Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

N-{2-[[1,1'-bi(cyclopropyl)]-2-yl]phenyl}-3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamide; sedaxane

EC Number: -
CAS Number: 874967-67-6

CLH-O-0000001412-86-280/F

Adopted

15 March 2019

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CLH-O-0000001412-86-280/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: ***N*-{2-[[1,1'-bi(cyclopropyl)]-2-yl]phenyl}-3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamide; sedaxane**

EC Number: **-**

CAS Number: **874967-67-6**

The proposal was submitted by **France** and received by RAC on **25 April 2018.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

France has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **4 June 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **3 August 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Ruth Moeller**

Co-Rapporteur, appointed by RAC: **Peter Hammer Sørensen**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **15 March 2019** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	*N*-{2-[[1,1'-bi(cyclopropyl)]-2-yl]phenyl}-3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamide; sedaxane		874967-67-6	Carc. 2 Aquatic Acute 1 Aquatic Chronic 2	H351 H400 H411	GHS08 GHS09 Wng	H351 H410		M=1	
RAC opinion	TBD	*N*-{2-[[1,1'-bi(cyclopropyl)]-2-yl]phenyl}-3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamide; sedaxane		874967-67-6	Carc. 2 Aquatic Acute 1 Aquatic Chronic 2	H351 H400 H411	GHS08 GHS09 Wng	H351 H410		M=1	
Resulting Annex VI entry if agreed by COM	TBD	*N*-{2-[[1,1'-bi(cyclopropyl)]-2-yl]phenyl}-3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamide; sedaxane		874967-67-6	Carc. 2 Aquatic Acute 1 Aquatic Chronic 2	H351 H400 H411	GHS08 GHS09 Wng	H351 H410		M=1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The substance *N*-{2-[[1,1'-bi(cyclopropyl)]-2-yl]phenyl}-3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamide; sedaxane (Syngenta code CYN524464) is a new active substance (broad-spectrum seed treatment fungicide) in the meaning of Regulation EC 1107/2009 and has no history of previous classification and labelling. The substance is a mixture of the trans isomers (SYN508210) and cis isomer (SYN508211). Each isomer constitutes a racemate of enantiomers. The purity is 960 g/kg with a purity range in the studies from 94.2 to 99.6%.

The dossier submitter (DS) proposed a classification as Carc. 2 – H351, Aquatic Acute 1 – H400, and Aquatic Chronic 2 – H411. Formerly, no classification as regards to carcinogenicity was proposed in the conclusion of the peer review of the pesticide risk assessment of the active substance sedaxane (EFSA, 2012). In 2011, U.S. EPA classified sedaxane "Likely to be carcinogenic to humans" based on the presence of multiple site tumours in two species. Following a request of the European Commission for reconsideration and confirmation of the conclusion of the toxicological assessment, sedaxane was re-discussed at the Pesticide Peer Review Meeting in November 2012 with the conclusion that classification as Carc. 2 – H351 would be required (EFSA, 2013).

The applicant (Syngenta) then has generated numerous mechanistic studies and performed mode of action (MoA) analysis for liver, thyroid and uterine tumours according to the WHO/IPCS Framework for analysing the relevance of a cancer MoA for humans. The proposed MoAs are reported *in extenso* in Appendix 1-3 of the CLH report and have been evaluated by the dossier submitter. During the public consultation, additional data have been submitted by the applicant to be considered in the RAC opinion making process.

Sedaxane is a succinate dehydrogenase inhibitor (SDHI). During public consultation, the DS highlighted that a high concern regarding the use of as fungicides in agriculture has been recently been raised by researchers and clinicians from French institutes with respect to the carcinogenic potential linked to the SDH inhibition (Benit *et al*., 2018). ANSES set up an emergency expert group to analyse the alert issued, and to identify whether immediate actions or additional risk management measures for the active substances and related products containing SDHI active substances should be taken.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Sedaxane has no physical properties warranting classification under CLP. It is not flammable, explosive or oxidising.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC supports the DS's proposal for **no classification of sedaxane regarding physical hazards**.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity oral route

Sedaxane was tested for acute oral toxicity in female HanRcc:WIST rats according to Up and Down Procedure (OECD TG 425, GLP) after an initial limit test with 5000 mg/kg bw by gavage in one female. The main test was conducted with 1 animal at 175 mg/kg bw, 1 animal at 550 mg/kg bw, 4 animals at 1750 mg/kg bw and 6 animals at 5000 mg/kg bw. The LD_{50} after single oral administration to female rats was estimated to be 5000 mg/kg bw with an approximately 95% profile likelihood confidence interval of 2513 to 9210 mg/kg bw. Since the acute oral LD_{50} was > 2000 mg/kg bw no classification was proposed by the DS for acute oral toxicity.

Acute toxicity dermal route

Sedaxane was tested for acute dermal toxicity (semi-occlusive) in HanRcc:WIST rats (5/sex) according to OECD TG 403 (GLP) at a dose level of 5000 mg/kg bw. The LD_{50} was > 5000 mg/kg bw. Since the acute dermal LD_{50} was > 2000 mg/kg bw no classification was proposed for acute dermal toxicity.

Acute toxicity inhalation route

Aerosolised Sedaxane was tested for acute inhalation toxicity (nose-only) in HanRcc:WIST rats (5/sex) according to OECD TG 402 (semi-occlusive, GLP) with 5.244 mg/L mean gravimetric exposure concentration for 4 hours. Gravimetric measurements of particle size distribution yielded MMAD of 3.02 and 2.97 µm and standard deviations of 2.84 and 2.87. Since the acute inhalation LC_{50} of aerosolised sedaxane of 5.244 mg/L was > 5 mg/L for dust/mists no classification was proposed by the DS for acute inhalation toxicity.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

As described above, three guideline acute toxicity studies investigating the effects of a single dose of sedaxane via oral, dermal and inhalation routes are available. In addition there is a guideline acute oral (gavage) neurotoxicity test in HanRcc:WIST rats (10/sex/dose) according to OECD TG 424 (GLP) with gavage dosing of 0, 30, 250, and 2000 mg/kg bw available.

Table: *Overview of LD50/LC50 values or mortalities in acute toxicity studies with sedaxane.*

	Acute oral	**Acute dermal**	**Acute inhalation**
Rat	4/7 females dosed 5000 mg/kg bw killed in extremis day 1 and 1/7 females died day 2; no death (0/4) at 1750 mg/kg bw; Estimated LD_{50} of 5000 mg/kg bw	No deaths; LD_{50} > 5000 mg/kg bw males and females	No deaths, LC_{50} > 5.244 mg/L males and females

	Acute oral	Acute dermal	Acute inhalation
	(95% PL confidence interval = 2513-9210 mg/kg bw females)		
Rat neurotoxicity	4/10 males and 3/10 females dosed 2000 mg/kg bw killed in extremis day 1		
Criteria Category 4	300-2000 mg/kg bw	1000-2000 mg/kg bw	1-5 mg/L (dusts and mists, 4 h)
	Not fulfilled	Not fulfilled	Not fulfilled

Taking into account the data on acute toxicity by the oral, dermal, and inhalation routes, and the acute neurotoxicity by oral route, and with reference to the numeric criteria of Annex I, 3.1.2.1, table 3.1.1 of CLP, RAC is of the opinion that sedaxane does not meet the criteria for classification for acute toxicity via the oral, dermal and inhalation route and **no classification is proposed for acute toxicity**.

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

Summary of the Dossier Submitter's proposal

In the standard single dose oral and dermal acute toxicity studies there was no evidence of specific target organ toxicity. In the acute inhalation toxicity study, minimal clinical signs consistent with aerosol inhalation were observed at the limit concentration of 5.244 mg/L (bradypnea 3 h from the start of exposure, rales as of 4 h) which fully recovered by day 3. There were no macroscopic findings or weight changes of the lungs at necropsy.

Sedaxane was tested in an acute neurotoxicity study in HanRcc:WIST rats (10/sex/dose) according to OECD 424 (GLP) at dose levels of 0, 30, 250, and 2000 mg/kg bw by gavage. Transient clinical signs of generalised toxicity were noted at non-lethal doses and the treatment did not produce any evidence of neurotoxicity, effects on brain weights, and there were no treatment-related neurohistopathological findings.

The dossier submitter concluded that there is no evidence from single or repeated dose studies (including acute neurotoxicity studies) of any clinical signs or other adverse effects indicative of specific target organ toxicity following single exposures to sedaxane at non-lethal doses meeting the classification criteria for specific target-organ toxicity category 1, 2 or 3. Therefore, no classification was proposed for STOT SE.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

In the standard single dose studies, oral and dermal routes, there was no evidence of specific target organ toxicity. At non-lethal oral doses clinical observations included ruffled fur, hunched

posture, slight sedation, poor coordination and ventral recumbency, resolving symptom free after few days. No clinical signs of systemic toxicity were noted after dermal dosing. After acute inhalation exposure of the limit dose of 5.244 mg/L there were no macroscopic pathology findings. Transient clinical signs comprised effects on breathing (bradypnea and rales), decreased spontaneous activity, hunched posture and ruffled fur, and transient, slight retardation in bodyweight gain or marginal to moderate bodyweight loss.

In the acute neurotoxicity study only slight clinical signs of general toxicity were observed. Treatment-related findings were noted at 250 and 2000 mg/kg bw, and included reduced activity, decreased rearing and a decreased body weight in males and females at 2000 mg/kg bw and a lower body weight gain in males at 250 and 2000 mg/kg bw, and a decreased food consumption in males and females at 250 and 2000 mg/kg bw. These findings were transient as there was no evidence of treatment related findings subsequent to day 8 of the study. Clinical signs observed in the FOB at 2000 mg/kg bw on day 1 seem to reflect the actual clinical condition of the animals. The treatment did not produce any evidence of neurotoxicity, effects on brain weights, and there were no treatment-related neurohistopathological findings.

Classification for STOT SE category 3 (respiratory tract irritation and narcotic effects) is primarily based on human data, if available, animal data can be included in the evaluation. No human data are available. Taking into account available acute toxicity (inhalation) animal data as described above, RAC concludes that the criteria for classifying in STOT SE 3 for transient target organ effects as provided in Annex I, 3.8.2.2.1 and 3.8.2.2.2 of CLP are not met.

