.38 (23)
Mean food consumption (g/animal/day) LD 0–7	36.0±1.05 (23)	35.3±1.01 (23)	34.1±1.14 (23)	34.0±0.80 (23)
Mean food consumption (g/animal/day) LD 7–14	50.8±0.84 (23)	50.3±1.08 (23)	50.7±0.86 (23)	50.3±1.00 (23)
Mean food consumption (g/animal/day) LD 14–21	55.9±1.76 (23)	58.1±1.12 (23)	54.7±1.06 (23)	54.5±1.35 (23)

Values are mean ± S.E. (*n*). Means for gestation include only dams found sperm positive and with pups at termination of gestation.
* Statistically different from control, Dunnett's Test $p \leq 0.05$; ** Statistically different from control, Dunnett's Test $p \leq 0.01$

Table 124: Reproductive Performance

Observation	Dietary Level of BYI 02960			
	Control	**120 ppm**	**500 ppm**	**1200 ppm**
No. of Animals Co-housed [a]	30	30	30	30
No. of Animals Mated	30	30	30	30
Maternal Wastage				
No. of Dams not Pregnant	3	1	1	0
No. of Dams that Delivered Dead Pups	1	0	0	2
No. of Dams with Pre-Mature Delivery	0	0	0	0
Mating Index	100.0	100.0	100.0	100.0
Fertility Index (No. of pregnant females/No. of inseminated females X 100)	90.0	96.7	96.7	100.0
Gestation Length (days) [b]	21.7±0.10 [22.0] (21.0–22.0)	21.7±0.13 [22.0] (20.0–23.0)	21.8±0.13 [22.0] (21.0–23.0)	21.9±0.16 [22.0] (20.0–23.0)

[a] Number of animals assigned to each dietary level.
[b] Values are mean ± S.E., [median], and (range).
Values were not statistically different from control, $p \leq 0.05$ or $p \leq 0.01$

During gestation, findings considered incidental and unrelated to the test substance included red vaginal discharge on GD 13 (1 mid-dose dam), fused digits (1 control dam), and hair loss (1 to 3 dams from all dietary levels, including controls), which is a common finding associated with nest-building behavior in pregnant rats. During lactation, findings that are considered incidental and unrelated to the test substance included fused digits (1 control dam) and areas of hair loss (1 to 4 dams at all dietary levels, including control).
During gestation, there was a statistical decrease (-7%), relative to controls, in mean body weight on GD 20 and a statistical decrease (-21%) in overall mean weight gain during gestation (GD 0-20) at the 1200 ppm dietary level. There were statistical decreases in body weights on LD 0 and 4 (-4% and -6%, respectively), relative to controls, at the 1200 ppm dietary level, and non-statistical decreases (-4% to -6%) that continued through termination on LD 21 at the 1200 ppm dietary level. There were no effects on body weight or body weight gain during gestation or lactation at any other dietary level.

Offspring

Table 125: The results of the offspring

Dose (ppm)	**0**		**120**		**500**		**1200**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Litter size and viability	No substance-related findings							
Clinical signs	There were no test substance-related clinical signs during lactation or postweaning in males or females at any dietary level.							
Body weight								
PND 17							d (-5%)	d (-5%)
PND 21							d (-5%)	d (-4%)
PND 4-17							ds(-7%)	d (-6%)
PND 11-17							ds(-10%)	ds (-7%)

Dose (ppm)	**0**		**120**		**500**		**1200**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Body weight Post weaning (PND 28-70)	No difference in body weight after weaning for males and females							
Developmental landmarks - Balanopreputial separation - Vaginal opening - Surface righting - Pupil constriction	No substance-related findings							
Detailed observational battery	No substance-related findings							
Motor activity PND 13 PND 17 PND 21 PND 60			 i i	d i			i i i	 i
Auditory startle habituation PND 23 PND 60								 is
Learning and memory - Passive avoidance - Water maze	There were no differences in acquisition or retention attributable to exposure to the test substance in either sex at any dietary level							
Ophthalmology	No substance-related findings							
Gross Pathology	No substance-related findings							
Terminal body weight PND 21 PND 75 (non-perfused)				 d (-4%) d (-5%)		 d (-7%) 	 d (-8%) d (-5%)	 d (-8%) d (-5%)
Brain weight PND 75 (non-perfused)							 d (-5%)	
Gross Brain measurements	No substance-related findings							
Micropathology brain measurements	No substance-related findings							

i/d: increased/decreased
is/ds: statistically significantly increased/decrease

Table 126: Litter Size and Viability

Observation	Dietary Level of BYI 02960			
	Control	120 ppm	500 ppm	1200 ppm
No. of Litters	23	23	23	23
Total No. of Pups Born	269	265	275	255
Total No. of Pups Missing	0	0	0	1
Litters with Pups Missing	0	0	0	1
Total No. of Pups Found Dead	2	2	0	0
Litters with Pups Found Dead	2	2	0	0
Total No. of Pups Cannibalized	0	0	0	0
Litter with Pups Cannibalized	0	0	0	0
Litter Size	11.7±0.34 [12.0] (8.0–15.0)	11.5±0.39 [12.0] (8.0–14.0)	12.0±0.36 [12.0] (8.0–15.0)	11.1±0.38 [11.0] (7.0–14.0)
Stillborn Pups Number % Mean±S.E. [Median] (Range)	 1 0.4 0.0±0.04 [0.0] (0.0–1.0)	 0 0.0 0.0±0.00 [0.0] (0.0–0.0)	 0 0.0 0.0±0.00 [0.0] (0.0–0.0)	 2 0.8 0.1±0.06 [0.0] (0.0–1.0)
Mean No. of Viable Pups Birth Day 4 (Pre-cull) [a] Day 4 (Post-cull) [b] Day 21	 12 12 8 8	 12 11 8 8	 12 12 8 8	 11 11 8 8
Live Birth Index [c]	99.6±0.43 [100.0] (90-100)	100.0±0.00 [100.0] (100-100)	100.0±0.00 [100.0] (100-100)	99.3±0.51 [100.0] (91–100)
Viability Index [c]	99.3±0.51 [100.0] (91–100)	99.1±0.64 [100.0] (89–100)	100.0±0.00 [100.0] (100–100)	100.0±0.00 [100.0] (100–100)
Lactation Index [c]	100.0±0.00 [100.0] (100–100)	100.0±0.00 [100.0] (100–100)	100.0±0.00 [100.0] (100–100)	99.5±0.54 [100.0] (88–100)

[a] Before standardization (culling) [b] After standardization (culling) [c] Values are mean ± S.E., [median], (range)
Values were not statistically different from control, $p \leq 0.05$ or $p \leq 0.01$

Table 127: Mean (± S.E.) Preweaning Pup Body Weights and Body Weight Change (g)

Postnatal Day	Dietary Level of BYI 02960							
	Control	120 ppm	500 ppm	1200 ppm	Control	120 ppm	500 ppm	1200 ppm
	Males				Females			
0	5.9±0.13 (23)	5.9±0.10 (23)	6.0±0.10 (23)	6.0±0.10 (23)	5.6±0.11 (23)	5.7±0.10 (23)	5.7±0.08 (23)	5.7±0.11 (23)
4 [a]	9.9±0.25 (23)	9.8±0.20 (23)	9.9±0.24 (23)	10.0±0.26 (23)	9.5±0.23 (23)	9.5±0.20 (23)	9.4±0.21 (23)	9.5±0.28 (23)
4 [b]	10.0±0.25 (23)	9.8±0.20 (23)	9.9±0.24 (23)	9.9±0.26 (23)	9.5±0.24 (23)	9.5±0.20 (23)	9.4±0.21 (23)	9.4±0.28 (23)
11	25.4±0.48 (23)	24.9±0.46 (23)	25.7±0.44 (23)	24.5±0.49 (23)	24.5±0.44 (23)	24.3±0.46 (23)	24.6±0.42 (23)	23.7±0.47 (23)
17	38.9±0.65 (23)	37.9±0.55 (23)	39.2±0.56 (23)	36.8±0.60 (23)	37.5±0.56 (23)	37.0±0.59 (23)	37.6±0.47 (23)	35.8±0.54 (23)
21	47.9±1.04 (23)	48.4±0.85 (23)	47.6±0.66 (23)	45.6±1.02 (23)	46.3±1.01 (23)	47.2±0.96 (23)	45.4±0.65 (23)	44.4±0.83 (23)
Body Weight Change								
	Males				Females			
4–17	28.9±0.56 (23)	28.1±0.45 (23)	29.3±0.41 (23)	26.9±0.51* (23)	27.9±0.50 (23)	27.5±0.48 (23)	28.2±0.33 (23)	26.3±0.43 (23)

4–21	37.9±0.88 (23)	38.6±0.73 (23)	37.7±0.57 (23)	35.7±0.87 (23)	36.8±0.88 (23)	37.7±0.82 (23)	36.0±0.56 (23)	34.9±0.68 (23)
11–17	13.6±0.31 (23)	13.0±0.20 (23)	13.5±0.23 (23)	12.3±0.25** (23)	13.0±0.29 (23)	12.7±0.22 (23)	13.0±0.21 (23)	12.1±0.18* (23)
11–21	22.5±0.75 (23)	23.5±0.47 (23)	22.0±0.52 (23)	21.1±0.68 (23)	21.9±0.77 (23)	22.9±0.56 (23)	20.8±0.51 (23)	20.7±0.58 (23)

Values are mean ± S.E. (*n*)
[a] Before standardization (culling). * Values were statistically different from control, $p \leq 0.05$
[b] After standardization (culling). ** Values were statistically different from control, $p \leq 0.01$

Table 128: Mean (±S.D.) Motor Activity Data (total activity counts for session)

Test Day	Dietary Level of BYI 02960			
	Control	120 ppm	500 ppm	1200 ppm
Males				
PND 13	55±55 (20)	66±82 (20)	63±66 (20)	114±130 (20)
PND 17	146±117 (20)	260±104 (20)	173±114 (20)	221±144 (20)
PND 21	307±119 (20)	367±132 (20)	331±149 (20)	367±123 (20)
PND 60	503±142 (20)	518±108 (20)	505±121 (20)	529±111 (20)
Females				
PND 13	74±141 (20)	88±81 (19)	68±60 (20)	62±40 (20)
PND 17	201±152 (20)	149±112 (19)	243±121 (20)	227±200 (20)
PND 21	303±88 (20)	333±140 (19)	308±132 (20)	322±119 (20)
PND 60	656±143 (20)	666±233 (19)	624±202 (20)	790±232 (20)

Values are mean ± S.D. (*n*) Values were not statistically different from control, $p \leq 0.05$

Table 129: Auditory Startle Reflex Peak Amplitude Data (g, mean ± S.D.)

Block		Dietary Level of BYI 02960			
		Control	120 ppm	500 ppm	1200 ppm
Males					
PND 23	Block 1	44±18	48±13	43±18	46±22
	Block 2	43±16	50±13	42±23	41±15
	Block 3	39±16	48±15	40±23	42±14
	Block 4	40±17	48±14	38±17	41±15
	Block 5	37±16	45±15	37±14	35±12
	Avg. For Total Session	41±15	48±12	40±18	41±13
	No. Of Animals	20	20	20	20
	Body Weight	60	60	60	57
PND 60	Block 1	270±115	301±159	241±151	324±162
	Block 2	255±161	299±190	203±141	289±215
	Block 3	200±140	245±157	175±136	214±183
	Block 4	187±119	214±140	152±130	177±163
	Block 5	145±92	187±131	134±116	144±108
	Avg. For Total Session	211±115	249±145	181±127	230±153
	No. Of Animals	20	20	20	20

Block		Dietary Level of BYI 02960			
		Control	**120 ppm**	**500 ppm**	**1200 ppm**
	Body Weight	292	291	280	285
Females					
PND 23	Block 1	39±22	38±16	35±21	45±17
	Block 2	39±22	36±16	36±18	43±17
	Block 3	36±19	37±18	34±18	43±17
	Block 4	32±14	37±17	31±18	39±16
	Block 5	29±15	34±15	28±14	36±16
	Avg. For Total Session	35±17	36±14	33±17	41±15
	No. Of Animals	20	20	20	20
	Body Weight	57	58	56	57
PND 60	Block 1	102±74	133±75	140±91	186±129
	Block 2	94±82	121±94	127±84	181±126 ***
	Block 3	71±73	108±86	113±76	160±116 ***
	Block 4	68±54	84±58	96±65	132±87 ***
	Block 5	58±33	74±46	76±50	122±83 ***
	Avg. For Total Session	78±57	104±65	110±68	156±102 ***
	No. Of Animals	20	20	20	20
	Body Weight	185	180	184	174

Values are mean ± S.D. *** Statistically different from control, ANOVA $p \leq 0.05$.

Body weight was non-statistically decreased in high-dose males and females on PND 17 and 21 (-4% to -5%). Although these differences from control are small and not statistically significant, they are attributed to the test substance because of the relationship with dose (both genders and at multiple time points). These lower body weights were comparable to the 4–6% lower body weight for the high-dose dams throughout lactation. Body weight was not affected by the test substance in either sex at any other dietary levels. Body weight gain for PND 4–17 was statistically decreased in high-dose males (-7%). Body weight gain for the shorter period of PND 11–17 was statistically decreased in high-dose males (-10%), females (-7%). There were similar non-statistical trends in body weight gain for PND 4–17 in high-dose females (-6%), for PND 4–21 and PND 11–21 in both sexes. These differences from control are attributed to the test substance. Body weight gain was not affected by the test substance in either sex at the 120 and 500 ppm dietary levels. There was no difference in body weight after weaning for males and females at any dietary level.

A non-statistical increase in both motor and locomotor activity was measured in high-dose males on PND 13. These differences from control are considered test substance-related, but are also partially attributed to a low mean average activity for controls (55 and 5, for motor and locomotor, respectively), which was below the range for historical controls (59–72 and 6–11, respectively) in the last five studies conducted at this laboratory. An increase in motor activity was measured in low- and high-dose males on PND 17. These differences from control are not attributed to the test substance, since there was no dose-related pattern and the increases were not observed in mid-dose males or in females. Additionally, the increases were not observed on any subsequent test occasions, and means for all groups were within the range of historical controls. An increase in locomotor activity was measured in low-, mid-, and high-dose males on PND 17. These differences from control are not attributed to the test substance, since there was no dose-related trend, increases

were not observed in females and the means were within the range of historical controls. Lastly, very slight increases in locomotor activity were measured in low-dose males on PND 21 and high-dose females on PND 60. These are not considered test substance-related since motor activity for these same animals was generally comparable to controls, other dose levels were not similarly affected, and the differences were seen in only one sex.

In the auditory startle habituation test startle amplitude for all 50 trials was statistically increased two-fold in high-dose females (156 g), compared to controls (78 g) on PND 60. This was reflected as a non-statistical increase in response amplitude for the first block of trials, and a statistical increase in blocks 2–5, relative to control. This finding is considered to be related to the test substance. There was a non-statistical trend involving a slight increase in average startle amplitude in all five blocks of trials, in females at the low- and mid-dose, relative to controls, on PND 60. This difference from control is not thought to be test substance-related since the slight increase was similar in both dose levels with a 4-fold increase in dose and the mean average for startle response amplitude in control animals (78 g) is below the range for historical controls (87–116 g) in the last five studies conducted in this laboratory. There were no differences in startle amplitude in male and female weanlings or in adult males at any dietary level age. Startle latency and habituation were not affected by the test substance in males or females at any dietary level, on any test occasion.

Test substance-related mean terminal body weight changes for PND 21 pups were noted in males at 1200 ppm dietary level (non-statistically decreased, 8%, as compared to controls) and in females at 120, 500, and 1200 ppm dietary levels (non-statistically decreased, -5, -7%, and -8%, respectively, as compared to controls).
Terminal body weights are collected once at termination from a sub-group of animals in each dose level (i.e., those animals scheduled for necropsy; 10/sex/dietary level). In-life body weights are collected from all animals at selected time points throughout the in-life phase of the study (e.g., mean litter weight is calculated using 23 litters/level). Although the sub-group of animals weighed at termination did indicate an apparent effect on body weight, in-life data clearly indicates no effect on body weight (compared to controls) in females from the 120 and 500 ppm dietary levels.
There was no test substance-related terminal body weight change noted at any dietary level in the PND 75 (± 5 days) perfused rats.
There was a slight trend of decline in terminal body weights at 1200 ppm males (-5%, as compared to controls) in the PND 75 (± 5 days) non-perfused rats. There was a decrease (-5%) in terminal body weights observed in 120 and 1200 ppm females; however, these changes were not considered test substance-related due to lack of dietary relationship or the changes were small.

There was a decrease (-5%) in fresh brain weights at 1200 ppm males as compared to controls in the PND 75 (± 5 days) Rat**,** however, this change was not considered directly related to test substance administration since it was related to the decrease in terminal body weights.
The brain weight range for the non-perfused males was 1.769 g to 2.050 g and the range for the non-perfused 1200 ppm treated males was 1.739 g to 1.993 g with only two animals with a brain weight outside the control range.

Acceptability
The study is considered acceptable.

Conclusions

Dietary exposure to BYI 02960 from GD 6 through LD 21 to mated female Wistar rats resulted in treatment-related effects (maternal: decreased body weight gain. Offspring: decreased body weight gain, slightly increased in motor and locomotor activity, slightly increased auditory startle habituation) evident only at 1200 ppm (102 mg/kg bw/day) in maternal animals and offspring. The maternal NOAEL was 500 ppm (42.4 mg/kg bw/d) based on decreased body weight and body weight gain. The developmental NOAEL was 500 ppm (42.4 mg/kg bw/d) based on decreased offspring body weight and body weight gain. The developmental neurotoxicity NOAEL was 500 ppm (42.4 mg/kg bw/d) based on increased startle amplitude in females only on PND 60 and increased motor and locomotor activity on PND 13 in males only.

4.11.2.2 Human information

4.11.3 Other relevant information

4.11.3.1 *In vitro* endocrine disruption assays

Study 1

Study design and results

Type of study: Estrogen receptor transactivation assay

Indicator cells	Endpoint	Response	Dose range	Reference
Human Cell Line (HeLa-9903)	Estrogen receptor alpha (hERα) binding	- (all conc)	10^{-10} – 10^{-3} M in steps of 10	Willoughby, Sr., J.A. (2015a)

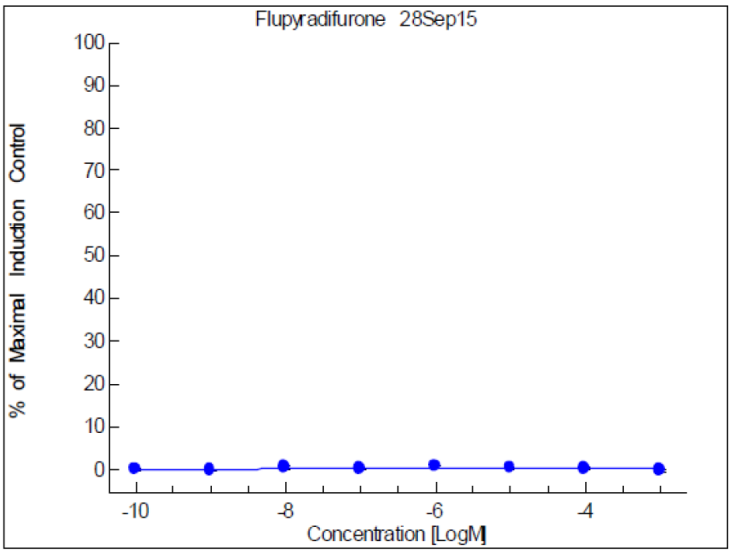


Figure 5: Representative binding plot for flupyradifurone in the estrogen receptor transactivation assay (6 duplicates/concentration)

In two independent runs of the transcriptional activation assay, flupyradifurone did not result in an increase in luciferase activity at any of the concentrations tested.

Acceptability
The study is considered acceptable.

Conclusions
Flupyradifurone is not an agonist of human estrogen receptor alpha (hERα) in the HeLa-9903 model system.

Study 2

Study design and results

Type of study: Androgen Receptor Binding assay

Indicator cells	Endpoint	Response	Dose range	Reference
Rat prostate cytosol	Androgen receptor binding	+/- 10^{-3} - 10^{-4} – 10^{-10}	10^{-10} – 10^{-3} M in steps of 10	Willoughby, Sr., J.A. (2015b)

Table 130: Results of the Androgen receptor binding assay

Test Substance	Concentration (Log[M])	Specific Binding (%) (run 1)	Standard Deviation (run 1)	Specific Binding (%) (run 2)	Standard Deviation (run 2)	Specific Binding (%) (run 3)	Standard Deviation (run 3)
Flupyradifurone	-3	76.1	0.7	74.9	2.6	73.6	1.3
	-4	100.3	1.8	101.0	1.7	99.7	0.4
	-5	104.5	0.6	101.9	2.4	102.9	2.1

(BYI 02960)	-6	106.2	2.7	102.5	3.6	105.0	1.4
	-7	107.5	1.2	109.2	4.2	104.4	1.5
	-8	98.1	3.4	97.8	0.9	99.4	2.8
	-9	100.1	2.0	101.2	2.1	100.6	0.6
	-10	101.7	2.2	103.6	2.7	102.8	3.4
R1881 (NSB)	-6	0.0	2.6	0.0	1.2	0.0	0.4
	-7	3.7	0.3	1.9	0.1	1.6	0.4
	-8	14.9	0.5	12.9	0.4	11.9	0.8
	-9	59.8	1.6	58.3	1.4	57.0	1.2
	-10	99.2	0.3	97.0	3.9	92.6	2.2
	-11	107.2	3.8	108.4	5.9	101.1	0.3
Dexamethasone	-3	7.8	0.3	6.1	0.6	6.3	0.1
	-4	37.4	1.4	38.5	0.2	35.6	1.1
	-5	87.2	0.8	86.5	1.0	85.4	1.8
	-6	102.0	1.9	100.2	3.1	97.2	2.5
	-7	106.1	2.6	103.6	1.9	103.9	1.6
	-8	105.6	2.1	107.4	2.3	102.9	2.7
	-9	104.9	3.7	104.2	3.4	101.2	2.5
	-10	100.5	0.8	109.5	18.9	120.7	27.6

The androgen receptor binding assay was performed two times, both included three independent runs. The outcome of the first three runs was not valid, due to an error in the preparation of the stock solution of flupyradifurone, which resulted in concentrations that were ~15% higher than intended. The outcome of the second trio of runs was considered valid. The first run was negative, the second and third tested equivocal. The receptor binding in all three runs was very close to the cutoff value of >75% (76.1%, 74.9%, and 73.6%, respectively). Only the highest concentration (10^{-3} M) was equivocal, the lower concentrations were all negative.

(It is also interesting to mention the results of the first three runs, which were not included in the results. Although they were not taken into account due to an error, the only deviation was the ~15% higher concentration of flupyradifurone. In all three runs the highest concentration (10^{-3} M) tested equivocal, with mean specific bindings of 71.4%, 74.0%, and 73.7%. This indicates that the observed effect is weak, but reproducible.)

Acceptability
The study is considered acceptable.

Conclusions
The overall result of the androgen receptor binding assay was categorized as equivocal.

Study 3

Study design and results

Type of study: Steroidogenesis assay

Indicator cells	Endpoint	Response	Dose range	Reference
Human Cell Line H295R	Testosterone and estradiol production	+/- 10^{-4} - 10^{-5} – 10^{-10}	10^{-10} – 10^{-4} M in steps of 10	Franz-Jonas, B.L. (2015)

Table 131: Results for Testosterone

Concentration (µM)	Fold Change over SC Run 1		Fold Change over SC Run 2		Fold Change over SC Run 3	
	Mean	SD	Mean	SD	Mean	SD
0.0001	0.93	0.07	1.04	0.03	1.03	0.05
0.001	0.92*	0.04	0.97	0.03	1.02	0.03
0.01	0.96	0.02	1.03	0.02	1.01a	0.06a
0.1	0.93	0.05	1.01	0.02	1.03	0.05
1	0.93	0.12	1.04	0.02	1.01	0.04
10	0.92*	0.04	0.97a	0.05a	0.95	0.04
100	0.85*	0.02	0.87	0.03	0.81**	0.04

SC = Solvent Control
SD = Standard Deviation
a = Average of five replicates. One value was not reportable (NR) due to sample error.
*= Statistically Significant ($p<0.05$, Kruskal-Wallis/Dunn's test)
**= Statistically Significant ($p<0.05$, Dunnett's test)

Table 132: Results for Estradiol

Concentration (µM)	Fold Change over SC Run 1		Fold Change over SC Run 2		Fold Change over SC Run 3	
	Mean	SD	Mean	SD	Mean	SD
0.0001	0.93	0.07	1.03	0.04	1.06	0.03
0.001	0.91*	0.04	1.01	0.05	1.04	0.03
0.01	0.95	0.03	1.05	0.04	1.03a	0.08a
0.1	0.93	0.06	1.03	0.03	1.04	0.05
1	0.94	0.11	1.04	0.03	1.01	0.02
10	0.93	0.04	0.99a	0.07a	0.94	0.04
100	0.74*	0.01	0.82**	0.03	0.75***	0.04

SC = Solvent Control
SD = Standard Deviation
a = Average of five replicates. One value was not reportable (NR) due to sample error.
*= Statistically Significant ($p<0.05$, Kruskal-Wallis/Dunn's test)
**= Statistically Significant ($p<0.05$, Dunnett's test of log transformed data)
***= Statistically Significant ($p<0.05$, Dunnett's test)

The steroidogenesis assay was performed in three independent runs with concentrations of 10^{-10} to 10^{-4} M. A decrease in the production of testosterone was observed at the highest concentration in 2 out of 3 runs. This decrease was small, but significant (0.85-fold and 0.81-fold). In the first run, a decrease in testosterone was also observed at concentration levels of 10^{-5} and 10^{-9} M, but this was not reproducible in the other runs.

The production of estradiol was significantly inhibited at the highest concentration in all three runs (0.74, 0.82, 0.75-fold). There was also a decrease in the first run at 10^{-9} M, which was not reproducible.

Overall, the response of flupyradifurone in the steroidogenesis assay was positive according to the study guideline.

Acceptability
The study is considered acceptable.

Conclusions
A slight but statistically significant inhibition was consistently observed at the highest concentration tested (100 µM) for both testosterone and estradiol. Therefore, flupyradifurone is categorized as positive based upon the assay results of the steroidogenesis assay.

4.11.4 Summary and discussion of reproductive toxicity

The results of the reproduction toxicity and teratogenicity studies are summarised in *Table 133*.

Table 133: Summary of reproduction toxicity and teratogenicity studies

Type of study	Species		NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Critical effects	Reference
Reproduction toxicity						
A range-finding 1-generation toxicity study	rat	parental	17.5	60	Body weight (gain) ↓, food consumption ↓ , spleen weight ↓	Milius, A.D. 2010
		reproduction	147.5	-	-	
		Offspring	17.5	61	Body weight (gain) ↓, brain weight ↓	
2-generation toxicity study	Rat	parental	6.4	32	Body weight gain ↓ Liver and thyroid weight	Milius, A.D.. 2011

Type of study	Species		NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Critical effects	Reference
		reproduction	32	122.1	↑, centrilobular hypertrophy ↑	
		Offspring	6.4	32	Estrous cycle ↓, litter size ↓, implants ↓ Body weight ↓, physical development ↓	
Developmental neurotoxicity in the rat	Rat	maternal	42.4	102	body weight gain ↓	Gilmore R.; 2012
		developmental	42.4	102	body weight gain ↓	
		developmental neurotoxicity	42.4	102	motor/locomotor activity and startle habituation ↑	
Embryo/fetal toxicity and teratogenicity						
teratogenicity study	rat	maternal	50	150	Body weight ↓, liver weight ↑	Langrand-Lerche, A. D. 2010
		developmental	50	150	short costal cartilage ↑	
Maternal tolerability study	rat	maternal	30	-	-	Langrand-Lerche. C., 2012
teratogenicity study	rabbit	maternal	15	40	Body weight gain ↓	Kennel, P. 2012
		developmental	40	-	-	

In the rangefinder one generation rat reproduction study, BYI 02960 was administered continuously in the feed to Wistar rats (10 animals/dose/sex) at nominal dietary concentrations of 0, 200, 700, and 2000 ppm. There were no test substance-related effects on any reproductive parameter (e.g. mating, fertility, or gestation indices, days to insemination, gestation length, or the median number of implants) at any dietary level tested. The parental systemic LOAEL is 700 ppm (60.0 mg/kg bw/day females), based on decreased body weight, decreased body weight gain, alterations in food consumption during premating, and decreased spleen weights in females. The parental systemic NOAEL is 200 ppm (17.5 mg/kg bw/day).

The reproductive NOAEL is 147.5 mg/kg bw/day based on no reproductive findings observed at the highest dose tested.

Declines in absolute pup weight were observed beginning post-natal day (PND) 14 and continuing to PND 21 in the 700 and 2000 ppm dietary groups with significance only observed for the females. Body weight gain for the males and females was declined in both the 700 and 2000 ppm dose group (9.7% and 10.8%, respectively).

Significant changes in brain weight in the 2000 ppm dose group and slight changes in the 700 ppm dose group were observed (decreased absolute and increased relative) and are considered to be secondary to body weight declines observed in the pups at these same dose groups. There were no test substance-related gross necropsy findings observed at any dietary level tested. Based on these findings, the offspring LOAEL is 700 ppm (60.9 mg/kg bw/day) and the offspring NOAEL is 200 ppm (17.5 mg/kg bw/day).

In the rat two-generation reproduction study, BYI 02960 was administered continuously in the diet to Wistar rats (30 animals/dose/sex) at nominal dietary concentrations of 0, 100, 500, and 1800 ppm. A significant decrease in body weight, but <10% was observed in the mid-dose of the F1 females and a non-significant decrease in body weight gain was found in the parental animals (21 % in females) and the F1 animals (16 % in females). Therefore the parental systemic LOAEL is considered 500 ppm (32 mg/kg bw/d) based on decreased body weight gain in females. The parental systemic NOAEL is 100 ppm (6.4 mg/kg bw/d. The reproductive NOAEL was 500 ppm (39.6 mg/kg bw/day females) based on decreased estrous cycle number, litter size, and the number of implants observed in the F_1 generation at the highest dietary level tested (1800 ppm =143.4 mg/kg bw/day in females). A significant decrease in body weight, but <10% was observed in the mid-dose of the F2 pups. The offspring LOAEL is therefore 500 ppm (32 mg/kg bw/day) based on maternal effects leading to secondary effects on pup weight, and physical development. The offspring NOAEL is 100 ppm (6.4 mg/kg bw/day).

In a rat developmental study, BYI 02960 was administered daily by gavage to groups of 25 pregnant Sprague-Dawley female rats per dose-group at 0, 15, 50 and 150 mg/kg/day from gestation day (GD) 6 to 20. Minimal maternal toxicity was observed at the high-dose level, manifested as decreased body weight gain and reduced food consumption. The mean absolute liver weight was increased in the high dose group. Based on these findings the NOAEL for maternal toxicity was set at 50 mg/kg bw/day. The increased incidence of parietal incomplete ossification showed no dose response, the unossified cervical centrum and the incomplete ossification of the sternebra was within the historical background. An increase of a short costal cartilage was seen at the high-dose. Based on these findings, the NOAEL for developmental toxicity was set at 50 mg/kg bw/day.

In a complementary study, where groups of 23 sperm-positive female Sprague-Dawley rats were exposed to BYI 02960 by oral gavage from gestation day (GD) 6 to 20 at 0, 20 and 30 mg/kg/day, no maternal toxicity was observed up to 30 mg/kg/day. Therefore, based on these two studies, it can be concluded that the NOEL for maternal toxicity was 30 mg/kg/day.

In a rabbit developmental study, groups of 23 time-mated pregnant female New Zealand White rabbits were administered BYI 02960 by oral gavage from gestation day (GD) 6 to 28 at 0, 7.5, 15 and 40 mg/kg/day. The effects on (corrected) body weight gain and food consumption were slight and mainly transient, however considered adverse (>10%).The NOAEL for maternal toxicity and developmental toxicity was set at 15 mg/kg bw/day.

In a developmental neurotoxicity study, dietary exposure to BYI 02960 from GD 6 through LD 21 to mated female Wistar rats resulted in treatment-related effects (maternal: decreased body weight gain. Offspring: decreased body weight gain, slightly increased in motor and locomotor activity, slightly increased auditory startle habituation) evident only at 1200 ppm (102 mg/kg bw/day) in maternal animals and offspring. Therefore, the NOAEL for both maternal animals and offspring is 500 ppm (42.4 mg/kg bw/day) in this developmental neurotoxicity study.

Three *in vitro* studies were performed to determine whether flupyradifurone possesses endocrine disrupting properties that may explain the effects observed in the 2-generation study. Of these studies, the estrogen receptor transactivation assay was negative, the androgen receptor binding assay equivocal and the steroidogenesis assay was positive. The effects observed in these studies occurred only at relatively high concentrations, but were reproducible in repeat runs. No

metabolites of flupyradifurone were tested. Based on these results, it cannot be confirmed nor excluded that flupyradifurone is an endocrine disruptor.

4.11.5 Comparison with criteria

Effects on sexual function and fertility

The effects on fertility were limited to reduced number of estrous cycles of the F1 females at the high dose in the 2-generation study. This effect was seen in connection with a reduced number of implantation sites and pups. These effects were observed in dams which showed reduced body weight (16%) in the pre-mating period. Such effects on oestrus cycle, implantation sites and pups were not observed in the parental generation which had a 5% lower exposure during the pre-mating period. The body weight was also reduced compared to the controls but only for 10%. No such effect was observed in the range-finding 1-generation test with even higher exposure levels. Therefore it remains unclear whether the effects in the F1 females is an effect on fertility only observed in the second generation or a developmental effect due to in utero exposure but only observed during mating. The effect may be secondary to the reduced maternal weight. The effect of reduced bodyweight was studied by Chapin (1993) in Sprague-Dawley rats using feed restriction. A reduction of body weight by 30% resulted in an increase of estrous cycle length by 22% at week 8-9. Also the number of corpora lutea/dam (significant) and implants/dam was reduced although not significant. At lower levels of body weight reduction, these effects were not significant. A study by Terry (2005) in Sprague-Dawley rats showed a feed restriction depending effect on estrous length and corpora lutea. However, the effect was limited in rats that still gained weight as in the study with flupyradifurone. Another study by Tropp (2001) showed that the effect of food deprivation on rats varies between strains. A study by Cooper (1967) on Wistar rats showed a dose-dependent increase of estrous length with feed restriction although the relation with body weight is not clear. A study by Carney et al. (2004) found a decrease in the number of estrous cycles of the F1 generation after 50% feed restriction (30-43% lower body weight post weaning), but not after 30% feed restriction (6-19% lower body weight post weaning). They also found a slight decrease in litter sizes in the 50% feed restricted group, but all values were well within historical control ranges. They reported no effect on litter size in the 30% feed restricted group.

Overall, it is clear that reduced body weight results in an increase in estrous length and a reduction in developing eggs. However, whether the observed level of reduced weight can explain the effects in F1 females remains unclear and should preferentially be determined by using feed restricted control animals. Therefore, it is unclear whether the observed effects on the F1 females are secondary to the reduced body weights. As a result, we consider that the criteria for category 2 are met. As it is unclear whether this should be considered an effect on fertility or development, no specification is proposed (H361).

Developmental effects

As explained under “Effects on sexual function and fertility”, effects on fertility were observed only in the F1 generation females, and thus may be developmental effects due to in utero exposure that are only observed during mating.

The reduced pup weights observed in the generation studies at dose levels also inducing reduced maternal weight are considered limited effects in presence of maternal toxicity and do not warrant

classification. The developmental effects in the developmental study in rats were limited to reduced ossification at the highest dose level in presence of reduced maternal body weight gain. As the reduced ossification is considered a limited effect secondary to the maternal toxicity, this effect does not warrant classification.

Developmental effects reported in a developmental neurotoxicity study were decreased body weight gain, slightly increased motor and locomotor activity (males), slightly increased auditory startle habituation (females), evident only at 102 mg/kg bw/day. The maternal LOAEL was the same, maternal effects seen at this concentration were decreased body weight and body weight gain. The limited developmental effects in this study are considered to be secondary to the maternal toxicity.