According to the criteria, STOT SE Categories 1 and 2 are assigned on the basis of findings of "significant" or "severe" toxicity. "Significant" means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. "Severe" effects are generally more profound and serious and of considerable adverse nature with significant impact on health. Based on available animal studies as outlined above and the classification criteria according Annex I, 3.8.2.1. and applicable guidance value ranges for single exposure provided in table 3.8.2 of CLP, there is no evidence for signs of specific non-lethal target organ toxicity meeting the classification criteria. **RAC concludes that classification of sedaxane for STOT-SE is not warranted.**

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

Sedaxane was tested for acute skin irritation in New Zealand White rabbits (3/group) according to OECD 404 (GLP) with 0.5 g topically applied for 4 hours semi-occlusive. There were no signs of skin irritation or corrosion and the DS concluded that classification was not applicable.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

In a guideline compliant *in vivo* skin irritation study according to OECD 404 in NZW rabbits no signs of irritation and corrosive effects were noted on the treated skin of any animal at any of the measuring intervals (1, 24, 48, 72 h) and no clinical signs were observed. The mean score for erythema and oedema was zero at all the time points. The primary irritation index was

calculated by totalling the mean cumulative scores at 24, 48 and 72 hours and then dividing by the number of data points. The primary irritation index was 0.00. No evidence for skin corrosion/irritation in humans is available. Taking into account the above animal data with reference to the criteria of Annex I, 3.2.2.6 of CLP, RAC is of the opinion that sedaxane does not meet the criteria and **no classification is proposed for skin corrosion/irritation**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

Sedaxane was tested for acute eye irritation in New Zealand White rabbits (3/group) according to OECD 405 (GLP) with 0.1 g single exposure to the rabbit eye. The instillation into the eye resulted in mild, early-onset and transient ocular changes, reversible within 72 hours after treatment. No classification was proposed by the dossier submitter.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

In a guideline compliant *in vivo* eye irritation study according to OECD 405 in NZW rabbits, only mild, early-onset and transient ocular changes reversible within 72 hours were recorded. No abnormal findings were observed in the treated eye of any animal 72 hours after treatment. The individual mean scores for all three animals were:

- corneal opacity and iris effects: 0, 0, 0,
- conjunctivae redness: 0.33, 0.67, 0.33,
- conjunctival chemosis: 0, 0, 0.

Taking into account the above animal data with reference to the criteria of Annex I, 3.3.2.6., table 3.3.1 and 3.3.2 of CLP, RAC is of the opinion that sedaxane does not meet the criteria and **no classification is proposed for eye damage/irritation.**

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

No specific animal or human data was available on respiratory sensitisation. There was no evidence of respiratory irritation or indication of sensitisation observed in the single dose inhalation toxicity study. There is no reported evidence of respiratory sensitisation in humans available. The dossier submitter did not propose a classification of sedaxane as respiratory sensitiser.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

No data are available in both human and animals. In agreement with the dossier submitter, **RAC does not propose to classify sedaxane as a respiratory sensitiser due to lack of data**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The dossier does not contain any human data. Sedaxane was tested for skin sensitisation in the Local Lymph Node Assay (LLNA) in female CBA/Ca mice (5/group) according to OECD TG 429 (GLP) with 0, 10, 25 and 50% concentrations applied topically to the ear of mice in each group for 3 consecutive days. Based on the analysed level of T-lymphocyte proliferation in the lymph nodes draining the site of chemical application, the test material was considered to be a non-sensitiser under the conditions of the test. No classification was proposed by the dossier submitter.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

In a LLNA performed on CBA/Ca mice, sedaxane was negative with 10, 25 and 50% w/w in acetone/olive oil 4:1 resulting in an increase in isotope incorporation of less than 3-fold at all concentrations (stimulation index: 10% - 1.12; 25% - 0.96; 50% - 0.71). The validity of the protocol used was confirmed with a concurrent positive control (hexylcinnamaldehyde). Consequently, the test substance was not considered a skin sensitiser under the conditions of the study.

Taking into account the negative LLNA result with reference to the criteria of Annex I, 3.4.2.2. of CLP, **RAC does not propose classification for skin sensitisation.**

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

Summary of the Dossier Submitter's proposal

The toxicity of sedaxane following repeated exposure has been evaluated by the oral route of administration in rats, dogs and mice, including lifetime studies in rats and mice. In addition, dermal toxicity was evaluated in rats in a 28-day study (no adverse effects were seen). Sedaxane is generally of a low order of toxicity in all species tested in repeat dose studies. The main evidence of systemic toxicity in all species was on body weight and food consumption. The most consistent effect in all species was seen in the liver (with most marked effects seen in the rat), indicating that this is a target organ for sedaxane.

There was some evidence for effects in the thyroid (rats only). In the carcinogenicity study in rats (Anonymous, 2010) at the 52-week interim kill, minimal-mild follicular cell hypertrophy was seen at the highest dose (3600 ppm ~ 240 mg/kg bw/d).

On basis of the mode of action, the DS concluded the only target organ at doses below the guidance cut-off value for category 2 was the liver. Based on the nature of the effects and the doses at which these occurred, liver effects did not warrant classification.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

In accordance with the guidance on the application of the CLP criteria, the effects for justifying classification for STOT RE were not features of exposure to sedaxane at doses below or equal to the guidance cut-off values for category 2. The only target organ at such doses was the liver with changes not sufficiently severe or reproducible to warrant classification. The observations related to the 28 days study (rats) where increased absolute and relative liver weights and hepatic enzyme CYP450 induction; CYP2B and CYP3A, increased cholesterol and triglycerides. In the rabbit developmental toxicity study increased liver weight was observed in the 200 mg/kg bw/d dose group.

Rat

In a 28 day oral study in rats (Anonymous, 2010), statistically significant increased liver weight was seen at the high and mid doses (5000 ppm (~ 437 mg/kg bw/d) and 2000 ppm (~ 180 mg/kg bw/d) respectively). Histopathologically, hepatocellular centrilobular hypertrophy was seen, together with hepatic enzyme induction (specifically CYP2B and CYP3A) as evidenced by immunoblotting and increased PROD activity. This was further confirmed in subsequent *in vitro* studies (Anonymous, 2016, Vardy, 2016) and investigative studies on stored tissues from longer-term studies (Anonymous, 2013).

Similar increases in liver weight and histopathological findings were seen in rats following 90 days exposure (Anonymous, 2009) at dietary inclusion levels of 325 and 168 mg/kg bw/d, and following exposure for up to 2 years (Anonymous, 2010) at 218/261 mg/kg bw/d. Increased liver weight and centrilobular hypertrophy were also seen in the two-generation reproductive study in rats at the highest dose of 120 mg/kg bw/d (Anonymous, 2010).

Dermal rat

Dermal administration of sedaxane up to the limit dose of 1000 mg/kg bw/d for 28 days did not result in any adverse local or systemic effects in rats.

Mouse

In mice, treatment for 90 days resulted in increased liver weight only at the highest dose tested at 7000 ppm (~ 1300 mg/kg bw/d) (Anonymous, 2008) but there were no accompanying histopathological changes.

Dog

In dogs, liver weights were higher than control values in male and female dogs after one year at 200 mg/kg bw/day (Anonymous, 2009) in the absence of associated histopathological findings. In the 90-day dog study (Anonymous, 2008) there were no effects on liver weights and no histopathology findings in the liver in doses up to 400 mg/kg bw/d.

Rabbit

In the developmental toxicity study in rabbits (Anonymous, 2010) increased liver weight was also seen at 200 mg/kg bw/d.

Mode of action studies showed that the liver and thyroid effects in rats were secondary to the activation of the CAR/PXR nuclear receptors in the liver and are less relevant to humans. This will be discussed in detail in the carcinogenicity section.

Table: *Summary of studies relevant for STOT RE assessment and the guidance values*

Study	**Cut off values Cat. 1/2**	**Effects at doses below guidance cut-off values**
28-d rat study Anonymous, 2010 Annex I. 3.12.1.1	30/300	Category 1: Lowest dose = 45.9/47.6 mg/kg bw/d Category 2: At 45.9/47.6 mg/kg bw/d : No adverse effects At 182.7 and 179.6 mg/kg bw/d: Body weights: slightly ↓(7%) in males Liver effects: ↑absolute and relative weights , ↑ cholesterol and triglycerides, Pentoxyresorufin (PROD) markedly ↑, slight ↑ EROD activity, ↑CYP 2B and CYP 3A
90-d rat study Anonymous, 2009 Annex I. 3.12.1.2	10 / 100	Category 1: Lowest dose = 24.8 mg/kg bw/d Category 2: No adverse effects at 24.8 mg/kg bw/d
90-d neurotoxicity study Anonymous, 2009b Annex I. 3.12.1.3	10 / 100	Category 1: Lowest dose = 19.7/24.3 mg/kg bw/d Category 2: No adverse effects at 19.7/24.3 mg/kg bw/d and 66/79.7 mg/kg bw/d
90-d mouse study Anonymous, 2008 Annex I. 3.12.1.5	10 / 100	Category 1: Lowest dose = 80/112 mg/kg bw/d Category 2: No adverse effects at 80/112 mg/kg bw/d
90-d dog study Anonymous, 2008 Annex I. 3.12.1.6	10 / 100	Category 1: Lowest dose = 50 mg/kg bw/d Category 2: No adverse effects at 50 mg/kg bw/d
1-year dog study Anonymous, 2009 Annex I. 3.12.1.7	2.5 / 25	Category 1: Lowest dose = 15 mg/kg bw/d Category 2: No adverse effects at 15 mg/kg bw/d and 50 mg/kg bw/d
2-year rat study Anonymous, 2010 Annex I. 3.9.1.1	1.25 / 12.5	Category 1: Lowest dose = 11/14 mg/kg bw/d Category 2: No adverse effects at 11/14 mg/kg bw/d
18-month mouse study Anonymous, 2010	1.7 / 17	Category 1: Lowest dose = 25 mg/kg bw/d Category 2: Lowest dose = 25 mg/kg bw/d

Study	Cut off values Cat. 1/2	Effects at doses below guidance cut-off values
Annex I. 3.9.1.2		
2-generation study Anonymous (2010) Annex I. 3.10.1.1	10/100	Category 1: Lowest dose = 16 mg/kg bw/d Category 2: No adverse effects at 16 mg/kg bw/d and 41 mg/kg bw/d
Rat developmental study Anonymous (2009) Annex I. 3.10.1.2	60/600	Category 1: No adverse effects at 25 mg/kg bw/d Category 2: At 100 mg/kg bw/d and 200 mg/kg bw/d Dams: ↓ body gain and food consumption, Foetuses: no adverse effects
Rabbit developmental study Anonymous, (2010) Annex I. 3.10.1.3	45/450	Category 1: No adverse effects at 25 mg/kg bw/d Category 2: No adverse effects at 100 mg/kg bw/d At 200 mg/kg bw/d Dams: ↓ body gain and food consumption, ↓ defaecation ↑ liver weight Foetuses: ↓ foetal body weight, increased incidence of 13th full rib(s).