4.11.6 Conclusions on classification and labelling

Flupyradifurone (BYI 02960) needs to be classified as Reproductive toxicant Cat. 2 H361 according to Regulation (EC) 1272/2008.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Effects on sexual function and fertility

The DS proposed classification for reproductive toxicity Category 2 (Repr. 2; H360) based on the results of a two-generation study in rats. In this study, a reduced number of estrous cycles of the F1 high dosed females in connection with a reduced number of implantation sites and pups were observed. These effects were found in dams with reduced body weight of 16 % in the pre-mating period.

Such effects were not found in parental generation which had a 5 % lower exposure during the pre-mating period although the body weights of these animals were also reduced compared to the controls, but only by 10 %.

In the range-finding one-generation test with even higher exposure levels no effects on oestrous cycle, implantation sites and pups were observed. Therefore the DS was not sure where these effects in the F1 females were on fertility which were only observed in the second generation or whether these effects are developmental effects due to in utero exposure but only observed during mating.

As the effects described may be secondary to reduced maternal body weight, the DS presented the findings from five studies investigating the impacts of reduced body weight on estrous length, number of corpora lutea and implantation rate. Brief descriptions of these studies are provided below:

Chapin (1993): Feed restriction in Sprague-Dawley rats led to reductions in body weight by 30 % which were accompanied by in an increase of estrous cycle length by 22 % at week 8-9, in a significant reduction of the number of corpora lutea/dam and in a non-significant reduction

of implants/dam.

Terry (2005): A feed restriction dependent effect on estrous cycle length and corpora lutea in Sprague-Dawley rats, limited to rats still gaining weight as observed in the study by Milius (2011) using flupyradifurone.

Tropp (2001): The effect of food reduction on rats varied between strains.

Copper (1967): Feed restriction led to a dose-dependent increase of estrous cycle length in Wistar rats but there was no clear relationship between body weight and estrous length.

Carney *et al*. (2004): In the F1 generation a decrease in the number of estrous cycles and a slight decrease (which was well within historical control ranges) in litter sizes after 50 % feed restriction were observed whereas after 30 % feed restriction no such effects were described.

According to the DS, it is clear that reduced body weight results in an increase in estrous cycle length and in a reduction in the number of developing eggs but it is not clear whether the observed effects on the F1 females are secondary to the reduced body weights. Besides, it is not clear whether the described effects should be considered an effect on fertility or on development (due to *in utero* exposure). Therefore no specification on fertility or development is suggested resulting in a proposal to classify flupyradifurone for Repr. 2; H361. Regarding additional *in vitro* studies performed to determine whether flupyradifurone possesses endocrine disrupting properties, the DS considered the estrogen receptor transactivation assay as negative, the androgen receptor binding assay as equivocal and the steroidogenesis assay as positive. Effects observed in these studies occurred only at relatively high concentrations, but were reproducible in repeated runs. No metabolites of flupyradifurone were tested. Therefore, the DS concluded that based on these results, it can neither be confirmed nor excluded that flupyradifurone is an endocrine disruptor.

Developmental effects

According to the DS, no classification for developmental toxicity would be warranted (based solely on the outcome of the three developmental toxicity GLP-compliant gavage studies) as the limited effects on reduced pup weights observed in the generation studies at dose levels also inducing reduced maternal weight are considered to be limited effects in presence of maternal toxicity. Further, the effects observed in the developmental studies on rats and rabbits and in the developmental neurotoxicity rat study were also related to maternal toxicity (observed in the high dose groups).

Comments received during public consultation

Two industry comments were of the opinion that flupyradifurone does not meet the criteria to classify flupyradifurone for Repr. 2. They point out that the limited effects on reproductive parameters are clearly associated with reduced maternal body weight. After re-evaluation of the estrous cycle data using the method of Goldman and additional statistical analyses, industry confirmed that the marginal variations of the parameters observed at the high dose correlate well with reduced body weight. Therefore in absence of other effects, industry concluded that no classification for reproductive/developmental toxicity is warranted. Industry also commented on the results of additional *in vitro* endocrine disruptor assays in relation to the effects observed in the two-generation study in rats. According to them, there is no evidence of estrogen receptor activation by flupyradifurone. Further, the high-concentration effects in the androgen receptor binding and steroidogenesis assays were equivocal and

inconsistent with an androgen antagonist mode of action. Industry also pointed out that no effects were observed consistent with a disruption of the hypothalamic-pituitary-gonadal mode of action. Industry also submitted qualitative comparisons of flupyradifurone with other substances. Overall, Industry concluded on no evidence of estrogen receptor activation and that the high-concentration effects in the androgen receptor binding and steroidogenesis assays are equivocal.

Four MSCAs, however, agreed with the proposed classification as Repr. 2; H361 without a specification (f or d letters) as it is not clear if the concern on reproductive toxicity may be related to fertility or development. In relation to the weight of evidence in favour of classification as Repr. 2, one MSCA pointed towards (equivocal) indications of endocrine activity *in vitro*.

Assessment and comparison with the classification criteria

The assessment of reproductive toxicology is based on a one-generation dose range-finding study, a two-generation study, three developmental toxicity studies, one developmental neurotoxicity study and three endocrine disruptions assays.

Assessment of sexual function and fertility

A one-generation dose range-finding study, a two-generation study and a developmental neurotoxicity study, all conducted in rats with flupyradifurone (purity 96.2 %), were available.

A **one-generation** GLP-compliant **dose range-finding** pilot study was performed in 10 Wistar rats/sex/dose in order to determine appropriate dietary levels for a two-generation study; the substance was administered continuously (*ad libitum*) at nominal dietary concentrations of 0, 200 (M: 14.5 mg/kg bw/day; F: 17.5 (premating), 15.8 (gestation), 17.5 mg/kg bw/day (lactation)), 700 (M: 50.1 mg/kg bw/day; F: 60.0 (premating), 48.8 (gestation), 60.9 mg/kg bw/day (lactation)), and 2 000 ppm (M: 147.5 mg/kg bw/day; F: 168.9 (premating), 164.4 (gestation), 182.3 mg/kg bw/day (lactation)).

There were no treatment-related deaths or clinical signs. In females, declines in body weight and in body weight gain (non-statistically significant at the mid-dose and statistically significant at the high dose) were observed as well as significant decreases in absolute and relative spleen weight at the high dose and decreases in absolute but increases in relative brain weight. In males, very slightly decreased body weight gain and decreased food consumption during premating in the high dose group were found as well as increases in the liver weight in the mid- and high-dose group and increases in absolute and relative spleen weights at the high dose. Declines in absolute pup weight in both sexes (reaching statistical significance for females) were observed in the 700 (60.9 mg/kg bw/day) and 2 000 (182.3 mg/kg bw/day) ppm dietary groups, beginning on PND 14 and continuing to PND 21 as well as declines in body weight gain. There were no substance-related gross necropsy findings at any dietary level tested.

In a GLP-compliant **two-generation study**, similar to OECD TG 416, Wistar rats were exposed to a nominal dietary concentration of 0, 100 (6.4 to 7.8 mg/kg bw), 500 (32.0 to 42.2 mg/kg bw) and 1800 (117.4 to 168.8 mg/kg bw) ppm.

Statistically significant decreases in body weight during premating, gestation and lactation were observed in parenteral females (at the highest dose level), in F1 females (at mid dose (< 10 %) and at high dose) and in high dose F1 males throughout exposure. Decreases in body

weight gain were found in high-dose parenteral (non-significant) and mid- and high-dose F1 females during premating (non-significant) and in high-dose F1 females during gestation (significant) as well as in mid- and high-dose F1 males during premating (significant). During gestation, in high-dose F1 animals of both sexes increased food consumption was observed (statistically significant (g/kg/day), non-significant (g/animal/day)) whereas during pre-mating the food consumption (g/animal/day) in high-dose parental and F1 females was statistically significant decreased.

In high-dose F1 females, a statistically significant decrease (2.9 vs. 3.5 in controls) in the number of oestrous cycles (parallel to significant weight loss), a concomitant non-significant increase in cycle length (4.4. vs. 4.0 in controls) and a significant decline in the total number of implantation sites (-17 %) without a clear dose-response relationship was noted.

Historical control data were not provided. Therefore, the decrease in the number of oestrous cycles, the increase in the cycle length and the decline in the total number of implantation sites cannot be related to previous study results.

Table: *Estrous cycle length and periodicity*

Observation	Dose Group (ppm)			
	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
P-Generation				
Number of Estrous Cycles (S.E.)	3.5 (0.1)	3.7 (0.1)	3.4 (0.2)	3.4 (0.1)
Estrous Cycle Length (S.E.)	4.4 (0.3)	4.3 (0.1)	4.3 (0.2)	4.3 (0.1)
F_1-Generation				
Number of Estrous Cycles (S.E.)	3.5 (0.2)	3.3 (0.2)	3.3 (0.2)	2.9* (0.2)
Estrous Cycle Length (S.E.)	4.0 (0.2)	4.1 (0.1)	4.4 (0.2)	4.4 (0.1)

* Statistically different from control, $p \leq 0.05$ Data taken from Table 6 in the study report.

Table: *Reproductive performance*

Observation	Dose Group (ppm)			
	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
P-Generation – F_1-Offspring				
Number Cohoused	30	30	30	30
Number Mated	30	29	29	30
Number of Animals Delivered	29	27	28	28
Number of Animals with Implants	29	27	28	28
Mating Index	100.0	96.7	96.7	100.0
Fertility Index	96.7	93.1	96.6	93.3
Gestation Index	100.0	100.0	100.0	100.0
Mean Number Days to Insemination (S.E.) Median	3.4 (0.67) 3.0	3.3 (0.59) 3.0	3.2 (0.52) 3.0	3.1 (0.59) 3.0
Mean Gestation Length (days) (S.E.) Median Gestation Length (days)	22.1 (0.11) 22.0	22.1 (0.12) 22.0	22.1 (0.12) 22.0	22.0 (0.12) 22.0
Total number of implantation sites (Median)	311 (11.0)	285 (11.0)	298 (10.5)	289 (10.0)
F_1-Generation – F_2-Offspring				
Number Cohoused	30	30	30	30
Number Mated	29	30	29	30
Number of Animals Delivered	27	28	28	29
Number of Animals with Implants	27	28	28	29
Mating Index	96.7	100.0	96.7	100.0
Fertility Index	93.1	93.3	96.6	96.7
Gestation Index	100.0	100.0	100.0	100.0
Mean Number Days to Insemination (S.E.) Median	2.3 (0.23) 2.0	3.7 (0.45) 4.0**	2.6 (0.24) 2.5	2.1 (0.18) 2.0
Mean Gestation Length (days) (S.E.) Median Gestation Length (days)	22.0 (0.09) 22.0	22.1 (0.15) 22.0	21.9 (0.09) 22.0	22.0 (0.08) 22.0
Total number of implantation sites (Median)	305 (12.0)	314 (11.0)	323 (11.0)	281 (10.0**)

[a]Data obtained from Table 6 in the study report ** Statistically different from control, $p \leq 0.01$

Industry provided a qualitative evaluation of the individual relationship between body weight gain and the oestrous cycle. Their analysis concluded that animals that weighed less immediately prior to pairing were more likely to have fewer estrous cycles (relative to controls). This relationship was less apparent in the P0 generation than in the F1 females since the difference in bodyweight at the end of the pre-mating period (10 weeks) between the controls and high-dose animals in the P0 generation (10 %) was smaller compared to the F1 generation (15.9 %). Industry claimed that the difference in bodyweight reduction between the two generations is likely to be due to the increased duration of exposure of the F1 animals (i.e. through gestation, lactation and puberty) compared to the P0 animals. However, a relationship between body weight decrease and a decreased reproductive success is difficult to ascertain from the analysis provided by Industry. Besides, RAC supports the DS's opinion that as the original study was performed and analysed according to OECD TG 416, there is no reason to consider the Goldman *et al*. (2007) approach more appropriate for analysing the study than the approach outlined in the guideline.

With regard to offspring, no effects on the litter size, pup viability, sex ratio or lactation index were observed in the F1 pups. In the high-dose F2 pups, a significant decrease in the median litter size (outside the performing laboratory´s historical control range, according to the applicant) was noted while the mean litter size was not statistically significantly decreased.

However, the decrease in the litter size paralleled the reduced body weight gain during gestation and a statistically significantly decreased number of implantation sites in the high-dose F1 females. Industry claimed a clear relationship between early gestation day (GD0) body weight and the number of implantations for both the P0 and F1 generations. GD0 was selected in the analysis to exclude the influence of the litter size. According to this analysis (table below), a clear partitioning effect occurs at a body weight greater than 220 g (the median body weight used as the basis for the grouping) in the F1 generation data. Animals with larger bodyweights generally had more implantation sites, particularly in the control animals. Linear regression between implantation site and GD0 bodyweight in controls and treated animals at the top dose (P0 and F1) had a regression coefficient of R^2 = 96 % and 80 % respectively (data not shown). The linear regression values in F1 and P0 animals were not given in the absence of partitioning at the 220 g body weight cut-off. However, RAC agrees that bodyweight may have a significant influence over implantation site number, as indicated by the overall control values below (range: 9.6 to 12.2) and the correlation (data not shown). RAC notes that the influence of body weight (and probably of body weight gain during gestation) on implantation sites differed between controls and treated animals (slope of the linear regression line through the control data was 0.061 vs. 0.019, respectively). Therefore, RAC cannot exclude a direct relation to the treatment on implantation sites (primarily in F1 generation) in addition to a secondary influence of the body weight and body weight gain.

Table 2. Separation of Implantation data within groups by bodyweight

P_0 Generation	Median BW (g)	Grouping Criteria	Mean BW (g)	Mean # of Implants
Control	237.8	≥ median	258.4	11.7
		< median	219.7	9.6
1800 ppm	217.4	≥ median	229.1	10.4
	(91.4% of control)	< median	203.8	10.2
F_1 Generation	**Median BW (g)**	**Grouping Criteria**	**Mean BW (g)**	**Mean # of Implants**
Control	246.1	≥ median	257.4	12.2
		< median	229.9	10.3
1800 ppm	203.5	≥ median	199.7	9.9
	(82.8 % of control)	< median	191.2	9.6

Table: *Litter parameters*

Observation	Dose Group (ppm)			
	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
P Generation				
Total number of pups born	297	277	282	281
Number stillborn	0	2	0	0
Sex Ratio Day 0 (% male)	55.1	53.0	54.7	49.6
Mean litter size Day 0 Median	10.2 11.0	10.3 11.0	10.1 10.0	10.0 10.0
Birth index	95.0	97.1	93.3	97.4
Live birth index	100.0	99.0	100.0	100.0
Viability index	98.0	99.3	99.7	98.1
Lactation index	99.1	99.5	100.0	99.1
F_1-Generation				
Total number of pups born	291	300	311	266
Number stillborn	1	0	1	4
Sex Ratio Day 0 (% male)	51.6	50.6	50.6	47.7
Mean litter size Day 0 Median	10.8 11.0	10.7 11.0	11.1 10.5	9.2 **10.0***
Birth index	95.6	92.7	96.4	94.8
Live birth index	99.7	99.7	99.2	98.2
Viability index	99.3	99.7	99.4	97.2
Lactation index	99.1	100.0	100.0	98.7

[a] Data obtained from Table 6 and 20 in the study report * Statistically different from control, $p \leq 0.05$

Statistically significantly decreased body weight was observed in F1 litters at high dose and in F2 litters at mid dose (beginning at PND 14) and high dose (beginning at PND 7). Regarding the pups, Industry provided a similar analysis and concluded that the decrease in bodyweight at GD0 (> 15 %) had a strong influence on implantation sites, and consequently, the number of pups. In summary, for the three endpoints affected (number of oestrous cycles, number of implantation sites and number of pups), Industry argued that these were an effect of the body weight rather than of the treatment. RAC concurs that the reduced body weight gain results in an increase in oestrous length and in a reduction in the number of developing eggs but, as pointed out by the DS, it is not clear whether the observed effects on the F1 females are secondary to the reduced body weight. Besides, it is not clear whether the described effects should be considered an effect on fertility or on development (due to *in utero* exposure).

There was a significant delay in preputial separation and a non-significant delay in vaginal patency in the F1 pups, which might be secondary to effects of lower body weight as they were found in parallel with decreased pup weight. This effect was not specifically analysed by Industry but it was also considered related to decreased body weights exceeding the maximum tolerated dose. Reduced organ weights (thymus, spleen) in F1 and F2 litters of the high dosed group were also considered to be secondary to the decreased body weights. There were no treatment-related macroscopic abnormalities or histopathological findings in pups. Reduced brain weight was also observed but not consistently between studies. When observed, effects were not associated with e.g. clinical signs or histopathological findings. Summing up, some uncertainty remains whether the observed effects are only secondary effects to lower body weight.

Any other effects on reproductive performance (e.g. treatment-related effect on sperm parameters) were not found.

Assessment of development

Three developmental toxicity GLP-compliant gavage studies (two in Sprague-Dawley rats, one in New Zealand White rabbits) and one GLP-compliant developmental neurotoxicity study similar to OECD TG 426 were available. All these studies were performed with flupyradifurone (purity 96.2 %).

One of the rat studies was performed to provide additional information on maternal tolerability of flupyradifurone and to determine more precisely the NOAEL for maternal toxicity. In this study, concentrations of 0, 20 and 30 mg/kg bw/day were fed. The other study was similar to OECD TG 414, administering 0, 15, 50 and 150 mg/kg bw/day of flupyradifurone. The doses of the substance given to rabbits were 0, 7.5, 15 and 40 mg/kg bw/day.

In none of the three studies were treatment-related mortality or clinical signs observed. Treatment did not affect (e.g.) the pregnancy rate, the number of live foetuses or the number of implantation sites per dam, the percentages of pre and post implantation losses, the number of early and late resorptions, foetal deaths or the percentage of male foetuses.

In the OECD-compliant rat study, an increased incidence of a short costal cartilage at the high-dose and of parietal incomplete ossification without any dose response relationship was found. The increased incidence of unossified cervical centrum and of the incomplete ossification of the sternebrae was within the historical control data generated from 15 in-house Sprague-Dawley rat studies (2001-2008).

In rabbits, the skeletal variations (e.g. increased incidence of incomplete or unossified hyoid centrum, incomplete ossification of the 5^{th} and 6^{th} sternebrae, incomplete ossification of pubis) were within the in-house historical control data, generated from 14 developmental toxicity studies with New Zealand White rabbits.

In an oral developmental neurotoxicity study with Wistar rats the effects were limited to the highest dose. In maternal animals, decreased body weight gain was observed. In the offspring, not only decreased body weight gain (> 10 %) but also slightly increased motor and locomotor activity in males and slightly increased auditory startle habituation in females was observed. Flupyradifurone was given ad libitum (except during neurobehavioral testing) at concentrations of 0, 120, 500, 1 200 ppm with adjustment during lactation to maintain a more consistent dosage throughout the exposure period what resulted in an average mean daily intake of 0, 10.3, 42.4 and 102 mg/kg bw/day.

As a conclusion, since no teratogenic effects in the absence of maternal toxicity were observed in the studies in rats and rabbits, RAC agrees with the DS that based on the outcome of the developmental studies no classification for developmental toxicity is warranted.

In vitro endocrine disruption assays

In an Estrogen Receptor Transactivation Assay conducted in two independent runs with human cells (Cell Line Hela-9903) flupyradifurone did not act as an agonist of the estrogen receptor alpha (hERα).

The overall result of an Androgen Receptor Binding Assay, which was performed two times due to an error in the preparation of the stock solution of flupyradifurone as a result of which one

testing was not considered valid, was categorised as equivocal.

In a Steroidogenesis Assay performed in three independent runs using human cell line H295R, a small but significant decrease in the production of testosterone in 2 out of 3 runs and a decrease in the production of estradiol in all three runs were observed at the highest test concentration (100 µM). Therefore, flupyradifurone is categorised as positive based upon the results of this assay.

Besides, it has to be pointed out that although the parent compound was only tested at rather high doses, the positive results in the *in vitro* Steroidogenesis Assay (which was reproducible) might be related to the observed effects. Disturbed steroidogenesis can interfere with the balance of androgens and estrogens, with possible effects on the oestrous cycle and follicular function. In addition, the *in vitro* tests were not performed with any metabolites of flupyradifurone although the substance is extensively metabolised.

Conclusion on reproductive toxicity

It is known that reduced body weight and body weight gain may result in an increase in estrous cycle length and in a reduction in the number of developing eggs, as described by the DS. There is also no doubt that maternal toxicity affects pup development (e.g. reduced body weight, reduced body weight gain, decreased oestrous cycle number, decreased litter size and decreased number of implantation sites in the F1 generation).

The reduced number of implantation sites and the slight decrease in median implantation sites (and thus decrease in litter size) in the F1 generation is considered a secondary effect due to decreased body weight and body weight gain. The effect was not observed in the P0 generation dams and the total number of implantation sites between the P0 and F1 generations in the control (311 and 305 in P0 and F1, respectively) and high-dose (289 and 281 in P0 and F1, respectively) are similar. Therefore, this slight decrease is likely due to the decreased body weight in the P0 females, which was observed as early as day 7 after initiation of exposure. No other effects were noted in female organ weights or histopathology in the two-generation reproduction study. Developmental toxicity studies in rats and rabbits did not show any significant decreases in the number of implantation sites in dams that showed a significant decrease in body weight, nor in any other changes in reproductive indices, tissue weights, or histopathology.

Although the decreased body weight and body weight gain at doses indicative of parental and offspring's toxicity may be an explanation for the reproductive effects observed, it is not clear whether all reproductive findings in the offspring are due to the parental decrease in body weight and body weight gain (e.g. delay in preputial separation and vaginal patency, reduced brain, thymus and spleen weights). It is doubtful whether the reduction in body weight is severe enough to cause an increase in estrous length (see Carney *et al*. (2004) and Chapin *et al.* (1993)). In the CLP Guidance (3.7.2.5.5), it is stated that the presence of maternal toxicity shall not be used to negate findings of embryo/foetal effects, unless it can be clearly demonstrated that the effects are secondary non-specific effects.

The slight effects on increased motor/locomotor activity and auditory startle habituation were noted at the same dose level in the offspring but they are considered by RAC as secondary to general toxicity. However, since the pesticidal mode of action of flupyradifurone is based on nicotinic acetylcholine receptor (nAChR) agonist property and no MoA studies have been

provided, it cannot be excluded that neurotoxic effects observed in offspring are related to the above properties. Besides, a significant decrease of brain weight was consistently observed in offspring (on day 21) in the two-generation study. In the developmental neurotoxicity study, the high dosed males showed a decrease of brain weight of 5% on PND 75. However, since the decrease in brain weight cannot be related to the effects on nAChR and since there is no indication of a MoA, RAC does not consider these effects sufficient to support a classification for reproductive toxicity for flupyradifurone.

Taken together, although there are uncertainties on the extent of the maternal toxicity as well as on the mode of action of flupyradifurone to explain the reproductive effects (in particular the increased length of the estrous cycles and the decreased number of implantation sites in the F1 high dosed females), the data supports that they were due to maternal body weight reductions. In conclusion, **RAC does not consider classification for fertility or development warranted**.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Acute oral neurotoxicity

Study 1

Characteristics

reference	:	Garcin, J.C. 2011	exposure	:	Once by gavage
type of study	:	Acute neurotoxicity study	doses	:	Initial study : 0, 50, 200 and 800 mg/kg bw Follow-up study : 0, 20 and 35 mg/kg bw
year of execution	:	2011	vehicle	:	methylcellulose 400 (0.5%)
test substance	:	BYI 02960 (batch number 2009-000239; purity 96.2%)	GLP statement	:	Yes
Route	:	Oral	guideline	:	According to OECD 424
species	:	Rat, Wistar Rj:WI (IOPS HAN)	acceptability	:	Acceptable
group size	:	Initial study: 12/sex/dose Follow-up study: 12 females/dose	**NOAEL**	:	35 mg/kg bw

Study design

The study was performed according to OECD 424. Initial study was performed with dose levels of 0, 50, 200 and 800 mg/kg bw with male and female rats (12/sex/dose). The follow-up study was performed in order to establish the NOAEL for the effects observed at all dose levels in the initial study, using the dose levels of 0, 20 and 35 mg/kg bw. Based on the higher sensitivity of females in

the initial study, the follow-up study was performed with females only (12/dose). The animals in the follow-up study were sacrificed without necropsy.

Results

The results are summarised in *Table 134* (initial study) and *Table 135* (follow-up study).

Table 134: The results of the initial study

Dose (mg/kg bw/d)	**0**		**50**		**200**		**800**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality								2/12	
Clinical signs	No substance-related findings								
Body weight (gain)	No substance-related findings								
Functional observation battery	See table below								
Motor activity measurements									
- *spontaneous motor activity, 60-min session, day 1*									
					ds	ds	ds	ds	
- 10 minutes							ds	ds	
- 60 minutes									
Pathology	No substance-related findings								
macroscopy	No substance-related findings								
microscopy	No substance-related findings								
Neuropathology	No substance-related findings								

i/d: increased/decreased
is/ds: statistically significantly increased/decreased
dr: dose response

Table 135: The results of the follow-up study

Dose (mg/kg food)	**0**	**20**	**35**
	f	**f**	**f**
Mortality	No mortality		
Clinical signs	No substance-related findings		
Body weight (gain)	Not evaluated in the study		
Functional observation battery			
- quantitative	No substance-related findings		
parameters	No substance-related findings		

Dose (mg/kg food)	**0**	**20**	**35**
	f	**f**	**f**
- home cage	No substance-related findings		
- open field	No substance-related findings		
- sensorimotor			
Motor activity measurements	No substance-related findings		
Pathology			
macroscopy	Not evaluated in the study		
Neuropathology	Not evaluated in the study		

Initial study

During the initial study, one high-dose female died during neurobehavioral testing conducted at the time of peak effect after dosing. Another high-dose female was found dead on study day 5.
One week after dosing, mean absolute body weight gain was statistically significantly lower in high-dose animals of both sexes (-40% and -46% in males and females, respectively, compared to controls). Body weight gain recovered after day 7 and overall, was comparable to controls for the duration of the study.

In the high-dose animals, findings associated with treatment were observed at the time-of-peak effect (2 hours after dosing), including piloerection, lower muscle tone, rapid respiration, low arousal, tremors, myoclonic jerks, chewing, repetitive licking of lips, gait incoordination, flattened or hunched posture, dilated pupils, impaired (uncoordinated or slow) righting reflex, impaired flexor and tail pinch responses and reduced rectal temperature. Automated measures of motor activity were also reduced in both sexes, compared to controls.

In the mid-dose group, at the time-of-peak effect after dosing, treatment-related observations included piloerection, rapid respiration, gait incoordination and flattened body posture in both sexes, with a higher incidence of tremors in both sexes. In addition, automated measures of motor activity were reduced during the first 10-min interval of the session, while activity for the entire test session was comparable to controls.

At the low-dose level, the only treatment-related effects were limited to higher incidences of piloerection (both sexes) and dilated pupils (females only) at the time-of-peak effect after dosing.

All effects were reversible, with none observed at later time points of the study.

There were no macroscopic or microscopic treatment-related observations in either sex at any dose level.

Table 136: Summary of significant changes in open field observations during the initial study at time peak-effect

BYI 02960 Dose levels (mg/kg)	**0**	**50**	**200**	**800**
Male				
Piloerection	4/12	8/12	12/12	12/12
Rapid respiration	1/12	0/12	9/12	12/12
Tremors	0/12	0/12	4/12	8/12
Myoclonic jerks	0/12	0/12	1/12	5/12
Chewing	0/12	0/12	0/12	5/12
Convulsions	0/12	0/12	0/12	0/12
Gait incoordination	0/12	0/12	3/12	7/12
Rearing (mean ± S.D.)	7.2 ± 2.4	7.8 ± 3.4	4.3 ± 3.6	2.7 ± 2.8
Low arousal	1/12	1/12	6/12	11/12
Repetitive licking of lips	1/12	0/12	0/12	2/12
Flattened or hunched posture	3/12	2/12	8/12	11/12
Female				
Piloerection	4/12	7/12	11/12	12/12
Rapid respiration	0/12	0/12	6/12	12/12
Tremors	0/12	0/12	4/12	10/12
Myoclonic jerks	0/12	0/12	0/12	7/12
Chewing	0/12	0/12	1/12	4/12
Convulsions	0/12	0/12	0/12	1/12
Gait incoordination	0/12	0/12	5/12	5/12
Rearing	12.3 ± 4.6	10.7 ± 3.3	9.9 ± 4.6	2.8 ± 2.8
Low arousal	0/12	0/12	1/12	8/12
Repetitive licking of lips	0/12	1/12	3/12	8/12
Flattened or hunched posture	0/12	0/12	3/12	11/12

Table 137: Summary of significant changes in sensory reactivity during the initial study at time peak-effect

BYI 02960 Dose levels (mg/kg)	**0**	**50**	**200**	**800**
Male				
Right pupil: dilated	0/12	1/12	4/12	6/12
Left pupil: dilated	0/12	1/12	4/12	6/12
Incoordianted or slow surface righting reflex	0/12	0/12	4/12	6/12
Abnormal tail pinch response	1/12	4/12	2/12	4/12
Female				
Right pupil: dilated	1/12	6/12	7/12	10/12
Left pupil: dilated	1/12	6/12	7/12	10/12
Incoordianted or slow surface righting reflex	0/12	1/12	2/12	5/12

Abnormal tail pinch response	3/12	2/12	4/12	8/11

Table 138: Significant changes in motor activity during the initial study at time peak-effect

BYI 02960 Dose levels (mg/kg)	**0**	**50**	**200**	**800**
Male				
First interval (10 minutes) (mean ± S.D.)	107 ± 37	120 ± 37	64 ± 32 *	31 ± 17 *
Total activity (60 minutes) (mean ± S.D.)	194 ± 47	254 ± 118	163 ± 92	97 ± 29 *
Female				
First interval (10 minutes) (mean ± S.D.)	136 ± 31	111 ± 28	72 ± 29 *	37 ± 23 *
Total activity (60 minutes) (mean ± S.D.)	293 ± 156	272 ± 154	199 ± 125	115 ± 72 *

* : Statistically different ($p \leq 0.05$) from the control

Follow-up study

No treatment-related effects were evident at either dose tested in the follow-up study that was performed to establish a NOAEL for effects observed at 50 mg/kg.

Acceptability

The study is considered acceptable.

Conclusions

Based on piloerection and dilated pupil in the initial study a LOAEL is 50 mg/kg. The NOAEL is therefore based on the follow-up study and set at 35 mg/kg bw in rats.

Semichronic oral neurotoxicity

Study 1

Characteristics

reference	:	Garcin, J.C. 2011	exposure	:	Dietary 90 days
type of study	:	90-day Neurotoxicity study	doses	:	0, 5.7/6.9, 29.4/34.8, 143/173 mg/kg bw/d (M/F)
year of execution	:	2011	vehicle	:	None
test substance	:	BYI 02960 (batch number 2009-000239; purity 96.2%)	GLP statement	:	Yes
Route	:	Oral (diet)	guideline	:	According to OECD 424
Species	:	Rat, Wistar Rj:WI (IOPS HAN)	acceptability	:	Acceptable
group size	:	12/sex/dose	**NOAEL sys** **NOAEL neuro**	:	29.4 mg/kbg bw/d 143 mg/kg bw/d

Study design

The study was performed according to OECD 424. The study was performed with dose levels of 0, 5.7/6.9, 29.4/34.8, 143/173 (M/F) mg/kg bw/d with male and female rats (12/sex/dose). Animals were observed for clinical signs daily, with body weight and food consumption measured weekly. A detailed physical examination was performed once during the acclimatization phase and weekly

throughout the study. Neurotoxicity assessment, consisting of a Functional Observational Battery (FOB) and automated measurement of motor activity, was performed on all animals on 5 occasions (pre-study, Study weeks 2, 4, 8 and 13 - 14). Ophthalmological examinations were performed on all animals during the acclimatization phase and on all animals of all dose groups during Week 13. All animals were subjected to a complete necropsy (macroscopic examinations). At least 6 animals from each group were perfused for neuropathological investigation. The brain was weighed and a range of tissues from the nervous system was collected, fixed and examined microscopically.

Results

Table 139: The results of the study

Dose (mg/kg bw/d) (M/F)	**0**		**5.7/6.9**		**29.4/34.8**		**143/173**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	No mortality throughout the study								
Clinical signs	No substance-related findings								
Body weight (gain)							d	ds	
Food consumption							d	d	
Ophthalmological examination	No substance-related findings								
Neurobehavioral assessment	No substance-related findings								
Motor activity measurements - *spontaneous motor activity*	No substance-related findings								
(Neuro-) Pathology -*Terminal body weight*							d	d	
- *brain to terminal body weight ratio*								is	
- *enlarged liver*								4/6	
macroscopy	No treatment-related macroscopic findings were observed.								
microscopy	No treatment-related microscopic findings were observed.								

i/d: increased/decreased
is/ds: statistically significantly increased/decreased
dr: dose response

Up to and including the highest dose tested of 2500 ppm, there was no treatment-related mortality, clinical signs or ophthalmological changes during the study and no treatment-related effects were observed in any of the neurotoxicology endpoints, including neuropathological examinations, in either sex.
There were no treatment-related effects in either sex at 5.7/6.9 and 29.4/34.8 mg/kg bw/d.

At 143/173 mg/kg bw/d body weight parameters were clearly affected in both sexes, especially during the first week of treatment, when mean body weight remained static in females and the mean body weight gain was 50% lower in males as compared to controls. Overall at the end of the study, mean body weight gain at this dose level represented 85% and 79% of the control values in males and females, respectively. Consequently, mean body weight remained lower compared to controls throughout the study in both sexes (- 7% to - 10% in males and - 5% to - 9% in females).
Mean food consumption was significantly lower in both sexes during the first week of treatment (- 18% and - 29% in males and females, respectively) and remained slightly lower in both sexes throughout the study (up to - 14% and up to - 13% in males and females, respectively, the effect being statistically significant on several occasions).
At necropsy, mean terminal body weight was 7% lower in both sexes, compared to controls. In remaining animals not used for neuropathology, enlarged liver was observed in 4/6 females.

Acceptability
The study is considered acceptable.

Conclusions
Body weight and food intake effects were seen at the highest dosage. The systemic NOAEL is therefore 29.4/34.8 mg/kg bw/d for males and females respectively.

There was no evidence of neurotoxicity at dietary levels as high as 143 mg/kg bw/d (males), therefore this is considered to be a NOAEL for neurotoxicology.

Summary

The results of the neurotoxicity studies are summarised in *Table 140*.

Table 140: Summary of neurotoxicity studies

Type of study	Species	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Critical effects	Reference
Acute neurotoxicity in the rat	Rat	35	50	Piloerection, dilated pupil	JC Garcin, 2011
90-day neurotoxicity in the rat	Rat	29.4 (systemic)	143	Reduced body weight and food intake.	JC Garcin, 2011
		143 (neuro)	-	None	
Developmental neurotoxicity in the rat	Rat	42.4 (maternal)	102	Maternal: decreased body weight gain	Gilmore R.; 2012
		42.4 (developmental)	102	Offspring: decreased body weight gain	
		42.4 (developmental neurotoxicity)	102	Develompental neurotoxicity: slightly increased motor/locomotor activity and startle habituation.	

Type of study	Species	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Critical effects	Reference

In an acute neurotoxicity study, BYI 02960 was administered by gavage in a single dose to non-fasted young adult Wistar rats at 0, 50, 200 and 800 mg/kg bw. Compound-related effects were observed at all dose concentrations in both sexes. The only treatment-related effects at 50 mg/kg were limited to higher incidences of piloerection in both sexes and dilated pupils in females only. A follow-up study was performed in order to establish a clear NOAEL for findings observed at all dose levels in the initial study. In this follow-up study, females only were used as they were equal or more sensitive than males at higher dose levels and were administered BYI 02960 at 0, 20 or 35 mg/kg. No treatment-related effects were evident at either dose tested. The dose level of 35 mg/kg of BYI 02960 was considered to be the overall NOAEL for both sexes.
In a 90-day neurotoxicity study, through approximately 13 weeks of continuous dietary exposure to BYI 02960 at 0, 100, 500 or 2500 ppm, there were no neurotoxic treatment-related findings apparent at any dietary level in either sex. Based on these findings, a NOAEL of 2500 ppm was established for the rat (equating to 143 mg/kg bw/day for males). Body weight and food intake effects were seen at the highest dosage. The systemic NOAEL is therefore 29.4/34.8 mg/kg bw/d for males and females respectively.
In a developmental neurotoxicity study, dietary exposure to BYI 02960 from GD 6 through LD 21 to mated female Wistar rats resulted in treatment-related effects (maternal: decreased body weight gain. Offspring: decreased body weight gain, slightly increased in motor and locomotor activity, slightly increased auditory startle habituation) evident only at 1200 ppm (102 mg/kg bw/day) in maternal animals and offspring. The maternal NOAEL was 500 ppm (42.4 mg/kg bw/d) based on decreased body weight and body weight gain. The developmental NOAEL was 500 ppm (42.4 mg/kg bw/d) based on decreased offspring body weight and body weight gain. The developmental neurotoxicity NOAEL was 500 ppm (42.4 mg/kg bw/d) based on increased startle amplitude in females only on PND 60 and increased motor and locomotor activity on PND 13 in males only.

4.12.1.2 Immunotoxicity

Study 1

Characteristics

Reference	:	Repetto M, 2011	exposure	:	diet 28-days
type of study	:	28-day Immunotoxicity study	dose	:	0, 125, 600 and 3000 ppm (equivalent to app. 0, 10, 50 and 230 mg/kg bw/d)
year of execution	:	2011	vehicle	:	acetone (positive control: cyclophosphamide)
test substance	:	Flupyradifurone (Batch 2009-	GLP statement	:	Yes

		000239, purity 96.2%)			
Route	:	Oral	guideline	:	U.S.E.P.A., OPPTS Series 870, Health Effects Testing Guidelines, N° 870.7800 (August 1998)
Species	:	Female Wistar rats	acceptability	:	Acceptable
group size	:	10 females/dose	**NOAEL**	:	Systemic NOAEL of 50 mg/kg bw/d Immunotoxic NOAEL of >230 mg/kg bw/d

Study design

This study was conducted according to U.S. E.P.A., OPPTS Series 870, Health Effects Testing Guidelines N° 870.7800 (August 1998). Female Wistar rats (10/group) were administered flupyradifurone continuously via dietary administration for at least 28 days at concentrations of 0, 125, 600 and 3000 ppm (equivalent to approximately 0, 10, 50 and 230 mg/kg bw/d). A similarly constituted group received untreated diet and a similarly constituted group received the positive control 3.5 mg/kg bw/d cyclophosphamide (immunosuppressive agent) for at least 28 days.

All animals were immunized with Sheeps Red Blood Cell (SRBC) antigen by intravenous injection of 2.5 x 10^8 SRBC/animal via the tail vain.

Mortality and clinical signs were observed daily, food consumption and body weight were recorded once weekly. Blood samples were collected of each surviving animal on study day 30 (just before necropsy) for specific anti-SRBC immunoglobulin M (IgM) analysis. All animals were necropsied, gross pathology observations were performed and spleen and thymus weighed.

Results

The flupyradifurone was homogeneous distributed through the diet and the concentrations were acceptable in the diet, in addition the formulation at 3000 ppm was found stable up to 34 days at room temperature in rodent diet.

One animal showed automutilation of both forelimbs and was killed for humane reasons.

Table 141

Dose (mg/kg bw/d)	**0**	**10**	**50**	**230**
n=10	**f**	**f**	**f**	**f**
Mortality	No treatment related mortality			
Clinical signs	No substance-related findings			
Body weight (mean)				
Day 1				
Day 8				**ds** (11%)
Day 15				**ds** (8.3%)
Day 22				**d**
Day 29				**d**
Food consumption				
Day 8				**d**
Day 15				**d**
Day 22				**d**
Day 29				**d**
Gross Pathology				

Dose (mg/kg bw/d)	**0**	**10**	**50**	**230**
n=10	**f**	**f**	**f**	**f**
macroscopy	No macroscopic finding was noticed.			
Mean spleen weight	No substance-related findings			
Mean thymus weight				
Clinical pathology				
SRBC-specific Ig-M	No substance-related findings			

CPP = cyclophosphamide (positive control)
ds/is statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls

Mean body weight at 3000 ppm was reduced by 6 to 11% from Study Day 8 to 29 when compared to the control group. The effect was statistically significant on the intervals Day 1 to 8 and Day 8 to 15. There was no body weight gain/day between Study Day 1 and 8. Body weight gain was then similar to control animals at the following intervals. Between Study Day 1 and 29, the cumulative body weight was reduced by 23%, when compared to the control group.
At 600 and 125 ppm, body weight parameters were unaffected by treatment.

At 3000 ppm, mean food consumption was reduced by approximately 34% on Study Day 8 and by 9 to 13% onwards, when compared to control group although not statistically significant.
At 600 and 125 ppm, food consumption was unaffected by treatment.

The spleen and thymus weight changes were considered to be incidental and not treatment-related (no dose response in the organ to bw ratio).

Table 142: Mean organ weights and organ/terminal body weight ratios.

Mean organ weight ±SD at scheduled sacrifice (% change when compared to controls)				
Sex	**Female**			
Dose level mg/kg bw/d	**0**	**10**	**50**	**230**
Mean absolute spleen weight (g)	0.762 ±0.122	0.743 ± 0.110	0.732 ± 0.135	0.720 ± 0.096
Mean spleen to body weight ratio (%)	0.316 ± 0.038	0.312 ±0.037	0.301 ± 0.039	0.319 ±0.036
Mean absolute thymus weight (g)	0.544 ±0.130	0.505 ± 0.075	0.591 ±0.154	0.522 ± 0.104
Mean thymus to body weight ratio (%)	0.226 ±0.048	0.212 ± 0.033	0.242 ± 0.053	0.230 ± 0.036

No statistically significant difference was noted in anti-SRBC IgM concentrations up to 3000 ppm. A high inter-individual variability was noted in all the groups as usually observed with SRBC sensitization. No dose-effect relationship was seen, the variations observed were due to only few animals.

Table 143: Mean SRBC-specific IgM

SRBC-specific IgM (u/mL) mean ± standard deviation

(% change when compared to controls)				
Dose level mg/kg bw/d	**0**	**10**	**50**	**230**
Study Day 30	**40516 ± 23875**	**62920 ± 41649 (+ 55%)**	**34286 ± 30710 (- 15%)**	**33354 ± 26016 (- 18%)**

In the positive controls (cyclophosphamide at a dose level of 3.5 mg/kg/day) there was no change in mean terminal body weight in treated animals when compared to the controls. The principal findings noted at the macroscopic examination were atrophic/small spleen and/or thymus (6/10 and 5/10 respectively) which correlated with significantly lower mean absolute and relative spleen and thymus weights (- 34% to - 32% and - 28% to - 27% respectively).

Table 144: Mean spleen weights

Mean spleen weight ±SD at scheduled sacrifice (% change when compared to controls)		
Sex	**Female**	
Dose level of Cyclophosphamide (mg/kg/day)	0	3.5
Mean absolute spleen weight (g)	0.762 ± 0.122	0.500** ± 0.049 (- 34%)
Mean spleen to body weight ratio (%)	0.3161 ± 0.0380	0.2136** ± 0.0176 (- 32%)

** : p ≤0.01

Table 145: Mean thymus weights

Mean thymus weight ±SD at scheduled sacrifice (% change when compared to controls)		
Sex	**Females**	
Dose level of Cyclophosphamide (mg/kg/day)	0	3.5
Mean absolute thymus weight (g)	0.544 ± 0.130	0.389** ± 0.063 (- 28%)
Mean thymus to body weight ratio (%)	0.2259 ± 0.0482	0.1656** ± 0.0202 (- 27%)

** : p ≤0.01

In the positive controls the mean anti-SRBC IgM concentration was markedly lower when compared to controls (- 93%).

Table 146: Mean SRBC-specific IgM

SRBC-specific IgM (u/mL) mean ± standard deviation (% change when compared to controls)		
Dose level of	0	3.5

Cyclophosphamide (mg/kg/day)		
Study Day 30	**40516 ± 23875**	**2732 ± 1737 (- 93%)**

Acceptability
The study is considered acceptable.

Conclusions
The mean body weight and mean food consumption was reduced at 3000 ppm (230 mg/kg bw/d). For the immunological response, no relevant change was noted in anti-SRBC IgM concentrations up to 3000 ppm (230 mg/kg bw/d) and no significant change was noted in the spleen and thymus weight.
The systemic flupyradifurone NOAEL is 600 ppm (equivalent to approximately 50 mg/kg bw/d), and the immunotoxic NOAEL is >3000 ppm (equivalent to approximately 230 mg/kg bw/d).

4.12.1.3 Specific investigations: other studies

4.12.2 Biokinetic study

The biokinetic study below is performed to assess if the Cmax concentration of flupyradifurone can be determined in a certain period.

Study 1

Characteristics

Reference	:	Odin-Feurtet M., 2010	exposure	:	diet 7-days
type of study	:	Biokinetic in the plasma of rats following 7 day exposure through the diet	dose	:	400 ppm (equivalent to app. 22.6 mg/kg bw/d in males and 32.4 mg/kg bw/d in females)
year of execution	:	2009-2010	vehicle	:	- (dry mixing with the diet)
test substance	:	flupyradifurone (Batch 2009-000239, purity 96.2%)	GLP statement	:	Not GLP but in a GLP laboratory
Route	:	Oral	guideline	:	-
Species	:	Rat Rj: WI (IOPS HAN)	acceptability	:	Acceptable as indicative study
group size	:	5 male, 5 female	**NOAEL**	:	-

Study design
The plasma concentration of flupyradifurone was assessed at three time points after continuous dietary administration for seven days. Groups of 5 male and 5 female Wistar rats received the diet

admixed with the test substance at a concentration of 400 ppm (equating approximately to 22.6 mg/kg body weight/day in males and 32.4 mg/kg body weight/day in females).
During the first 15 days of acclimatization, the daily light/dark rhythm was 7 a.m. - 7 p.m. Rats being known to preferably eat during the dark period, the daily light/dark rhythm of rats was changed from pre-study Day 16, *i.e.* 14 days prior to first day of treatment, onwards, *i.e.* dark period was from 2 a.m. to 2 p.m. and light period was from 2 p.m. to 2 a.m., in order to allow the blood sampling during the working day. Clinical signs were observed daily. Body weights were measured just prior to the start of admixed food administration (Study Day 1) and on Study Day 8. Food consumption was recorded on Study Days 1 - 4, 4 - 7 and 7 - 8.
Whole blood was collected on heparin for plasma preparation from treated animals at three time points on Study Day 8, 6 hours after the start of feeding (8 am), 12 hours (2 pm) and 15 hours (5 pm). As the compound is rapidly absorbed, this should correspond to the period where the blood concentration in BYI 02960 should be higher. Blood samples collected at later time points should be lower. This should provide enough information to know when blood samples should be collected during subchronic or long term studies.The day after the end of the treatment period, animals were sacrificed without necropsy.

Results
There were no mortalities and no treatment-related clinical signs observed during the study. The few clinical signs observed (hair loss observed on two females) were considered not to be related to flupyradifurone administration. Body weight evolution and food consumption were similar to the one observed in the other repeated dose studies.

The individual and mean concentrations of flupyradifurone in plasma samples were used to identify the appropriate maximal concentration (Cmax) and the corresponding time point of collection for the long-term studies.
The results obtained indicated a small sex difference in the plasma concentrations of flupyradifurone, values being slightly higher for females than for males.
In male rats, a Cmax of 8.3 mg/L was measured at the time of collection of 8 a.m. In female rats, a Cmax of 9.4 mg/L was measured at the time collection of 2 p.m. However, in view of the inter-individual variability, the concentrations of flupyradifurone in plasma were considered similar between the three times of blood collection for both sexes (between 7.8 and 8.3 mg/L for males and between 8.8 and 9.4 mg/L for females).

Table 147: Plasma concentration of flupyradifurone in male

Animal reference	Time collection	Plasma concentration of flupyradifurone		
		Individual data (mg/L)	Mean (mg/L)	SD
TT1M4982	8 a.m.	6.14	8.3	1.3
TT1M4983		8.63		
TT1M4984		8.59		
TT1M4985		8.56		
TT1M4986		9.77		
TT1M4982	2 p.m.	5.87	7.8	1.3
TT1M4983		7.14		
TT1M4984		8.73		
TT1M4985		8.36		

TT1M4986		8.90		
TT1M4982	5 p.m.	4.73	8.0	2.0
TT1M4983		9.98		
TT1M4984		8.30		
TT1M4985		7.77		
TT1M4986		9.05		

Table 148: Plasma concentration of flupyradifurone in female

Animal reference	Time collection	Plasma concentration of flupyradifurone		
		Individual data (mg/L)	Mean (mg/L)	SD
TT1F4987	8 a.m.	7.76	9.0	1.3
TT1F4988		9.33		
TT1F4989		8.66		
TT1F4990		11.0		
TT1F4991		8.28		
TT1F4987	2 p.m.	8.16	9.4	1.2
TT1F4988		9.86		
TT1F4989		9.15		
TT1F4990		11.1		
TT1F4991		8.56		
TT1F4987	5 p.m.	7.56	8.8	1.6
TT1F4988		9.85		
TT1F4989		7.91		
TT1F4990		11.2		
TT1F4991		7.67		

Using the experimental study design, blood samples collected between 8 a.m. and 5 p.m. are adequate for measuring flupyradifurone concentrations in plasma around the C_{max} for both sexes.

Acceptability

The study is not performed in GLP compliance. It was performed according to standard operation procedures of the GLP compliant laboratory but the results were not subjected to specific quality assurance inspections. The study is considered acceptable as supplementary study.

Conclusions

In conclusion, in the environmental conditions of the long-term studies (light: 7 a.m. to 7 p.m. & dark period: 7 p.m. to 7 a.m.) and since animals start eating food when the light is switched off, the blood collection can be performed between 1 a.m. and 10 a.m. in order to measure the maximum concentration of flupyradifurone in the ADME studies.

4.12.2.1 Human information

4.12.2.2 Summary and discussion

4.12.2.3 Comparison with criteria

Not applicable.

4.12.2.4 Conclusions on classification and labelling

Not applicable.

5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental hazards of Flupyradifurone, (Figure 6) were assessed in the Draft Assessment Report, (13th February 2014) concerning the placing of plant protection product on the market. The DAR is publicly available via the EFSA web site (http://dar.efsa.europa.eu/dar-web/provision). From now on flupyradifurone will be referred to as BYI 02960.

The summaries included in this proposal are copied from the DAR (and its addenda and assessment reports when these contain updated information). For an overview of the hazard property being evaluated, all reliable information relating to that property has been summarised in a table. Detailed information is included for the key studies used to derive the classification. For more details the reader is referred to the DAR and its addenda.

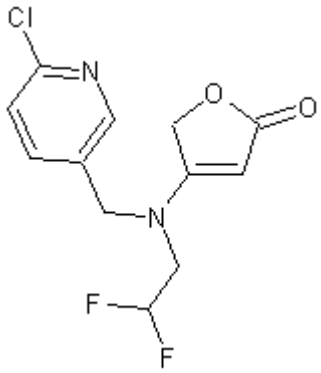
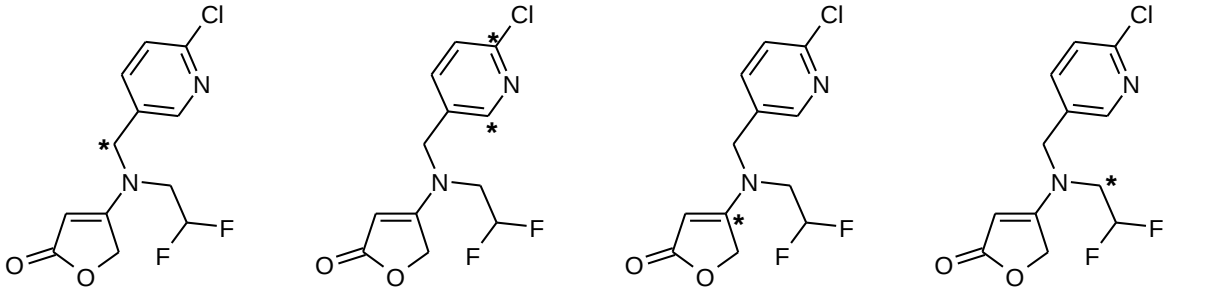


Figure 6: ***Chemical structure of BYI 02960 (flupyradifurone).***

5.1 Degradation

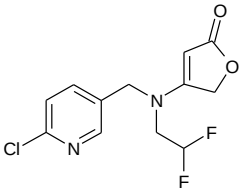
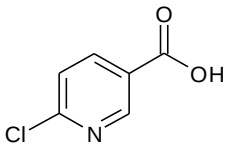
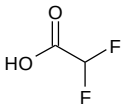
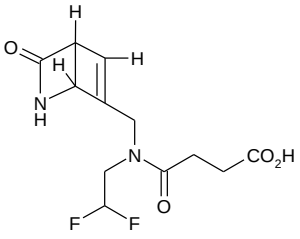
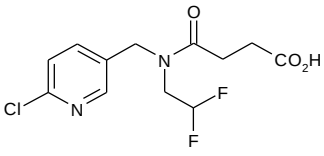
The stability and degradation of BYI 02960 was examined in several studies. In these studies labelled BYI 02960 was used and in Figure 7 below these radiolabeled positions are shown. Table 149 shows BYI 02960 and its metabolites that were identified in the studies reported in the DAR.
No ready biodegradability test was performed. Five different water/sediment studies were submitted in the dossier. The other studies submitted in the DAR were used as supporting studies, these studies are not directly used for classification and labelling purposes. These are six aerobic and three anaerobic soil studies. Furthermore, there are several soil photolysis & field studies mentioned in the DAR. These soil photolysis and field studies are believed not to influence the classification and are therefore not discussed; please refer to the DAR Volume 3 B8 if required. An overview of the relevant degradation studies is shown in Table 150.



[Pyridinyl-methyl-^{14}C] BYI 02960 (PYM-^{14}C) | [Pyridine-2,6-^{14}C] BYI 02960 (PYR-^{14}C) | [Furanone-4-^{14}C] BYI 02960 (FUR-^{14}C) | [Ethyl-1-^{14}C] BYI 02960 (ETH-^{14}C)

Figure 7: ***The structure of BYI 02960 (flupyradifurone) and the positions of the different radiolabels****. * indicates position of radiolabel.*

Table 149: "List of concordances for names and designations of compounds". (Unnumbered table from the DAR, p. 4 Vol. 3 B8.)

compound designation	**Reference Code**	**Molecular weight / formula**	**Structure**
Flupyradifurone (active substance)	BYI 02960	288.68 $C_{12}H_{10}ClF_2N_2O_2$	
M27 6-chloroniotinic acid	BYI 02960-CNA BCS-AA35572	157.56 $C_6H_4ClNO_2$	
M44 difluoroacetic acid	BYI 02960-DFA BCS-AA56716	96.03 $C_2H_2F_2O_2$	
M47	BYI 02960-azabicyclosuccinamide BCS-CS64875	288.25 $C_{12}H_{14}F_2N_2O_4$	
M48	BYI 02960-succinamide BCS-CR74729	306.69 $C_{12}H_{13}ClF_2N_2O_3$	

ANNEX 1 – BACKGROUND DOCUMENT TO RAC OPINION ON 4-[(6-CHLORO-3-PYRIDYLMETHYL)(2,2-DIFLUOROETHYL)AMINO]FURAN-2(5H)-ONE; FLUPYRADIFURONE

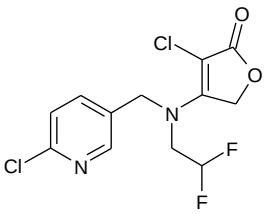
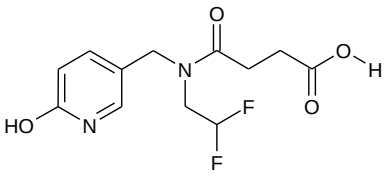
compound designation	**Reference Code**	**Molecular weight / formula**	**Structure**
The following are minor metabolites in environmental matrices and are not considered in PEC calculations			
M01	BYI 02960-chloro BCS-CD27046	323.13 $C_{12}H_{10}Cl_2F_2N_2O_2$	
M49	BYI 02960-deschlorohydroxysuccinamide DHS	288.25 $C_{12}H_{14}F_2N_2O_4$	

Table 150: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Stability			
Hydrolysis [furanone-4-14C] BYI 02960, JMAFF (2000), EPA 161-1, PMRA DACO Number 8.2.3.2, OECD 111 (2002)	Hydrolytically stable	50°C, pH = 4, 7 & 9	Mislankar and Woodard 2011
Photodegradation in Water – 1 [furanone-4-14C] BYI 02960, OPPTS 835.2240, 2008; Japanese JMAFF New Test Guidelines, 2000 Canada PMRA DACO Number 8.2.3.3.2	DT_{50} = 13.8 hours	25°C, pH 7 Metabolites: BYI 02960-succinamide (39.6% AR at 28 hours), and BYI 02960-azabicyclosuccinamide (25.9% AR at 35 hours) and BYI 02960-deschlorohydroxysuccinamide (DCHS, 2.5% at 35 hours).	Hall 2011
Photodegradation in Water – 2 BYI 02960, Phototransformation of Chemicals in Water, Part A: Direct Phototransformation, UBA, Berlin, Germany (Dec 1992); Test Method: ECETOC (Polychromatic Light Source)	Average $DT_{50} \approx 33$ hours (10 to 14 days during periods of main use, i.e. in spring to summer)	25°C, pH = 4, 7 & 9 $\Phi = 0.000138$	Heinemann 2011
Photodegradation in Water – 3 [furanone-4-14C] BYI 02960, JMAFF (2000)	DT_{50} = 14 hours	25°C, pH = 7 (8.2) Metabolites: BYI 02960-succinamide (38.2% AR at 28 hours), and BYI 02960-azabicyclosuccinamide (14.3% AR at 28 hours) and BYI 02960-deschlorohydroxysuccinamide (DCHS, 2.2% at 22 hours).	Hall, L.R., 2011
Photodegradation in Air BYI 02960, AOPWIN™ (U.S. EPA, 2008) software	DT_{50} = 4.4 – 13.1 hours	Estimated values (modelled)	Hellpointer 2010
Aquatic Simulation Studies			

Method	Results	Remarks	Reference
Water-Sediment – 1 Anaerobic, Dark, [Pyridine-2,6-^{14}C] BYI 02960, incl. OECD 308	Total system, DT_{50} > 1000 days (Lawrence, KS) & DT_{50} = 415.0 days (Pikerville, NC)	BYI 02960 is considered stable (Geometric mean DT50 ≥ 664 days)	Xu 2012
Water-Sediment - 2 Aerobic, Dark, [Pyridine-2,6-^{14}C] BYI 02960, incl. OECD 308	Total system, DT_{50} = 193.1 & 246.9 days	2 systems, 3 different labeled positions	Hellpointner & Unold 2012
Water-Sediment – 3 Aerobic, Dark, [Furanone-4-^{14}C] and [Ethyl-1-^{14}C] BYI 02960 BYI 02960, incl. OECD 308	Total system, DT_{50} = 202.4 – 285.0	2 systems, 2 different labeled positions	Menke & Unold 2012
Water-Sediment - 4 Metabolite: BYI 02960-DFA Aerobic, Dark, [1-^{14}C]	Total system, DT_{50} = 248.1	2 systems The metabolite BYI 02960-DFA is considered stable	Hellpointner & Unold 2012
Water-Sediment - 5 Aerobic, Microcosm, BYI 02960 (tech.), partly OECD 308	Total system, DT50 = 92.7	Many remarks of RMS, incl. DT_{50} are specific and are **not to be generalized** to other small outdoor waters across the EU.	Burns 2012
Soil degradation studies			
Soil, Aerobic – 1 Dark, [pyridinyl-methyl-^{14}C] BYI 02960, OECD 307 (2002), US EPA OPPTS 835.4100 (2008), OECD 106 (2001)	DT50 = 68.8 days, measured geometric mean	Average (4 soils) of 47.65% CO_2 formed at 120 days	Menke 2011
Soil, Aerobic – 2 Dark, [Furanone-4-^{14}C] BYI 02960, OECD 307 (2002), US EPA OPPTS 835.4100 (2008)	DT50 = 56.2 days, NB: ~30% NER	4 European soils	Menke & Unold 2011
Soil, Aerobic – 3 Dark, [Furanone-4-^{14}C] BYI 02960, OECD 307 (2002), US EPA OPPTS 835.4100 (2008)	DT50 = 228 days silt loam & DT50 = 65.7 days sandy loam with NB: ~30% NER	2 US soils	Ripperger 2011

Method	Results	Remarks	Reference
Soil, Aerobic – 4 Dark, [Ethyl-1-^{14}C] BYI 02960, OECD 307 (2002), US EPA OPPTS 835.4100 (2008)	DT50 = 41.6 days, measured geometric mean	3 European soils, metabolite Difluoroacetic acid (DFA) ~20% end of the study (118 days) Average of 34.03 % 14-CO_2 formed at 118 days*	Menke & Unold 2011
Soil, Aerobic – 5 Dark, [Pyridine-2,6-^{14}C] BYI 02960, OECD 307 (2002), US EPA OPPTS 835.4100 (2008)	DT50 = 37.4 days	1 European soil, 57.4% 14-CO_2 of AR formed at 117 days	Menke & Unold 2011
Soil, Aerobic – 6 Dark, [Pyridine-2,6-^{14}C] BYI 02960, OECD 307 (2002), US EPA OPPTS 835.4100 (2008)	DT50 = 242 days (Sanger, ~26% NER) and 56.3 days (Springfield, ~ 11% NER)	2 US soils, metabolite 6-chloronicotinic acid (6-CNA) (8.6% end of study), 28.15% 14-CO_2 of AR formed at 120 days**	Shepherd 2011
Soil, Anaerobic – 1 Dark, [Pyridine-2,6-^{14}C], [furanone-4-^{14}C] and [ethyl-1-^{14}C] BYI 02960, OECD 307 (2002), US EPA OPPTS 835.4100 (2008)	DT50 = 633.7 days	1 soil, metabolite Difluoroacetic acid (DFA) & others <5% AR	Menke & Unold 2012
Soil, Anaerobic – 2 Dark, [Pyridine-2,6-^{14}C] BYI 02960,OECD 307 (2002), US EPA OPPTS 835.4100 (2008)	No degradation during anaerobic phase, aerobic phase DT50 = 152-165 days	1 US soil, metabolite 6-chloronicotinic acid (6-CNA) (12%, end of study) & BYI 02960-chl (2%, end of study), End of the aerobic phase, there was 6.1% 14-CO_2.	Mislankar and Woodard 2012
Soil, Anaerobic – 3 Dark, [Pyridine-2,6-^{14}C] BYI 02960,OECD 307 (2002), US EPA OPPTS 835.4100 (2008)	No degradation during anaerobic phase, aerobic phase reported as minor, no DT50, terminated.	1 US soil.	Woodard 2012

* 42.3 (DD) + 25.9 (AX) + 33.9 AR (HF)/ 3 = 34.03% average ^{14}-CO_2 formation

* * 20.2 (Springfield) + 36.1 (Sanger) / 2 = 28.15% average ^{14}-CO_2 formation

5.1.1 Stability

Hydrolysis

Study 1 of 1 (Hydrolysis) *DAR reference STUDY IIA, 7.5/01*

reference	:	Mislankar, S., Woodard, D., 2011		purity	:	radiochemical purity 99%.
study type	:	hydrolysis		nominal concentration	:	1.0 mg/L
year of execution	:	2009		pH	:	4, 7, 9
GLP statement	:	yes		temperature	:	50°C
guideline	:	JMAFF (2000), EPA 161-1, PMRA DACO Number 8.2.3.2, OECD 111 (2002)		conclusion	:	hydrolytically stable
test substance	:	[furanone-4-^{14}C] BYI 02960		acceptability	:	**acceptable**

The hydrolytic stability of flupyradifurone was assessed at pH 4, 7, and 9 at 50˚C according to OECD TG 111. A preliminary test at 50˚C showed that no volatiles were formed during hydrolysis, confirmed by the recoveries.

The test showed that BYI 02960 was stable, with minimal degradation for pH.4 (92.1% ± 3.6 AR at day 5), pH 7 (96.6% ± 0.4 AR at day 5) and pH 9 (95.8% ± 1.1 AR at day 5). Three transformation products were identified (at all pH values), each maximally present at 2.7% at the end of the test (day 5). No DT_{50} values were presented since minimal degradation was observed in the pH 4, 7 and 9 buffer solutions.

The mass balances were complete throughout the study for all pH test systems, with the mean material balance ranging from 95.8 to 100.7%.

Flupyradifurone is considered to be hydrolytically stable under environmental conditions. It is concluded that hydrolysis is not relevant for the degradation of the substance in the environment.

Photodegradation in water

Study 1 of 3 (Photodegradation in water) *DAR reference STUDY IIA, 7.6/01*

reference	:	Hall, L.R., 2011	incubation time	:	up to 35 hours
study type	:	aqueous photolysis	nominal concentration	:	~1.0 mg/L
year of execution	:	2010	pH/temperature	:	pH 7/temp 25°C
GLP statement	:	Yes	light intensity	:	680 W/cm2
guideline	:	OPPTS 835.2240, 2008; Japanese JMAFF New Test Guidelines, 2000 Canada PMRA DACO Number 8.2.3.3.2	conclusion	:	photolytic DT_{50} under study conditions 13.8 hours
test substance	:	[furanone-4-^{14}C] BYI 02960	acceptability	:	**Acceptable**
purity	:	radiochemical purity 99.3%			

The aqueous phototstability of [FUR-^{14}C] BYI 02960 was assessed at pH 7, at 25˚C according to the Japanese JMAFF Guideline OPPTS 835.2240. Artificial irradiation (xenon lamp, >290 nm,

quartz and Suprax® filter) was applied for 35 hours, no longer as the DT_{75} was exceeded at this time point.

In the irradiated test systems, [FUR-^{14}C] BYI 02960 decreased from 98.1% of AR at time 0 (mean of replicates) to 8.4% of AR at 35 hours of irradiation. The major degradates included BYI 02960-succinamide (max. 39.6% of AR at 28 hours) and BYI 02960-azabicyclosuccinamide (maximum 25.9% of AR at 35 hours). A minor degradate was identified as BYI 02960-deschlorohydroxysuccinamide (DCHS, maximum of 2.5% at 35 hours) which is considered as intermediate between the two major photodegradates. No other single detected component exceeded 3% of AR at 35 hours. The determined dissipation time DT50 is 13.8 hours.

The mass balances were complete throughout the study, with the mean material balance of the irradiated [^{14}C] BYI 02960 test systems being 100.6% and individually ranging from 99.3 to 101.6%.

RMS Remarks:The study is acceptable and results are reliable. Only the FUR BYI 02960 was tested and not another radiolabel. The other ring structure is also photolytically active. A test with this radiolabel might result in different metabolites. Therefore, in principle PYR BYI 02960 should also be tested. However, for BYI 02960 in the aqueous photolysis study, two major degradates BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide were identified and contained the same number of carbons as the parent compound as demonstrated in the pathway. Thus the bond between the two rings (furanone and chloropyridine) was not broken during the course of this study. In addition, the bond between the difluoroethyl side chain and the amino moiety remained intact. Therefore conducting this study with only one radiolabel position is sufficient for tracking all the major degradates formed in the photolyis of BYI 02960.

Study 2 of 3 (Photodegradation in water) *DAR reference STUDY IIA, 7.6/02*

reference	:	Heinemann, O., 2011	incubation time	:	500 min
study type	:	aqueous photolysis, quantum yield	nominal concentration	:	5.03 mg/L in water
year of execution	:	2011	temperature	:	temp 25°C
GLP statement	:	Yes	pH	:	4, 7, 9
guideline	:	Phototransformation of Chemicals in Water, Part A: Direct Phototransformation, UBA, Berlin, Germany (Dec 1992); Test Method: ECETOC (Polychromatic Light Source)	quantum yield	:	Φ = 0.000138
test substance	:	BYI 02960	acceptability	:	**acceptable**
purity	:	99.4%			

The aqueous phototstability of BYI 02960 was assessed at pH 4,7 & 9, at 25˚C according to the ECETOC method using polychromatic light. Not more than 10% degradation was measured after a maximum irradiation period of 500 minutes. This indicated moderate degradability of BYI 02960. A mean quantum yield of Φ = 0.000138 was calculated on the basis of UV absorption data and the degradation kinetics determined from both experiments.

The estimates based on the two modelling concepts (Zepp & Cline or Frank & Kloepffer) are comparable. Both estimates consider the quantum yield Φ and the absorption in the UV-VIS spectrum being in the range of wavelengths relevant for the environment. Environmental direct phototransformation half-lives of BYI 02960 in sunlight exposed surface water layers are estimated to fall in a range of 10 to 14 days during periods of main use, i.e. in spring to summer. This assessment does not consider other potential mechanisms which may enhance the degradation in natural water, e.g. by indirect photolytic processes.

The registrant reported varying environmental DT50 for direct phototransformations, depending on the season and latitude, for instance, the best case is a DT50 = 10.3 hours at 30th degree latitude in summer and the worst case is a DT50 = 195 hours at 60th degree latitude in winter. The average is DT50 = 32.96 hours (about 33 hours).

The results show that direct transformation in aqueous solution may contribute to the dissipation of BYI 02960 from the environment.

Study 3 of 3 (Photodegradation in water) *DAR reference STUDY IIA, 7.6/03*

reference	:	Hall, L.R., 2011	incubation time	:	28 hours
study type	:	aqueous photolysis in sterile natural water	nominal concentration	:	1 mg/L
year of execution	:		pH/temperature	:	8.2 / 25°C
GLP statement	:	Yes	light intensity	:	680 W/cm^2
guideline	:	JMAFF (2000)	conclusion	:	DT_{50} in sterile buffer pH 7 14 hours days, equivalent to <3.8 calendar days at Tokyo (35°N)
test substance	:	[furanone-4-^{14}C] BYI 02960	acceptability	:	**Acceptable**
purity	:	radiochemical purity 99.3%			

The aqueous photostability of BYI 02960 was assessed at pH 8, at 25˚C according to the Japanese JMAFF. Artificial irradiation (xenon lamp, >290 nm, quartz and Suprax® filter) was applied for 28 hours, no longer as the DT_{75} was exceeded at this time point.

In the irradiated test systems, flypuradifurone (BYI 02960) decreased from 95.1% of AR at time 0 (mean of replicates) to 17.2% of AR at 28 hours of irradiation. The major degradates included BYI 02960-succinamide (max. 38.2% of AR at 28 hours) and BYI 02960-azabicyclosuccinamide (maximum 14.3% of AR at 28 hours). A minor degradate was identified as BYI 02960-deschlorohydroxysuccinamide (DCHS, maximum of 2.2% at 22 hours), which is considered an intermediate leading to the formation of BYI 02960-azabicyclosuccinamide. A group of components ("polar mixture") that eluted near the HPLC column's void volume was shown by TLC to consist of multiple components, and none of these components exceeded 7.3% of the applied radioactivity. The determined dissipation time DT50 is 14.0 hours. Based on this value the half-life of BYI 02960 under environmental conditions is calculated to be, e.g., 3.8 days at Tokyo, Japan (latitude 35°N). See also Figure 8, proposed degradation pathway in water/sediment systems.

The mass balances were complete throughout the study, with the mean material balance of the irradiated test systems being 98.1% and individually ranging from 95.7 to 99.7%.

RMS Remarks:

The RMS evaluated the photolysis study and agrees that photolysis is an important process in the aqueous environment. No major difference caused by indirect photolysis. Results are not used for EU risk assessment.

Study 1 of 1 (Photodegradation in air)

reference	:	Hellpointer, E., 2010	purity	:	n.a.
year of execution	:	2010	test system	:	theoretical calculation
GLP statement	:	n.a.	hydroxyl rate constant	:	0.5-1.5 × 10^6 molecules (radicals)/cm^3
guideline	:	n.a.	half-life in air	:	4.4 – 13.1 hours
test substance	:	BYI 02960	acceptability	:	**acceptable**

Based on the estimation method according to structure-activity relationship (SAR) methods developed by Roger Atkinson and co-workers the chemical lifetime of the BYI 02960 in the air was assessed by the program AOPWIN^{TM}, version 1.92a (U.S. EPA, 2008). The half-life time ($t_{1/2}$) was estimated within a range of 4.4 hours (short-term scenario) to 13.1 hours (long-term scenario), depending on the mean concentration of hydroxyl radicals present in the troposphere.

In addition, BYI 02960 is susceptible to reactions with ozone, however, that attack and its resulting chemical half-life is considered to be slower by a factor of 2 to 10.