RAC concludes, in accordance with the proposal by the dossier submitter that **no classification is justified for STOT RE.**

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Sedaxane has been tested in a series of *in vitro* and *in vivo* genotoxicity assays. *In vitro*, the substance was negative for gene mutations in bacteria (Ames test) and in mammalian cells (L5178Y TK+/- mouse lymphoma), as well as for chromosomal aberrations in human primary lymphocyte cultures. *In vivo,* up to the limit dose, sedaxane was not clastogenic in the mouse bone marrow micronucleus assay, and in two rat liver UDS assays the compound gave equivocal results in one and negative results in the other study. The DS did not propose classification for germ cell mutagenicity.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

In an OECD TG 471 and GLP -compliant Reverse Mutation Test using bacteria, sedaxane was tested in the *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 or in the *Escherichia coli* strains WP2 uvrA/pKM101 and WP2 uvrA/pKM101. No increase in the number of revertant colonies were observed in any of the six tested strains following treatment with

sedaxane at dose levels up to the recommended maximum test concentration of 5 mg/plate in two independent experiments each in the presence or absence of metabolic activation (S9 mix). The validity of the protocol used was confirmed with a concurrent positive control. Sedaxane was also negative for mammalian gene mutations in the *in vitro* mouse lymphoma assay according to OECD TG 476 and GLP. No mutations were observed at the thymidine kinase locus using the cell line L5178Y in two independent experiments up to cytotoxic concentration range, each in the presence and absence of S9 metabolic activation system. The validity of the protocol used was confirmed with a concurrent positive control. In an OECD TG 473 and GLP -compliant Chromosome Aberration Test in Human Lymphocytes, sedaxane was negative for cytogenicity. No increase in structural chromosomal aberrations was observed in two independent experiments up to cytotoxic concentrations (50% reduction in mitotic index at the highest concentrations), each in the presence and absence of an exogenous metabolic activation system S9 mix. The validity of the protocol used was confirmed with a concurrent positive control.

In vivo, sedaxane was tested in the mammalian erythrocyte micronucleus assay in NMRI mice according to OECD TG 474, and GLP compliant. No increase in micronuclei was detected in mice treated with a single dose of sedaxane up to the recommended limit dose for 24 hours (500, 1000, 2000 mg/kg bw) and for 48 hours (2000 mg/kg bw). No toxicity to the bone marrow was evident based on the unchanged PCE ratio, thus it was not demonstrated that the target organ was reached. It is noted that the available toxicokinetic studies suggest bone marrow exposure, as there was a rapid oral absorption (at least 87% of the administered dose) and a wide distribution throughout the body (peak plasma concentrations at 1-5 hours for low and high dose level) with extensive metabolism via demethylation, hydroxylation, oxidation and conjugation. Rapid and extensive elimination was detected with the majority of the administered dose (>85%) excreted within 48 hours mainly via faeces. Also, the validity of the micronucleus assay protocol used was confirmed with a concurrent positive control. Thus, since the substance was tested at the recommended limit dose and no cytogenicity is expected based on the *in vitro* results, the result is considered acceptable and suggested that sedaxane is not genotoxic *in vivo* in the micronucleus assay. In addition, there are two available Unscheduled DNA Synthesis (UDS) tests with mammalian liver cells *in vivo* in rats according to OECD TG 486 and GLP compliant. The first test in Sprague Dawley rats was equivocal with all the parameters assessing mutagenicity (NNGC, N-C and % in repair) increased at the limit dose of 2000 mg/kg bw at the 16 hour harvest time point, exceeding the Historical Control Data (HCD) but not reaching statistical significance. The second study in Wistar rats was negative for DNA repair up to the limit dose of 2000 mg/kg bw. The UDS assay is an indicator test only indirectly showing DNA lesions and there is not sufficient information to conclude on the induction of gene mutation by the substance. No germ cell data are available.

RAC concludes that sedaxane was negative for *in vitro* genotoxicity and is unlikely to be genotoxic *in vivo.* Thus the classification criteria of Annex I, 3.5.2 of CLP are not met, which would require a positive evidence obtained from *in vivo* somatic cell mutagenicity, or positive results from other *in vivo* genotoxicity assays supported by *in vitro* mutagenicity results, in order to classify in category 2. Therefore, RAC agrees with the DS that **classification for germ cell mutagenicity is not warranted**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS proposed to classify sedaxane as Carc. 2. Oral administration of sedaxane to rats and mice for two years resulted in increased incidences of three types of tumours: malignant uterine

adenocarcinoma, benign thyroid tumours and hepatocellular adenoma in rats, and hepatocellular adenomas and adenomas/carcinomas (combined) were reported in mice. The tumour findings indicated that sedaxane has a carcinogenic potential. Additional factors were also taken into account by the DS when assessing the overall level of concern and for making the decision on the category of classification.

Two carcinogenicity studies were included in the CLH report for sedaxane, one 2-year chronic/carcinogenicity study according to OECD TG 453 in rats and one OECD TG 451 -compliant study in mice. No classification with regard to carcinogenicity was proposed in the Conclusion on the peer review of the pesticide risk assessment of the active substance sedaxane (EFSA, 2012). In 2011, US-EPA classified sedaxane as "Likely to be Carcinogenic to Humans" based on the presence of the three tumour types at multiple sites in two species observed in these two studies. Following a request from the European Commission to re-consider the toxicological assessment and confirm the conclusions on sedaxane, carcinogenicity was re-discussed at the Pesticides Peer Review Meeting 98 in November 2012 and it was concluded that the overall pattern of tumours in rats and mice suggests that classification of sedaxane as Carc. 2, H351 would be required (EFSA, 2013). Following this, the pesticide applicant has generated numerous mechanistic studies for the assessment of modes of action for liver, thyroid, and uterine tumours. The putative MoAs and their human relevance were assessed using the framework developed by the International Programme on Chemical Safety (IPCS) and the International Life Science Institute (ILSI).

In the rat study in the high dose of 3600 ppm (218/261 mg/kg bw/d males/females), a statistically significantly increased incidence in malignant uterine adenocarcinomas was observed. This incidence was within the historical control data range from the laboratory, however, the incidence was far above the HCD mean and above the incidence of nine out of ten historical controls. At this dose, there was also an increased incidence of hepatocellular adenomas and thyroid follicular cell adenomas in the males. The tumour incidences were accompanied by a marked effect on body weight in males and females, which by the end of study represented a 23.5% and 49.6% decrease in body weight gain in males and females, respectively. There was no other evidence of toxicity since the survival rate was not affected and there was no difference in clinical observations between the groups. The DS considered the effects at high dose relevant for assessing the carcinogenic potential of sedaxane.

In the mouse study, statistically significantly higher incidences of hepatocellular adenomas and adenomas/carcinomas (combined) were reported. These incidences were slightly above the historical control range of the laboratory.

The Pesticide applicant had performed a range of non-guideline MoA studies for sedaxane-induced hepatocellular adenoma/carcinoma and thyroid follicular cell adenomas, these included:

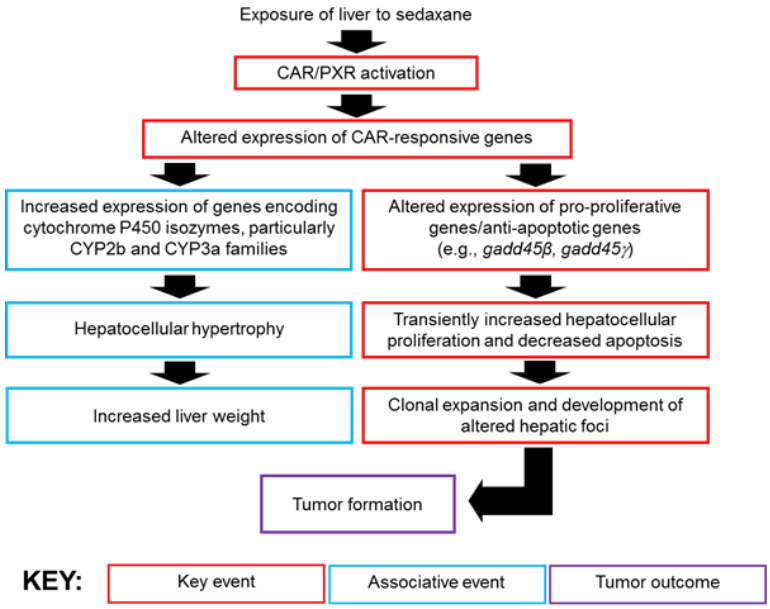
- Dose-range finding study in mice to investigate liver tumour mode of action,
- A mouse study to assess liver pathology (weight, Ki67, BrdU, mRNA levels, biochemical analysis, toxicogenomics),
- A rat study to evaluate effects of sedaxane on the liver and thyroid,
- A rat hepatocyte culture study to assess effects on hepatocellular proliferation (measured as the change in replicative DNA synthesis [S-phase of the cell cycle]) and cytochrome P450 enzyme activities),
- A study on cultured male human hepatocytes to assess effects on hepatocellular proliferation (measured as the change in replicative DNA synthesis [S-phase of the cell cycle]) and cytochrome P450 enzyme activities,
- A mouse study to analyse liver samples from 28- and 90-day dietary studies with sedaxane for protein and cytochrome P450 content and selected enzyme activities,
- CAR3 Transactivation assay with mouse, rat and human CAR,

- Human PXR assay on agonist activity directed against human, rat and mouse PXR,
- *In vitro* study on effect on rat thyroid peroxidase activity.

Based on the results of the MoA studies, the applicant had proposed the following mode of action for the observed liver tumours in rats and mice that were considered not relevant to humans:

Sedaxane treatment resulted in the activation of the Constitutive Androstane Receptor (CAR) and/or Pregnane X Receptor (PXR) in the liver. This resulted in the altered expression of CAR-responsive genes that promoted a pro-proliferative and anti-apoptotic environment in the liver and an early transient increase in hepatocellular proliferation. Increased hepatocellular foci as a result of clonal expansion of spontaneously mutated cells in the mouse and rat resulted in slight increases in liver tumour incidences compared to concurrent controls. This MoA was supported by a series of associative events including: increased expression of genes encoding cytochrome P450s, increased microsomal (endoplasmic reticulum) proliferation and hepatocellular hypertrophy and increased liver weight. The MoA hypothesis as postulated by the pesticide applicant is represented below, with the identified causal key events and associative events:

Figure: *Proposed MoA for liver tumours in rats and mice*

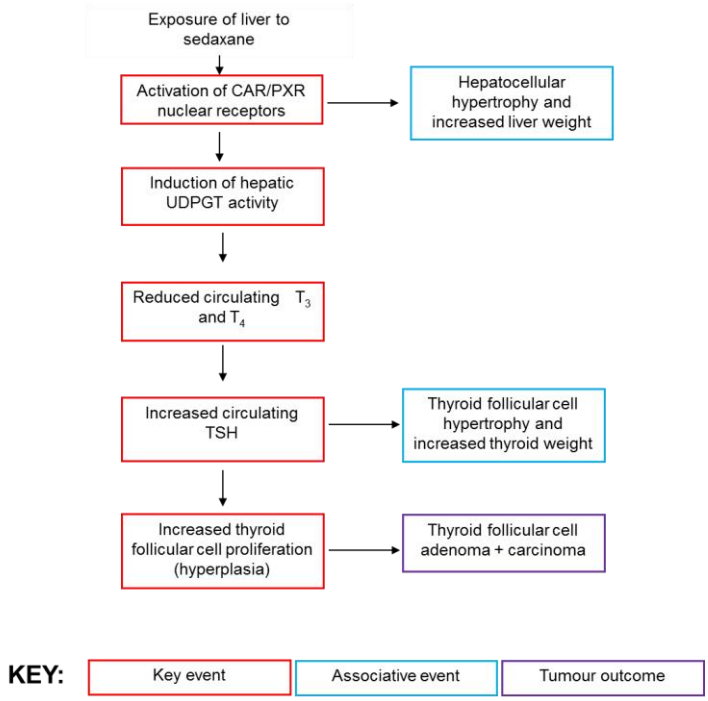


The DS concluded that the available data provided enough evidence to support the postulated MoA (CAR activation) for the liver tumours observed in rodent males. Similar to phenobarbital (known CAR inducer), sedaxane did not induce DNA replication (prerequisite for tumour formation) in human hepatocytes following induction of human CAR, in contrast to rat. Due to this qualitative difference, the liver tumours as a result of CAR-activation by sedaxane were considered to be of little relevance to humans.