As a consequence of the short half-life in air, no long-range transport of BYI 02960 in the atmosphere is likely to occur nor an accumulation of BYI 02960 in the environmental compartment air. From the low vapor pressure of the substance it is concluded that very low, if any, quantities of BYI 02960 are expected to enter the atmosphere from volatilization.

RMS Remarks:
The study is acceptable. The RMS considers the endpoint based on the 12 hour OH radical concentration, in line with FOCUSAIR, reliable. The atmospheric half-life of BYI 02960 is estimated to be 4.4 hours. BYI 02960 is not considered to have potential for long range transport based on this result.

5.1.2 Biodegradation

Biological degradation of BYI 02960 (flupyradifurone) in aquatic systems was investigated in studies on anaerobic and aerobic biodegradation under dark laboratory conditions using natural water-sediment systems, as well as soil systems. **For a full summary, please refer to section 5.1.3.**

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

A ready biodegradability test is not performed (DAR, section B.8.4.3.1, p.191), but BYI 02960 is considered “not readily biodegradable”.

5.1.2.3 Simulation tests

Aquatic simulation studies

Four different water/sediment studies were submitted in the dossier: an anaerobic water sediment study for BYI 02960, two aerobic water sediment studies (one with one radiolabel and one with two radiolabels) for BYI 02960 and a water/sediment study for the metabolite difluoroacetic acid (DFA).Additionally, one field microcosm study was submitted. Remark: The non-extractable residue (NER) formed during the experiment was only reported in a study-overview table (in addition to the text) when exceeding about 20% or more.

Study 1 of 4 (degradation in water-sediment systems) *DAR reference STUDY IIA, 7.8.2/01*

reference	:	Xu, T., 2012	incubation time	:	102 days
year of execution	:	2010	nominal concentration	:	0.233 and 0.203 mg/L
GLP statement	:	Yes	temperature	:	24 ± 2 °C
guideline	:	incl. OECD 308	DT_{50}	:	415 and > 1000 days
test substance	:	[Pyridine-2,6-^{14}C] BYI 02960	metabolites	:	< 5% (unidentified)
purity	:	radiochemical purity 99%	acceptability	:	**acceptable supplementary**
test system	:	see materials			

The **anaerobic biotransformation** of [pyridine-2,6-^{14}C] BYI 02960 was studied in two pond water/sediment systems. One system was collected from a pond near Lawrence, KS, USA and the other was taken from a pond in Pikeville, NC, USA. The study was conducted for 102 days in the dark at 24 ± 2 °C. BYI 02960 was applied at a rate of 0.233 and 0.203 mg a.i./L for Lawrence, KS and Pikeville, NC. The treatment rate was based an application rate of 410 g a.i./ha. The test systems consisted of an Erlenmeyer flask containing 50 g (dry weight) sediment and 150 mL pond water, i.e. a sediment/water ratio of 1:3. Eight sampling intervals were conducted and included 0, 7, 14, 21, 29, 43, 70 (71 for Pikeville, NC), and 102 days post treatment. The water samples were filtered and analyzed by direct sample injection into HPLC. The sediment was extracted sequentially at ambient temperature, followed by two aggressive extractions using a microwave extractor at 70 ºC. Appropriate volumes of both the ambient and aggressive extracts were concentrated and analyzed by HPLC coupled to a ^{14}C detector to characterize ^{14}C-BYI 02960 residues. Identification of the BYI 02960 was achieved by co-chromatography and liquid chromatography-electrospray ionization mass spectrometry (LC-ESI/MS).

The anticipated test conditions (incubation temperature, anaerobicity and microbial viability) were maintained, and material balances for both test systems were complete throughout the study.

Lawrence test system: The radioactive residues in the water phase decreased from an average of 99.1% at Day 0 to 48.6% at Day 7 and declined to 25.1% at the end of the study (Day 102). The radioactive residues in the sediment increased from an average of 8.8% on Day 0 to 46.8% at Day 7 and increased to 70.4% at Day 43, and 68.9% at the end of the study. Unextractable residues were increasing from 0.2% at Day 0 to 4.9% at the end of the study. Volatile compounds remained low with $^{14}CO_2$ ≤0.1% and organic volatiles below the LOQ. No major degradates were formed in the test systems with Lawrence, KS sediment. Total minor unidentified ranged from 1.3 – 5.3% throughout the study.

Pikeville test system: The radioactive residues in the water phase decreased from an average of 87.3% at Day 0 to 48.4% at Day 7 and further declined to 18.5% by the end of the study (Day 102). The radioactive residues in the sediment increased from an average of 11.4% on Day 0 to 49.2% at Day 7 and continued to increase to 78.7% at Day 71 and 64.8% at the end of the study. Unextractable residues were increase from 0% at Day 0 to 12% at the end of the study. Volatile compounds remained low with $^{14}CO_2$ ≤0.1% and organic volatiles below the LOQ. No major degradates were formed in the test systems. Total minor unidentified ranged from 0.0 -. 0.7% throughout the study.

BYI 02960 dissipated from the water phase to the sediment with a half-life of 7.2 and 9.6 days for Lawrence and Pikeville test system, respectively. The half-lives of BYI 02960 in the sediment under anaerobic conditions were 415 days for Pikeville sediment and greater than 1000 days for Lawrence sediment. **Thus the compound is regarded as stable under anaerobic conditions, no major metabolite was formed**

RMS Remarks:

The study is acceptable. It is noted that the two systems are in the low range of organic carbon content as prescribed in the OECD guideline. The water/sediment system was tested under anaerobic conditions, however the redox potential and dissolved oxygen measurements show that the system is not completely anaerobic. The RMS agrees with the applicant that BYI 02960 stable under anaerobic conditions. Results of this anaerobic study are not used for further assessment in the aquatic environment. The study is accepted as supplementary information.

Study 2 of 4 (degradation in water-sediment systems) *DAR reference STUDY IIA, 7.8.3/01*

Reference	:	Hellpointner, E., Unold, M., 2012	incubation time	:	119 days
year of execution	:	2011	nominal concentration	:	0.040 mg/L
GLP statement	:	yes	temperature	:	20 ± 1 °C
guideline	:	incl. OECD 308	DT_{50}	:	193.1 days (HW, 25% NER) and 246.9 days (AW), entire system
test substance	:	[Pyridine-2,6-^{14}C] BYI 02960	metabolites	:	<1.8% AR
purity	:	radiochemical purity >99%	acceptability	:	**acceptable**

test system	:	see materials			

The **aerobic transformation** of [pyridine-2,6-^{14}C] BYI 02960 was studied in two different water/sediment systems (Hönniger and Angler Weiher) for a maximum of 119 days in the dark at 20 ± 1 °C. In a supplementary test, the degradation of [PYR-^{14}C] BYI 02960 was studied in samples which were sterilized by gamma radiation and (separate vessels) by steam pressure. The test systems consisted of laboratory microcosm flasks attached to traps for collection of CO_2 and volatile organic compounds. Individual flasks filled a volume ratio of water to sediment of 3:1 were treated with [PYR-^{14}C] BYI 02960 with an application rate of 20.9 µg/batch corresponding to approx. 40 µg/L water assuming a maximum field application rate of 400 g BYI 02960/ha. During incubation the supernatant water was agitated gently.

Duplicate test systems were processed and analyzed after 0, 3, 7, 14, 28, 63, 91, and 119 days of incubation. The water samples were analyzed for radioactivity after centrifugation. Prior to radio-HPLC analysis, a concentration step was performed. The sediment samples were extracted at ambient temperature three times with acetonitrile/water (70/30, v/v), followed by one extraction step with pure acetonitrile. Afterwards, the sediment was extracted once more using a microwave-accelerated solvent extraction (aggressive organic extract) with 80 mL acetonitrile/water (70/30, v/v). The combined extracts from the ambient extractions and the aggressive extraction were analyzed by LSC and, after a concentration step, via radio-HPLC. Identification of BYI 02960 residues was by HPLC-MS, HPLC-MS/MS, NMR and HPLC co-chromatography.

The non-extractable residues (NER) in sediment samples were determined, and those from Hönniger Weiher in case of the last sampling date were separated into humin, humic acid and fulvic acid fractions.

The test conditions outlined in the study protocol were maintained throughout the study, and the material balance in the two test series was complete (on average 98.9% for HW and 98.0% for AW test system). The complete material balance demonstrates that no significant portion of radioactivity dissipated from the vessels or was lost during processing.

The radioactivity in the water phase of HW test systems decreased to 12.9% of AR, and in the water phase of AW test systems to 24.6% of AR at study termination. Extractable ^{14}C sediment residues in test systems from HW increased from 1.9% of the applied radioactivity at DAT-0 to 59.8% at DAT-63 and declined to 53.0% towards the end of the study. Extractable ^{14}C residues in the sediments from AW increased from 1.1% of the applied radioactivity at day 0 to 49.7% at study termination. The maximum amount of non-extractable ^{14}C residues (NER) in the sediment was 25.0% of AR for test systems from HW and 13.6% of AR for AW (DAT-119). The fractionation of NER of HW resulted in portions of 4.5 and 5.4% of AR within the humic acids, whereas similar amounts of radioactivity were associated with the fulvic acids (9.1 and 9.3% of AR) or strongly integrated into the insoluble humin of the soil matrix (10.7 and 9.8% of AR). At the end of the study, 6.8% and 8.5% of AR were present as $^{14}CO_2$ in the test systems from HW and AW,

respectively. Organic volatile compounds were not detected in significant amounts (< 0.1% of AR in all test systems).

In the water phase of HW and AW, the amount of [PYR-^{14}C] BYI 02960 decreased from 96.7 and 96.5% of AR at day 0 to 11.4 and 22.3% at study termination, respectively. In the sediment phase of HW, the amounts of BYI 02960 increased from 1.8% of AR at day 0 to a maximum of 59.4% at DAT-63, followed by a decrease to 52.6% towards the end of the study. In the AW sediment the amounts of BYI 02960 increased from 1.0% of AR at day 0 to 49.5% at study termination. Not any major transformation products were to be detected in the water phase and the sediment of both test systems.

The dissipation of BYI 02960 from the water phases was mainly characterized by rapid partitioning into the sediment. The best-fit kinetic models for the determination of trigger values were the First Order Multi-Compartment (FOMC) kinetic model for HW and the Double First-Order in Parallel (DFOP) kinetic model for AW with DT_{50} values of 8.5 and 34.5 days, respectively. The corresponding modelling endpoints were calculated using the DFOP kinetic model. The DT_{50} values for the slow degradation phase were 63.0 and 63.6 days for the water phase of HW and AW, respectively. In the entire water/sediment systems, BYI 02960 was degraded slowly which was best described using single first-order kinetics (SFO). **The estimated DT_{50} values were 193.1 and 246.9 days for the entire water/sediment test system from HW and AW, respectively.** The only major transformation products were carbon dioxide and formation of NER.

In the supplemental test, i.e. the test systems sterilized by gamma radiation or steam pressure and then incubated for 0, 60 or 120 days, no CO_2 was formed (≤ 0.1% AR). The amount of radioactivity in the water phase, predominantly represented by parent compound, was about two times higher than in that from the microbial active samples. Further, there was a clear trend that NER formation in sediment was lower than in the microbial active test flasks, especially seen for both test systems HW and AW sterilized by steam pressure. This shows that the NER were at least partly formed by microbial processes.

RMS Remarks:
The RMS finds the water/sediment study acceptable. The modelling endpoints for water phase are not used for exposure modelling since they are not level PII true degradation values. Level PI whole system values are considered acceptable for use in environmental exposure modelling.

Study 3 of 4 (degradation in water-sediment systems) *DAR reference STUDY IIA, 7.8.3/02*

reference	:	Menke, U., Unold, M., 2012	incubation time	:	120 days
year of execution	:	2009-2010	nominal concentration	:	0.04 mg/L
GLP statement	:	yes	temperature	:	20 ± 2 °C
guideline	:	incl. OECD 308	DT_{50}	:	208.2 (FUR-^{14}C, HW, 22.6% NER) and 202.4 days (ETH-^{14}C,HW, 26.6% NER) 246.1 days (FUR-^{14}C, AW) and 285.0 days (ETH-^{14}C,HW)

test substance	:	[Furanone-4-^{14}C] and [Ethyl-1-^{14}C] BYI 02960	metabolites	:	Difluoroacetic acid (DFA) ≤0.9% AR & minor metabolites ≤1.1% AR
purity	:	radiochemical purity >98% [FUR] >99% [ETH]	acceptability	:	**acceptable**
test system	:	see materials			

The **aerobic transformation** of [FUR-^{14}C] and [ETH-^{14}C] BYI 02960 was studied in two water/sediment systems, Hönniger Weiher (HW) and Angler Weiher (AW) for a maximum of 120 days in the dark at 20 ± 2°C. The test systems consisted of laboratory microcosm flasks attached to traps for collection of CO_2 and volatile organic compounds. Individual flasks filled a volume ratio of water to sediment of 3:1 were treated with [^{14}C] BYI 02960. The actual application rates were 20.86 and 20.80 µg/batch for label FUR and label ETH test systems from Hönniger Weiher and 20.63 and 20.53 µg/batch for label FUR and label ETH test systems from Angler Weiher, corresponding to approx. 40 µg/L water assuming a field application rate of 400 g BYI 02960/ha. During incubation the supernatant water was agitated gently.

Duplicate test systems were processed and analyzed after 0, 1, 3, 7, 14, 30, 45/44, 60/58, 87/86 and 120 days of incubation for test systems from HW and AW (both labels), respectively. The water samples were decanted and analyzed for radioactivity after centrifugation and filtration. Prior to HPLC analysis, a concentration step was performed. The sediment samples were extracted three times with acetonitrile/water (70/30, v/v), followed by one extraction step with pure acetonitrile, all at ambient temperature (ambient organic extracts). Afterwards, the sediment was extracted once more using a microwave-accelerated solvent extraction (aggressive organic extract) with 80 mL acetonitrile/water (70/30, v/v). The combined extracts from the ambient extractions and the aggressive extract were analyzed by LSC and via HPLC after a concentration step. For selected samples, the HPLC analysis was confirmed by TLC. Identification of the two test items was achieved by HPLC-MS, HPLC-MS/MS, NMR and/or Co-chromatography. The major metabolite detected in label ETH test systems was characterized by HPLC-MS. The non-extractable residues (NER) in sediment samples were determined, and those from the last sampling date were separated into humin, humic acid and fulvic acid fractions.

The test conditions outlined in the study protocol were maintained throughout the study, and the mean material balances in all four test series ranged from 98.2 – 100.4% of AR. The complete material balance demonstrates that no significant portion of radioactivity dissipated from the vessels or was lost during processing.

The radioactivity in FUR and ETH test systems from HW water decreased steadily from 96.0 and 97.2% of AR at day 0 to 14.3 and 14.2% at study termination. The radioactivity in FUR and ETH test systems from AW water decreased from 97.9 and 98.3% of AR at day 0 to 37.3 and 44.1% towards the end of the study. Extractable ^{14}C sediment residues in FUR and ETH test systems from HW increased from 4.2 and 3.8% of AR at day 0 to 54.7 and 54.1% at study termination, respectively. Extractable ^{14}C residues in FUR and ETH sediments from AW increased from 2.2 and 2.4% of AR at day 0 to maxima of 37.7 and 40.1% of AR at study termination. The maxima of non-extractable ^{14}C residues (mean values of duplicates) in the sediments were 22.6 and 26.6% of AR for FUR and ETH test systems from HW, and 17.9 and 15.2% of AR for FUR and ETH test systems from AW, respectively.

At the end of the study period, 3.9 and 1.5% of AR were present as $^{14}CO_2$ in systems from HW, and 5.5 and 0.9% of AR were present as $^{14}CO_2$ in systems from AW, each for FUR and ETH label,

respectively. The total amount mainly accounted for of $^{14}CO_2$ trapped in soda lime, but as well for the amount of $^{14}CO_2$ present in the water phase (all sampling intervals) and sediments (only DAT-120). Organic volatile compounds amounted to ≤ 0.2% of the applied radioactivity in both systems and for both labels.

In the water phase from HW, BYI 02960 decreased from 95.5 and 96.8% of AR at day 0 to 14.3 and 14.1% at study termination for FUR and ETH test systems, respectively. In AW water of FUR and ETH test systems, BYI 02960 decreased from 97.4 and 97.9% of AR at day 0 to 35.6 and 36.8% at study termination. In the sediment phase of FUR and ETH test systems from HW, BYI 02960 increased from 4.1 and 3.7% of AR at day 0 to maximum amounts of 58.7 and 58.3% at DAT-60 or DAT-45, and then slightly declined to 54.3 and 52.6% towards study termination, respectively. In AW sediment (FUR and ETH, respectively) BYI 02960 increased from 2.2 and 2.3% of AR at day 0 to 37.6 and 38.9% at study termination.

DFA (difluoroacetic acid) was observed as a degradation product of [ETH] BYI 02960 in the water phases and in the sediment extracts of both water/sediment systems. In the water phases it accounted for up to 1.1% (HW) and 6.0% (AW) of AR, in the sediment extracts, DFA accounted for only 0.8% and 0.9% of AR, respectively. Two very minor metabolites were detected in the water phases of ETH test systems (≤ 1.1% of AR), and three very minor metabolites were detected in the water phases of FUR test systems (≤ 1.0% of AR). In the sediments, one very minor metabolite was detected in the ETH test systems (≤ 0.5% of AR) and the FUR test systems (≤ 0.1% of AR). The maximum amount of the non-characterized radioactivity was 0.5% of AR for all test systems and compartments.

The dissipation of BYI 02960 from the water phase was mainly characterized by a fast translocation into the sediment. The best-fit kinetic model for the determination of trigger values was the DFOP kinetic model with DT_{50} values of 9.8 and 9.4 days for FUR and ETH test systems from HW, and DT_{50} values of 59.2 and 66.2 days for FUR and ETH test systems from AW. The corresponding modelling endpoints were also calculated using the DFOP kinetic model. The DT50 values for the slow degradation phase were 48.5 and 50.2 days for the water phase of HW, but 123.8 and 117.5 days for the water phase of AW.

In the entire water/sediment systems, BYI 02960 was degraded slowly, the degradation was best described using single first-order kinetics (SFO). The estimated DT_{50} values were 208.2 and 202.4 days for FUR and ETH systems from HW, and 246.1 and 285.0 for FUR and ETH test systems from AW. These values are to be used as trigger values and modelling endpoints.

Remarks RMS:

The RMS finds the water/sediment study acceptable. The modelling endpoints for water phase are not used for exposure modelling since they are not level PII true degradation values. Level PI whole system values SFO kinetics are considered acceptable for use in modelling.

Study 4 of 4 (degradation in water-sediment systems) *DAR reference STUDY IIA, 7.8.3/03*

reference	:	Hellpointner, E., Unold, M., T., 2012	incubation time	:	99 days
year of execution	:	2011	nominal concentration	:	4.7 µg DFA per test system

GLP statement	:	yes	temperature	:	19.2 ± 0.1 °C
guideline	:	incl. OECD 308	DT50	:	**109.0 days (HW) and 567.2 days (AW)**
test substance	:	[1-^{14}C] BYI 02960-DFA	metabolites	:	<2% AR (minor metabolites)
Purity	:	radiochemical purity > 99.5%	acceptability	:	**acceptable**
test system	:	see materials			

The **aerobic transformation of the metabolite [1-^{14}C] DFA** was studied in two water/sediment systems Hönniger Weiher (HW) and Angler Weiher (AW) for a maximum of 99 days in the dark at 19.2 ± 0.1 °C. The application rate of 4.7 µg DFA per test system was the ten-fold overdose of the application rate calculated based on the use rate of the parent compound BYI 02960 (400 g/ha) and the maximum amount of BYI 02960-DFA formed in the parent aerobic aquatic metabolism study (6.9%). The test systems consisted of laboratory microcosm flasks attached to traps for the collection of CO_2 and volatile organic compounds. Entire vessels filled with either 88.4 g (HW) or 210.3 g (AW) dry weight sediment and 520 mL of supernatant water (volume ratio of water to sediment: 3:1) were treated with [1-^{14}C] BYI 02960-DFA. During incubation the supernatant water was in smooth motion.

Samples were taken after 0, 7, 19, 33, 61, 79 and 99 days of incubation. The water layers were decanted and centrifuged. The sediment samples were extracted stepwise with acetonitrile/water mixtures at ambient temperature (ambient organic extracts). Afterwards, the sediment was extracted once more using a microwave-accelerated solvent extraction (aggressive organic extract). The extracted sediment phase was air-dried or freeze-dried, homogenized and combusted in an oxidizer in order to determine the non-extractable residues (NER). The water phases and the combined extracts of the ambient extraction steps were analyzed by LSC and TLC in order to determine the amounts of the test item and its transformation products. The aggressive extracts were only analyzed by LSC since they contained only low amounts of radioactivity. Identification of the test item in application solution was achieved by HPLC-MS, HPLC-MS/MS and NMR.

The test conditions outlined in the study protocol were maintained throughout the study. The mean material balances in the two test series ranged from 97.7 to 105.8% for test systems from Hönniger Weiher and from 97.7 to 107.6% for test systems from Angler Weiher.

The radioactivity in the water phase of HW test systems decreased steadily from 97.6% at day 0 to 34.6% of AR at study termination. The radioactivity in the water phase of AW test systems decreased from 100.0% of AR at day 0 to 80.1% at DAT-7 and varied between 75.0% (DAT-79) and 83.8% (DAT-33) afterwards. Extractable sediment ^{14}C residues in test systems from HW increased from 2.8% of at day 0 to 27.7% of AR at DAT-61 and declined to 24.1% towards the end of the study. Extractable ^{14}C residues in the sediment from AW accounted for 1.5% of at day 0 and varied between 16.2% (DAT-7) and 17.2% of AR (DAT-79) afterwards. The maximum of non-extractable ^{14}C residues (NER) in the sediment were 15.8% and 6.5% of AR for test systems HW and AW at DAT-61, respectively. The maximum of $^{14}CO_2$ in the test systems from HW and AW were 25.1% and 7.5% of AR, respectively. The majority of total $^{14}CO_2$ accounted for volatile $^{14}CO_2$ trapped by the soda lime. A very minor portion included in those values was $^{14}CO_2$ present dissolved in the water phases (all sampling dates) and sediments (only DAT-99). Organic volatile compounds were not detected in significant amounts (< 0.1% of the applied radioactivity in all test systems).

In the water phase of HW, BYI 02960-DFA decreased from 93.8% of AR at day 0 to 32.3% at study termination. In the water of AW, BYI 02960-DFA decreased from 95.4% of AR at day 0 to 78.3% at DAT-7 and varied between 72.3% (DAT-79) and 81.1% (DAT-33) afterwards. In the sediment phase of HW, BYI 02960-DFA increased from 2.8% of AR at day 0 to a maximum of 25.2% at DAT-79 and then declined 22.7% towards the end of the study. In the sediment phase of AW, BYI 02960-DFA accounted for 1.5% of AR at day 0 and varied between 13.3% (DAT-33) and 16.5% (DAT-79) afterwards. Just minor transformation products were observed in the water and sediment phase of both test systems.

The dissipation of DFA from the supernatant water phase was characterized by translocation into the sediment and by degradation. This was best described using the DFOP kinetic model with DT_{50} values of 54.2 and 583.9 days for Hönniger Weiher and Angler Weiher, respectively. The corresponding modelling endpoints were either determined using the DFOP kinetic model (Hönniger Weiher), resulting in a DT_{50} value of 75.3 days for the slow degradation compartment, or the SFO kinetic model (Angler Weiher) with a DT_{50} value of 371.5 days. **In the entire water/sediment systems, -DFA was degraded slowly which was best described using the DFOP kinetic model. The estimated DT_{50} values were 106.5 and 967.1 days for test systems from Hönniger Weiher and Angler Weiher. The corresponding modelling endpoints were determined using the SFO kinetic model with estimated DT_{50} values of 109.0 and 567.2 days for test systems from Hönniger Weiher and Angler Weiher,.**

Remarks RMS:

The RMS considers the water/sediment study acceptable. The modelling endpoints for water phase are not used for exposure modelling since they are not level PII true degradation values. Level PI whole system values are considered acceptable for use in modelling.

Field investigation study

Study 1 of 1 (degradation in a microcosm system) *DAR reference STUDY IIA, 7.8.3/04*

reference	:	Burns, E., 2012	incubation time	:	148 days
year of execution	:	2010	nominal concentration	:	10 ang 100 µg/L
GLP statement	:	yes	temperature	:	ambient
guideline	:	OECD Guidance Document "Simulated Freshwater Lentic Field Tests (Outdoor Microcosms and Mesocosms)", April 2006 OECD 308, but only in part	DT_{50}	:	95.1 days (system specific and not to be generalized, see text)
test substance	:	BYI 02960 (tech.)	metabolites	:	Not reported
purity	:	96.2%	acceptability	:	**acceptable**
test system	:	see materials			

The fate of BYI 02960 (tech.) was determined in pond water and sediment in outdoor microcosms as an aquatic model ecosystem for lentic aquatic freshwater systems with different trophic levels. For this purpose, 4 microcosms were treated with two different concentrations of the test item. During the consecutive months samples of water and sediment were taken and the content of the

test item in these compartments was analyzed. Additionally several parameters of water and sediment were monitored to characterize the system.

In November 2009 a pond with the shape of a stub pyramid (bottom 53 m^2, surface 80 m^2) was filled with a layer of natural sediment (0.15 m height) and water (0.7 m height). The walls and bottom of the pond is made of plastic foil. The sediment originated from a drinking water reservoir system, the water was composed of local ground water and water from a natural pond. Twenty-five enclosures (= microcosms) made of polycarbonate (diameter 0.97 m. height 0.9 m) were inserted into the pond on May 4, 2010 (= about 4 weeks before application). Overall, the microcosms are representative for small stagnant water bodies.

The test substance BYI 02960 (tech.) (Batch ID: 2009-000239) was applied once on June 02, 2010 onto the water surface of four microcosms. Two treatment levels, 10 and 100 µg a.i./L, were tested with two replicates each:. Two microcosms were kept untreated as controls. The microcosms were investigated for a period of 148 days after the treatment. Several times during the study period water and sediment samples were taken and analyzed to demonstrate the initial test concentrations and the fate of the test substance in these compartments. The physic-chemical water parameters were also evaluated. Furthermore the abundance of filamentous algae und the turbidity of the water was assessed.

The results of initial concentrations demonstrated that the nominal concentrations had been applied. A steady decline of BYI 02960 in the water phase was shown. At the end of the study (= 5 months after application) the a.i. concentration was 22% of applied amount at the test concentration of 10 µg a.s./L, and 40% of applied amount at 100 µg a.s./L concentration level. The analyzed concentrations of BYI 02960 in the sediment did not show a clear trend during the entire study period, although a slight increase of BYI 02960 concentrations was observed towards the end of the study. For the 10 µg a.s./L-level, 2.3 to 7.5% of applied amount was found in the sediment; for the 100 µg a.s./L-level, 1.9 to 11.4 % of the applied amount were detected in the sediment.

The half-life calculations for BYI 02960 in the microcosm water using SFO kinetics model resulted on average (arithmetic mean) for both concentration level in 80.6 days, and in the entire system (water + sediment) in 95.1 days. A calculation for the fate in sediment was not given, since the fate of BYI 02960 in this matrix did not show a clear pattern of decline.

Remarks RMS: (for the full remarks please refer to the DAR B.8.4.3, p. 236)

Concluding: The study can be used for risk assessment but only in case i) the half-lives derived are calculated back to 20 °C (as well as possible, the weekly temperature measurements should preferably be supplemented, e.g. with temperature measurements nearby and correlating these to the experiment, because DT_{50} is a function of temperature and ii) as one of the 2-4 systems providing DT_{50} values to be used for calculating a geometric mean DT_{50}. **The DT_{50} values of this study are system specific and cannot be generalized to other small outdoor waters across the EU.**

Soil degradation studies

Six degradation studies in aerobic soil were submitted and three anaerobic soil studies. Remark: The non-extractable residue formed during the experiment was only reported in a study-overview table (in addition to the text) when exceeding about 20% or more.

Aerobic

Study 1 of 6 (degradation in aerobic soil) *DAR reference STUDY IIA, 7.1.1/01*

Reference	:	Menke, U., 2011	study type	:	aerobic soil degradation
year of execution	:	2007-2009	incubation time	:	120 days
GLP statement	:	yes	nominal concentration	:	0.53 mg / kg soil
Guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008) OECD106 (2001)	temperature	:	20°C
test substance	:	[pyridinyl-methyl-^{14}C] BYI 02960	DT50	:	68.8 days
Purity	:	> 99%	metabolites	:	<3% AR
Soils	:	four soils; sandy loam, silt loam, loam, clay loam	acceptability	:	**acceptable**

The biotransformation and time dependent sorption of [pyridinylmethyl-^{14}C] BYI 02960 was studied in four European soils: Laacher Hof AXXa (AX), Höfchen am Hohenseh (HF), Hanscheiderhof Plot 611 (HN), and Dollendorf II (DD) for a maximum period of 120 days under aerobic conditions in the dark at approx. 20°C and 55% WHC_{max} (max. water holding capacity). BYI 02960 was applied at the nominal rate of 0.53 mg/kg soil, which is equivalent to 200 g/ha field application rate.

At each sampling date the soil samples were shaken for 24 hours with 400 mL $CaCl_2$-solution in order to measure the time-dependent desorption of the test item. Subsequently they were extracted by shaking at ambient temperature and in a microwave at 70 °C with acetonitrile/water mixtures, and the BYI 02960 residues were analyzed and quantified by TLC with HPLC as the confirmatory method.

Material balances were complete throughout the study, and the test item declined from 97.1, 96.1, 96.5 and 93.1% AR at DAT-0 to 37.1, 24.5, 50.2 and 28.7% in soils AX, HF, HN and DD, respectively, at the end of the study. Applying double first-order kinetics a half-life (geometric mean) of 68.8 days was calculated for BYI 02960 in the tested soils under aerobic conditions.

The registrant reported the mineralization of [PYM-^{14}C] BYI 02960 in this study as high. At the end of the study (DAT-120) up to 45.3 (AX), 58.6 (HF), 29.4 (HN) and 57.3% AR (DD) of $^{14}CO_2$ were generated. This is an average of 47.65% CO_2 formation. Volatile organic compounds were negligible (≤ 0.1% AR). With the exception of carbon dioxide only very minor transformation products (all were below 3% AR) were detected. Non-extractable ^{14}C-residues (NER) increased from 1.0, 1.5, 1.9 and 4.1% AR at DAT-0 to 12.6, 13.2, 16.8 and 12.5% AR at the end of the study period.

RMS Remarks:
The aerobic soil degradation, apart of the time dependent sorption study, is acceptable and the results are reliable.

Study 2 of 6 (degradation in aerobic soil) *DAR reference STUDY IIA, 7.1.1/02*

reference	:	Menke, U., Unold, M. (2011)	study type	:	aerobic degradation

year of execution	:	2008	incubation time	:	120 days
GLP statement	:	yes	nominal concentration	:	1.07 mg/kg
guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008)	temperature	:	10°C or 20°C
test substance	:	[Furanone-4-^{14}C] BYI 02960	DT_{50}	:	56.2 days, NB: ~30% NER
purity	:	> 98 %	metabolites	:	<2% AR
soils	:	Four soils; sandy loam, silt loam, silt loam, silty clay	acceptability	:	**acceptable**

The biotransformation of [furanone-4-^{14}C] BYI 02960 was studied in four European soils: Laacher Hof AXXa (AX), Höfchen am Hohenseh (HF), Hanscheiderhof Plot 611 (HN), and Dollendorf II (DD) for a maximum period of 120 days under aerobic conditions in the dark at approx. 20 °C and 55% WHC_{max} (max. water holding capacity). BYI 02960 was applied at the nominal rate of 1.07 mg/kg dry weight of soil, which is equivalent to 400 g/ha field application rate.

At each sampling date, the soil samples were extracted with 2 x 80 mL acetonitrile/water (50/50, v/v), 1 x acetonitrile/water (80/20, v/v) and 1 x 80 mL acetonitrile by shaking at ambient temperature. Another extraction step was performed with acetonitrile/water (80/20, v/v) at 70°C using a microwave. The BYI 02960 residues and transformation products were analyzed and quantified by HPLC. TLC was used as confirmation method.

Material balances were complete throughout the study, and the test item declined from 96.9, 95.9, 96.4 and 94.3% of AR at DAT-0 to 37.3, 20.2, 45.2 and 26.9% at the end of the study for soils AX, HF, HN and DD, respectively. Applying double first-order kinetics a half-life (geometric mean) of 56.2 days was calculated for [furanone-4-^{14}C] BYI 02960 in the tested soils under aerobic conditions.

The mineralization of [furanone-4-^{14}C] BYI 02960 in this study was high. At the end of the study (DAT-120) up to 27.6 (soil AX), 38.9 (soil HF), 18.0 (soil HN) and 32.0% AR (soil DD) of $^{14}CO_2$ were generated. Volatile organic compounds were negligible (≤ 0.1% AR). Non-extractable ^{14}C-residues (NER) increased from 2.4, 3.8, 3.3 and 4.6% of AR at DAT-0 to 27.8, 33.6, 31.0 and 34.1% AR at the end of the study period for soils AX, HF, HN and DD, respectively. The major portions of NER radioactivity were found in the insoluble humin fraction.

RMS Remarks:
The aerobic soil degradation study is acceptable and the results are reliable.

Study 3 of 6 (degradation in aerobic soil) *DAR reference STUDY IIA, 7.1.1/03*

reference	:	Ripperger, R.J. (2011)	study type	:	aerobic degradation
year of execution	:	2010	incubation time	:	120 days
GLP statement	:	yes	nominal concentration	:	1.07 mg/kg
guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008)	temperature	:	20°C
test substance	:	[Furanone-4-^{14}C] BYI 02960	DT_{50}	:	228 days silt loam (16% NER) & 65.7 days sandy loam, NB:

					~30% NER
purity	:	> 98 %	metabolites	:	≤3.6% AR (non characterized)
soils	:	Two soils; silt loam, sandy loam	acceptability	:	**acceptable**

The biotransformation of [FUR-^{14}C] BYI 02960 was studied in two US soils: silt loam Springfield, NE, and sandy loam Sanger, CA, for a maximum period of 120 days under aerobic conditions in the dark at approx. 20 °C and soil moistures maintained between pF 2.0 to 2.5. Gamma irradiated samples were investigated along with microbial active test systems. BYI 02960 was applied at the rate of 1.1 µg a.i./g soil, equivalent to 410 g a.i./ha assuming a 2.5 cm soil depth.

Duplicate test systems were analyzed at 0, 3, 7, 14, 28, 60, 92, and 120 days of incubation. The 50-g soil samples were extracted by shaking with acetonitrile:water (70:30) and acetonitrile (100%), followed by microwave extraction at 70 °C(aggressive extract) using acetonitrile:water (70:30). Extract aliquots were concentrated and analyzed by HPLC. Identification of the transformation products was performed by LC/MS, co-chromatography, and/or retention time comparison with reference standards.

Material balances were complete throughout the study, and the test item declined from 98.0 and 99.4% and of the applied amount at day 0 to 67.3 % and 30.8% of the applied at the end of the study. The first-order half-life of BYI 02960 in silt loam was 228 days. The first-order half-life in the sandy loam was 65.7 days.

The registrant reports that BYI 02960 mineralizes relatively rapidly under aerobic condition to $^{14}CO_2$ (12.3% Springfield, 36.1% Sanger) and becomes increasingly bound to soil (16.4% Springfield, 30.6% Sanger, CA) by study end. The amount of $^{14}CO_2$ and bound residue formed in gamma-irradiated soils was significantly less than in non-sterile soils indicating a biological component to degradation and the formation of non-extractable residues from BYI 02960. Additionally, soil fractionation shows that even with extraction using strong base, BYI 02960 related residues remain bound to the solid (humin) fraction indicating very strong and irreversible binding to soil.

RMS Remarks:
The aerobic soil degradation study is acceptable and the results are reliable.

Study 4 of 6 (degradation in aerobic soil) *DAR reference STUDY IIA, 7.1.1/04*

reference	:	Menke, U., Unold, M., (2011)	study type	:	aerobic degradation
year of execution	:	2009-2011	incubation time	:	118 days
GLP statement	:	Yes	nominal concentration	:	1.07 mg/kg
guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008)	temperature	:	20°C
test substance	:	[Ethyl-1-^{14}C] BYI 02960	DT_{50}	:	41.6 days
purity	:	> 98 %	metabolites	:	Difluoroacetic acid (DFA) ~20% end of the study
soils	:	three soils, silt loam, loamy sand and clay loam	acceptability	:	**acceptable**

The biotransformation of [ethyl-1-^{14}C] BYI 02960 was studied in three European soils: Laacher Hof AXXa (AX), Höfchen am Hohenseh (HF), and Dollendorf II (DD) for a maximum period of approx. 120 days under aerobic conditions in the dark at approx. 20°C and 55% WHC_{max} (max. water holding capacity). BYI 02960 was applied at the nominal rate of 1.07 mg/kg dry weight of soil, which is equivalent to 400 g/ha field application rate.

At each sampling date, the soil samples were extracted with 2 x 80 mL acetonitrile/water (50/50, v/v), 1 x acetonitrile/water (80/20, v/v) and 1 x 80 mL acetonitrile by shaking at ambient temperature. Another extraction step was performed with acetonitrile/water (80/20, v/v) at 70°C using a microwave. The BYI 02960 residues and transformation products were analyzed and quantified by LCS and radio-HPLC. Radio-TLC was used as confirmation method.

Material balances were complete throughout the study, and the test substance declined from 96.0, 96.6 and 97.1% of AR at DAT-0 to 17.7, 39.6 and 23.8% of AR at the end of the study for soils DD, AX and HF, respectively. Applying double first-order kinetics a half-life (geometric mean) of 41.6 days was calculated for BYI 02960 in the tested soils under aerobic conditions.

The mineralization of [ETH-^{14}C] BYI 02960 was high. At the end of the study (DAT-118), up to 42.3 (DD), 25.9 (AX) and 33.9% AR (HF) of $^{14}CO_2$ were generated. Volatile organic compounds were not detected in significant amounts ($\leq$ 0.1% AR).

In addition to carbon dioxide, one major transformation product was detected in the extracts of all three soils. It was identified as difluoroacetic acid (DFA) via HPLC-MS and accurate mass determination. DFA reached maximum values of 30.2, 22.0 and 33.8% of AR on DAT-45 or DAT-48 in soils DD, AX and HF, respectively. Towards the end of the study, the levels of DFA declined to 17.0, 16.3 and 23.8% of AR in soils DD, AX and HF, respectively.

Non-extractable ^{14}C-residues increased from 2.7, 2.8 and 3.2% of AR at DAT-0 to 17.9, 14.3 and 15.4% AR at the end of the study period for soils DD, AX and HF, respectively.

Remarks RMS:
The aerobic soil degradation study is acceptable and the results are reliable.

Study 5 of 6 (degradation in aerobic soil) *DAR reference STUDY IIA, 7.1.1/05*

reference	:	Menke, U., Unold, M., (2011)	study type	:	aerobic degradation
year of execution	:	2010	incubation time	:	117 days
GLP statement	:	yes	nominal concentration	:	1.07 mg/kg
guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008)	Temperature	:	20°C
test substance	:	[Pyridine-2,6-^{14}C] BYI 02960	DT_{50}	:	33 or 37.4 days
purity	:	> 98 %	metabolites	:	<2.5% AR
soils	:	One soil; silt loam	acceptability	:	**acceptable**

The biotransformation of [pyridine-2,6-^{14}C] BYI 02960 was studied in one soil, Höfchen am Hohenseh 4a (HF, Burscheid, Germany), a silt loam soil of pH 6.5 ($CaCl_2$) and 2.4% organic carbon content. The incubation was conducted for max. 117 days under aerobic conditions in the dark at ca. 20°C and 55% WHC_{max} (max. water holding capacity). BYI 02960 was applied at the nominal rate of 106.7 µg/ 100 g soil, which is equivalent to a field application rate of 400 g/ha.

At each sampling date, i.e. 0, 1, 4, 7, 14, 29, 48, 61, 90 and 117 days after treatment (DAT), the duplicate soil samples were extracted with 2 x 80 mL acetonitrile/water (50/50, v/v), 1 x acetonitrile/water (80/20, v/v) and 1 x 80 mL acetonitrile by shaking at ambient temperature. Another extraction step was performed with acetonitrile/water (80/20, v/v) at 70°C using a microwave. The BYI 02960 residues and transformation products were analyzed and quantified by LCS and radio-HPLC. Radio-TLC was used as a confirmatory method.

Material balances were complete throughout the study (ranged on mean from 94.1 to 100.3% of AR), and the test substance declined from 96.7% at DAT-0 to 24.6% of AR at the end of the study.

The mineralization of [PYR-^{14}C] BYI 02960 was high. At the end of study (DAT-117), $^{14}CO_2$ accounted for 57.4% of AR. Volatile organic compounds were not detected in significant amounts ($\leq$ 0.1% AR).

Only minor transformation products (all mean values were $\leq$ 2.5% of AR) were detected in the extracts and were characterized by their retention time. The transformation product assigned to ROI 2 reached a mean maximum level of 2.0% of AR at DAT-48. The maximum amount of the second transformation product (ROI 3) was detected on DAT-7 (2.5% of AR). Towards the end of the study, the amounts decreased below the detection limit. ROI 3 was identified as 6-chloronicotinic acid (6-CNA) by co-chromatography. The third minor transformation product was detected only once (DAT-48, 0.3% of AR). Non-extractable ^{14}C-residues increased from 3.3% of AR at DAT-0 to 16.7% of AR at the end of the study period.

Conclusions

The registrant reported that current the laboratory study demonstrated that [PYR-^{14}C] BYI 02960 is degradable in soils under aerobic conditions with a DT_{50} of 33 days. An overall summary of the "best-fit" trigger values for all soils is given in the DAR, Table B.8.1.2.1-21, p.105 and here the overall geometric mean is reported at 37.4 days.

Remarks RMS:
The aerobic soil degradation study is acceptable and the results are reliable.

<u>Study 6 of 6 (degradation in aerobic soil)</u> *DAR reference STUDY IIA, 7.1.1/06*

reference	:	Shepherd, J.J. (2011)	study type	:	aerobic degradation
year of execution	:	2010	incubation time	:	120 days
GLP statement	:	yes	nominal concentration	:	1.1 mg/kg
guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008)	Temperature	:	20°C
test substance	:	[Pyridine-2,6-^{14}C] BYI 02960	DT_{50}	:	242 days (Sanger, ~26% NER) and 56.3 days (Springfield, ~ 11% NER)

purity	:	> 98 %	metabolites	:	6-chloronicotinic acid (6-CNA) (8.6% end of study)
soils	:	Two soils; silt loam, sandy loam	acceptability	:	**acceptable**

The biotransformation of [pyridine-2,6-^{14}C] BYI 02960 was studied in two US soils, silt loam Springfield, NE, and sandy loam Sanger, CA, for a maximum period of 120 days under aerobic conditions in the dark at approx. 20 °C and soil moistures maintained between pF 2.0 to 2.5. Gamma irradiated samples were investigated along with microbial active test systems. BYI 02960 was applied at the rate of 1.1 µg a.i./g soil, equivalent to 410 g a.i./ha assuming a 2.5 cm soil depth.

Duplicate test systems were analyzed at 0, 3, 7, 14, 28, 60, 92, and 120 days of incubation. The 50-g soil samples were extracted by shaking with acetonitrile:water (70:30) and acetonitrile (100%), followed by microwave extraction at 70 °C(aggressive extract) using acetonitrile:water (70:30). Extract aliquots were concentrated and analyzed by HPLC. Identification of the transformation products was performed by LC/MS, co-chromatography, and/or retention time comparison with reference standards.

Material balances were complete throughout the study, and the test item declined from 97.4 and 98.6% and of the applied amount at day 0 to 65.5 and 30.0% of the applied at the end of the study. In the silt loam, extractable ^{14}C-residues decreased from 99.3% of the applied amount at day 0 to 67.0% of the applied at the end of the study. Non-extractable 14C-residues increased from 0.7% of the applied amount at day 0 to a maximum of 11.3% at day 120. At study termination, evolved $^{14}CO_2$ reached 20.2%, and radioactive volatile organics were not detected. Total unidentified radioactivity ranged from 0.0% to 2.4% of the applied amount.

In the sandy loam, extractable ^{14}C-residues decreased from 99.9% of the applied amount at day 0 to 40.0% of the applied at the end of the study. One major degradate was identified as 6-chloronicotinic acid (6-CNA), which formed 6.9% on day 14, reached a maximum of 17.1% on day 60, and declined to 8.2% by the end of the study.

In the sterile silt loam, extractable 14C-residues decreased from 99.1% of the applied amount at day 0 to 89.4% of the applied at the end of the study. Non-extractable 14C-residues increased from 0.9% of the applied amount at day 0 to a maximum of 6.2% at day 122. At study termination, evolved $^{14}CO_2$ reached 0.4%, and radioactive volatile organics were not detected.

In the sterile sandy loam, extractable 14C-residues decreased from 99.8% of the applied amount at day 0 to 85.8% of the applied at the end of the study. Non-extractable ^{14}C-residues increased from 0.2% of the applied amount at day 0 to a maximum of 9.3% at day 122. At study termination, evolved $^{14}CO_2$ reached 0.9%, and radioactive volatile organics were not detected.

[PYR-^{14}C] BYI 02960 mineralizes relatively rapidly under aerobic condition to $^{14}CO_2$ (20.2% Springfield, 36.1% Sanger) and becomes increasingly bound to soil (11.3% Springfield, 25.5% Sanger, CA) by study end. Total unidentified radioactivity ranged from 0.0% to 2.4% (Springfield) and 0.0% to 3.0% (Sanger) of the applied amount.

The amount of $^{14}CO_2$ and bound residue formed in gamma-irradiated soils was significantly less than in non-sterile soils indicating a biological component to degradation and the formation of non-extractable residues from BYI 02960. Additionally, soil fractionation shows that even with extraction using strong base, BYI 02960 related residues remain bound to the solid (humin) fraction indicating very strong and irreversible binding to soil.

Remarks RMS:
The aerobic soil degradation study is acceptable and the results are reliable.

Conclusions

The current laboratory study demonstrated that BYI 02960 is degradable in soils under aerobic conditions with "best fit" DT_{50} values of 242 and 56.3 The overall geometric mean is reported at 234.9 days for Springfield soil and 56.8 for Sanger soil.

Study 1 of 3 (degradation in anaerobic soil) *DAR reference STUDY IIA, 7.1.2/01*

reference	:	Menke, U., Unold, M. 2012	study type	:	anaerobic degradation
year of execution	:	2009-2011	incubation time	:	up to 123 d
GLP statement	:	yes	nominal concentration	:	1.1 mg/kg
guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008)	temperature	:	20°C
test substance	:	[Furanone-4-^{14}C] and [Ethyl-1-^{14}C] and [Pyridine-2,6-^{14}C] BYI 02960	DT_{50}	:	633.7 days
purity	:	radiochemical purity 98-99 %.	metabolites	:	Difluoroacetic acid (DFA) & others <5% AR
soil	:	One soil; silt loam	acceptability	:	**Acceptable**

The present laboratory study investigated the degradation of BYI 02960 in one soil (silt loam) under flooded anaerobic conditions. [Pyridine-2,6-^{14}C], [furanone-4-^{14}C] and [ethyl-1-^{14}C] BYI 02960 (equivalent to label PYR, FUR, and ETH, respectively) were applied at of 110.1, 104.6 and 105.9 µg/100 g soil (dry matter), i.e. equivalent to 103.2, 98.0 and 99.2% of the nominal application rate of 400 g/ha.

The soil in duplicate test flasks/interval was maintained under aerobic conditions for 30 days in the dark at 20 ± 2°C and approx. 55% of the maximum water holding capacity. Following the aerobic phase, the samples were flooded with oxygen-depleted de-ionized water (approx. 3 cm layer above soil level), set under nitrogen atmosphere, and maintained in the dark at 20 ± 2°C under anaerobic conditions for max. 123 days. During the aerobic study phase, air-permeable traps were attached for the collection of CO_2 and volatile organics (static test system design). At start of the anaerobic study phase, the trap systems were replaced by sealable two-valve glass stoppers connected with plastic gas sampling bags.

Soil samples and water layers were separated by decanting to allow for individual analysis. The soil was extracted four times with at ambient temperature (combined as "ambient organic extract"). Subsequently, the soil was extracted once at an elevated temperature ("aggressive extract"). BYI 02960 residues in water layers were directly analyzed by reversed phase HPLC; the soil extracts were subjected to solvent exchange prior to analysis (all labels). TLC was employed as second contrasting separation method for the confirmation of the results. A limit of quantification (LOQ) of equal or better than 0.9% of the applied radioactivity (% AR) was calculated for HPLC radioactivity detection within the sample matrices.

For all three radiolabels complete material balances found at all sampling intervals demonstrated that no significant portion of radioactivity dissipated from the flasks or was lost during processing. During the 30 days of aerobic incubation $^{14}CO_2$ accounted for up to 26.7 (PYR), 15.9 (FUR) and 6.9 % of AR (ETH). During the anaerobic incubation phase, mineralization to $^{14}CO_2$ was negligible

(< 0.1 % of AR). Organic volatiles were not observed in the aerobic or in the anaerobic study phase (< 0.1 % AR at all sampling intervals).

The radioactivity extractable from soil decreased to 55.9, 55.2 and 82.0% of AR towards the end of the aerobic incubation phase (DAT-30), and further to 41.5, 40.2 and 53.4% of AR until the end of the anaerobic incubation period for labels PYR, FUR and ETH, respectively.

Within the aerobic phase of the study (30 days) the percentages of BYI 02960 in the entire systems decreased to 53.7, 52.6 and 54.7% of AR for labels PYR, FUR and ETH, respectively. During the anaerobic incubation period (i.e. flooded state) the portions of BYI 02960 slightly decreased further to 47.8, 47.2 and 47.7% of AR for labels PYR, FUR and ETH, respectively.

Only one transformation product exceeded 5% of AR over the entire study period. It was detected in the test systems of label ETH and it was identified as difluoroacetic acid (DFA). DFA levels increased to a level of 25.1% of AR during the initial 30 days of aerobic conditions. During the anaerobic phase, the amounts of DFA remained more or less stable (24.2 - 26.2% of AR).

In the aerobic incubation phase, non-extractable residues (NER) in soil increased from 2.7 to 12.9% (label PYR), 3.1 to 25.6% (Label FUR) and 2.8 to 10.8% of AR (Label ETH). During the anaerobic incubation phase the NER slightly increased further.

Applying single first order kinetics (SFO) to the BYI 02960 residues in the entire systems during the anaerobic phase of the study, the estimated DT_{50} values range from 581.8 to 693.2 days (geometric mean: 633.7 days).

Based on the results obtained within this study it can be expected that the amounts of BYI 02960 and its only significant metabolite DFA remain stable under flooded field conditions. Degradation would be expected to continue whenever the conditions become aerobic.

Remarks RMS:
The anaerobic soil degradation study is acceptable and the results are reliable.

Study 2 of 3 (degradation in anaerobic soil) *DAR reference STUDY IIA, 7.1.2/02*

reference	:	Mislankar, S. Woodard, D., 2012	study type	:	anaerobic degradation
year of execution	:	2011	incubation time	:	up to 121 d
GLP statement	:	yes	nominal concentration	:	1.17 mg/kg
guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008)	temperature	:	20°C
test substance	:	Pyridine-2,6-^{14}C] BYI 02960	DT50	:	No degradation during anaerobic phase, aerobic phase = 152-165 days (see text)
purity	:	radiochemical purity 99 %.	metabolites	:	6-chloronicotinic acid (6-CNA) (12%, end of study) & BYI 02960-chl (2%, end of study)
soil	:	Loamy sand, Sanger California, USA	acceptability	:	**acceptable**

The anaerobic biotransformation of [pyridine-2,6-^{14}C] BYI 02960 was studied in a loamy sand (pH 6.7 in 0.01M CaCl2, organic carbon 0.45%) from Sanger, California, USA. During the first phase of the study, the soil was maintained under aerobic conditions for 30 days in the dark at 20 ± 1 °C and

at soil moisture of 55% maximum water holding capacity. Following the aerobic phase, the samples were flooded with water (water:soil ratio 3:1, w/w) and maintained in the dark under anaerobic conditions for 121 days at 20 ± 1 °C. [PYR-^{14}C] BYI 02960 was applied at a rate of 1.17 µg a.i./g, dry soil, equivalent to an application rate of 410 g a.i./ha.

Samples were analyzed at 0, 14 and 32 days of aerobic incubation, and at 0, 14, 30, 45, 59, 91 and 121 days of incubation following flooding (post treatment) of the samples (anaerobic phase). The water was decanted from each test system and the soil was extracted by a shaking method. In addition, aggressive extraction was conducted. The water layer, ambient extract and the microwave extracts were analyzed by HPLC. Identification of the parent compound and major metabolite was achieved by mass spectrometry (LC/ESI/MS) and co-chromatography using an authentic standard.

The average total material balance in the soil/water system for BYI 02960 was 96.5% ± 1.8% of AR. Non-extractable (bound) residues in soil increased from 0.6% at day 0 to 15.7% at day 32. At the end of the aerobic phase, 6.1% of the applied radioactivity was present as CO_2. No volatile organic compounds were present. The concentration of BYI 02960 in the aerobic phase decreased to 61.4% of AR at day 32.

In the anaerobic phase, radioactivity in the combined water and ambient extract decreased from 60.2% at day 0 to 35.8% by the end of the study. Aggressive extractions with both acetonitrile:water and methanol:water ranged from 3.1% to 2.9% of the applied radioactivity in the study, indicating that residue left after ambient and aggressive extraction was not easily extractable. Since non-extractable residues did not change during aerobic (15.7%) and anaerobic phases (16.1%), no further characterization was conducted. No CO_2 or volatile organic compounds were produced during the anaerobic phase of the study.

During the anaerobic phase, the concentration of BYI 02960 in soil decreased from 51.1% at day 0 to 26.2% of the applied amount at study termination. One major metabolite, 6-chloronicotinic acid (6-CNA), was detected during the aerobic phase of the study and it reached maximum of 12% (water/sediment) at day 0 of anaerobic phase and remained steady (12 to 14 %) throughout anaerobic phase. One minor metabolite, BYI 02960-chloro was detected during the aerobic phase and accounted for 2.8% at day 32. During the anaerobic phase, it remained steady (2%).

The observed DT_{50} values for BYI 02960 in the aerobic, then anaerobic soil/water system were determined using single first-order kinetics (SFO), first-order multi compartmental (DFOP) and double first-order in parallel (FOMC) and half-lives were 152 days, 164 days and 584 days, respectively for the anaerobic phase. BYI 02960 degrades moderately under aerobic conditions and remains more or less stable during anaerobic phase in soil.

Remarks RMS:
The anaerobic soil degradation study is acceptable and the results are reliable.

Study 3 of 3 (degradation in anaerobic soil) *DAR reference STUDY IIA, 7.1.2/03*

reference	:	Woodard, D., 2012	study type	:	anaerobic degradation
year of execution	:	2011	incubation time	:	up to 60 d, terminated
GLP statement	:	yes	nominal concentration	:	1.1 mg/kg
guideline	:	OECD 307 (2002) US EPA, OPPTS 835.4100 (2008)	Temperature	:	20°C

test substance	:	Pyridine-2,6-^{14}C] BYI 02960	DT50	:	No degradation during anaerobic phase, aerobic phase reported as minor, no DT50, terminated
purity	:	radiochemical purity 100 %.	metabolites	:	see results
soil	:	sand clay loam, Springfield, Nebraska, USA	Acceptability	:	**Acceptable**

The anaerobic biotransformation of [pyridine-2,6-^{14}C] BYI 02960 was studied in sandy clay loam (pH 6.5 in 0.01 M $CaCl_2$, organic carbon 1.9%) from Springfield, NE, USA. During the first phase of the study, the soil was maintained under aerobic conditions for 29 days in the dark at 20 ± 2°C and at soil moisture of 55% maximum water holding capacity. Following the aerobic phase, the samples were flooded with water (water:soil ratio 3:1, w/w) and maintained in the dark under anaerobic conditions for 60 days at 20 ± 2 °C. The study was terminated after the 60 day sampling due to a failure of: the temperature control which resulted in a temperature of 50°C and compromised the remaining samples.

[PYR-^{14}C] BYI 02960 was applied at a rate of 1.1 µg a.i./g, dry soil, equivalent to an application rate of 410 g a.i./ha.

Samples were analyzed at 0, 14 and 29 days of aerobic incubation, and at 0, 19, 31, 45 and 60 days of incubation following flooding (post treatment) of the samples (anaerobic phase). The water was decanted from each test system and the soil was extracted using a shaking method. In addition, an aggressive extraction was conducted. The water layer, ambient extract and the microwave extracts were analyzed by HPLC using a flow-through 14C detector. Identification of the parent compound was achieved by mass spectrometry (LC/ESI/MS) and co-chromatography using an authentic standard.

The material balance for the study was complete (on average 105.8% ± 3.2%, mean range = 100.0 to 109.4%). Extractable [^{14}C] residues in soil decreased from 99.7% at day 0 to 79.0% by day 29. Non-extractable (bound) residues in soil increased from 0.3% at day 0 to 9.8% at day 29. At the end of the aerobic phase, 16.2% of the applied radioactivity was present as CO_2. No volatile organic compounds were present. In the aerobic phase the concentration of BYI 02960 decreased from 99.7% of the applied amount at day 0 to 79.0% at day 29. In the anaerobic phase, radioactivity in the combined ambient and aggressive extracts remained steady from 69.5% at day 0 to 71.7% by the end of the study. Aggressive extractions ranged from 6.7% to 11.7% of the applied radioactivity in the study, indicating that residue left after ambient and aggressive extraction was not easily extractable. NER and CO_2 in soil remained more or less constant during the anaerobic phase of the study, and no volatile organic compounds were produced.

BYI 02960 degrades moderately under aerobic conditions and its residues remain more or less stable during anaerobic phase in soil.

The study was terminated after the 60 day sampling due to a failure of the temperature control which resulted in a temperature of 50°C and compromised the remaining samples, this does not affect the interpretation of results of the study.

Remarks RMS:
The anaerobic soil degradation study is acceptable and the results are reliable, altough the study was terminated after 60 days due to a failure of the temperature control. The study did not complete the

guidance subscribed 120 days, however the same trend was observed as in the other anaerobic degradation studies submitted. Limited degradation of the test substance was observered.

Combined summary of Study 1 to 6 (rate of degradation in soil) *DAR reference STUDY IIA, 7.2.1/01-06*

The laboratory studies presented in the paragraph before (route of degradation in soil) were also designed to derive information on the rate of degradation of BYI 02960 and its significant metabolites under standardized laboratory conditions in soil. In study IIA 7.2.1/01 to /06 the methods and results of the respective kinetics calculations were described in more detail. In addition, a separate experimental degradation study was performed with 6-CNA, a major aerobic soil metabolite. For more information please refer to the DAR, Volume 3 B8. These studies are believed not to influence the classification and are therefore not discussed in more detail, please refer to the DAR Volume 3 B8 if required.

5.1.3 Summary and discussion of degradation

The stability and degradation of BYI 02960 was examined in several studies.

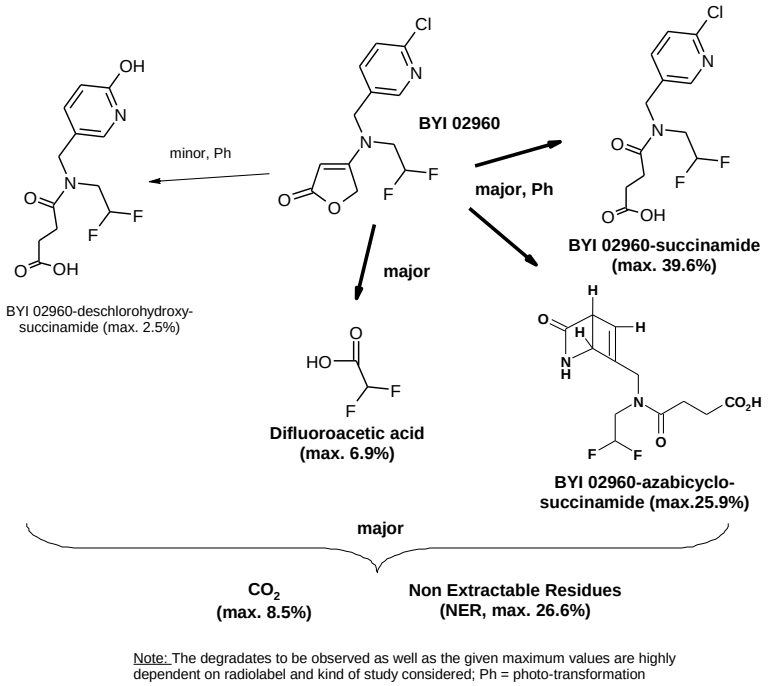
There were one hydrolysis study and four photolytic degradation studies. BYI 02960 is considered to be hydrolytically stable at pHs 4, 7 and 9. Three photolytic degradation studies in water reported half-lives varying from about 14 hours to 33 hours. The fourth photolytic degradation study is in air and reports DT_{50} = 4.4-13.1 hours.

No ready biodegradability test was performed, but in the DAR BYI 02960 is considered to be “Not Readily Biodegradable”.

Five different water-sediment studies were submitted in the dossier. All water-sediment studies show predominantly dissipation of the parent compound and little ultimate or primary degradation, as can be seen from the little amounts of CO_2 and metabolites formed: first, an anaerobic water sediment study with a half-life DT_{50} ≥415 days was reported. During this study, volatile $^{14}CO_2$ was low, i.e. ≤0.1%. Not more than 5% metabolites in total were formed, these were unidentified.
Then two aerobic water sediment studies (one with one label and one with two labels) with half-lives DT_{50} = 193.1 – 248.1 days were reported. For both studies not more than 8.5% of AR was present as $^{14}CO_2$ in the system at the end of the study period. One of the studies reported the formation of difluoroacetic acid (DFA, ≤0.9% AR) and furthermore, for both studies no major metabolites were found (<1.8% AR). In several samples of both studies, high NER formation was observed (up to 26.6%).
The fourth water sediment study was with the metabolite DFA (DT_{50} = 248.1 days). In this study only minor metabolite formation was observed (<2% AR) and a maximum of 25.1% $^{14}CO_2$ formation at the end of the study.
These four studies are all reliable and accepted and they are used as the key studies for classification purpose. BYI 02960 does not have a has a half-life shorter than 16 days in any of these studies (or degradation of >70% within 28 days) and is therefore considered to be not rapidly degradable (according to CLP guidance V4.0 nov 2013, p. 518).

A final water-sediment study was an outdoor microcosm study (DT_{50} = 92.7 days). This study was marked by the RMS as system specific and its results should not be generalised. Therefore, this study is not used as a key study for classification and labeling purpose.

In Figure 8 below, the proposed routes are shown for the degradation pathway in water-sediment systems of BYI 02960.



Note: The degradates to be observed as well as the given maximum values are highly dependent on radiolabel and kind of study considered; Ph = photo-transformation

Figure 8: ***Proposed route of degradation of BYI 02960 in water/sediment systems (BYI 02960 rapidly partitioned into the sediment phase, DFA is mainly present in water phase). (Figure B.8.4.4-01 in the DAR, p. 242 Vol. 3 B8.)***

Six different aerobic soil degradation studies were submitted in the DAR dossier. These studies are not directly used for classification and labelling purposes, but considered supportive studies. The half-lives reported vary from DT50 = 37.4 to 242 days. Some studies show high levels of non-extractable residue (NER, up to 30%) and the maximal reported CO_2 formation was 57.4% (study number 5, at 117 days). All but one (study number 4, 20% metabolite DFA) report little metabolite formation (≤ 8.6% of AR). Therefore, it is important to note that the half-lives may be rather dissipation times than actual degradation times. Two of three anaerobic soil studies (also supportive studies) show no degradation during the anaerobic phase, one of those studies was terminated and no DT50 was determined. The other study has a DT50 = 152-165 days and formation of 6-chloronicotinic acid (6-CAN, 12%) and BYI 02960-Chl (2%) were seen at the end of the study. The third does not differentiate between the anaerobic and aerobic phase, it reports a DT50 = 633.7 days and formation of the metabolite Difluoroacetic acid (DFA) (and <5% formation of other metabolites). In the DAR, it is mentioned that the clear bi-phasic degradation kinetics indicates that the compound is less available for biotransformation with time, probably due to a time-dependent sorption behavior in soil (confirmed by the high reported formation of NER in many of the studies). The BYI 02960 residues remain stable under anaerobic conditions and degrades (or dissipates) moderately to slowly in aerobic soil.

In Figure 9below the proposed routes are shown for the degradation pathway in soil systems of BYI 02960.

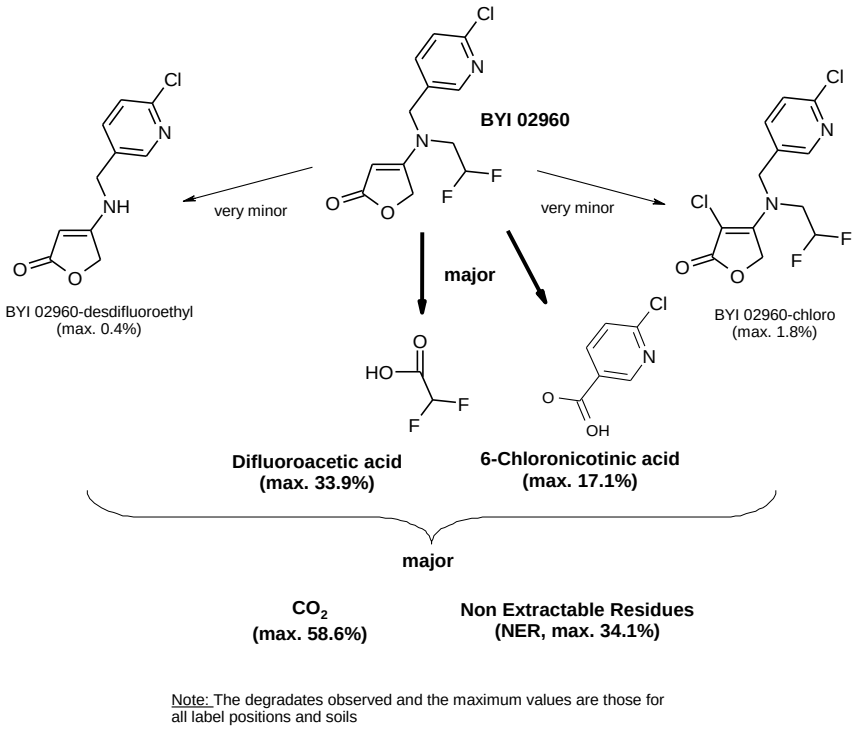


Figure 9: ***Proposed degradation pathway of BYI02960 in soil (Figure B.8.1.3-01 in the DAR, p.136 Vol. 3 B8.)***

Soil photolysis & Field studies

These studies are believed not to influence the classification and are therefore not discussed; please refer to the DAR Volume 3 B8 if required.

5.2 Environmental distribution

Studies on the environmental distribution of BYI 02960 are to support the classification and are believed not to be of direct influence on the classification itself. They are therefore briefly summarized in Table 151 and the paragraphs below; please refer to the DAR Volume 3 B1-B5 if required. BYI 02960 is a compound with good water solubility (~3 g/L), low volatility and high to moderate hydrophobicity (see log $K_F$oc and log Kow values in ***Table 151***). This implies that BYI 02960 will most likely be found in the water and soil/sediment compartment in the environment, more than in the air.

Table 151: Summary of relevant information on environmental distribution of BYI 02960

Method	Results	Remarks	Reference
Solubility in water EC A.6, OECD 105 GLP:Y	pH 4 (buffer) 3.2 g/L at 20°C pH 9 (buffer) 3.0 g/L at 20°C In distilled water: pH 7, 3.2 g/L at 20°C		Wiche and Bogdoll 2011
Vapour pressure EC A.4, OECD 104	Extrapolated: 9.1 x 10-7 Pa for 20 °C 1.7 x 10-6 Pa for 25 °C 2.6 x 10-5 Pa for 50 °C		Smeykal 2008
Volatility, Henry's law constant Calculated	Henry's law constant at 20 °C in distilled water (pH: 7.0) 8.2 x 10-8 Pa.m3.mol-1 Henry's law constants at 20 °C at different pH values: at pH 4: 8.2 x 10-8 Pa.m3.mol-1 at pH 9: 8.8 x 10-8 Pa.m3.mol-1	A vapour pressure of 9.1 x 10-7 Pa (20 °C) and the follwing water solubility values at pH 4: 3.2 g/L pH 9: 3.0 g/L were used to calculate the Henry's law constant.	Bogdoll and Eyrich 2011
Partition co-efficient, ECA.8 OECD 117	1-octanol / water (25 °C, pH 7) Kow = 16, log Kow = 1.2		Bogdoll and Stunk 2011
Dissociation constant (pKa), OECD 112	No dissociation occurs in aqueous solutions in the pH-range 1< pH <12		Wiche and Bogdoll 2011
Surface tension (technical active substance), EC A.5, OECD 115	Technical substance: 69.1 mN/m at 20°C		Eyrich and Bogdoll 2011
Log Koc OECD 106	$K_F$oc = 74.9- 107.0 (21 ±1°C)		Menke and Telscher 2008
Log Koc OECD 106	$K_F$oc = 74.9- 107.0 / 85.2 – 132.2 (20 ±1°C) *!		Stroech 2011

*! The Characteristics table of this study in the DAR (p 146 Vol 3, B8) seems to have a mistake in the reported $K_F$oc value. Namely, the $K_F$oc in the Characteristics table doesn't match to the $K_F$oc values mentioned later in the study information (DAR p.151 Vol 3, B8, Table B8.2.1 14). As it is

exactly the same as the study before, it is suspected to be a copy-paste error in the Characteristics table and the values of the study description are reported here instead.

5.2.1 Adsorption/Desorption

Four studies have been reported on the adsorption, desorption and mobility in soil in the DAR. The Koc of BYI 02960 ranges from 74.9-107.0 in the first two studies. The other two examine ad- and desorption and do not report a combined Koc value. The Metabolite 6-CAN has a reported Koc of 70-258 and metabolite DFA has a Koc = 70-258. This means that BYI 02960, 6-CAN and DFA all have a high potential to sorb, with a (depending on the soil type) low to slight mobility in soils.

5.2.2 Volatilisation

In the DAR the vapour pressure (9.1 x 10^{-7} Pa, 20°C) and volatility (8.2 x 10^{-8} $Pa.m^3.mol^{-1}$, pH 7, 20°C, calculated) are reported as low (p.6, Vol. 3 B1-B5).

5.2.3 Distribution modelling

No studies were reported in the DAR, which specifically address environmental distribution modelling.

5.3 Aquatic Bioaccumulation

There are no experimental studies found in the DAR for bioaccumulation.

5.3.1 Aquatic bioaccumulation

BYI 02960 has a log Kow of 1.2 according to OECD 117 (HPLC method).

5.3.1.1 Bioaccumulation estimation

There are no estimation studies are available.

5.3.1.2 Measured bioaccumulation data

There are no measured bioaccumulation data found in the DAR (Vol 3 B9).

5.3.2 Summary and discussion of aquatic bioaccumulation

The log Kow is used an indicator of bioaccumulation potential in the absence measured BCF data.

The log Kow of BYI 02960 is reported as 1.2, which is below the cut-off value of log Kow = 4. Consequently, BYI 02960 is not considered to have bioaccumulative potential.

5.4 Aquatic toxicity

The aquatic toxicity of BYI 02960 was examined in several studies. There are four acute fish, four acute aquatic invertebrate and one short term algae and plants studies. Also, there are one chronic fish and three chronic aquatic invertebrate and one chronic algae and plants studies reported in the DAR and shown in this chapter. There is one amphibian toxicity study (frogs), however, this is believed not to influence the classification and therefore not discussed; please refer to the DAR Volume 3 B9 if required. An overview of the relevant aquatic toxicity studies is shown in Table 152.

Several metabolites were studied as well, being 6-CAN (6-chloronicotinic acid), DFA (difluoroacetate), BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide. These are all less toxic than the parent compound BYI 02960 (lowest reported EC50 >1 mg/L nominal, 48h, 6-CAN, *Chironomus tentans*) and are believed not to influence the classification and are therefore not discussed. The data is shown in Chapter 7.2: "The Aquatic Toxicity of Metabolites of BYI 02960" and please refer to the DAR Volume 3 B9 if more information is required.