The applicant had proposed the following MoA for the observed thyroid tumours in rats that were considered not relevant to humans:

The activation of the CAR/PXR nuclear receptors by sedaxane led to an induction of hepatic UDP-glucuronosyltransferase (UDPGT), resulting in an increased conjugation and excretion of triiodothyronine (T3) and thyroxine (T4) and in a decrease in serum T3 and T4 levels. A compensatory increase in thyroid stimulating hormone (TSH) levels secreted via the hypothalamus-pituitary-thyroid (HPT) axis resulted in a chronic proliferative stimulus of thyroid follicular cells by TSH prompting hypertrophy and hyperplasia that eventually progressed to form follicular cell adenomas and/or carcinomas. This MoA hypothesis is represented below, with the identified causal key events and associative events:

Figure: *Proposed MoA for thyroid tumours in rats*



The DS concluded that the available data provided plausible evidence to support the postulated MoA (CAR-mediated induction of hepatic UGT activity for the slightly increased incidence of thyroid adenomas observed in high dose male rats. The increased activity of hepatic UDPG-transferase resulted in an increased clearance of thyroid hormone levels (T4), resulting in thyroid stimulation. Such a mechanism/effect cannot be directly extrapolated to humans due to a T4 binding protein that greatly reduces susceptibility to plasma T4 depletion. The thyroid effects observed in rats were therefore considered of insufficient concern for classification (with a reference to the ECHA guidance on the application of the CLP Criteria).

The applicant had performed also a range of non-guideline mechanistic studies and an OECD TG 440 study for sedaxane-induced uterine adenocarcinomas:

- *In vitro* dopamine D2S receptor binding assay (assessed by a displacement of [3^H]methyl-spiperone, a known binder of the dopamine receptor),
- Determination of the oestrous cycle stage based on the microscopic examination of the vagina, uterus, and ovary of female rats exposed to sedaxane,
- Visualisation and quantification of dopaminergic neurons in the TIDA region of the hypothalamus from control female Wistar rats of different ages (Tyrosine hydroxylase (TH), immunohistochemistry and RNAscope™ *in situ* hybridisation),
- Examination of brain samples for hypothalamic TH expression via immunohistochemistry and *in situ* hybridization on stored tissue from the 2-year rat study,
- Radio- or enzyme-immunoassay for prolactin, leptin and adiponectin using frozen 1-year (52-week) serum samples,
- Uterotrophic assay according to OECD TG 440 with gross examination of the uterus and recording of uterine weights.

Based on the results the applicant had proposed a mode of action for uterine adenocarcinomas:

Sedaxane treatment induced a large and sustained reduction in body weight gain which was suggested by the applicant to lead to lower amounts of adipose tissue and consequently to lower blood levels of leptin. Reductions in body weight gain and in the following adipose tissue throughout the animals' lifetime were suggested to cause a delay in the normal age-related loss

of the tuberoinfundibular dopaminergic (TIDA) neurons of the hypothalamus. The suggested retention of a greater number of functional TIDA neurons in aging sedaxane-treated rats would result in continued production of dopamine, which would suppress prolactin release from the anterior pituitary. The delayed and/or diminished prolactin drive in the mammary gland was considered to explain the lower incidence of mammary gland fibroadenomas. The suppressed circulating prolactin levels in blood resulted in a delay in reproductive senescence as the oestrous cycles were more regular in the aging sedaxane-treated rats as compared to the aging control rats. Thereby the cumulative exposure of the uterus was higher to oestrogen and lower to progesterone in the aged sedaxane-treated females as compared to the controls, which in turn lead to a pro-proliferative estrogenic stimulation of the uterine endometrial cells of the sedaxane-treated rats. Over the time, the estrogenic proliferative drive on the uterus was considered to lead to an increased spontaneous incidence of uterine adenocarcinomas.

The DS however considered the experimental data did not provide sufficient evidence to support the postulated mode of action of rat uterine tumours induced by sedaxane, due to several deficiencies identified:

- *Key event 1:* A significant treatment-related decrease in body weight gain was supported by the experimental data (50% lower in high dose females as compared to controls at the end of the 2-year rat study). However, since adipose tissue was not measured in the 2-year rat study, there was no evidence for a sedaxane induced decrease. Furthermore, decreased body weight gain was considered not to be a specific initiating event for uterine tumours - such tumours were not systematically observed as a result of exposure to substances at high doses even if significant reductions in body weight gain were observed.
- *Associated event 1*: The statistically non-significant decrease in the mean leptin values observed in the high dose female rats sacrificed at 1 year may indicate a decrease in adipose tissue. However, this was not supported by the mean values for adiponectin, which were not affected by the treatment.
- *Key event 2*: In the 2-year brain samples, both protein and mRNA staining supported an increased TH expression in the TIDA region of the high dose group at 2 years. However according to the DS, it did not automatically mean an age-related decrease in dopaminergic signalling.
- *Key event 3*: There was no direct experimental data to support a "suppression of age-related increase in prolactin" (i.e.: decreased prolactin level) and the only measurements performed at 1-year time point did not show any treatment effect.
- *Associated events 2 and 3*: the DS considered that experimental data supported associative event 3 and to a lesser extent associative event 2. There was a tendency (not statistically significant) for decreased pituitary adenomas and a statistically significant decrease in the incidence of mammary fibroadenomas in the high dose rat females.
- *Key event 4*: The blinded histopathology re-evaluation of the vagina, ovaries and uterus from existing histology slides from a 90-day rat study and the 2-year rat study did not support key event 4, as no differences in oestrous cyclicity measurements were observed in young animals (i.e., from 13 weeks up through the 52-week sacrifice). At 2-year time point, a similar high rate of senescence (repetitive pseudopregnancy or persistent anoestrus) was recorded in all groups.
- *Associated event 4*: According to the blinded histopathology re-evaluation of the vaginas from existing histology slides from the 2-year rat study, the incidence of vaginal mucification was only slightly lower in 3600 ppm group compared to control group (21/51 vs 29/50).
- *Key event 5*: From the experimental data there was no supportive evidence of increased total number of oestrous cycles and proliferation. There might have been differences in oestrous cycles between 1 year and 2 years, but this allegation was not substantiated by experimental data and the putative higher oestrogen:progesterone ratio has not been

objectified. Furthermore, no histopathological findings indicative of overt estrogenic stimulation (as squamous metaplasia or endometrial hyperplasia) was observed at 1-year or 2-year sacrifice.

In the absence of an established MoA that could be considered not relevant to humans, the DS considered classification for carcinogenicity warranted. Overall, the DS considered classification as Carc. 2; H351 warranted, since the uterine tumours were limited to a very high dose level in a single species.

Comments received during public consultation

Five Member States Competent Authorities (MSCA) provided comments to carcinogenicity classification proposal.

Three MSCA supported the proposed classification as Carc. 2:

One MSCA considered the MoA for liver tumours (via the CAR/PXR pathway) and thyroid tumours (CAR-mediated hepatic UGT activation) sufficiently supported by the mechanistic data, but concluded that the human relevance of the uterine tumours observed cannot be excluded based on the uncertainty involved in the MoA. Therefore, the MSCA shared the opinion of the dossier submitter that classification of sedaxane as Carc. 2 is warranted based on the uterine tumours observed in female rats.

Another MSCA supported the classification as Carc. 2 based on a significant increase in incidences of uterine adenocarcinomas in female rats, liver adenomas in male rats, liver adenomas in male mice and liver carcinomas in male mice, and raised several issues to be critically discussed, in particular:

- The definition of "sufficient" evidence was partially met (CLP Annex I, 3.6.2.2.3), due to the 2-fold increase in liver carcinomas in mice over the concurrent control and HCD supported with the occurrence of liver adenomas in two species, mice and rats accordingly,
- Observed tumour may also occur in humans (uterus, liver, thyroid),
- Incidences of observed tumours are outside the HCD including follicular adenoma in male rats, liver adenoma in male rats, liver adenoma in male mice, liver carcinoma in male mice,
- The tumours are not spontaneous tumour types (liver tumours ware observed in Crl:CD-1(ICR), but not in B6C3F1 mice),
- Multiple site response in male rats was observed,
- Uterine tumours in female rats and liver tumours in male mice progressed to malignancy,
- The postulated MoA for uterine tumours, in support of the position of DS, the submitted experimental data were inconclusive to substantiate the postulated MoA (for further details see RCOM).

The third MSCA concurring with the DS' conclusion provided further considerations in relation to the mechanistic data for uterine tumours, i.e. further uncertainties have been highlighted such as the lack of experimental evidence for decrease in adipose tissue or the role of leptin levels in dopaminergic signalling, also the fact that a role and causality for prolactin levels in uterine adenocarcinoma has not been proven.

Two MSCA considered classification for carcinogenicity not warranted:

In contrast to the DS, one MSCA considered that the increased incidence of uterine carcinoma in female rats provide weak and inconsistent evidence not sufficient to warrant carcinogenicity classification, because most importantly, the slightly statistically significant increased incidence was within the range of HCD from the test laboratory during the period (2002-2012) and uterine adenocarcinoma is a common finding in aging Wistar rats.

The other MSCA also highlighted that the incidence (17%) was within the range of historical control data (0-19%) and that RITA Wistar rat data (0-28%) can be used as evidence of high rate of spontaneous tumours. Moreover, a significant body weight decrease (50%) in animals at the top dose interferes with the interpretation of the study.

Industry provided also comments, the pesticide applicant and an industry trade association:

The trade association did not agree with the DS' assessment on the uterine tumours because the overall weight of evidence demonstrated that the observed uterine tumours are not relevant to human due to fundamental differences in physiological control of reproductive senescence between humans and rats. It was considered that the key events for the proposed MoA are well-described in the scientific literature, and the shift in tumour incidence was dependent on a marked and sustained deficit in body weight gain occurring in the female rats. The different tumour outcomes observed at 1200 ppm and 3600 ppm sedaxane indicated that the observed dose-response for the decrement in body weight gain translates into a dose response and threshold for the consequential shift in tumour incidence.

The pesticide applicant highlighted the non-relevance of the uterine tumours in rats being based on the fundamental physiological differences between humans and rats with regard to reproductive senescence as well as the role of prolactin during reproductive cycles. The uterine tumours observed in the 2-year carcinogenicity study at the high dose as a consequence of an increased duration of a persistent oestrous state would not be observed in humans (for further details see BD). To complete the overall assessment as presented in the CLH report and to address the data gaps noted by the DS, the applicant has recently completed additional investigations into the proposed MoA for the observed shift in tumour profile in rats treated with a structurally related SDHI, isopyrazam. According to the CLH dossier, isopyrazam showed a similar uterine tumour profile in the 2-year carcinogenicity as sedaxane (i.e. increased uterine tumours with a concomitant decrease in mammary gland fibroadenomas and pituitary adenomas). The applicant considered the new isopyrazam data provide convincing evidence supporting the MoA for sedaxane. The additional data submitted consisted of the following:

- 18-month Investigative Dietary Study in the Female Han Wistar Rat on the structural analogue isopyrazam and one of its metabolites,
- OECD summary of the 18-Month Investigative Dietary Study in the Female Han Wistar Rat on isopyrazam,
- Detailed weight of evidence document describing the MoA and human non-relevance of uterine tumours,
- Short summary addressing the data gaps identified in the MoA by the DS in the CLH report.

In the view of the applicant, the new data confirmed the proposed MoA in rats and the overall database demonstrated that the observed shift in tumour profile, including the higher incidence of uterine tumours, has no relevance to human health. The applicant had slightly changed the initially proposed MoA (e.g.: initial key event: decreased food utilisation versus decreased bodyweight):

The DS considered the newly submitted mechanistic study with isopyrazam and acknowledged that the data substantiated some key events not previously observed in the data package with sedaxane (e.g.: decreased adipose tissue and statistical decreased plasma leptin and prolactin). The proposed initial key event however was still considered a broad event. It was further raised by the applicant that the described not typical pattern of response of sedaxane and isopyrazam with decreased food utilisation and body weight deficit sustained throughout the entire lifetime of the study, could be linked to their common fungicidal mode of action (SDH inhibitors) and inhibition of succinate dehydrogenase could be the molecular initiating event (however there is

no specific supporting data). The dossier submitter highlighted still a range of remaining uncertainties in the data package (for details please see BD).

In conclusion, the dossier submitter was still of the opinion that the experimental data do not provide sufficient evidence to support the postulated mode of action of rat uterine tumours induced by sedaxane. It was further pointed out that an alternative potential mode of action through SDH inhibition and accumulation of succinate (considered as oncometabolite) could not be ruled out in respect to the alert recently raised by researchers and clinicians from French institutes (Benit, 2018), who established that SDH inhibitors readily inhibit the earthworm and the human enzyme, thereby raising a new concern because the loss of function, partial or total, of SDH activity caused by genetic variants causes severe human neurological diseases, or leads to the development of tumours and/or cancers. The proposal for classification therefore was maintained.

Assessment and comparison with the classification criteria

Sedaxane was tested in two OECD guideline compliant chronic/carcinogenicity studies, one OECD TG 453 study in Crl:WI (Han) rats and one OECD TG 451 study in Crl:CD-1 (ICR) mice.

Rat

In rats, 0, 200 (11/14 mg/kg bw/d males/females), 1200 (67/86 mg/kg bw/d males/females) or 3600 (218/261 mg/kg bw/d males/females) ppm sedaxane was administered to groups of 52 rats per sex via the diet for at least 104 consecutive weeks. In addition, four smaller groups of 12 animals per sex were included and dosed for 52 weeks. There were no statistically significant differences in mortality between the control and any other groups for males and no treatment related effect for females for both the 104-weeks carcinogenicity groups and the 52-weeks toxicity dosing groups. There were no increases in clinical observations, which could be attributed to test substance treatment.

Males and females treated at the high dose of 3600 ppm showed a consistent and lower body weight and weight gain compared to their respective controls throughout the treatment period. The reduced cumulative body weight gain throughout the study in the high dose represented a maximum of 23.5% decrease in males and 49.6% decrease in females at termination (reduction of terminal body weight in females by 37%). Lower values for food consumption (for females throughout the study, for males week 1-7) and reduced food utilisation (reported for week 1-13 in the CLH report) were noted in males and females at 3600 ppm. At 1200 ppm, body weight and body weight gain were also decreased in females but not in males (consistently lower weight gain than control from week 66 to the end of the study, terminal weight reduced by 8%).

Dose related higher adjusted liver weights were observed in males and females of the mid and high dose (both in the 104-weeks and 52-weeks groups), correlating with micropathology findings of centrilobular hepatocyte hypertrophy and hepatocyte pigmentation. Also selected changes in clinical chemistry parameters were observed, involving higher total protein, albumin and globulin levels in males and females considered to be treatment related and indicating adaptive changes in the liver (see table 3.9.1.1-9 of Annex I to CLH report), further changes included higher gamma glutamyl transferase (GGT) for the high dose males, higher cholesterol levels in high dose females, higher phosphate levels in high dose males, high glucose levels in mid and high dose males, and higher prothrombin for high dose males.

In the thyroid, follicular cell hypertrophy was observed in both sexes at 52 weeks with higher incidences of follicular cell hyperplasia after the 104 weeks in the 3600 ppm males, colloid basophilia and desquamation of the follicular epithelium at 1200 and 3600 ppm for both sexes. The incidence of diffuse C-cell hyperplasia was decreased in both sexes receiving 3600 ppm.

The incidence of mucification of the vagina and mammary gland lobular hyperplasia were decreased in females receiving 3600 ppm compared to controls. A blinded histopathology re-evaluation of the vagina, ovaries and uterus has been performed and according to this re-evaluation, the incidence of vaginal mucification was only slightly lower in 3600 ppm group compared to control group (21/51 vs 29/50).

Non-neoplastic findings

Table: *Non-neoplastic findings in the 2-year carcinogenicity study of sedaxane in rats*

	Males				**Females**			
Sedaxane (ppm)	**0**	**200**	**1200**	**3600**	**0**	**200**	**1200**	**3600**
Mortality	43/52 (83%)	40/52 (77%)	43/52 (83%)	44/52 (85%)	44/52 (85%)	35/52 (67%)	37/52 (71%)	44/52 (85%)
Body weight gain								
0-1	49.1	50.7	45.4**	33.3**	23.2	22.6	20.5**	12.4**
0-3	112.1	116.8	109.2	83.0**	54.0	55.1	50.6	35.4**
0-13	232.5	247.9*	228.0	187.3**	106.1	108.1	102.2	72.9**
0-52	370.3	387.3	360.9	300.7**	163.5	165.6	151.1**	108.1**
0-104	464.9	509.7*	447.9	355.7**	262.6	259.2	232.7*	132.4**
(week 104)	(-)	(+9.6%*)	(-3.6)	(-23.5%**)	(-)	(-1.3%)	(-11.4%*)	(-49.6%**)
Food intake (g/rat/day)								
-1	19.5	19.7	19.2	19.7	16.4	16.5	16.7	17.0*
1	22.7	23.0	21.6*	19.5**	16.6	16.7	16.2	14.4**
7	21.6	21.8	21.3	20.3*	17.1	17.5	16.8	15.2**
13	20.7	21.2	20.8	20.3	17.0	17.0	17.1	14.6**
28	21.0	21.7	21.9	21.6	16.6	17.5*	17.1	14.1**
52	22.3	22.4	21.5	21.6	18.4	18.2	17.3	15.5**
104	22.2	22.4	21.0	21.3	18.5	19.7	18.2	16.2*
Food utilisation (g/100 g diet)								
1-4	21.3	22.1	21.5	16.8**	13.7	13.6	12.8*	10.1**
5-8	8.4	8.6	7.7	9.3	4.8	5.3	5.4	5.4
9-13	5.4	6.1	5.9	4.1*	2.8	2.6	2.8	1.5**
1-13	11.4	12.0*	11.4	9.6**	6.8	6.8	6.6	5.3**
Liver								
Liver weights adjusted (g)	18.10	18.06	20.21** (11.7%)	24.20** (33.7%)	11.56	12.05	12.65** (9.4%)	14.64** (26.6%)
Hypertrophy	0/52	0/52	8/52**	16/52***	0/52	0/52	1/52	38/52***
Eosinic cell focus	8/52	7/52	15/52	25/52***	2/52	10/52*	12/52**	14/52**
Hepatocyte pigment	0	1	0	1	2	3	1	15
Thyroid gland								
Desquamation. epithelial follicular	7	8	11	16	2	5	9*	14**
Basophilia colloid	7	9	12	16+	3	6	11*	17***
Diffuse C-cell hyperplasia	27	27	24	10***	29	31	27	5***
Focal follicular cell hyperplasia	7	8	8	16+	0	4+	0	4+
Vagina and mammary gland								
Vagina mucification*					29/52 (15/52)	(22/52)	29/52 (16/52)	21/52 (3/52**)
Mammary gland lobular hyperplasia	7/43	1/43	1/45*	4/41	34/52	34/50	32/51	21/52*

Histology re-evaluation corrected the originally reported incidences (in brackets) with 29/52, 29/52, and 21/52 for control, mid and high dose, respectively (Annex I, 3.9.4.11).

*, ** and ***: Statistically-significantly different from control with $p<0.05$, $p<0.01$ and $p<0.001$, respectively. (Dunnett's test or Fisher's Exact Test).

Neoplastic findings

A statistically significant increased incidence (by pairwise test $p<0.01$) of uterine adenocarcinoma in females at 3600 ppm (17.3%) and statistical significant reduction in mammary gland tumours (27% control vs 0% for the high dose) and a numerical, not significant, decrease in anterior pituitary tumours was observed for this high dose. For the mammary and pituitary tumours, these are toxicologically not significant based on the direction of change, but, based on the comparison with the control group, may reflect a result of treatment. Uterine adenocarcinomas were statistically significant also by trend test. There were no treatment-related effects on the uterus at the 52-week interim sacrifice, and no non-neoplastic micropathology changes to the uterus in the 104-week study groups. No increase in pre-neoplastic glandular endometrial hyperplasia was recorded. HCD for uterine adenocarcinoma for the testing laboratory have been submitted for the years in 2002-2012 (± 5 years from start of the sedaxane study) ranged from 0-19% with a mean of 7% for 10 studies. In addition, RITA historic control data have been provided as well, for studies of 22 to 25 months of duration, showing a range of 0-28%. RAC notes that the concurrent control is the most important control and that HCD can be used as supportive information for assessment of study results, in particular for assessing any limitations of the concurrent control group and assessing the range of normal for the endpoint. HCD should be from the same laboratory with comparable housing and feeding conditions, strain, animal supplier, and similar time periods (± 4-5 years). Therefore, the RITA data are not considered relevant for the assessment. Concerning the Charles River HCD, RAC acknowledges that the sedaxane related incidence of 17% is within the range of the HCD and that the concurrent control incidence of zero appears low. However, not only the range but also the distribution of the HCD is important. In agreement with the DS, it is noted that 2 of the 10 HCD studies had also a control incidence of zero, thus the concurrent control is considered reliable. Furthermore, the incidence of 17% exceeded the control incidence of 9 out of 10 HCD studies, thus the sedaxane treatment group does not appear to reflect the normal range of variation. It cannot be excluded that the single historical control incidence at the upper range was an outlier. In addition, the dossier submitter pointed out that another structurally related substance of the group of SDH-inhibitors isopyrazam also induced uterine adenocarcinoma at a comparable dose level, this concomitant with reduced mammary gland tumours, i.e. a similar pattern of effects. RAC notes that isopyrazam carcinogenicity data are not available to RAC and not subject for assessment in relation to the current CLH proposal. The brief information provided by the dossier submitter could indicate that the two substances may have a common MoA due to their similar chemical structure.