Table 152: Summary of relevant information on aquatic toxicity

Acute Aquatic Toxicity Studies			
Method	**Results**	**Remarks**	**Reference**
Acute Fish – 1 96 hours, Rainbow trout *(Oncorhynchus mykiss)*, BYI 02960, FIFRA 72-1, OPPTS 850.1075, OECD 203	LC50, 96h >74.2 mg a.s./L m.m.	Static, stock solution in DMF	Matlock & Lam (2010a)
Acute Fish – 2 96 hours, Fathead minnow *(Pimephales promelas)*, BYI 02960, FIFRA 72-1, OPPTS 850.1075, OECD 203	LC50, 96h >70.5 mg a.s./L m.m.	static, stock solution in DMF	Matlock & Lam (2010b)
Acute Fish – 3 96 hours, Common carp *(Cyprinus carpio)*, BYI 02960, FIFRA 72-1, OPPTS 850.1075, OECD 203	LC50, 96h >100 mg a.s./L nominal	Static, measured concentration was 101-120% of the nominal concentration, stock solution in DMF	Bruns (2011a)
Acute Fish – 4 96 hours, Sheepshead minnow (*Cyrpinodon variegatus*), BYI 02960, FIFRA 72-3, OPPTS 850.1075, OECD 203	LC50, 96h >83.9 mg a.s./L m.m.	Static, stock solution in DMF	Banman & Lam (2009b)
Acute Aquatic Invertebrate – 1 48 hours, *Daphnia magna*, FIFRA 72-2, OPPTS 850.1010, OECD 202	EC50, 48h >77.6 mg a.s./L m.m.	Static, stock solution in DMF	Banman & Lam (2009a)
Key Study Acute Aquatic Toxicity	EC50, 48h = 61.7 µg a.s./L nominal = 0.0617 mg a.s./ L	Static, measured concentrations were 97-107% of the	Bruns (2011e)

Acute Aquatic Invertebrate – 2 48 hours, *Chironomus riparius*, OPPTS 850.1300, OECD 202		nominal concentration	
Acute Aquatic Invertebrate – 3 96 hours, Eastern oyster *(Crassostrea virginica)*, OPPTS 850.1025	EC50, 96h >29 mg a.s./L	Flow through, stock solution in DMF	Gallagher et al. (2009a)
Acute Aquatic Invertebrate – 4 96 hours, Salt water mysid *(Americamysis bahia)*, OPPTS 850.1035	EC50, 96h = 0.26 mg a.s./L	Static, acceptable for juveniles only	Gallagher et al. (2009b)
Short term Algae & Plants – 1 96 hours, *Pseudokirchneriella subcapitata*, FIFRA 123-2, OPPTS 850.5400, OECD 201 (2006)	ErC50 & EyC50, 72 hours >80 mg a.s./L nominal	Static, stock solution in DMF, measured concentrations were 111-120% of the nominal concentration	Banman & Lam (2010)
Short term Algae & Plants – 2 7 days, Duckweed *(Lemna gibba G3)*, FIFRA 123-2, OPPTS 850.4400, OECD 221 (2006)	Lowest NOEC = 34.2 mg a.s./L m.m. Lowest EC50 >67.7 mg a.s./L m.m.	Semi-static, stock solution in DMF	Banmann (2010)
Chronic Aquatic Toxicity Studies			
Method	**Results**	**Remarks**	**Reference**
Long Term Fish – 1 35 days, ELS test, Fathead minnow (*Pimephales promelas*), FIFRA 72-4, OPPTS 850.1400, OECD 210	NOEC, 35 days = 4.41 mg a.s./L m.m.	Flow trough, stock solution in DMF	Matlock & Lam (2011)
Long term Aquatic Invertebrate – 1 21 days, *Daphnia magna*, EPA 72-4, OPPTS 850.1300, EEC C.20, OECD 211	NOEC, 21 days = 3.2 mg a.s./L nominal	Semi-static, stock solution in DMF, measured concentrations were 102-117% of the nominal concentration	Riebschläger (2011)
Key Study Chronic Aquatic Toxicity Long term Aquatic Invertebrate – 2 28 days, *Chironomus riparius*, OECD 219	NOEC, 28 days = 10 µg a.s./L nominal = 0.010 mg a.s. /L (→ 4.1 µg a.s. / L m.m.)	Static, stock solution in DMF, measured concentrations decline to a mean 41% in overlying water, still NOEC based on nominal concentration!	Bruns (2011g)
Long term Aquatic Invertebrate – 3 28 days, Saltwater mysid *(Mysidopsis bahia)*, OPPTS	NOEC, 28 days = 13.2 µg a.s./L m.m. = 0.0132 mg a.s./L m.m.	Flow through, stock solution in DMF	Claude et al. (2011)

850.1350			

5.4.1 Fish

There are four short-term and one long-term test available on the toxicity of BYI 02960 to fish.

5.4.1.1 Short-term toxicity to fish

Study 1 of 4 (short-term toxicity to fish) *DAR reference STUDY IIA, 8.2.1.1/01*

Reference	:	Matlock D. & Lam C.V. (2010a)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Acute toxicity study	species	:	Rainbow trout (*Oncorhynchus mykiss*)
year of execution	:	2009	exposure duration	:	96 hours
GLP statement	:	Yes	nominal concn.	:	5, 10, 20, 40 and 80 mg a.s./L
Guideline	:	FIFRA 72-1, OPPTS 850.1075, OECD 203	dosing method	:	Static; stock solution in DMF
test substance	:	BYI 02960 technical, batch 2009-000239 = Technical Product	acceptability	:	Acceptable
Purity	:	96.2%	96-h LC50	:	>74.2 mg a.s./L

(no summary available)

Methods

A 96-hour acute toxicity test in juvenile rainbow trout (*Oncorhynchus mykiss*) (1 replicate of ten fish per concentration, not fed for 48 hours prior to testing) was conducted under static conditions with BYI 02960 technical at test concentrations of 5, 10, 20, 40 and 80 mg a.s./L. It was reported that numerous preliminary trials demonstrated that a solvent stock would be needed to achieve maximum solubility in dilution water. The test solutions were prepared by adding aliquots of a stock solution in DMF to test water (max 0.1 mL/L DMF). The test included untreated and solvent controls (0.1 mL/L DMF). The concentrations of BYI 02960 were analytically confirmed by LC-MS/MS in samples of all test solutions taken at the start and end of exposure.

Results

The measured concentrations represented 72-99% of nominals at the start and 69-92% at the end of exposure. Test endpoints were based on mean measured concentrations (3.52, 8.31, 19.0, 35.1 and 74.2 mg a.s./L). Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. No mortality and clinical signs were observed in any of the treatments and the controls. The 96-hour LC50 is >74.2 mg a.s./L.

Conclusion
96-hour LC50 >74.2 mg a.s./L.

Guidelines and limitations

The study is acceptable.

Study 2 of 4 (short-term toxicity to fish) *DAR reference STUDY IIA, 8.2.1.2/01*

Reference	:	Matlock D. & Lam C.V. (2010b)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Acute toxicity study	species	:	Fathead minnow (*Pimephales promelas*)
year of execution	:	2009	exposure duration	:	96 hours
GLP statement	:	Yes	nominal concn.	:	5, 10, 20, 40 and 80 mg a.s./L
Guideline	:	FIFRA 72-1, OPPTS 850.1075, OECD 203	dosing method	:	Static; stock solution in DMF
test substance	:	BYI 02960 technical, batch 2009-000239	acceptability	:	Acceptable
Purity	:	96.2%	96-h LC50	:	>70.5 mg a.s./L

(no summary available)

Methods

A 96-hour acute toxicity test in juvenile fathead minnow (*Pimephales promelas*) (1 replicate of ten fish per concentration, not fed for 48 hours prior to testing) was conducted under static conditions with BYI 02960 technical at test concentrations of 5, 10, 20, 40 and 80 mg a.s./L. It was reported that numerous preliminary trials demonstrated that a solvent stock would be needed to achieve maximum solubility in dilution water. The test solutions were prepared by adding aliquots of a stock solution in DMF to test water (max 0.1 mL/L DMF). The test included untreated and solvent controls (0.1 mL/L DMF). The concentrations of BYI 02960 were analytically confirmed by LC-MS/MS in samples of all test solutions taken at the start and end of exposure.

Results

The measured concentrations represented 80-96% of nominals at the start and 85-98% at the end of exposure. Test endpoints were based on mean measured concentrations (4.29, 9.00, 19.4, 34.3 and 70.5 mg a.s./L). Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. No mortality and clinical signs were observed in any of the treatments and the controls. The 96-hour LC50 is >70.5 mg a.s./L.

Conclusion
96-hour LC50 >70.5 mg a.s./L.

Guidelines and limitations
The study is acceptable.

Study 3 of 4 (short-term toxicity to fish) *DAR reference STUDY IIA, 8.2.1.2/02*

Reference	:	Bruns E. (2011a)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Acute toxicity study	species	:	Common carp (*Cyprinus carpio*)
year of execution	:	2011	exposure duration	:	96 hours

GLP statement	:	Yes	nominal concn.	:	100 mg a.s./L (limit test)
Guideline	:	FIFRA 72-1, OPPTS 850.1075, EEC C.1, OECD 203	dosing method	:	Static; stock solution in DMF
test substance	:	BYI 02960 (tech), batch BYI 02960-01-03 (original batch number 2009-000239)	acceptability	:	Acceptable
Purity	:	96.2%	96-h LC50	:	>100 mg a.s./L

(no summary available)

Methods

A 96-hour acute toxicity limit test in juvenile carp (*Cyprinus carpio*) (2 replicates of 15 fish, not fed for 48 hours prior to testing) was conducted under static conditions with BYI 02960 technical at a nominal test concentration of 100 mg a.s./L. The test solutions were prepared by adding aliquots of a stock solution in DMF to test water (max 0.1 mL/L DMF). The test included untreated and solvent controls (0.1 mL/L DMF). The concentrations of BYI 02960 were analytically confirmed by LC-MS/MS in samples of all replicates taken at the start, after 48 hours and at the end of exposure.

Results

The measured concentrations represented 101-120% of nominals at the start and 97-104% after 48 hours and at the end of exposure. The value of 120% for 1 replicate was the average of two injections, one giving 147% of nominal and the other one giving 92% of nominal. The value for 147% of nominal may be considered an outlier, since the results clearly demonstrated that the test substance was stable under the test conditions, and measured concentrations in the same replicate were 101-104% of nominal after 48 and 96 hours. Test endpoints were based on nominal concentrations, which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. No mortality and clinical signs were observed in the treated and control replicates. The 96-hour LC50 is >100 mg a.s./L.

Conclusion
96-hour LC50 >100 mg a.s./L.

Guidelines and limitations
The study is acceptable.

Study 4 of 4 (short-term toxicity to fish) *DAR reference STUDY IIA, 8.11.1/01*

Reference	:	Banman C.S. & Lam C.V. (2009b)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Acute toxicity study (marine species)	species	:	Sheepshead minnow (*Cyprinodon variegatus*)
year of execution	:	2009	exposure duration	:	96 hours
GLP statement	:	Yes	nominal concn.	:	5, 10, 20, 40 and 80 mg a.s./L
Guideline	:	FIFRA 72-3, OPPTS 850.1075, OECD 203	dosing method	:	Static; stock solution in DMF
test substance	:	BYI 02960; technical, batch 2009-000239	acceptability	:	Acceptable

Purity	:	96.2%	96-h LC50	:	>83.9 mg a.s./L

(no summary available)

Methods

A 96-hour acute toxicity test on juvenile sheepshead minnow (*Cyprinodon variegatus*; saltwater species) (1 replicate of ten fish per concentration, not fed for 48 hours prior to testing) was conducted under static conditions with BYI 02960 technical at test concentrations of 5, 10, 20, 40 and 80 mg a.s./L. It was reported that numerous preliminary trials demonstrated that a solvent stock would be needed to achieve maximum solubility in dilution water. The test solutions were prepared by adding aliquots of a stock solution in DMF to test water (max 0.1 mL/L DMF). The test included untreated and solvent controls (0.1 mL/L DMF). The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated LC-MS/MS method in samples of all test solutions taken at the start and end of exposure.

Results

The measured concentrations represented 100-113% of nominals at the start and 102-111% at the end of exposure. Test endpoints were based on mean measured concentrations (5.6, 10.4, 21.0, 40.4 and 83.9 mg a.s./L). Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. No mortality and clinical signs were observed in any of the treatments and the controls. The 96-hour LC50 is >83.9 mg a.s./L.

Conclusion
96-hour LC50 >83.9 mg a.s./L.

Guidelines and limitations
The study is acceptable.

5.4.1.2 Long-term toxicity to fish

Study 1 of 1 (long-term toxicity to fish) *DAR reference* STUDY IIA, 8.2.4/01

reference	:	Matlock D. & Lam C.V. (2011)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Chronic toxicity study (ELS)	species	:	Fathead minnow (*Pimephales promelas*)
year of execution	:	2010	exposure duration	:	35 days
GLP statement	:	Yes	nominal concn.	:	0.625, 1.25, 2.5, 5 and 10 mg a.s./L
guideline	:	FIFRA 72-4, OPPTS 850.1400, OECD 210	dosing method	:	Flow-through; stock solution in DMF
test substance		BYI 02960 technical, batch 2009-000239	acceptability	:	Acceptable
purity	:	96.2%	NOEC	:	4.41 mg a.s./L

Methods

A 35-day fish early life stage flow-through study was undertaken with fathead minnow. Newly fertilised eggs (<24 hours post fertilisation, four replicate glass vessels with 35 eggs each per concentration) were exposed to BYI 02960 technical at test concentrations of 0.625, 1.25, 2.5, 5 and 10 mg a.s./L, with untreated and solvent control (DMF, 0.1 mL/L). On test day 5, when at least 90% of all viable control eggs had hatched, alevin were thinned to 20 per replicate. The total duration of the exposure period was 35 days. Samples for analytical confirmation of test concentrations using a sufficiently validated LC-MS/MS method were taken on day 0 and thereafter once weekly from selected replicates, with additional measurements on day 15 and 22. Hatching, mortality and fish behaviour was observed daily, and fish length and dry weight were measured at the end of the test.

Results

The mean measured concentrations of BYI 02960 technical were 0.619, 1.11, 2.05, 4.41 and 8.40 mg a.s./L, with limited and acceptable variation between replicates and time points. During the test, the pH was in the range 7.6-8.0, the oxygen concentration was 76-98% of the saturation value, and the temperature was in the range 24.6-25.0. In all treatments and the controls, hatch started on day 3. The observations of scoliosis and being at the water surface were infrequent, did not represent a dose-relationship and are considered to be unrelated to the treatment. The only adverse treatment related effect was reduced larval survival at the highest concentration. The reduced larval survival at 0.619 mg a.s./L is considered to be unrelated to the treatment in the absence of a dose-relationship. The NOEC is 4.41 mg a.s./L.

Conclusion

NOEC 4.41 mg a.s./L.

Guidelines and limitations

The study is acceptable.

5.4.2 Aquatic invertebrates

There are four short-term and three long-term tests available on the toxicity of BYI 02960 to aquatic invertebrates.

5.4.2.1 Short-term toxicity to aquatic invertebrates

Study 1 of 4 (short-term toxicity to aquatic invertebrates) *DAR reference STUDY IIA, 8.3.1.1/01*

reference	:	Banman C.S. & Lam C.V. (2009a)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Acute toxicity study	Species	:	*Daphnia magna*
year of execution	:	2009	exposure duration	:	48 hours
GLP statement	:	Yes	nominal concn.	:	80 mg a.s./L

guideline	:	FIFRA 72-2, OPPTS 850.1010, OECD 202	dosing method	:	Static; stock solution in DMF
test substance		BYI 02960 technical, batch 2009-000239	Acceptability	:	Acceptable
Purity	:	96.2%	48 h-EC50	:	>77.6 mg a.s./L

A 48-hour acute toxicity test in *Daphnia magna* (<24 h old, 6 replicates of five daphnia each for the treatment and each of the controls) was conducted under static conditions with BYI 02960 technical at a nominal test concentration of 80 mg a.s./L, with untreated control and solvent control (0.1 mL/L DMF). The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated LC-MS/MS method in samples of all test solutions taken at the start and end of exposure.

Results

The measured concentration was 102% of nominal at the start and 92% at the end of exposure. Test endpoinst were based on the mean measured concentration (77.6 mg a.s./L). Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 202 guideline. No mortality and signs of intoxication were observed in any replicate. The 48-hour EC50 is >77.6 mg a.s./L.

Conclusion

48-hour EC50 >77.6 mg a.s./L.

Guidelines and limitations

The study is acceptable.

Study 2 of 4 (short-term toxicity to aquatic invertebrates), *DAR reference STUDY IIA, 8.3.1.2/01*

reference	:	Bruns E. (2011e)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Acute toxicity study	species	:	*Chironomus riparius*
year of execution	:	2011	exposure duration	:	48 hours
GLP statement	:	Yes	nominal concn.	:	3.125, 6.25, 12.5, 25, 50 and 100 µg a.s./L
guideline	:	OPPTS 850.1300, OECD 202	dosing method	:	Static; direct addition to test water
test substance	:	BYI 02960 (tech.), batch 2009-000239 (batch code BYI 02960-01-03)	acceptability	:	Acceptable
purity	:	96.2%	48 h-EC50	:	61.7 µg a.s./L

Methods

A 48-hour acute toxicity test in *Chironomus riparius* (2-3 day old first instar larvae, 4 replicates of 10 larvae each for the treatments and the control) was conducted under static conditions with BYI 02960 technical at nominal test concentrations of 3.125, 6.25, 12.5, 25, 50 and 100 µg a.s./L, with untreated control. The test solution of the highest concentration was prepared by adding the test substance directly to the test water. Lower concentrations were prepared by serial dilution. A small amount of feed (0.01 mL of an aqueous fish food suspension) was added immediately after addition of the larvae. The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated LC-MS/MS method in samples of all test solutions taken at the start and end of exposure.

Results

The measured concentrations were 97-103% of nominal at the start and 101-107% at the end of exposure. Test endpoints were based on nominal concentrations, which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 202 guideline. At 0, 3.125, 6.25, 12.5, 25, 50 and 100 µg a.s./L, respectively, immobility was 0%, 0%, 0%, 2.5%, 2.5%, 30% and 85%. The reported 48-hour EC50 (Probit analysis) was 61.7 µg a.s./L (95% CI 41.4-109 µg a.s./L. At 100 µg a.s./L, surviving larvae showed reduced immobility.

Conclusion

48-hour EC50 61.7 µg a.s./L.

Guidelines and limitations

The study is acceptable.

Study 3 of 4 (short-term toxicity to aquatic invertebrates), *DAR reference* STUDY IIA, 8.11.1/02

reference	:	Gallagher S.P. et al. (2009a)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Acute toxicity study (marine organism)	species	:	Eastern oyster (*Crassostrea virginica*)
year of execution	:	2009	exposure duration	:	96 hours
GLP statement	:	Yes	nominal concn.	:	0.94, 1.9, 3.8, 7.5, 15 and 30 mg a.s./L
guideline	:	OPPTS 850.1025	dosing method	:	Flow through; stock solutions in DMF
test substance	:	BYI 02960 technical, batch 2009-000239	acceptability	:	Acceptable
purity	:	96.2%	96-h EC50	:	>29 mg a.s./L

Methods

A 96-hour shell deposition test in Eastern oyster (*Crassostrea virginica*) (1 replicate of 20 oysters per concentration) was conducted under flow-through conditions with BYI 02960 technical at test

concentrations of 0.94, 1.9, 3.8, 7.5, 15 and 30 mg a.s./L. The test included untreated and solvent controls (0.1 mL/L DMF). The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated HPLC/UV method in samples of all test solutions taken at the start, after 48 hours and at the end of exposure.

Results

The measured concentrations represented 94-100% of nominals. Test endpoints were based on mean measured concentrations (0.90, 1.8, 3.6, 7.3, 15 and 29 mg a.s./L). Water quality parameters (pH, oxygen concentration, temperature and salinity) were in accordance with the OPPTS 850.1025 guideline (pH 8.1-8.2, dissolved oxygen ≥6.9 mg/L(≥86% of saturation), 19.2-21.1°C, salinity $20^{0}/_{00}$). The validity criteria of OPPTS 850.1025 were satisfied (mortality <10%, dissolved oxygen >60%, no evidence of spawning, >2 mm shell growth in controls). No mortality was observed in any of the treatments and the controls. At 0.90, 1.8, 3.6, 7.3, 15 and 29 mg a.s./L, respectively, inhibition of shell growth was 2.2, 2.9, -13, 13, -19 and 13%. None of the differences between the treated groups and the pooled control were statistically significant at the 5% uncertainty level. The 96-hour EC50 is >29 mg a.s./L.

Conclusion

96-hour EC50 >29 mg a.s./L.

Guidelines and limitations

The study is acceptable.

Study 4 of 4 (short-term toxicity to aquatic invertebrates), *DAR reference* STUDY IIA, 8.11.1/03

reference	:	Gallagher S.P. et al. (2009b)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Acute toxicity study (marine organism)	species	:	Salt water mysid (*Americamysis bahia*)
year of execution	:	2009	exposure duration	:	96 hours
GLP statement	:	Yes	nominal concn.	:	0.13, 0.22, 0.36, 0.60 and 1.0 mg a.s./L
guideline	:	OPPTS 850.1035	dosing method	:	Static; test substance dissolved in test water
test substance	:	BYI 02960 technical, batch 2009-000239	acceptability	:	Acceptable for juveniles (not tested whether these are most sensitive life-stage)
purity	:	96.2%	96-h LC50	:	0.26 mg a.s./L

Methods

A 96-hour acute toxicity test on juveniles (<24 h old) of the saltwater mysid *Americamysis bahia* (2 replicates of 10 mysids per concentration) was conducted under static conditions with BYI 02960 technical at test concentrations of 0.13, 0.22, 0.36, 0.60 and 1.0 mg a.s./L. The test included untreated controls. The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated HPLC/UV method in samples of all test solutions taken at the start, after 48 hours and at the end of exposure.

Results

The measured concentrations represented 94-98% of nominals. Test endpoints were based on mean measured concentrations (0.12, 0.21, 0.35, 0.58 and 0.98 mg a.s./L). Water quality parameters (pH, oxygen concentration, temperature and salinity) were in accordance with the OPPTS 850.1035 guideline (pH 8.1-8.2, dissolved oxygen ≥6.5 mg/L (≥89% of saturation), 23.2-25.7°C, salinity $20^0/_{00}$). No mortality was observed in the controls. At 0.12, 0.21, 0.35, 0.58 and 0.98 mg a.s./L, respectively, mortality was 5, 60, 50, 100 and 100%. Signs of toxicity were noted at 0.21 mg a.s./L and above. The 96-hour LC50 was 0.26 mg a.s./L (95% CI 0.12-0.58 mg a.s./L.

Conclusion

96-hour LC50 0.26 mg a.s./L.

Guidelines and limitations

The study result is acceptable for juvenile mysids. The OPPTS 850.1035 guideline states that a range-finding test should be conducted to determine which life stage (juvenile or young adult) is to be utilized in the definitive test. The definitive test should be conducted on the mysid life stage (juveniles or young adults) which is most sensitive to the test substance. The report did not state that a range-finding test was conducted with juveniles and young adults, hence it is unclear whether the juveniles tested were the most sensitive life stage.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Study 1 of 3 (long-term toxicity to aquatic invertebrates), *DAR Reference STUDY IIA, 8.3.2.1/01*

reference	:	Riebschläger T. (2011)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Chronic toxicity study	Species	:	*Daphnia magna*
year of execution	:	2011	exposure duration	:	21 days
GLP statement	:	Yes	nominal concn.	:	0.8, 1.6, 3.2, 6.4, 12.8 and 25.6 mg a.s./L
guideline	:	EPA 72-4, OPPTS 850.1300, EEC C.20, OECD 211	dosing method	:	Semi-static; stock solution in DMF
test substance	:	BYI 02960 technical, batch BYI02960-01-03 (original batch 2009-000239)	Acceptability	:	Acceptable

purity	:	96.2%	21-day NOEC	:	3.2 mg a.s./L

Methods

A 21-day chronic reproductive toxicity test in *Daphnia magna* (<24 h old, 10 replicates with one daphnia each per concentration) was conducted according to OECD 211 under semi-static conditions (renewal every 2-3 days) with BYI 02960 technical at nominal test concentrations of 0.8, 1.6, 3.2, 6.4, 12.8 and 25.6 mg a.s./L, with untreated control and solvent control (0.1 mL/L DMF). The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated HPLC-MS/MS method in samples of all fresh test solutions of day 0, 9 and 19 and in the corresponding aged solutions. Endpoints reported were parent mortality, total number of offspring per parent animal, parent age at first offspring emergence, neonates behaviour and length and dry weight of parent daphnia at the end of the test.

Results

The measured concentrations of BYI 02960 were 102-109% of nominal in fresh solutions and 102-117% in the corresponding aged solutions. Test endpoints were based on nominal concentrations, which is acceptable. Water quality parameters and the number of offspring in the controls were in accordance with the requirements of the OECD 211 guideline.

No sublethal effects were observed in neonates. Treatment related statistically significant adverse effects were recorded on reproduction, as measured by the cumulative number of offspring per living female, at and above 12.8 mg a.s./L and on growth (body length) at and above 6.4 mg a.s./L, whereas body mass was statistically significantly reduced at the highest concentration of 25.6 mg a.s./L only. The NOEC is 3.2 mg a.s./L.

A study with the reference item potassium dichromate was performed about 4 months earlier and gave a 24-h EC50 of 1.0 mg/L, which is acceptable (OECD 202 (2004): 0.6-2.1 mg/L).

Conclusion

NOEC 3.2 mg a.s./L.

Guidelines and limitations

The study is acceptable.

Study 2 of 3 (long-term toxicity to aquatic invertebrates), *DAR Reference STUDY* STUDY IIA, 8.3.2.2/01

reference	:	Bruns E. (2011g)	water solubility a.s.	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Chronic toxicity study	species	:	*Chironomus riparius*
year of execution	:	2010	exposure duration	:	28 days

GLP statement	:	Yes	nominal concn.	:	1.25, 2.50, 5, 10, 20 and 40 µg a.s./L
guideline	:	OECD 219	dosing method	:	Static (spiked water)
test substance	:	BYI 02960; technical, batch 2009-000239 = Technical Product	acceptability	:	Acceptable
a.s. content	:	96.2%	28-day NOEC	:	10 µg a.s./L

Methods

The chronic toxicity of BYI 02960 technical to *Chironomus riparius* (2-3 days old, 1st instar larvae) was assessed in a 28-day water/sediment system under static conditions using the spiked water method. The test was performed in 0.6 L glass beakers, with four replicates for each test concentration and the solvent control. The beakers were filled with 140 g wet sediment (artificial OECD 219 soil: 75.8% quartz sand, 4% peat (measured organic carbon content 2.0%), 20% kaolinite clay, 0.2% calcium carbonate) and 380 mL (about 6 cm) of overlying water, 7 days prior to treatment. Twenty midge larvae were added to each beaker one day prior to treatment. The water was gently aerated during the test, except for 24 hours following addition of the midges. On day 0, the test water was spiked just below the surface with an aliquot of a stock solution of the test substance in DMF, to provide nominal test concentrations of 1.25, 2.50, 5, 10, 20 and 40 µg a.s./L, with solvent control (0.1 mL/L DMF). Additional replicates were set up for analytical measurements on overlying water and pore water on day 0 (1 hour after treatment), 7 and 28 after treatment. Water samples were analysed by HPLC-MS/MS using a sufficiently validated method. Behaviour, mortality and emergence of the midges were monitored until test end.

Results

The measured concentrations of BYI 02960 in the overlying water were in the range 85-110% (mean 99%), 37-83% (mean 60%) and 30-52% (mean 41%) of nominal, after 1 hour, 7 days and 28 days, respectively. The test endpoints were based on nominal concentrations, which is acceptable. The measured concentrations of BYI 02960 in the porewater were in the range 0.4-1.0% (mean 0.6%), 1.0-3.0% (mean 2.0%) and 1.4-2.9% (mean 2.2%) of nominal, after 1 hour, 7 days and 28 days, respectively.

The biological test results are summarised in Table 153. Water quality parameters were in accordance with the OECD 219 guideline. All validity criteria of the OECD 219 guideline concerning emergence in the control and physico-chemical conditions during the test were satisfied. No emergence occurred at 40 µg a.s./L. Treatment related statistically significant adverse effects on emergence and development rate were recorded at 20 µg a.s./L. The NOEC was 10 µg a.s./L.

Table 153: Summary of biological results of 28-day chronic toxicity test with BYI 02960 technical on Chironomus riparius

Nominal conc. (µg a.s./L)	Emergence rate (%)	Development rate (1/d)
solvent control	90.0	0.058
1.25	87.5	0.058
2.5	92.5	0.057

5	83.8	0.058
10	88.8	0.059
20	52.5*	0.050*
40	0	0

* Statistically significant at 5% level

Conclusion

NOEC 10 µg a.s./L.

Guidelines and limitations

The study was performed according to OECD 219 (2004) and is acceptable.

Study 3 of 3 (long-term toxicity to aquatic invertebrates), *DAR Reference STUDY IIA, 8.11.1/04*

reference	:	Claude M.B. *et al.* (2011)	water solubility	:	3000-3200 mg/L at pH 4-9 & 20°C
type of study	:	Life cycle toxicity study (marine organism)	species	:	Saltwater mysid (*Mysidopsis bahia*)
year of execution	:	2010-2011	exposure duration	:	28 days
GLP statement	:	Yes	nominal concn.	:	4.6, 8.0, 13.9, 24.2 and 42 µg a.s./L
guideline	:	OPPTS 850.1350	dosing method	:	Flow through; stock solutions in DMF
test substance	:	BYI 02960 technical, batch BYI 2009-02960-01-03	acceptability	:	Acceptable
purity	:	96.2%	NOEC	:	13.2 µg a.s./L

Methods

A 28-day life-cycle toxicity test on juveniles (<24 h old) of the saltwater mysid *Mysidopsis bahia* was conducted under flow-through conditions with BYI 02960 technical at test concentrations of 4.6, 8.0, 13.9, 24.2 and 42 µg a.s./L. The test included untreated and solvent controls (0.1 mL/L DMF). The test was initiated with <24-h old juveniles (4 replicate chambers with 15 neonate mysids each for each concentration and the controls). On day 14 of the test, after mysids attained sexual maturity, male and female adults were paired in each treatment and control group, with a maximum of five reproductive pairs per replicate. Reproduction of the paired mysids was monitored through termination on day 28. Observations for mortality and signs of toxicity were conducted daily throughout the test. At test termination, the total body lengths and dry weights of all surviving first-generation mysids were measured. The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated HPLC/UV method in samples of all test solutions taken at the start of the test, weekly during the test and at test termination.

Results

The measured concentrations were in the range 76-103% of nominals. Test endpoints were based on mean measured concentrations (4.2, 7.8, 13.2, 23.6 and 40 µg a.s./L). Water quality parameters (pH, oxygen concentration, temperature and salinity) were in accordance with the OPPTS 850.1350 guideline (pH 7.9-8.1, dissolved oxygen ≥5.0 mg/L(≥68% of saturation), 24.5-26.4°C, salinity 19-21 $^0/_{00}$). No treatment related signs of intoxication were observed in any of the treatments. No treatment related mortality was observed in any treatment, and at test termination there were no biologically relevant and statistically significant differences in body length and dry weight between the treated groups and the controls. The results for reproduction are summarised in *Table 154*.

Table 154: Reproductive results of 28-day life cycle toxicity test with BYI 02960 technical on Mysidopsis bahia

Treatment (µg a.s./L)	no. of young/reproductive day	
	mean	standard deviation
negative control	0.396	0.119
solvent control	0.450	0.167
pooled control	0.423	0.137
4.2	0.358	0.250
7.8	0.267	0.145
13.2	0.240	0.090
23.6	0.156*	0.157
40	0.173*	0.113

** Statistically significant difference from control at 5% uncertainty level.*

At 4.2, 7.8, 13.2, 23.6 and 40 µg a.s./L, respectively, reproduction was reduced by 15, 37, 43, 63 and 59%, relative to the pooled control. The difference from the pooled control was statistically significant at the two highest test concentrations. The high variability of reproduction (see standard deviations in Table above) is associated with the test design, where total reproduction per replicate of 5 pairs is determined. Based on statistical significance, the NOEC concluded by the author of the report was 13.2 µg a.s./L. However, biological relevance should also be taken into consideration. The replicate mean reproduction in the controls was in the range 0.261-0.661 young per reproductive day. At 4.2 µg a.s./L (mean reproduction reduced by 15%), replicate mean reproduction was in the range 0.102-0.600 young per reproductive day, and 2 of 4 replicate means were below the control range. At 7.8 µg a.s./L (mean reproduction reduced by 37%), replicate mean reproduction was in the range 0.107-0.415 young per reproductive day, and 2 of 4 replicate means were below the control range. At 13.2 µg a.s./L (mean reproduction reduced by 43%), replicate mean reproduction was in the range 0.169-0.369 young per reproductive day, and 3 of 4 replicate means were below the control range. Based on the fact that there is a concentration-related trend in effects on reproduction, that at 7.8 and 13.2 µg a.s./L reproduction was reduced by 37-43% relative to the pooled control, and that reproduction in 2-3 out of 4 replicates was below the control range, it was proposed by the RMS to set the NOEC at 4.2 µg a.s./L. In response to this proposal, the applicant submitted two statements (Springer T, 2013, BCS doc M-452382-01-1; Bruns *et al.*, 2013, BCS doc M-452374-01-1). These statements are summarised below.

Statement by Springer (2013)

Springer (2013) suggested that the decision to designate 4.2 µg a.i./L as the NOEC might be related to current discussions on using EC10 values as a surrogate for the NOEC. The mean reproduction rate in the pooled controls was 0.423 offspring/female/day, with 95% confidence limits of 0.128 to 0.627. Based on this confidence interval, the smallest change in reproduction that can dependably be shown to be a decrease relative to the pooled controls is approximately 30% (i.e. 100-100*0.128/0.423). When using the value of 0.128 offspring/female/day as the within-group standard deviation during power analysis (based on 4 replicates and a type I error rate of 0.05), the smallest effect size that could be detected with a power of 0.8 is approximately a 73 % difference between the control and treatment group. Springer (2013) stated that this power is far less than is desirable, and indicates that that small changes in mysid reproduction cannot be dependably assessed using the standard experimental design described in test guideline. Springer also noted that the pooled control mean was strongly influenced by a single replicate from the solvent control, where reproduction had an unusually high value of 0.66.

Statement by Bruns et al. (2013)

Bruns *et al.* (2013) stated that "*the mean value of the solvent control has been dramatically impacted by an extraordinary high reproduction of one single breeding pair. The number of 29 young per reproductive female observed for this single breeding pair in the solvent control appears to be inconsistently high considering that the next highest value is 16 young per breeding pair. The fact that this value was extraordinary was checked by BCS using the control data of the 10 last mysid studies available in the BCS electronically archiving system DART. In none of the evaluated studies a control breeding pair produced 29 young within 14 reproduction days. Hence it is reasonable to exclude this number from the evaluation.*" The results excluding this single breeding pair are summarised in Table 155.

Table 155: Reproductive results of 28-day life cycle toxicity test with BYI 02960 technical on Mysidopsis bahia (excluding outlier in solvent control)

Mean measured concentration (µg a.i/L)	**Mean number of young produced per reproductive day ± SD**	**Mean percent of surviving females producing young[1]**	**Mean number of young per surviving female ± SD[1]**
Negative control	0.396 ± 0.119	100	5.2 ± 1.54
Solvent control[2]	0.352 ± 0.105	85.0	4.6 ± 1.36
Pooled control[2]	0.374 ± 0.106	92.3	4.9 ± 1.38
4.2	0.358 ± 0.250	92.9	4.9 ± 3.76
7.8	0.267 ± 0.145	88.9	3.5 ± 1.89
13.2	0.240 ± 0.090	77.8	3.1 ± 1.16
23.6	0.156 ± 0.157[*]	47.1[**]	2.1 ± 2.02[*]
40	0.173 ± 0.113[*]	50.0[**]	2.3 ± 1.47[*]

* Statistically significant decrease in reproduction and mean number of young per surviving female in comparison to the pooled control using Dunnett's test ($p \leq 0.05$).