Based on the information available in the CLH report, RAC considers that the increased incidence of uterine adenocarcinoma is potentially a treatment related effect.

A higher incidence of hepatocellular adenomas (10% vs 2% in control), thyroid follicular cell adenomas (15% vs 6% in control) and thyroid adenoma/carcinoma combined (17% vs 6% in control) in males at 3600 ppm was observed. The incidences clearly exceeded the concurrent control incidences and also the HCD from the testing laboratory including 5 prior or concurrent studies at CRL in 2002-2005 with tumour incidences ranging from 0-3% for hepatocellular adenomas, 2-11% for follicular cell adenoma and 0-6% for follicular cell carcinoma.

In line with the dossier submitter, RAC considers the increased incidence hepatocellular adenoma, thyroid follicular cell adenoma and adenoma/carcinoma combined in the high dose males a treatment related effect (the combined thyroid tumour increase was driven by the increased incidence of adenomas, i.e. no increase in malignant tumours).

Table: *Neoplastic findings in the 2-year carcinogenicity study of sedaxane in rats*

Tumour findings	**Sedaxane (ppm)**			
	0	**200**	**1200**	**3600**
Females (# animals examined: 52)				
Uterine adenocarcinoma[a]$	0	3 (6%)	2 (4%)	**9** ** **(17%)**
Uterine adenoma	0	0	1 (2%)	0
Mammary gland fibroadenoma	14 (27%)	9 (18%)	10 (20%)	**0** ***
Pituitary adenoma anterior lobe	23 (44%)	28 (56%)	20 (38%)	**16 (31%)**
Males (# animals examined: 52)				
Hepatocellular adenoma[b]	1^ (2%)	1 (2%)	1 (2%)	**5 (10%)**
Thyroid follicular cell adenoma[b]	3^ (6%)	3 (6%)	4 (8%)	**8 (15%)**
Thyroid follicular cell carcinoma[b]	0	0	2 (4%)	1 (2%)
Combined thyroid follicular cell adenoma and carcinoma	3^ (6%)	3 (6%)	6 (12%)	**9 (17%)**

**p<0.01; *** p<0.001, pairwise Fisher' Exact Test.

$ p<0.05, Positive trend by Peto Trend Test (Groups 1-4). P-value for linear trend including groups 1 to 4 = 0.002. P-value for linear trend including groups 1 to 3 = 0.22

a Historical control data from the testing laboratory including 10 studies started in CRL between 2002-2012, ranged from 0-19% mean 7% for uterine adenocarcinoma

No statistically significant differences from control group by Fisher's Exact Test (p<0.05)

^p < 0.05 Trend analysis (significance of trend denoted at control) by Exact Trend Test

b Historical control data from the testing laboratory including 5 prior or concurrent studies at CRL in 2002-2005 ranged from 0-3% for hepatocellular adenomas, 2-11% for follicular cell adenoma and 0-6% for follicular cell carcinoma

Based on the findings reported, RAC notes that the high dose of 3600 ppm (equal to about 218/261 mg/kg bw/d males/females), where tumour incidences were increased, induced marked reduction in body weight and weight gain. According to the OECD TG 453, "*unless limited by the physical-chemical nature or biological effects of the test chemical, the highest dose level should be chosen to identify the principal target organs and toxic effects while avoiding suffering, severe toxicity, morbidity, or death*". In the view of RAC, this condition is fulfilled with the selection of dose levels, as there was no increase in mortality or clinical observations that would indicate suffering, morbidity or severe toxicity. Although the body weight gain reduction exceeded the 10%, which is commonly given as a convention for the Maximum Tolerable Dose (MTD), RAC considers the MTD not exceeded and the findings relevant for classification and labelling. No other signs of severe toxicity were apparent and there is no general (causal) association of body weight reduction with higher tumour incidences. There was no excessive toxicity and associated cell necrosis in the target tissues (or any other tissue) that would indicated that regenerative cell proliferation might have occurred, neither was hyperplasia recorded. However, RAC takes note of the MoA hypothesized by the pesticide applicant, which postulates marked body weight reduction / feed utilisation as initial event, and considers further assessment warranted.

Mouse

In the mouse study, groups of 50 male and 50 female CD-1 mice were fed diets containing 0, 200 ppm (25/29 mg/kg bw/d), 1250 ppm (157/185 mg/kg bw/d) and 7000 ppm (900/1001 mg/kg bw/d) of sedaxane for a period of at least 80 weeks.

Non-neoplastic findings

The treatment of sedaxane in mice results in mild body weight decrease with maximum of 7% in males and 9% in females and increased adjusted liver weight in males up to 16%. All non-neoplastic histology findings were considered background findings associated with this age and strain of mice, on this kind of study at Charles River, Edinburgh.

Neoplastic findings

In male mice at 7000 ppm, the incidence of hepatocellular adenomas was numerically higher than in control or other male treatment groups (14%, 18%, 20%, 30% for control, low, mid and high dose, respectively), but there were no statistically significant differences by the Peto trend test or a pairwise Fishers Exact test. A comparison to historic control data, and the RITA database, clearly shows that the incidence at 7000 ppm was above the range of normal variability for male hepatocellular adenomas in this laboratory and strain of mice.

Similarly, in male mice at 7000 ppm, the incidence of hepatocellular carcinomas was numerically higher than incidence in the control (10%, 10%, 6%, 20% for control, low, mid and high dose, respectively), but was not statistically significantly different from the concurrent control value by the Peto trend test or a pairwise Fishers Exact test (including animals dying before week 49). A comparison to the historic control data range of the study-performing laboratory shows that the incidence of the high dose exceeded the background variability. However, it is acknowledged that the liver tumours is a relatively common finding in male CD-1 mice. In addition, the RITA control data showed a range up to 22%, but these data are of less relevance as HCD should be from the same laboratory, strain and similar housing and feeding conditions and similar time (± 4-5 years).

The incidences of hepatocellular adenomas and carcinomas in female mice in the current study were extremely low in all control and treated groups (maximum 1/50).

Considering the background variability, the incidence of liver tumours in male mice are of minor concern. RAC notes however a clear dose-response for adenoma, which is indicative of a treatment-related effect. For carcinoma, the incidence in the high dose exceeded the concurrent and historical control data. In addition, the findings were not related to excessive or significant toxicity as the body weight data and clinical findings were not indicating exceedance of the MTD. Therefore, it is concluded that a treatment-related effect on carcinoma induction cannot be ruled out either.

Table: *Neoplastic findings in mice treated with sedaxane*

	Dietary concentration sedaxane (ppm)				**Historical Control Incidence**	
	0	**200**	**1250**	**7000**	**Lab (Range)** [a]	**RITA (Range)** [b]
Adenoma						
No. Animals	50	50	50	50	30/150	
Intercurrent	1	2	1	3		
Terminal kill	6	7	9	12		
Total	7 (14%)	9 (18%)	10 (20%)	15 (30%)	10-28%	0.0 – 13.6%
Carcinoma						
No. Animals	50	50	50	50	11/150	
Intercurrent	1	0	0	4		
Terminal kill	4	5	3	6		
Total	5 (10%)	5 (10%)	3 (6%)	10 (20%)	6-10%	4.0 – 22.0%

RAC assessment of the mode of action for liver tumours

The findings included higher incidences of hepatocellular adenomas in male rats and in male mice and hepatocellular carcinomas in male mice. In summary, the following incidences were reported:

Table: *Overview of liver tumours observed in rodent carcinogenicity studies with sedaxane*

	Dietary Concentration of sedaxane (ppm)			
MALES Rats	**0**	**200**	**1200**	**3600**
Number examined	52	52	52	52
Hepatocellular adenoma[a]	1 (2%)	1 (2%)	1(2%)	5 (10%)

	Dietary Concentration of sedaxane (ppm)			
MALES Mice	**0**	**200**	**1250**	**7000**
Number examined	48	45	45	48
Hepatocellular adenoma[a]	7 (14%)	9 (18%)	10 (20%)	15*(30%)
Hepatocellular carcinoma[a]	5 (10%)	5 (10%)	3 (6%)	10 (20%)
Adenoma/carcinoma combined	9 (19%)	13 (29%)	12 (27%)	19* (40%)[1]

* $p < 0.05$ Pair-wise comparison: significance denoted at dose level by Fisher Exact Test, excluding animals died before week 49.
[1] RAC noted that the CLH report Annex I, Table 3.9.1.2-11 reports an incidence of 15/48* (40%), which however equals 31%. As 6 animals are stated bearing adenoma and carcinoma, RAC concludes a combined incidence of 19/48* (40%).

A range of mechanistic studies was performed by the pesticide applicant to support the hypothesis of a CAR-mediated MoA for liver tumours. The data have been assessed by the dossier submitter. The DS concluded that the data convincingly demonstrated the CAR-PXR mechanism being the most plausible mechanism for liver tumour formation. The dose ranges in the mechanistic assays were in the same order of magnitude as the doses used in the long term studies in rats and mice.

RAC assessment for the specific key and associative events for the MoA

Key event 1: CAR/PXR activation

Sedaxane was evaluated in an *in vitro* CAR3 reporter assay for its ability to activate CAR from rat, mouse and human, by a method that has previously been shown to detect known species-specific activators of this nuclear receptor. In addition, sedaxane was evaluated in PXR reporter assays in the rat, mouse and human. In each assay, model compounds that are known to activate the specific CAR or PXR receptors were also tested to confirm the performance of the assays. The results from the *in vitro* CAR and PXR transactivation assays demonstrate that sedaxane activates CAR from rat, mouse and human and PXR from rat origin.