** Statistically significant decrease in percent of surviving females producing young in comparison to the pooled control using Fisher's Exact test ($p \leq 0.05$).

[1] Calculated based on the total number of surviving females present at test termination. Females that died prior to test termination and the young that they produced were excluded from the calculation of the mean percent of females producing young and the mean number of young per female.

[2] One female in compartment 3, replicate D of the solvent control group had an inconsistently high number of young (the female produced 29 mysids whereas the next closest amount produced from a female out of the entire test was 16 young). Therefore, the data of this female was excluded and the statistics were reanalyzed.

Bruns *et al.* (2013) further argued that the discriminatory power of the chronic mysid shrimp study especially for the endpoint 'reproduction' is hampered by the natural high biological variability of this endpoint. BCS performed additional statistics of the data set by evaluating the endpoint 'reproduction' on the basis of the single breeding pairs instead of using the mean values for the replicates. This approach is covering the entire biological variation as observed for the single breeding pairs. The data set was used to calculate EC30 values as well as the corresponding confidence limits where possible. The EC30 was used due to the known standard deviation of control data as well as taking the statement of Springer (2013) into consideration. The results are summarised in Table 156. Bruns *et al.* concluded that all calculated EC30 values are slightly above the originally reported NOEC of 13.2 µg/L, and that in all cases the NOEC of 13.2 µg/L was within the calculated confidence limits of the EC30 values.

Table 156*: EC_{30} calculations based on the 10 previous control data sets (excluding outlier in solvent control)*

Data used for calculation	EC_{30}	R^2	P(F)	Lower 95% confidence limit	Upper 5% confidence limit
Historical control data of 10 previous studies - **Probit**	17.8 µg/L	0.74	0.061	-	-
Historical control data of 10 previous studies plus data minus concentration 40 µg/L - **Probit**	15.4 µg/L	0.96	0.019	10.6 µg/L	19.7 µg/L
Historical control data of 10 previous studies plus data minus concentration 40 µg/L - **Logit**	15.7 µg/L	0.97	0.018	10.9 µg/L	19.6 µg/L
Historical control data of 10 previous studies plus data minus concentration 40 µg/L - **Weibull**	16.1 µg/L	0.97	0.016	11.3 µg/L	19.5 µg/L

The coefficient of determination, r^2 ($0 <= r^2 <= 1$), gives the proportion of variance explained by the dose/response function.
if p(F) <= alpha, the selected significance level, (e.g., alpha = 0.05) the regression revealed significant results (= slope is significantly different from zero).

Bruns *et al.* presented historical control data from 10 chronic mysid studies available to the applicant (including those of the present study). Based on this control data, Bruns *et al.* concluded that 30% difference to the control mean value for the endpoint 'reproduction' is not a clear effect as

the controls are varying by about ±30%. Bruns *et al.* stated that the most important points which were concluded on the basis of this data are:

- The number of young per female is extremely variable: 0 – 45 young per female within 12-17 reproductive days.
- Number of Young produced per female and reproduction day ranges from 0 to 2.65
- The system is resulting in LOEC's which are ranging between 33 to 93% inhibition compared to controls
- The NOECs are ranging between -19 to 43% difference compared to the controls
- The concentrations below and including the NOEC range between -38.7 up to 47.3% difference compared to the controls.

Bruns *et al.* (2013) stated that this evaluation underpins the observation that the endpoint 'reproduction' is highly variable.

Lastly, Bruns *et al.* (2013) gave the following additional arguments supporting their case:

- The chronic mysid shrimp study is a problematic test system. In frequent cases studies have to be performed several times before a valid test (according to guideline recommendations) is available.
- The observed NOEC, as presented in the original report, is fulfilling the validity criteria for controls according to OPPTS 850.1350. This gives further support to the reliability of the presented NOEC of 13.2 µg/L.

Evaluation of statements by RMS

It is agreed that the solvent control breeding pair with 29 young per reproductive female is an outlier and may be eliminated from the data (although there was one breeding pair in the historical control data that produced an even higher number of 45 young in 17 reproduction days). The RMS performed a Dixon's outlier test on this data of 29 young per reproductive female using the reported results for 20 breeding pairs in the solvent control, and this gave a Q-value of 0.552, which is higher than the statistical Q value of 0.401 for n=20. Hence this test confirmed that the data for this breeding pair are exceptionally high and may be removed prior to statistical analysis. The evaluation below is based on data excluding this outlier. When excluding this outlier, at 4.2, 7.8, 13.2, 23.6 and 40 µg a.s./L, reproduction (expressed as mean number of young produced per reproductive day) was reduced by -3%, 29%, 33%, 62% and 52% relative to the pooled control. Statistical significance (5% uncertainty level) was reached only at the two highest concentrations. Since the reductions at 7.8 and 13.2 µg a.s./L (29-33%) were approximately equal to the relative standard deviation in the control (30%) and the solvent control (30%), it is insufficiently certain that the reductions of 29-33% at 7.8 and 13.2 µg a.s./L are a treatment related effect. Hence the NOEC may be set at 13.2 µg a.s./L.

The submitted historical control data originated from 3 different laboratories, and only 4 studies (inlcuding the present study) were performed at Wildlife. The studies were conducted between 1996 and 2008, whereas the present study was performed in 2011. Historical control data should preferably be recent data from the same laboratory where the study is performed. Therefore the use of the combined historical control data from all ten studies in EC30 estimations is considered to be a questionable procedure. However, these control data confirm the high variability of the reproduction rate: the relative standard deviation of the reproduction rate in 14 controls (4 studies had controls and solvent controls) was in the range of 5-73%, with a mean of 25%.

Conclusion

NOEC 13.2 µg a.s./L.

Guidelines and limitations

The study is acceptable. Comments on the interpretation of the study results were incorporated in the above summary. The conclusion as stated above is acceptable.

5.4.3 Algae and aquatic plants

There is one short term and one long term test available on the toxicity of BYI 02960 to aquatic invertebrates.

Study 1 of 1 (short-term toxicity to algae & aquatic plants), *DAR reference STUDY IIA, 8.4/01*

reference	:	Banman C.S. & Lam C.V. (2010)	water solubility	:	3000-3200 mg a.s./L at pH 4-9 & 20°C
type of study	:	Algae growth inhibition	species	:	*Pseudokirchneriella subcapitata*
year of execution	:	2010	exposure duration	:	96 hours
GLP statement	:	Yes	nominal concn.	:	5, 10, 20, 40 and 80 mg a.s./L
guideline	:	FIFRA 123-2, OPPTS 850.5400, OECD 201 (2006)	dosing method	:	Static; stock solution in DMF
test substance	:	BYI 02960 technical, batch 2009-000239	acceptability	:	Acceptable
purity	:	96.2%	72h-ErC50 & EyC50	:	>80 mg a.s./L

Methods

A 96-hour short-term toxicity test on green algae (*Pseudokirchneriella subcapitata*) (3 replicates for the treatment and the untreated and solvent controls, each containing 1 x 10^4 cells/mL at the start) was conducted with BYI 02960 technical at test concentrations of 5, 10, 20, 40 and 80 mg a.s./L. The solution of the highest concentration was prepared by adding an aliquot of a stock solution in DMF to test water (0.1 mL/L DMF). The lower concentrations were prepared by serial dilution of

this solution. The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated LC-MS/MS method in samples of all test solutions taken at the start and end of exposure.

Results

The measured concentrations were in the range 111-120% of nominal at the start and end of exposure. Test endpoints were based on nominal concentrations, which is acceptable. Physico-chemical parameters were in accordance with the OECD 201 guideline. The pH increased from 7.3-7.4 at the start to 10.1-10.3 at the end, but this is associated with CO_2 depletion due to strong cell growth in the controls and all treaments and does not invalidate the test. The mean growth factor in the controls was 144 within 72 hours. The mean CV of the section-by-section specific growth rates in the control was 13%. The CV of the average specific growth rate in the control during the 72-hour period was 1.0%. Hence the validity criteria of OECD 201 (2006) were satisfied. The % inhibition range for specific growth rate and area under the curve (biomass), respectively, were in the range -3.3 to -1.0% and -3.5 to -17.7%. The 72-h ErC50 and EbC50 was >80 mg a.s./L. The 72-h EyC50 was not reported, but clearly also in excess of 80 mg a.s./L.

Conclusion

72-h ErC50, EbC50 and EyC50 >80 mg a.s./L.

Guidelines and limitations

The study is acceptable.

Study 1 of 1 (long-term toxicity to algae & aquatic plants), *DAR reference STUDY IIA, 8.6/01*

Reference	:	Banmann C.S. *et al* (2010)	species	:	Duckweed (*Lemna gibba G3*)
type of study	:	Duckweed growth inhibition test	duration	:	7 days
year of execution	:	2010	nominal conc.	:	5, 10, 20, 40 and 80 mg a.s./L
GLP statement	:	Yes	dosing method	:	Semi-static; stock solution in DMF
guideline	:	FIFRA 123-2, OECD 221 (2006) & OPPTS 850.4400	acceptability	:	Acceptable
test substance	:	BYI 02960 technical, batch 2009-000239	Lowest NOEC	:	34.2 mg a.s./L
purity	:	96.2%	Lowest EC50	:	>67.7 mg a.s./L

Methods

A 7-day toxicity test on the growth of duckweed (*Lemna gibba G3*) (3 replicates per concentration, each containing three plants with four fronds each at test initiation) was conducted under semi-static conditions (renewal on day 3) with BYI 02960 technical at nominal test concentrations of 5, 10, 20, 40 and 80 mg a.s./L, with untreated and solvent control each tested in 3 replicates. The solution of the highest concentration was prepared by adding an aliquot of a stock solution in DMF to test water (0.1

mL/L DMF). The lower concentrations were prepared by serial dilution of this solution (including addition of solvent such that the final concentration of DMF was 0.1 mL/L in all treatments). The concentrations of BYI 02960 were analytically confirmed by a sufficiently validated LC-MS/MS method in samples of all test solutions taken at the start (fresh solutions), on day 3 (aged and fresh solutions) and at the end of exposure (aged solutions). On day 0, 3, 5 and 7, frond number and any change in plant development were recorded. Frond dry weight was recorded on day 0 and 7.

Results

The measured concentrations in the test solutions taken on day 0 (fresh), day 3 (aged and fresh) and day 7 (aged) were in the range 65-68%, 71-77%, 86-104% and 93-101%, respectively. The mean measured concentrations were in the range 80-86% of nominal: 4.02, 8.17, 16.0, 34.2 and 67.7 mg a.s./L. The test endpoints were based on mean measured concentrations. The concentrations and % inhibition are shown in *Table 157*.

Table 157: The toxicity of BYI 02960 technical to Lemna gibba

Mean measured conc. (mg a.s./L)	frond number			biomass		
	mean no. of fronds (day 7)	% inhibition growth rate	% inhibition yield	mean biomass (mg d.w.) (day 7)	% inhibition growth rate	% inhibition yield
Control	238	-	-	27.2	-	-
Solvent cont	217	-	-	25.2	-	-
Pooled con	228	-	-	26.2	-	-
4.02	208	3.0	8.4	23.8	3.6	9.0
8.17	215	2.0	5.5	24.9	2.0	4.8
16.0	191	5.9	16.0	23.0	4.9	12.3
34.2	219	1.5	3.7	28.0	-1.7	-6.8
67.7	169	10.3*	25.9*	21.5	7.7	17.9

* statistically significant difference from control at 5% level.

Water quality parameters (light intensity, pH and temperature) were in accordance with the OECD 221 guideline. The doubling time in the control was 1.65 days, hence the validity criterion of OECD 221 (2006) was satisfied (doubling time <2.5 days). Phytotoxic effects were not observed in any treatment.

All EC50 values are >67.7 mg a.s./L as effects at the highest test concentration were well below 50% for all parameters. No statistically significant effects on yield and growth rate based on biomass were recorded, hence the corresponding NOECs are 67.7 mg a.s./L. Based on statistically significant reductions of yield and growth rate based on frond number at the highest tested concentration, the corresponding NOECs are 34.2 mg a.s./L.

The report also calculated percentage inhibition values for cumulative biomass based on frond number: 13%, 10%, 19%, 8% and 29% at 4.02, 8.17, 16.0, 34.2 and 67.7 mg a.s./L, respectively, with statistical significance (5% level) reached at 16.0 and 67.7 mg a.s./L. The author of the report stated that the statistically significant reduction of 19% at 16.0 mg a.s./L was unrelated to the treatment, since the next higher dose level was unaffected, and since in a pre-test at 16 mg a.s./L the inhibition was -1.5% for fround counts. This is acceptable. In addition, this parameter is not required by OECD 221.

Conclusion

Semi-static test: lowest NOEC 34.2 mg a.s./L, lowest EC50 >67.7 mg a.s./L.

Guidelines and limitations

The test was performed in agreement with OECD 221 and is acceptable.

5.4.4 Summary and discussion of aquatic toxicity

Acute and chronic aquatic toxicity data are available for three trophic levels (plants & algae, invertabrates, fish). First the acute toxicity will be summarized.

Acute aquatic toxicity

Four different fish species are acutely tested, being Rainbow trout, Fathead minnow, Common carp and Sheepshead minnow. All studies have an LC50 >70.5 mg a.s./L (96h, m.m.) or higher.

Four different invertebrate species were also acutely tested, being the cladoceran *Daphnia magna*, the midge *Chironomus riparius* and, the marine species *Americamysis bahia* and the mollusc *Crassostrea virginica*.

The most sensitive organism of all the acute aquatic toxicity tests for BYI 02960 was *Chironomus riparius* with an EC50 = 0.0617 mg a.s./L (48h, nominal) and this is therefore used as the key study for classification (DAR reference STUDY IIA, 8.3.1.2/01). The chironomid study is based on nominal concentrations. The nominal EC50 = 0.0617 mg/L is within the criterium of Aquatic Hazard Category Acute 1, with an M-factor 10 ($0{,}01 < L(E)C50 \leq 0{,}1$) and it is to be expected that measured concentrations will be lower, falling within the same M-factor 10 range. Technically, the actual exposure concentration could even be lower leading to a higher M factor. Although the NOEC is based on a nominal concentration, the study itself is reliable and acceptable. The next most sensitive species is the salt water mysid, *Americamysis bahia* (DAR reference STUDY IIA, 8.11.1/03), with an EC50 = 0.26 mg a.s./L (96h, m.m.). The mysid test deviates from the protocol. The study is accepted, however, only for juveniles. Guidance prescribes that a range finding test with juveniles and young adults has to be conducted to determine the most sensitive life stage. It is not reported that this has been done, thus young adults might be more sensitive to BYI 02960. This study is also reliable and, as it lies close to the chironomid study, used as a supportive study for the proposed classification. Overall, taken into account the uncertainty that comes with nominal concentrations of the chironomid study and of the mysid life stage sensitivity, an M factor of 10 is believed to be safe and realistic for classification.

Finally, one short term algae toxicity test (ErC50 >80 mg a.s./L, 72h, nominal) was reported.

Chronic aquatic toxicity

There are five chronic aquatic toxicity studies available. One long term fish study (Fathead minnow) has a NOEC = 4.41 mg a.s./L (35 days, m.m.) and one long term duckweed study (lowest NOEC = 34.2 mg a.s./L, 7 days, m.m.). Three long term invertebrate studies show respectively a NOEC = 3.2 mg a.s./L (21 days, nominal) for *Daphnia magna*, NOEC = 0.010 mg a.s./L (28 days, nominal) for *Chironomus riparius* and NOEC = 0.0132 mg a.s./L (28 days, m.m.) for *Mysidopsis bahia*. The chronic tests confirm the high toxicity of the active substance to *Chironomus riparius*, which is again the most sensitive organism tested. This chronic toxicity test to *Chironomus riparius* (DAR Reference STUDY IIA, 8.3.2.2/01) is therefore used as a key study for the classification. The test is based on nominal concentrations. The nominal NOEC of 0.010 mg/L is exactly at the criterium of Aquatic Hazard Category Chronic 1, with an M-factor 10 and it is to be expected that measured concentrations will be lower, falling within the same M-factor 10 range (0.001 < NOEC ≤ 0.01 mg/L). Again, as explained for the acute test, the actual exposure concentration could even be lower leading to a higher M factor. Although the NOEC is based on a nominal concentration, the study itself is reliable and acceptable. Therefore, taken into account the uncertainty that comes with nominal concentrations, an M factor of 10 is believed to be safe and realistic for classification. The next most sensitive species are the mysids (DAR Reference STUDY IIA, 8.11.1/04*)* with a measured NOEC = 0.0132 mg a.s./L. This study is also reliable and, as it lies close to the chironomid study, used as a supportive study for the proposed classification.

Several metabolites were studied as well, being 6-CAN (6-chloronicotinic acid), DFA (difluoroacetate), BYI 02960-succinamide and BYI 02960-azabicyclosuccinamide. These are all less toxic than the parent compound BYI 02960 (lowest reported EC50 >1 mg/L nominal, 48h, 6-CAN, *Chironomus tentans*) and are believed not to influence the classification and are therefore not discussed; please refer to the DAR Volume 3 B9 if required. The data is shown in Chapter 7.2: "The Aquatic Toxicity of Metabolites of BYI 02960" and please refer to the DAR Volume 3 B9 if more information is required.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

BYI 02960 is not considered rapidly degradable. The key studies are four water-sediment simulation experiments, in which BYI 02960 neither has a half-life shorter than 16 days (or degradation of >70% within 28 days). Therefore, BYI 02960 is considered to be not rapidly degradable (according to CLP guidance V4.0 nov 2013, p. 518). The soil simulation studies, which are used as supportive studies, confirm that the compound does not show ultimate nor primary degradation with a half-life <16 days (or degradation of >70% within 28 days). The bioaccumulation potential of BYI 02960 is low. This is based on the log Kow of 1.2, which is below the cut-off value of log Kow = 4.

As there is adequate acute and chronic toxicity data available for all three trophic levels, classification is carried out according to table 4.1.0(b)(i) (according to CLP guidance V4.0 nov 2013, p. 524 & Table on page 525). The most sensitive species acute and chronically is *Chironomus riparius*, with an EC50 = 0.0617 mg a.s./L (48h, nominal) and a NOEC = 0.01 mg a.s./L (28 days,

m.m.). BYI 02960 is therefore classified as **Aquatic Acute 1,** M factor 10 and **Aquatic Chronic 1**, M factor 10.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

The proposed classification for BYI 02960 is Aquatic Acute 1, with an M factor 10 and Aquatic Chronic 1, with an M factor 10.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS proposed to classify flupyadifurone as Aquatic Acute 1 and Aquatic Chronic 1 with an M-factor of 10 for both acute and chronic.

Degradation

A hydrolysis study conducted according to OECD TG 111 and in compliance with GLP at pH 4, 7 and 9 at 50 °C for 5 days, showed that flupyradifurone is hydrolytically stable.

The photodegradation of radio-labelled flupyradifurone in water was examined in three studies. In two of them, performed according to Japanese JMAFF Guideline OPPTS 835.2240, flupyradifurone decreased from 95-98 % of AR at time 0 to 17-8 % of AR at 35 and 28 hours of irradiation. The determined dissipation time DT_{50} was 14 hours. The major degradants included flupyradifurone-succinamide and flupyradifurone-azabicyclosuccinamide. In the third study, carried out according to an ECETOC method using polychromatic light, not more than 10 % degradation was measured after a maximum irradiation period of 500 minutes.

Ready biodegradability tests were not available.

Four different water/sediment studies were submitted in the CLH dossier: two aerobic studies (one with one radiolabel (GLP, Hellpointner and Unold, 2012) and one with two radiolabels (GLP, Menke and Unold, 2012)), an anaerobic water sediment study (GLP, Xu, 2012), and a water/sediment study for the metabolite difluoroacetic acid (GLP, Hellpointner and Unold, 2012).

In Hellpointner and Unold (2012), the aerobic transformation of [pyridine-2,6-^{14}C] flupyradifurone was studied in two different water/sediment systems (Honniger and Angler Weiher) for a maximum of 119 days in the dark at 20 ± 1 °C. The dissipation of flupyradifurone from the water phases was mainly characterised by rapid partitioning into the sediment. The estimated DT_{50} values were 193.1 and 246.9 days for the entire water/sediment test system from Honniger and Angler Weihe, respectively. The only major transformation products were carbon dioxide (6.8 % and 8.5 %) and formation of non-extractable ^{14}C-residues (NER, 25 % and 13.6 %).

In Menke and Unold (2012), the aerobic transformation of two test substances with radiolabels in different positions (FUR-^{14}C and ETH-^{14}C) was studied in two water/sediment systems, Honniger Weiher (HW) and Angler Weiher (AW) for a maximum of 120 days in the dark at 20 ± 2 °C. The dissipation of the substance from the water phase was mainly characterised by a fast removal into the sediment.

In the entire water/sediment systems, flupyradifurone was degraded slowly (Xu, 2012).

The estimated DT_{50} values were 208.2 and 202.4 days for FUR and ETH test substances from HW, and 246.1 and 285.0 for FUR and ETH flupyradifurone from AW. Difluoroacetic acid was observed as a degradation product of ETH flupyradifurone in the water phases and in the sediment extracts of both water/sediment systems (Hellpointner and Unold, 2012).

The studies are all reliable and they are proposed by the DS as key studies for classification.

A final water-sediment study was an outdoor microcosm study (DT_{50} = 92.7 days, Burns, 2012). This study is not used as a key study for classification and labelling purpose but it still shows a low rate of degradation consistent with the other studies. Additional soil simulation studies and the anaerobic water-sediment study show similarly low levels of degradation. These studies are not directly used for classification and labelling purposes, but are considered supportive studies.

Based on the information above, the DS concluded that the substance is not considered to be rapidly degradable.

Bioaccumulation

For flupyradifurone, there was an available measured log K_{OW} of 1.2 according to OECD TG 117 (HPLC method). There are no measured BCF data.

Based on this information, the DS concluded that the substance has a low bioaccumulation potential.

Aquatic Toxicity

The DS provided acute and chronic aquatic toxicity data for the three trophic levels: fish, invertebrate, algae and aquatic plants. There is one amphibian toxicity study (frogs), however, this is believed not to influence the classification and is therefore not discussed.

The following table summarises the relevant studies on aquatic toxicity, all considered acceptable by the DS. The key studies used for the classification are indicated in bold.

Method	Results	DS Remarks	Reference
Acute Aquatic Toxicity Studies			
Acute Fish Rainbow trout *(Oncorhynchus mykiss)* FIFRA 72-1, OPPTS 850.1075, OECD TG 203	LC_{50}, 96 h > 74.2 mg a.s./L m.m.	Static, stock solution in DMF	Matlock & Lam, 2010a
Acute Fish Fathead minnow *(Pimephales promelas)* FIFRA 72-1, OPPTS 850.1075, OECD TG 203	LC_{50}, 96 h > 70.5 mg a.s./L m.m.	static, stock solution in DMF	Matlock & Lam, 2010b

Acute Fish Common carp *(Cyprinus carpio)* FIFRA 72-1, OPPTS 850.1075, OECD TG 203	LC_{50}, 96 h > 100 mg a.s./L nominal	Static, measured concentration was 101-120 % of the nominal concentration, stock solution in DMF	Bruns, 2011a
Acute Fish Sheepshead minnow (*Cyprinodon variegatus*) FIFRA 72-3, OPPTS 850.1075, OECD TG 203	LC_{50}, 96 h > 83.9 mg a.s./L m.m	Static, stock solution in DMF	Banman & Lam, 2009b
Acute Aquatic Invertebrate *Daphnia magna* FIFRA72-2, OPPTS 850.1010, OECD TG 202	EC_{50}, 48 h > 77.6 mg a.s./L m.m.	Static, stock solution in DMF	Banman & Lam, 2009a
Key Study **Acute Aquatic Invertebrate 48 hours, *Chironomus riparius* OPPTS 850.1300, OECD TG 202**	**EC_{50}, 48 h = 0.0617 mg a.s./ L nominal**	Static, measured concentrations were 97-107 % of the nominal	Bruns, 2011e
Acute Aquatic Invertebrate Eastern oyster *(Crassostrea virginica)* OPPTS 850.1025	EC_{50}, 96 h > 29 mg a.s./L	Flow through, stock solution in DMF	Gallagher *et al.,* 2009a
Acute Aquatic Invertebrate 96 hours, Salt water mysid *(Americamysis bahia)* OPPTS 850.1035	EC_{50}, 96 h = 0.26 mg a.s./L	Static, acceptable for juveniles only	Gallagher *et al.*, 2009b
Short term Algae & Plants *Pseudokirchneriella subcapitata* FIFRA 123-2, OPPTS 850.5400, OECD TG 201 (2006)	E_rC_{50} & E_yC_{50}, 72 h > 80 mg a.s./L nominal	Static, stock solution in DMF, measured concentrations were 111-120 % of the nominal concentration	Banman & Lam, 2010
Short term Algae & Plants 7 days, Duckweed *(Lemna gibba G3)* FIFRA 123-2, OPPTS 850.4400, OECD TG 221 (2006)	Lowest EC_{50} > 67.7 mg a.s./L m.m.	Semi-static, stock solution in DMF	Banmann, 2010
Chronic Aquatic Toxicity Studies			
Long Term Fish ELS test, Fathead minnow (*Pimephales promelas*) FIFRA 72- 4, OPPTS 850.1400, OECD TG 210	NOEC, 35 days = 4.41 mg a.s./L m.m.	Flow through, stock solution in DMF	Matlock & Lam, 2011
Long term Aquatic Invertebrate *Daphnia magna* EPA 72-4, OPPTS 850.1300, EEC C.20, OECD TG 211	NOEC, 21 days = 3.2 mg a.s./L nominal	Semi-static, stock solution in DMF Measured concentrations were 102-117 % of the nominal concentration	Riebschläger, 2011
Key Study **Long term Aquatic Invertebrate** **28 days, *Chironomus riparius*** **OECD TG 219**	**NOEC, 28 days = 0.010 mg a.s. /L nominal = (0.0041 mg a.s. / L m.m.)** ***NOEC = 0.00681 mg/L (geom. mean measured)**	Static, stock solution in DMF, measured concentrations decline to a mean 41 % in overlying water, still NOEC based on nominal concentration!	Bruns, 2011g
Long term Aquatic Invertebrate Saltwater mysid *(Mysidopsis bahia)* OPPTS 850.1350	NOEC, 28 days = 0.0132 mg a.s./L m.m.	Flow through, stock solution in DMF	Claude *et al.*, 2011
Short term Algae & Plants 7 days, Duckweed *(Lemna gibba G3)* FIFRA 123-2, OPPTS 850.4400, OECD TG 221 (2006)	Lowest NOEC = 34.2 mg a.s./L m.m.	Semi-static, stock solution in DMF	Banmann, 2010

*value not provided in the CLH report, but agreed by DS in the response to public consultation, from updated DAR December 2014, V3-B9 part 1 (see also Section "Additional key elements").

Acute aquatic toxicity

Tests was provided for four different fish species: Rainbow trout (*Oncorhynchus mykiss*), Fathead minnow (*Pimephales promelas*), Common carp (*Cyprinus carpio*) and Sheepshead minnow (*Cyrpinodon variegatus*). All studies have an LC_{50} > 70.5 mg a.s./L (96 h, m.m.).

Four different invertebrate species were also tested for the acute aquatic toxicity: the cladoceran *Daphnia magna*, the midge *Chironomus riparius*, the marine species *Americamysis bahia* and the mollusc *Crassostrea virginica*. The most sensitive organism was *Chironomus riparius* with an EC_{50} = 0.0617 mg a.s./L (48 h, nominal), therefore this was proposed as the key study for classification. The *Chironomus* study was based on nominal concentrations (acceptable because measured concentrations were 97-107 % of the nominal). The next most sensitive species is the salt water mysid *Americamysis bahia*, with an EC_{50} = 0.26 mg a.s./L (96 h, m.m.). The study is accepted only for juveniles, because it is not reported that a preliminary test with juveniles and young adults has been conducted to determine the most sensitive life stage (as the guidance prescribes). However, this study is reliable and used as a supportive study for the proposed classification, as its EC_{50} lies close to the value found in the *Chironomus* study.

Finally, one short term algae toxicity test (E_rC_{50} > 80 mg a.s./L, 72 h, nominal) and one *Lemna gibba* test (EC_{50} > 67.7 mg a.s./L, 72 h, m.m.) were reported.

Chronic aquatic toxicity

There are five chronic aquatic toxicity studies available.

One is a fish study (*Fathead minnow*) with a NOEC = 4.41 mg a.s./L (35 days, m.m.).

There are three invertebrate studies with respectively a NOEC = 3.2 mg a.s./L (21 days, nominal) for *Daphnia magna*, a NOEC = 0.010 mg a.s./L (28 days, nominal) for *Chironomus riparius* and a NOEC = 0.0132 mg a.s./L (28 days, m.m.) for *Mysidopsis bahia*.

Finally, a *Lemna gibba* study is reported; lowest NOEC = 34.2 mg a.s./L (7 days, m.m.).

The above chronic invertebrate toxicity studies confirm that *Chironomus riparius* is the most sensitive organism tested and therefore it is used as a key study for classification. The test proposed in the CLH report was based on the 28 d NOEC = 0.010 mg a.s./L nominal concentrations, although the mean measured concentrations declined to 41 % in overlying water after 28 days, (see also Section: "Assessment and comparison with the classification criteria"). The next most sensitive species is *Mysidopsis bahia* with a measured NOEC = 0.0132 mg a.s./L.

Metabolite aquatic toxicity

Several studies were available from the DAR and reported in the CLH report, related to the following metabolites: 6-CAN (6-chloronicotinic acid), DFA, (difluoroacetate), flupyradifurone-succinamide (in surface water) and flupyradifurone-azabicyclosuccinamide (in sediment).

According to these tests, all metabolites are less toxic than flupyradifurone. The lowest reported short–term value with 6-CAN was an EC_{50} > 1 mg/L (48 h, nominal) for *Chironomus tentans.* The most sensitive long term organism resulted *Daphnia magna*

with a NOEC value of 43.3 mg/L flupyradifurone-succinamide (21 days, nominal). None of these data influence the classification of flupyradifurone.

Comments received during public consultation

Five MSCAs commented on the environmental classification. Four of them agreed with the proposed environmental classification, one of them asked for additional information. MSCAs who have commented, recommended the use of the mean measured concentration for the chronic toxicity end point of the key study test. The NOEC value of the toxicity test with *Chironomus riparius* was 0.010 mg a.s./L (nominal), however, according to the updated DAR December 2014, V3-B9 part 1, it should be recalculate to 0.00681 mg/L (geom. mean measured). The DS agreed with the commenters, but points out that the updated measured concentrations do not influence the classification (0.001 < NOEC ≤ 0.01 mg/L).

In addition, the DS clarified that for the chronic toxicity test with *C. riparius*, the concentrations were measured in the overlying water and pore water (not on the sediment phase) and that it was unlikely that the experiment was performed in the dark.

Additional key elements

According to the updated DAR December 2014, V3-B9 part 1, the key study for chronic aquatic toxicity to *C. riparius* was updated with information on geometric mean measured concentration to derive the NOEC value. In the updated table, the second column with measured concentrations was added:

"Table B.9.2.2.1-03 Summary of biological results of 28-day chronic toxicity test with flupyradifurone technical on *Chironomus riparius"*

nominal conc. (µg a.s./L)	**geom. mean measured conc. (µg a.s./L)**	Emergence rate (%)	development rate (1/d)
solvent control	**solvent control**	90.0	0.058
1.25	**0.679**	87.5	0.058
2.5	**1.25**	92.5	0.057
5	**2.25**	83.8	0.058
10	**6.81**	88.8	0.059
20	**15.4**	52.5*	0.050*
40	**29.8**	0	0

* Statistically significant at 5 % level

The recalculated NOEC based on mean measured concentrations was 6.81 µg a.s./L. The study was performed according to OECD TG 219 (2004) and considered acceptable.

Based on the information available, RAC concludes that the recalculated NOEC based on measured concentrations (geometric mean) should be used for classification.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS proposal to consider flupyradifurone as not rapidly degradable.

The substance is hydrolytically stable at environmentally relevant pH and not ultimately degraded to a level greater than 70 % over 28 days in water/sediment simulation studies.

Bioaccumulation

RAC agrees with DS that flupyradifurone has a low potential to bioaccumulate in aquatic organisms. The basis for this is that log K_{OW} value of 1.2 is below the cut-off value of 4.

Aquatic toxicity

The most sensitive organism was *Chironomus riparius* with an EC_{50} = 0.0681 mg a.s./L (48 h, nominal, immobility) and therefore this was proposed as the key study for classification. The *Chironomus* EC_{50} was based on nominal concentrations. The study itself is reliable and the measured concentrations were 97-107 % of the nominal values, therefore it is acceptable that the EC_{50} is based on a nominal concentration. In conclusion, based on the EC_{50} = 0.0681 mg/L being in the range ($0.01 < L(E)C_{50} \leq 0.1$), **RAC agrees to classify flupyradifurone as Aquatic Acute 1; H400 with an M-factor 10**.

Chronic aquatic hazard

The lowest value reported was a NOEC of 0.010 mg/L (nominal concentration) for the invertebrate *Chironomus riparius*. In the CLH report, the DS highlighted that after 28 days the measured concentrations declined by an average of 41 % in the overlying water: the measured concentrations of flupyradifurone in the overlying water were in the range 85-110 % (mean 99 %), 37-83 % (mean 60 %) and 30-52 % (mean 41 %) of nominal, after 1 hour, 7 days and 28 days, respectively.

As already stated in the "Additional key elements" section, RAC agrees with the DS's final conclusion that the NOEC should be based on mean measured concentrations. Indeed, in the additional DAR report (December 2014) it is stated that "*during pesticide peer review meeting 124 in December 2014, it was agreed that the endpoints for Chironomus should be expressed in terms of mean measured concentration in the water column when the analytical measurement concentrations are outside of the range 80-120 % and when concentrations in the sediment are not available*".

Therefore, according to the above table B.9.2.2.1-03, reported as an update in the Public Consultation response, the DS concluded that for *Chironomus riparius*, the NOEC based on geometric mean measured concentrations is equal to 6.81 µg a.s./L. The initial proposal is however not affected, because this value is in the same range than the nominal value for classification as Aquatic Chronic 1 (NOEC ≤ 0.01 mg/L).

In conclusion, flupyradifurone is considered to be not rapidly degradable, and **RAC agrees to classify flupyradifurone as Aquatic Chronic 1; H410 with an M-factor 10** ($0.001 < NOEC \leq 0.01$ mg/L).

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7 ANNEXES

7.1 Clarification by the applicant on the possible endocrine mediated mode of action of flupyradifurone.

In the rat two-generation reproduction study conducted on Flupyradifurone changes were observed in offspring at the high dietary concentration of 1800 ppm. Effects seen on PPS and VO, two well-established markers of pubertal development, included a statistically significant delay (PPS) and a slight, non-statistical delay (VO) in F1 generation animals. In addition, variations in brain, thymus, and spleen weights in offspring of the 500 ppm (F2 generation) and 1800 ppm (F1 and F2

generations) dose groups were also observed. A slight, albeit significant, decrease in median implantation sites (and thus decrease in litter size) in the F1-generation was observed with 1800 ppm Flupyradifurone. It is the position of both Bayer CropScience (BCS) and the performing laboratory that the aforementioned effects are likely a result of treatment-induced reductions in BW and BWG rather than a direct, endocrine-mediated MOA. The discussion that follows defends this position for each of the observed changes in the context of available data on Flupyradifurone and in the publicly available literature (see below). This position from BCS should be taken into account during the expert consultation.

Preputial Separation

Toxicity testing guidelines for the rat two-generation reproduction toxicity study require that systemic toxicity is evident at the highest dose level, and dose levels should be chosen with the aim to induce some reproductive and/or systemic toxicity, but not death or severe suffering (OECD 416; OCSPP 870.3200). As such, effects on BW (reductions >10%) are the most common effects noted at maximum tolerated dose levels, and it is reasonable that delays in preputial separation may be observed as a result of general systemic toxicity rather than a consequence of an endocrine-mediated MOA (Stump et al., 2012). In the present study, animals exposed to 100 ppm or 500 ppm. Flupyradifurone achieved PPS in a time latency similar to concurrent controls and well within historical control data for Wistar Han rats in the performing laboratory (see Appendix 1). At the high dietary level of 1800 ppm, however, a statistically significant delay in PPS (3.9 days) was observed compared to controls. The observed delay in PPS in animals at 1800 ppm is likely due to significant BW loss and food consumption and not a result of an endocrine MOA associated with delayed PPS.