Associative event 1: Increased expression of genes encoding CYP2B/3A

In rat, the results from two 28-day studies with sedaxane and isomers support associative event 1. Increased PROD and testosterone 16β-hydroxylase activity are markers of CYP2B and cyp3A activity indicative of CAR and PXR activation. In mouse, data from RT-PCR and microarray analysis shows increased expression of hepatic CYP2B10 mRNA, CYP2Cc65 m RNA and PROD activity. Testosterone 6β-hydroxylase activity was noted.

Key event 2 and 3: Altered gene expression and altered expression of pro-proliferative genes/anti-apoptotic genes

Increase in Gadd45β mRNA and increase in xenobiotic metabolizing enzymes and other genes associated with associated with CAR/PXR activation were detected in the microarray assay in mice.

Associative event 2 and 3: Hepatocellular hypertrophy and increased liver weights

The 28-days and 90-day rat studies, and the 21-day liver MoA study in mouse together with the 90 days/80 weeks repeated dose toxicity study in mice confirm the findings in the carcinogenicity studies as increases in centrilobular hypertrophy (rats) and in liver weight (rats and mice) were observed. No histopathology including centrilobular hypertrophy or increased hepatic foci were noted in mice at any dose levels.

Key event 4: Transient increased hepatocellular proliferation and decreased apoptosis

In male rats, sedaxane induced a transient increase in hepatocellular proliferation measured by the BrdU labelling index. In male mice, sedaxane induced a slight increase in hepatocellular proliferation measured by Ki67 and BrdU labelling index.

Key event 5: Clonal expansion and development of altered hepatic foci

In the 2-year carcinogenicity study in rats, sedaxane led to increased eosinophilic cell foci.

Overall, RAC notes that in accordance to the available data, a good dose-concordance between the causal key events, associative events and the apical outcome (liver tumours) were observed in both male rats and male mice.

Key events in the animal MoA plausible in humans

To explore the species differences in response to sedaxane, an *in vitro* investigative study using primary hepatocytes isolated from male Wistar rats was conducted to assess the effects of sedaxane on PROD (CAR-marker) and BROD (PXR/CAR-marker) activities and hepatocellular proliferation and a similar experiment was conducted with isolated male human hepatocytes from one donor.

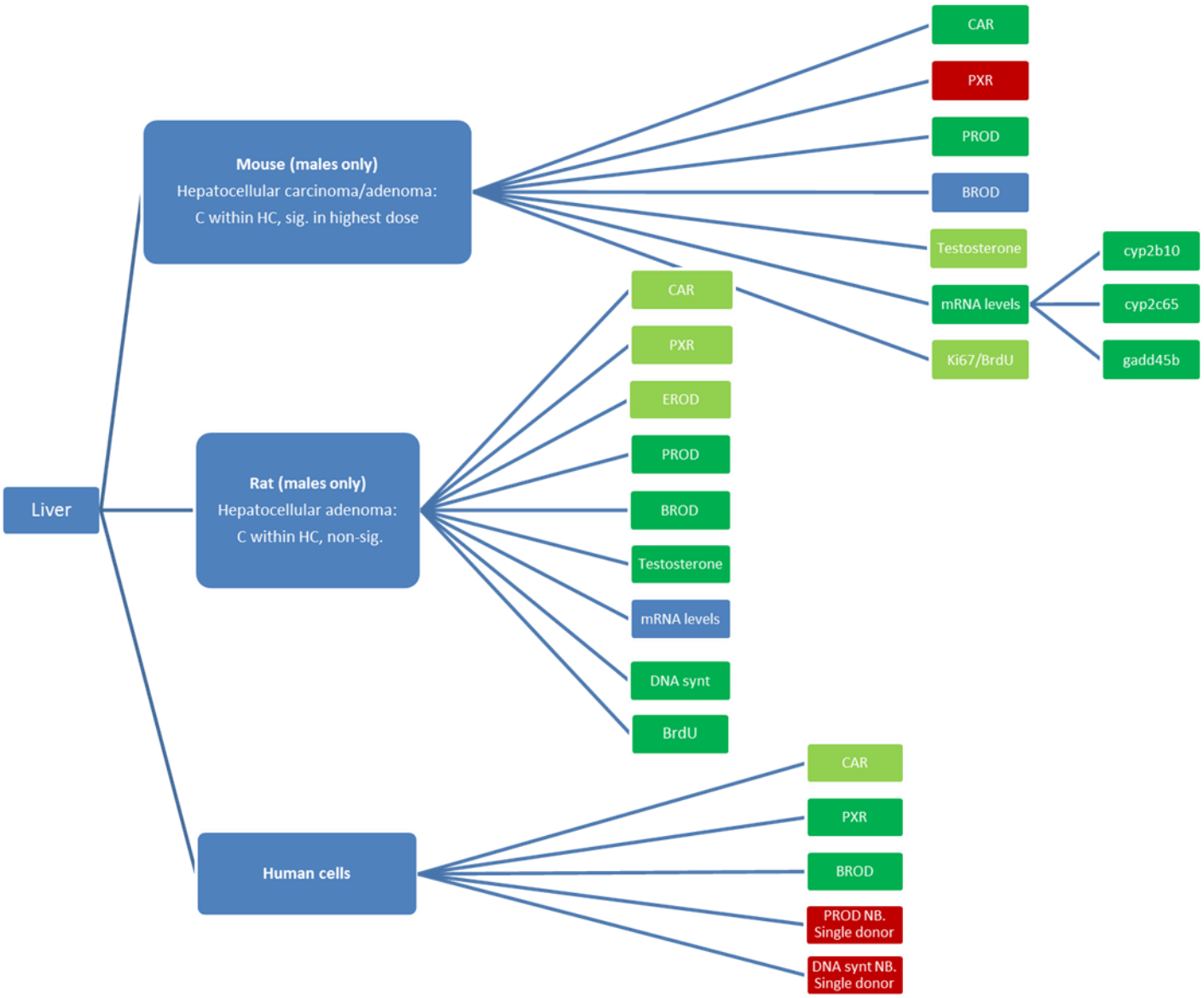
Table: *In vitro* comparison to the suggested MoA (CAR-PXR) with rat and human hepatocytes

Species	**CYP induction**	**Cell proliferation**
Rat Hepatocytes	↑ PROD activity and ↑ BROD activity	↑ S-phase labelling index
Human Hepatocytes **Only one male donor**	↑ BROD activity; no effect on PROD activity	None

The experiments showed a PXR/CAR activity (BROD activity) in human cells but negative activity for PROD activity (no CYP2B activity). Based on experimental data with one donor, the human hepatocytes have been shown to be non-responsive to sedaxane regarding the causal key event of cell proliferation. It was concluded by the pesticide applicant and supported by the DS that these data indicate that the tumourigenic MoA established for sedaxane in male rats and male mice, although likely operative in humans, may be expected to show qualitative differences between rodents and humans in their response to sedaxane, i.e. different in the critical key event cell proliferation that would ultimately lead to tumour formation. However as only one donor was tested, RAC acknowledges significant uncertainties remaining in the conclusion that the pattern of effects matches the known species differences that have been demonstrated for phenobarbital and other CAR activators as regards to a qualitative difference in the tumourigenic MoA for rodents (rats and mice) and humans. As an additional uncertainty it is noted, that no CAR-knock-out studies have been conducted.

The following figure presents a summary on the available evidence in support of a CAR-mediated MoA for liver tumour formation:

Figure: *Available data and strength of evidence for liver tumour MoA based on mechanistic studies in CLH report Appendix 2*



Colour coding and the relevant receptors, the enzymes and the gene expression:

Colour coding
Positive test
Slightly positive/less potent
Negative
Not tested

CAR	*PROD*	*CYP2B*
PXR/CAR	*BROD*	*CYP2B/3A*
AhR	*EROD*	*CYP1A*
PPAR		*CYP4A*

Overall, the available data for sedaxane support the proposed MoA to account for the higher incidences of liver tumours in male rats and male mice. The changes in the liver seem attributable to activation of CAR (with a possible lesser activation of PXR in rats), which results in a series of well-documented downstream events, ultimately leading to a higher incidence of tumours vs. the concurrent controls. The available data provide an indication that the causal event cell proliferation, and thus related adverse outcome liver tumours, of this MoA seem of less relevance to humans. RAC however points out that the key experiments in the mechanistic data package in support of the hypothesis that the tumours are likely not relevant for humans, including studies with CAR-knock-mice and convincing data on cell proliferation of more than one human hepatocyte donor would have increased RAC´s confidence in the assessment. As human liver cell

proliferation represents the key data to discount human relevance of the tumour findings observed in rodents, testing of only human donor is considered a significant uncertainty in the data package.

RAC assessment of alternative mechanisms for liver tumour formation

In addition to CAR/PXR activation, alternative MoA for induction of liver tumours in rodents or humans have been demonstrated with the data package. The DS did perform an assessment of alternative MoA in appendix 2 in the CLH report. Most important the exclusion of genotoxicity, peroxisome proliferation, AhR mediated and cytotoxicity MoA seem to be supported:

- Direct genotoxicity

It was considered that this MoA can be excluded as sedaxane has been demonstrated to be negative for genotoxicity in a comprehensive package of *in vitro* and *in vivo* assays. RAC agrees that a direct genotoxic MoA appears unlikely. Sedaxane has been tested negative in series of *in vitro* and *in vivo* genotoxicity assays and it is concluded that the substance is unlikely to be genotoxic (see section of germ cell mutagenicity).

RAC concludes that the data do not support a (direct) genotoxic MoA for liver tumours.

- Cytotoxicity / regenerative cell proliferation

Chronic cytotoxicity with subsequent regenerative cell proliferation is considered a mode of action by which tumour development can be enhanced. Following administration to rats and mice, sedaxane did not produce elevations in markers of hepatocyte damage nor was there any evidence of cytotoxicity or regenerative proliferation (the proliferation noted following treatment with sedaxane was transient and not sustained).

RAC concludes that the data do not support a cytotoxicity-mediated MoA.

- Peroxisome proliferator

Treatment with sedaxane did not increase male mouse hepatic peroxisomal fatty acid β-oxidation or lauric acid 12-hydroxylation activity (a marker of Cyp4a activity). Also, in a rat 28-day study, electron microscopy of the livers showed no evidence of peroxisome formation.

RAC concludes that the data indicate that peroxisome proliferator-MoA is unlikely.

- AhR-mediated

Treatment with sedaxane did not result in increased EROD activities in rats or in mice. In addition, no strong induction of CYP1A isoform expression of the magnitude seen with AhR activators was observed in mouse liver microarrays.

RAC concludes that the data indicate that AhR-mediate-MoA is unlikely.