Table 158: Mean Day to Criterion and Body Weight (g) at Preputial Separation

	Dose levels in ppm			
	0	100	500	1800
	Days to criterion			
Mean	42.6±0.38	42.7±0.44	43.3±0.31	46.5**±0.50
N	35	35	35	35
BW at criterion				
Mean	178±2.6	180±2.2	176±2.9	180±2.9
N	35	35	35	35

Statistically significant compared to control animals ($p \leq 0.05$).

A recent study concluded that while treatment-related delays in PPS may be indicative of specific anti-androgenic activity, impaired growth could also alter the onset of puberty (Melching-Kollmuss et al., 2014). The mean BW at the day of complete PPS was similar between the control animals (178 g) and 1800 ppm group (180 g), however, the mean BW at PND 21 was significantly decreased (-12.5%) compared to control animals (see Appendix 2); BW at PND 21 is often used as a covariate for evaluating the age at complete PPS (Stoker et al., 2000b). This significant decrease in BW at PND 21 was accompanied by significant decreases observed in maternal BW of the 1800 ppm group throughout gestation (GDs 0, 6, 13, and 20; see Appendix 3). As other sensitive anti-androgenic parameters investigated in this study such as male fertility, male reproductive organs (weights and histopathology) and sperm parameters (count, motility, or progressive sperm) were not affected at any dietary level of Flupyradifurone, it is likely that the effect on PPS was due to a non-specific effect linked to overt toxicity with decreased BW and retarded growth.

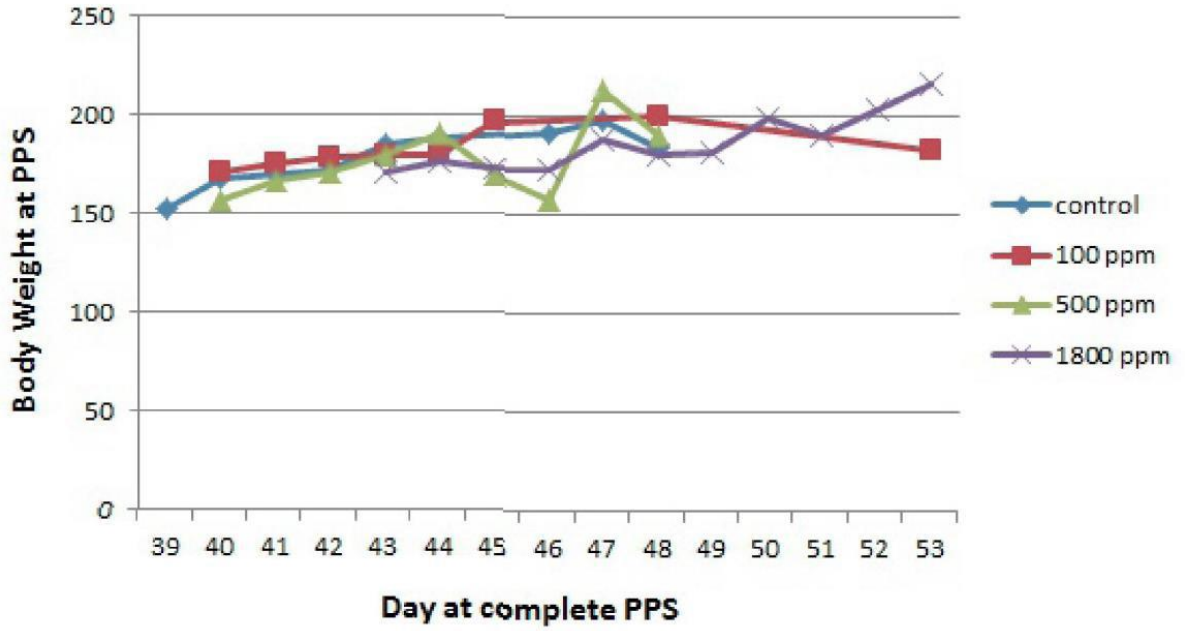

Figure 10: ***Mean Body Weight at Day of Complete Preputial Separation***

The influence of BW loss on reproductive endpoints has also been evaluated in rats by diet restriction. A significant delay in PPS (2.1 days) was observed in Wistar rats that were food-restricted and exhibited a mean body weight loss of 13% (Stoker et al., 2000), and Ashby and LeFevre (2000) reported that the day of PPS was dependent upon the BW at weaning, and not specifically to BW at the time of complete PPS. Consistent with these diet restriction studies, P-generation females in the high dietary dose group exhibited significantly reduced food consumption throughout premating, which is likely responsible for the observed decreases in BW and BWG in these dams and delayed PPS in their male offspring. By comparison, direct-acting anti-androgens do not inhibit food consumption or slow growth (Anderson et al., 1995), but they delay PPS and reduce the weights of the ventral prostate, seminal vesicle, epididymides, Cowper's glands, and levator ani plus bulbocavernosus muscles (Stoker, 2000b; Owens et al., 2006). Compounds that act as anti-androgens may also delay PPS via hypothalamic-pituitary or steroid hormone synthesis disruptions (for review see Stoker et al., 2000b), but in these cases, changes in testicular weights, testosterone and/or luteinizing hormone concentrations (not evaluated in the current study), and changes in accessory sex gland tissue weights would have accompanied the observed delay in PPS. Furthermore, anti-androgens such as vinclozolin, can decrease male pup anogenital distance (AGD) to an extent similar to female pups (Gray et al., 2001). None of these anti-androgenic changes were observed in the two-generation reproduction study or the rat developmental neurotoxicity study (Gilmore, 2012) conducted on Flupyradifurone. These data strongly support the conclusion that the delay in PPS observed in F1-generation males after exposure with high dietary concentrations of Flupyradifurone is due to significant BW loss and reduced food consumption and BWG over the exposure period, and not due to an endocrine-mediated MOA.

Vaginal Patency

F1-generation females showed a slight, non-significant delay in VO (1.0 day) in animals in the 1800 ppm dietary concentration group. The mean for complete VO was 34.8 days for the control females

and 35.8 days for the high dietary level females. It should be noted, however, that the day means to complete VO were within the historical control range (33.4 – 36.7 days, see Appendix 1) for all dietary levels of Flupyradifurone. The F1-generation females achieved VO with significantly reduced BW and BWG (-16.6% and -19%, respectively, compared to controls) during lactation, and no other disruptions in estrogen sensitive endpoints (i.e. tissue weights, estrous cyclicity, histopathological analysis, mating parameters) were observed in the remainder of the study. Furthermore, no other estrogen sensitive parameters were affected by Flupyradifurone in the rat developmental neurotoxicity study (Gilmore, 2012), the developmental toxicity studies (Langrand-Lerche, 2010 ; Kennel, 2012), or the chronic toxicity/carcinogenicity studies (Garcin, 2012 ; Kennel, 2012). Therefore, the slight delay of 1 day in VO after high dietary exposure to Flupyradifurone is most likely the result of decreased BW and BWG during lactation, as well as decreased maternal BW and BWG throughout premating, gestation, and lactation. Delays in VO, concurrent with observations of delayed PPS in males, is understood to indicate a general growth delay, rather than an endocrine MOA (Stump et al., 2012). A slight, but statistical, decrease in the number of estrous cycles in the F1-generation was observed, but is also attributable to BW loss, as irregular cycles are often noted in the immediate post-pubertal period (Goldman et al., 1985). Taken together, these points indicate that the non-statistically significant delay VO is not endocrine-mediated and is of no biological significance.

Table 159: Mean Day to Criterion and Body Weight (g) at Vaginal Patency

	Dose levels in ppm			
	0	100	500	1800
	Days to criterion			
Mean	34.8±0.78	34.1±0.48	34.4±0.43	35.8±0.60
N	34	35	35	35
BW at criterion				
Mean	113±3.5	106±2.2	102*±1.8	103*±2.0
N	34	35	35	35

*Statistically significant compared to control animals ($p \leq 0.05$).

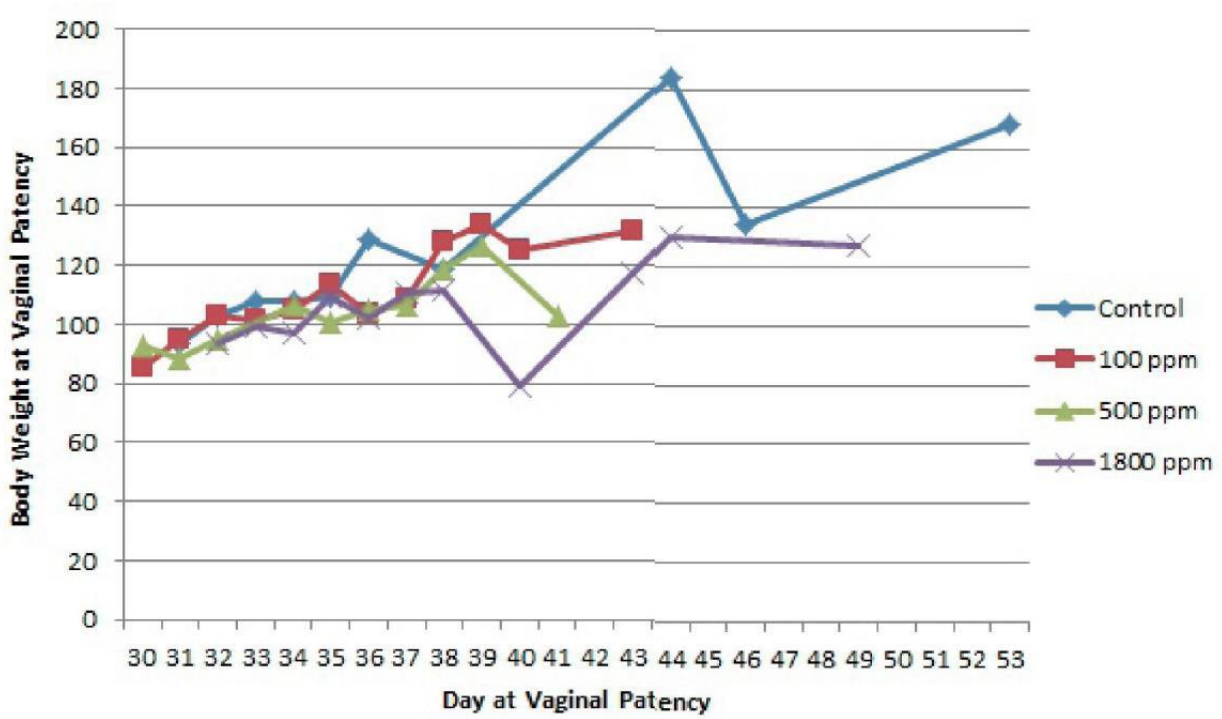

Figure 11: Mean Body Weight at Day of Complete Vaginal Patency

Organ Weight Changes in Pups

Organ weight changes in pups were observed in brain, thymus, and spleen in pups exposed to 1800 ppm Flupyradifurone. Similar to changes observed in PPS and VO, the pup organ weight changes were due to significant BW loss over the exposure period, and not due to an endocrine MOA. In F1-generation pups, birth weight was significantly decreased in both sexes, and an overall decrease in BWG throughout lactation was observed in F1- (-13.7%) and F2-generation (-13.8%) pups at the high dietary level. Pup terminal BWs were also decreased in males (-10%) and females (-12%). In both sexes and generations, absolute brain weights were decreased, while brain weight relative to BW was increased. A similar trend was observed thymus weights, while absolute spleen weights were decreased in F1 males and both sexes in the F2 generation.

Brain weights (see Appendix 4):

In F1 pups on PND21, a slight decrease in absolute brain weight was observed in males (-3.93%) and females (-3.3%) at 1800 ppm (only one male was outside the control range) and a slight increase in brain weight to body weight ratio was observed (+9.5% in males and +9.07% in females) due to lower body weight. Nine males from 7 litters and 12 females from 7 litters were outside the control range but they had lower body weight compared to control animals.

In F2 pups, a statistically significant decrease in absolute brain weight was observed in females only (-3%) with only one animal outside the control range. A statistically significant increase in brain weight to body weight ratio was observed in males (+11.5%) with 6 animals from the same litter outside the control range due to lower body weight and in females (+11.4%) with only one animal outside the control range.

Thymus weights (see Appendix 5):

F1 pups at 1800 ppm: Statistically significant thymus weight changes were observed in males for absolute weights (-11.3%, all animals within control range) and in females for thymus weight to body
weight ratio (+6.8% with 3 animals outside the control range due to lower body weight).
F2 pups at 1800 ppm: A statistically significant decrease in absolute thymus weight in females (- 10.96%) was noted with 12 animals from 5 litters outside the control range due to lower body weight. No significant changes were observed in thymus weight to body weight ratio.
Spleen weight (See Appendix 6):
F1 pups at 1800 ppm: A statistically significant decrease in absolute spleen weight was observed in males only (-17.98%) with all animals within the control range and no significant effect on spleen weight to body weight ratio.
F2 pups at 1800 ppm: A statistically significant decrease in absolute spleen weight was observed in both males (-14.98%) with 6 animals from one litter outside the control range due to lower body weight and females (-15.48%) with 6 animals from 3 litters outside the control range due to lower body weight. No significant effect on spleen weight to body weight ratio in both sexes.
The organ weight changes and reduction in pup terminal BWs seen in the present study are consistent with changes in the brain, thymus, and spleen observed in a food restriction reproduction study in which animals were given 30% less food daily relative to control groups (Carney et al., 2004). Animals receiving 30% less food (equivalent to 6-19% BW decrease) showed similar reductions in absolute organ weights with concomitant increases in relative organ weights in both male and female rats ; animals receiving only 10% less food, which did not change BW, showed no organ weight changes compared to control groups (Carney et al., 2004). Animals receiving 30% less food exhibited BW reductions similar in magnitude to those found in animals exposed to the high dietary level of Flupyradifurone in the two-generation reproduction study. These data support the notion that significant reductions in BW can influence organ weights. In addition, no microscopic changes were observed in the brain, thymus, or spleen in animals of either sex in the Flupyradifurone two-generation reproduction study. The rat DNT study (Gilmore, 2012) and rat subchronic neurotoxicity study (Garcin, 2011) conducted with Flupyradifurone did not produce any brain weight changes up to the high dietary levels of 1200 ppm and 2500 ppm, respectively. In both studies, BW and BWG declines were not as severe in magnitude as those seen in the two-generation reproduction study, supporting the conclusion that the organ weight changes observed in the reproduction study are likely due to reductions in BW and BWG. In a rat immunotoxicity study conducted with Flupyradifurone (Repetto, 2011), significant decreases in the absolute weight of the spleen and thymus were observed, but relative weights were unchanged for both organs. These results are consistent with other repeat-dose dietary toxicity studies conducted with Flupyradifurone and support the conclusion that the observed organ weight changes in pups are likely due to general systemic toxicity and decreased BW and BWG of the animals. The lack of response in endocrine-sensitive tissues in both sexes further supports these organ weight changes are due to significant decreases in BW and not an endocrine-mediated event.

Reduced Number of Implantation Sites

A slight, albeit significant, decrease in median implantation sites (and thus decrease in litter size) in the F1-generation was observed with 1800 ppm Flupyradifurone. This effect, however, was not observed in the P0-generation dams and the total number of implantation sites between the P0- and F1-generations in the control (311 and 305, P0 and F1, respectively) and high dose (289 and 281, P0 and F1, respectively) are similar. This slight decrease is likely due to the decreased BW in the P0 females, which was observed as early as day 7 after initiation of exposure. Significant BW decreases were maintained for the duration of the study, including the F1 females at birth, and continuing through mating and gestation of the F2 animals (see Appendix 3). Exposure to

exogenous chemicals that produce a reduction in the amount of BW gained during the first 9 days of pregnancy is an indication of the inherent toxicity of the chemical and may contribute to maternal toxicity and pregnancy loss (Cummings et al., 2000). No other effects were noted in female organ weights or histopathology in the two-generation reproduction study. Developmental toxicity studies in rats (Langrand-Lerche, 2010) and rabbits (Kennel, 2012) for Flupyradifurone did not show any significant decreases in the number of implantation sites in dams that showed a significant decrease in BW, nor in any other changes in reproductive indices, tissue weights, or histopathology. Taken together, the data supports that this finding was due to a decreased BW and not an endocrine-mediated MOA.

Table 160: Total Number of Implantation Sites Across Generations and Dietary Levels of Flupyradifurone

Observations	Dose levels in ppm			
	0	100	500	1800
	Po-generation 311			
Total number of implantation sites (Median)		285 (11.0)	298 (10.5)	289 (10.0)
	Fi-generation 305			
Total number of implantation sites (Median)		314 (11.0)	323 (11.0)	281 (10.0**)

Statistically different from control, $p < 0.01$

Summary and Conclusion

The two-generation reproduction study conducted in rats with Flupyradifurone reported changes in a few endpoints that are known to be influenced by endocrine MOAs. In light of this, EFSA questioned whether observed delays in PPS and VO, pup organ weight changes, and reductions in implantation sites observed at the highest dietary level of 1800 ppm might be endocrine mediated. Thorough evaluation of the available data on Flupyradifone and the peer-reviewed literature confirmed the initial position of BCS and the performing laboratory that these changes were secondary to decreases in BW and BWG. Several publications reporting studies that utilized food restriction to evaluate the effect of reduced BW and BWG on developmental and reproductive endpoints support this position. The mammalian toxicity database for Flupyradifurone does not indicate any anti-androgenic or anti-estrogenic activity, two known endocrine-mediated MOAs. Thus, the changes seen in the endpoints in question appear to occur secondary to overt toxicity associated with exposure to Flupyradifurone as evidenced by reductions in BW and BWG throughout all stages, including critical periods of development, of the two-generation reproduction study.

Appendix 1: Historical Control of Preputial Separation and Vaginal Patency for the Wistar Han Rat (Days)

*Study #	Vehicle/Excipient	Study Conduct (Start	Preputial Separation		Vagina 1 Patency	
			Fl	F2	Fl	F2
03-R72-PZ	Ethanol (Dietary)	6/03 - 4/04	40.7	40.7	33.4	33.6
03-R72-PT	Acetone (Dietary)	4/03 - 3/04	41.8	41.6	33.7	35.3
03-P72-SI	Dry Mix (Dietary)	9/03 - 3/04	42.3	-	33.8	-
04-R72-SJ	Dry Mix (Dietary)	4/04 - 2/05	42.4	-	35.1	-
06-R72-DI	Dry Mix (Dietary)	1/06 - 10/06	43.8	-	35.5	-
06-R72-DX	Acetone (Dietary)	3/06 - 12/06	41.0	-	34.8	-
06-R72-GY	Acetone (Dietary)	10/06 - 7/07	42.0	-	34.3	-
06-R72-HW	Dry Mix (Dietary)	11/06 - 8/07	42.6	-	34.0	-
07-R72-IH	Dry Mix (Dietary)	1/07 - 12/07	42.5	43.2	34.1	34.4
07-R72-MK	Acetone (Dietary)	11/07 - 10/08	43.5	44.0	35.5	36.7
08-R72-MQ	Dry Mix (Dietary)	1/08 - 10/08	44.3	-	36.0	-
08-R72-MX	Acetone (Dietary)	2/08 - 10/08	43.6	-	35.4	-
08-R72-OB	Acetone (Dietary)	7/08 - 4/09	44.4	-	34.8	-
Range			**40.7 - 44.4**		**33.4 - 36.7**	

Not applicable, either a one-generation study or only performed in the first generation. = All animals supplied by Charles River, Raleigh, NC.

Appendix 2: Mean (± Standard Error) pup weights (g) with percentage compared to control group mean

Males								
Lactation	Fl-generation				F2-generation			
	Dose levels in ppm				Dose levels in ppm			
	0	100	500	1800	0	100	500	1800
0	6.2±0.11	6.2±0.11 (100%)	6.0±0.10 (100%)	5.7**±0.12 (92%)	6.2±0.10	6.0±0.11 (97%)	6.0±0.11 (97%)	6.0±0.08
4[a]	10.4±0.24	10.4±0.24 (100%)	9.9±0.28 (95%)	9.4*±0.29 (90%)	10.3±0.23	9.7±0.23 (94%)	9.8±0.26 (95%)	9.8±0.22 (95%)
4b	10.4±0.25	10.4±0.24 (100%)	9.9±0.28 (95%)	9.4*±0.29 (90%)	10.3±0.23	9.7±0.22 (94%)	9.8±0.26 (95%)	9.8±0.21 (95%)
7	16.2±0.30	16.3±0.34 (101%)	15.5±0.41 (96%)	14.4**±0.39 (89%)	16.3±0.33	15.8±0.29 (97%)	15.5±0.38 (95%)	15.0±0.40 (92%)
14	32.5±0.42	32.1±0.62 (99%)	30.8±0.70 (95%)	28.2**±0.53 (87%)	32.4±0.64	31.5±0.48 (97%)	30.3*±0.51 (94%)	28.4**±0.76 (88%)
21	49.8±0.59	49.3±0.96 (99%)	48.0±1.00 (98%)	43.5**±0.78 (87%)	49.6±0.80	48.1± 0.86 (97%)	45.9**±0.74 (93%)	43.8**±1.19 (88%)

Females								
Lactation	Fl-generation				F2-generation			
	Dose levels in ppm				Dose levels in ppm			
	0	100	500	1800	0	100	500	1800
0	5.9±0.12	5.9±0.12 (100%)	5.7±0.11 (97%)	5.4*±0.11 (92%)	5.9±0.11	5.8±0.09 (98%)	5.7±0.10 (97%)	5.6±0.08
4[a]	10.0±0.28	10.1±0.28 (101%)	9.7±0.29 (97%)	9.1±0.27 (99%)	9.9±0.24	9.6±0.21 (97%)	9.4±0.24 (95%)	9.5±0.25 (96%)
4b	10.0±0.29	10.1±0.28 (101%)	9.7±0.29 (97%)	9.1±0.26 (99%)	9.9±0.24	9.6±0.21 (97%)	9.4±0.24 (95%)	9.5±0.25 (96%)
7	15.8±0.36	15.8±0.39 (100%)	15.1±0.41 (96%)	14.0**±0.35 (89%)	15.7±0.34	15.4±0.22 (98%)	15.0±0.35 (95%)	14.4*±0.39 (92%)
14	31.9±0.52	31.3±0.60 (98%)	30.0±0.71 (94%)	27.7**±0.51 (87%)	31.8±0.57	30.9±0.40 (97%)	29.6*±0.52 (93%)	27.5**±0.76 (84%)
21	48.4±0.72	47.8±0.80 (99%)	46.1±1.05 (95%)	42.2**±0.70 (87%)	47.8±0.77	46.8±0.71 (98%)	44.4**±0.74 (93%)	41.8**±1.07 (87%)

a: Before standardization (culling) b : After standardization (culling)

* Statistically different from control, $p < 0.05$ ** Statistically different from control, $p < 0.01$

Appendix 3: Mean body weight and body weight gains (± Standard Error) during premating, gestation and lactation with percentage compared to control group

Premating:

Observations/Study Week	Dose levels of Flupyradifurone			
	0	100	500	1800
P-generation Males				
Mean body weight (g) – Week 14	436.0±7.81	436.0±5.96 (100%)	441.3±7.77 (101%)	438.4±7.54 (100%)
Mean weight gain (g)	200.8	201.9 (100%)	200.0 (100%)	198.0 (99%)
P-generation Females				
Mean body weight (g) – Week 14 SD	237.4±3.33	235.5±3.09 (99%)	229.0±2.92 (96%)	213.3**±2.73 (90%)
Mean weight gain (g)	63.4	61.8 (97%)	50.4 (79%)	36.3 (57%)
Fl-generation Males				
Mean body weight (g) – Week 14 SD	454.5±8.27	456.0±7.51 (100%)	452.3±8.28 (99%)	410.8**±7.53 (90%)
Mean weight gain (g)	152.3	152.0 (100%)	166.2 (109%)	145.1 (95%)
Fl-generation Females				
Mean body weight (g) – Week 14 SD	242.1±3.04	237.3±4.06 (98%)	224.2**±2.90 (93%)	203.5**±2.49 (84%)
Mean weight gain (g)	52.2	51.5 (99%)	43.7 (84%)	41.2 (79%)

**Statistically different from control, $p < 0.01$

Gestation:

Observations/Study Day	Dose levels of Flupyradifurone			
	0	100	500	1800
P-generation Females				
Mean body weight (g) – Day 0	238.l±4.27	235.2±3.25 (99%)	232.2±3.10 (98%)	214.8**±3.06 (90%)
Mean body weight (g) – Day 6	255.0±3.65	250.2±3.07 (98%)	248.6±3.22 (97%)	228.1**±3.25 (89%)
Mean body weight (g) – Day 13	276.0±3.54	272.3±3.57 (99%)	267.9±3.63 (97%)	247.1**±3.06 (89%)
Mean body weight (g) – Day 20	333.6±5.68	326.5±4.28 (98%)	322.l±5.41 (97%)	301.1**±4.43 (90%)
Mean weight gain (g) – Days 0-20	95.6±3.36	91.3±2.59 (96%)	89.9±3.74 (94%)	86.4±2.87 (90%)
Fl-generation Females				
Mean body weight (g) – Day 0	243.4±3.27	238.3±4.82 (98%)	224.4**±3.01 (92%)	202.6**±2.85 (83%)
Mean body weight (g) – Day 6	257.6±3.09	251.0±4.57 (97%)	238.7**±2.86 (93%)	214.0**±3.23 (83%)
Mean body weight (g) – Day 13	277.8±3.33	270.0±4.54 (97%)	258.3**±2.80 (93%)	230.8**±2.98 (83%)
Mean body weight (g) – Day 20	335.l±4.52	327.4±5.28 (98%)	313.8**±4.01 (94%)	277.2**±4.28 (83%)
Mean weight gain (g) – Days 0-20	91.8±2.59	89.l±3.50 (97%)	89.4±2.86 (97%)	74.7**±2.11 (81%)

Statistically different from control, $p < 0.01$

Lactation:

Observations/Study Day	Dose levels of Flup yradifurone (in ppm)			
	0	100		1800
P-generation Females				
Mean body weight (g) – Day 0	262.8±3.22	256.l±2.95 (97%)	250.8±4.30 (95%)	233.3**±3.24 (89%)
Mean body weight (g) – Day 4	271.3±3.12	271.6±3.36 (100%)	265.8±3.91 (98%)	245.7**±3.66 (91%)
Mean body weight (g) – Day 7	277.4±2.81	276.7±3.05 (100%)	271.2±3.90 (98%)	251.6**±3.20 (91%)
Mean body weight (g) – Day 14	292.6±3.81	292.3±2.77 (100%)	287.2±4.22 (98%)	267.1**±3.17 (91%)
Mean body weight (g) – Day 21	285.8±3.67	281.4±2.80 (98%)	280.l±3.59 (98%)	264.3**±3.15 (92%)
Fl-generation Females				
Mean body weight (g) – Day 0	263.0±3.38	254.5±4.53 (97%)	242.5**±3.27 (92%)	218.4**±2.84 (83%)
Mean body weight (g) – Day 4	277.3±3.24	265.9±4.13 (96%)	256.0**±3.51 (92%)	225.1**±3.69 (81%)
Mean body weight (g) – Day 7	278.7±3.23	268.5±4.02 (94%)	260.9**±3.43 (94%)	231.3**±3.02 (83%)
Mean body weight (g) – Day 14	294.8±3.54	282.8±3.64 (96%)	272.2**±3.72 (92%)	244.3**±3.53 (83%)
Mean body weight (g) – Day 21	283.8±3.45	278.5±3.91 (98%)	261.6**±4.17 (92%)	247.1**±3.22 (87%)

Statistically different from control, $p < 0.01$

Appendix 4: Statistically significant brain weight changes in pups on day 21

F1 pups				
Absolute Brain weights				
Groups	Sex	MeantS.D. (N)	range	Comments
Control	Males	1.525 ± 0.0128 (28)	[1.312 - 1.721]	
	Females	1.475 ± 0.0118 (27)	[1.278 - 1.605]	
1800 ppm	Males	1.465** ± 0.0130 (25)	[1.312 - 1.735]	1 animal outside the control range
	Females	1.426* ± 0.0114 (27)	[1.315 - 1.587]	All animals within the control range
Brain weight to body weight ratio				
Control	Males	3.089 ± 0.0364 (28)	[2.557 - 3.665]	
	Females	3.088 ± 0.0462 (27)	[2.485 - 3.764]	
1800 ppm	Males	3.383** ± 0.0449 (25)	[2.808 - 3.906]	9 animals outside the control range (from 7 litters) due to lower body weight
	Females	3.396** ± 0.0549 (27)	[2.818 - 4.247]	12 animals outside the control range (from 7 litters) due to lower body weight
F2 pups				
Absolute Brain weight				
Groups	Sex	Mean ±S.D. (N)	range	Comments
Control	Males	1.505 ± 0.0085 (27)	[1.370 - 1.683]	
	Females	1.469 ± 0.0092 (27)	[1.264 - 1.594]	
1800 ppm	Males	1.474 ± 0.0115 (29)	[1.295 - 1.613]	8 animals outside the control range (from 4 litters) due to lower
	Females	1.425* ± 0.0116 (29)	[1.256 - 1.576]	1 animal outside the control range
Brain weight to body weight ratio				
Control	Males	3.073 ±0.0476 (27)	[2.597 - 4.376]	
	Females	3.114 ± 0.0629 (27)	[2.490 - 6.117]	
1800 ppm	Males	3.427** ± 0.0867 (29)	[2.656 - 5.283]	6 animals outside the control range from the same litter due to lower body weight
	Females	3.468** ± 0.0773 (29)	[2.471 - 4.812]	1 animal outside the control range

Appendix 5: Statistically significant thymus weight changes in pups on day 21

F1 pups				
Absolute Thymus weight				
Groups	Sex	MeantS.D. (N)	range	Comments

Control	Males	0.222 ± 0.0057 (28)	[0.135 - 0.296]	
	Females	0.219 ± 0.0052 (27)	[0.113 - 0.298]	
1800 ppm	Males	0.197* ± 0.0064 (25)	[0.160 - 0.294]	All animals within the control range
	Females	0.206 ± 0.0056 (27)	[0.150 - 0.299]	1 animal outside the control range
Thymus weight to body weight ratio				
Groups	Sex	Mean ±S.D.(N)	range	Comments
Control	Males	0.448 ± 0.0103 (28)	[0.340 - 0.556]	
	Females	0.455 ± 0.0090 (27)	[0.314 - 0.582]	
1800 ppm	Males	0.452 ± 0.0106 (25)	[0.346 - 0.593]	3 animals outside the control range due to lower body weight
	Females	0.486* ± 0.0090 (27)	[0.333 - 0.669]	3 animals outside the control range due to lower body weight
F2 pups				
Absolute Thymus weight				
Groups	Sex	Mean ±S.D. (N)	range	Comments
Control	Females	0.219 ± 0.0054 (27)	[0.149 - 0.320]	
1800 ppm	Females	0.195** ± 0.0056 (29)	[0.114 - 0.250]	12 animals outside the control range from 5 litters dam due to lower body weight – No significant effects on thymus weight to body weight ratio

Appendix 6: Statistically significant spleen weight changes in pups on day 21

F1 pups				
Absolute Spleen weight				
Groups	Sex	MeantS.D. (N)	range	Comments
Control	Males	0.228 ± 0.0082 (28)	[0.061 - 0.348]	
1800 ppm	Males	0.187** ± 0.0069 (25)	[0.092 - 0.325]	All animals within the control range
Spleen weight to body weight ratio				
Control	Males	0.456 ± 0.128 (28)	[0.170 - 0.684]	
1800 ppm	Males	0.428 ± 0.0121 (25)	[0.337 - 0.661]	All animals within the control range
F2 pups				
Absolute Spleen weight				
Groups	Sex	Mean ±S.D.(N)	range	Comments
Control	Males	0.227 ± 0.0062 (27)	[0.118 - 0.361]	
	Females	0.226 ± 0.0065 (27)	[0.126 - 0.320]	
1800 ppm	Males	0.193** ± 0.0082 (29)	[0.070 - 0.327]	6 animals outside the control range from one litter due to lower body weight - No significant effects on spleen weight to body weight ratio

	Females	0.191** ± 0.0088 (29)	[0.074 - 0.348]	6 animals outside the control range from 3 litters due to lower body weight - No significant effects on spleen weight to body weight ratio

General Comment

Effects unrelated to the endocrine system observed after chemical treatment often confound the interpretation of results of endpoints evaluated in standard reproductive toxicology studies (Carney et al., 2004; Laws et al., 2007; Marty et al., 2007; Laws et al., 2000; Stoker et al., 2000a; for review see Stoker et al., 2000b). For example, significant body weight loss, altered feed intake, and stress responses can contribute to changes in study endpoints that may be inaccurately attributed to an endocrine-mediated mechanism. Several peer-reviewed publications report studies that have been conducted utilizing food restriction, resulting in varying degrees of BW loss, to determine the influence of BW reduction on *in utero* and postnatal developmental endpoints (Carney et a., 2004; Chapin et al., 1993; Laws et al., 2000; Laws et al., 2007; Marty at al., 2007; Stoker et al., 2000a). Endocrine-related endpoints evaluated in these studies include PPS and sperm parameters in males, VO and estrous cyclicity in females, and organ weights and hormone concentrations in both sexes.

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7.2 The Aquatic Toxicity of Metabolites of BYI 02960.

According to the LoEP (List of Endpoints in the DAR) from fate and behaviour, the environmental occurring metabolites requiring further assessment by other disciplines (toxicology and ecotoxicology) are: 6-CNA, DFA, BYI 02960-succinamide and BYI 02960-azabicyclosuccinmaide (in surface water) and 6-CAN and DFA (in sediment).

Accepted acute data from the DAR on the toxicity of the relevant metabolites are summarized in Table 161 and the chronic data in Table 162.

Table 161: The acute toxicity of metabolites of BYI 02960 to aquatic life.

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Fish				
Rainbow trout (*Oncorhynchus mykiss)*	BYI 02960-succinamide	96 hr (static)	Mortality, EC_{50}	>100 ($_{nom}$)
Rainbow trout (*Oncorhynchus mykiss)*	DFA (BCS-AB60481 (tech.) = sodium difluoroacetate)	96 hr (static)	Mortality, EC_{50}	>10 ($_{nom}$)
Aquatic invertebrates				
Daphnia magna	DFA (BCS-AB60481 (tech.) = sodium difluoroacetate)	48 hr (static)	Mortality, EC_{50}	>10 ($_{nom}$)
Daphnia magna	6-CNA (IC-0)	48 h (static)	Mortality, EC_{50}	>95.1 ($_{mm}$)
Chironomus riparius	BYI 02960-succinamide	48 h (static)	Mortality, EC_{50}	>100 ($_{nom}$)
Chironomus riparius	BYI 02960-azabicyclosuccinamide, sodium salt	48 h (static)	Mortality, EC_{50}	>100 ($_{nom}$)
Chironomus tentans (most sensitive acute)	6-CNA	48 h (static)	Mortality, EC_{50}	>1 ($_{nom}$)
Algae				

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Pseudokirchneriella subcapitata	DFA (BCS-AB60481 (tech.) = sodium difluoroacetate)	72 hr (static)	Growth rate: E_rC_{50} and Yield: E_yC_{50}	>10 ($_{nom}$)
Pseudokirchneriella subcapitata	BYI 02960-succinamide	72 hr (static)	Growth rate: E_rC_{50} and Yield: E_yC_{50}	>10 ($_{nom}$)
Pseudokirchneriella subcapitata	6-CNA (IC-0)	72 hr (static)	Growth rate: E_rC_{50} Yield: E_yC_{50}	>100 ($_{nom}$) 85 ($_{nom}$)

Table 162: The chronic toxicity of metabolites of BYI 02960 to aquatic life.

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1] (mg/L)
Aquatic invertebrates				
Daphnia magna (most sensitive chronic)	BYI 02960-succinamide	21 d (semi-static)	Reproduction, NOEC	43.3 ($_{nom}$)
Sediment dwelling organisms				
Chironomus riparius	DFA (BCS-AB60481 (tech.) = sodium difluoroacetate)	28 d (static; water-spiked)	NOEC	100 ($_{nom}$)
Chironomus riparius	6-CNA (IC-0)	28 d (static; water-spiked)	NOEC	100 ($_{nom}$)