In conclusion on the MoA for liver tumours*, RAC is of the opinion that the available data provide evidence to support the postulated MoA – CAR activation - to be the underlying MoA of liver tumours observed in rodent males. However, uncertainty remains especially for the hepatocellular carcinoma in male mice, as no studies with CAR-Knock-Out mice have been performed and only one single donor for the human hepatocytes assay was available.*

RAC assessment of the mode of action for thyroid tumours in rats

The findings included higher incidences of thyroid follicular cell adenoma and carcinoma in male rats. The following incidences were reported:

Table: *Overview of thyroid tumours observed in rodent carcinogenicity studies with sedaxane*

MALES Rats	**Dietary Concentration of sedaxane (ppm)**			
Tumour findings	**0**	**200**	**1200**	**3600**
Number examined	52	52	52	52
Thyroid follicular cell adenoma[a]	3 (6%)	3 (6%)	4 (8%)	8 (15%)
Thyroid follicular cell carcinoma[a]	0	0	2 (4%)	1 (2%)
Combined thyroid follicular cell adenoma and carcinoma	3 (6%)	3 (6%)	6 (12%)	9 (17%)

a Historical control data from the testing laboratory including 5 prior or concurrent studies at CRL in 2002-2005 ranged from 0-3% for hepatocellular adenomas, 2-11% for follicular cell adenoma and 0-6% for follicular cell carcinoma.

The pesticide applicant has undertaken a series of investigative studies to determine the mode of action for sedaxane in the higher incidence of thyroid follicular cell adenomas in male rats. The studies have been assessed by the dossier submitter and it was agreed that the postulated MoA involving activation of the CAR/PXR nuclear receptors by sedaxane and consequent induction of hepatic UDP-glucuronosyltransferase (UDPGT) is the most plausible mechanism. The dose ranges in the mechanistic assays were in the same order of magnitude as the doses used in the long term studies in rats and mice.

RAC assessment for the specific key and associative events for the MoA

Key event 1: CAR/PXR activation

The results from the *in vitro* CAR and PXR reporter assays support that sedaxane activates CAR from rat, muse and human and PXR from rat.

Associative event 1: Hepatocellular hypertrophy and increased liver weight

In the 28-day, 90-day and 2-year rat studies, sedaxane induced increased liver weight and hepatocellular hypertrophy.

Key event 2: Induction of hepatic UGT

In the 28-day rat mechanistic study, sedaxane induced increased hepatic UGT activity.

Associative event 2: Thyroid follicular cell hypertrophy and increased thyroid weight

In the 28-day, 90-day and 2-year rat studies, sedaxane induced increased thyroid weight and thyroid follicular cell hypertrophy.

Key event 3: Reduced circulating T3 and T4

In the 28-day rat mechanistic study, total T3 showed a statistically significant decrease in one or both sedaxane treatment groups on days 2, 4, 8 and 15. However, total T4 was statistically significantly decreased by treatment with sedaxane only at day 2.

This key event is only weakly supported by the experimental data.

Key event 4: Increased circulating TSH

In the 28-day rat mechanistic study, a clear increase of circulating TSH was not observed after sedaxane treatment. A marginal TSH increase could possibly be present after 14-28 days of sedaxane treatment, but definitive increases in TSH levels for sedaxane treated groups were not discernible for the time points that were assessed in the study. The individual animal data were quire variant, while the positive control phenobarbital behaved as expected with a clear effect on thyroid hormones and TSH.

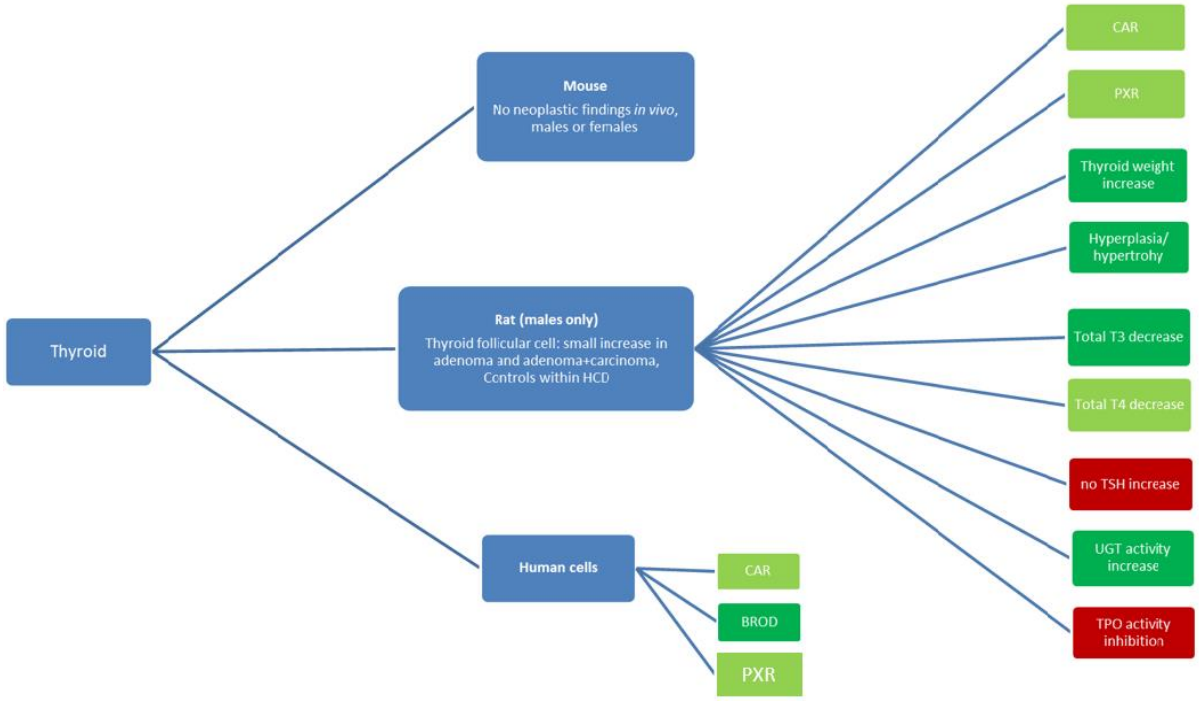
This key event is only weakly supported by experimental data.

Key event 5: Increased thyroid follicular cell proliferation

In the 2-year rat study, sedaxane induced thyroid follicular cell proliferation.

The following figure presents a summary on available data and strength of evidence in support of a UGT-mediated MoA for thyroid tumour formation:

Figure: *Available data and strength of evidence for thyroid tumour MoA based on mechanistic studies in CLH report Appendix 2*



RAC assessment of alternative mechanisms for thyroid tumour formation in rats

In addition to the MoA described, alternative modes of action for the induction of thyroid tumours exist, of which some can be assessed:

- Genotoxicity

One such alternative MoA is genotoxicity. This MoA can be excluded as sedaxane has been demonstrated to be negative for genotoxicity in a comprehensive package of *in vitro* and *in vivo* assays for genotoxicity (see previous section).

- Cytotoxicity

Generally, chronic cytotoxicity with subsequent regenerative cell proliferation is considered a MoA by which tumour development can be enhanced. Sedaxane and/or its metabolites reach the target organ as indicated by the toxicokinetic data (CLH report, Annex I, section 2), however direct toxicity on the thyroid gland is unlikely the cause. Following administration to rats and mice, there was no evidence of cytotoxicity. The observed organ weight increase and hypertrophy

is suggestive of a characteristic feedback-regulated increase in thyroid gland activity to meet higher demands of thyroid hormones.

RAC concludes the data do not suggest this cytotoxicity-mediated MoA.

- Direct inhibition of the thyroid hormone synthesis

A second alternative MoA is direct inhibition of the thyroid hormone synthesis. Organification of iodine via monoiodination of L-tyrosine is the first step in the synthesis of T3 and T4 and is catalysed by the enzyme thyroid peroxidase (TPO). Inhibition of TPO, in order to reduce circulating T3/T4, by compounds such as propylthiouracil (PTU) is exploited as a treatment for hyperthyroidism in humans, such as in Graves' disease. PTU has also been shown to induce thyroid follicular cell adenomas in rats (IARC, 2001). Sedaxane was evaluated for its potential to inhibit TPO *in vitro* across a concentration range up to 10 µM compared to the appropriate controls. Taken together with the evidence supporting the proposed MoA for thyroid tumours, these data provide compelling evidence that sedaxane lacks the intrinsic properties to interact with and inhibit TPO.

This MoA can be excluded for sedaxane as it was found not to be an inhibitor of male rat thyroid-derived TPO *in vitro*, whereas PTU was shown to be a potent inhibitor.

In conclusion on the MoA for thyroid tumours*, based on the assessment of the available data, RAC agrees with the dossier submitter that the CAR-mediated induction of hepatic UGT activity is the most plausible mechanism. This MoA might give rise to thyroid tumours in rodents. Such MoA based on enhancement of the metabolism and excretion of thyroid hormone (TH) by the liver, largely through induction of UGT enzymes, is considered less relevant to humans due to known differences in sensitivity. Two key events are only weakly supported by the data package, which leaves some uncertainty, but mild effects on thyroid hormone homeostasis have been noted. The available data permitted to rule out three alternative MoAs, i.e. genotoxicity, cytotoxicity, and inhibition of thyroid peroxidase (TPO). No other potentially alternative or additional MoA was identified but other operative pathways on thyroid hormone disruption, such as e.g. deiodinase inhibition or NIS inhibition, have not been investigated. The pattern of effects, hypertrophy and organ weight increase, is suggestive of a MoA involving disruption of TH homeostasis. Overall, some uncertainties on the underlying MoA are left.*

RAC assessment of the mode of action for uterine adenocarcinoma in rats

The DS assessed the MoA as postulated by the applicant. The dossier submitter considered the proposed MoA as not sufficiently demonstrated and thus did not analyse human relevance.

<u>RAC assessment of the postulated mode of action</u>

Initial key event "Decreased food utilisation", key event "Decreased adipose tissue" and associated "Decreased body weight"

The pesticide applicant considers the mechanism of secondary nature due to reduced food utilisation and body fat tissue. In the view of RAC, this is not sufficiently supported. A decrease in body weight and body weight gain throughout the 2-year carcinogenicity study was observed with 49.6% decrease in body weight gain in females at termination (reduction of terminal body weight in females by 37%). Food utilisation was measured only in the first 13 weeks of the study and was statistically significantly reduced. No direct evidence for decreased adipose tissue via measurement of whole body fat content is available for sedaxane.

Thus, RAC acknowledges that a marked reduction on body weight gain is reported for the carcinogenic dose level. However, RAC agrees with the dossier submitter and commenting MSCA that reduced body weight/food utilisation (leading to decrease in adipose tissue) is a very broad, commonly observed effect in chronic toxicity studies with the high doses, and usually not leading

to increased tumour formation. It has been raised by RAC that the marked decrease in body weight gain might have even masked a more pronounced tumourigenic effect.

Further assessment of the initial key event, i.e. a potential secondary versus direct effect on the dopaminergic system, see below section "RAC assessment of Initiating Key Event".

Key event "Decreased plasma leptin and signalling to the hypothalamus"

Further downstream of the postulated MoA, plasma leptin levels and adiponectin levels were measured as weight reduction and caloric restriction are related to increase in adiponectin and decrease in leptin and the hormones provide neuroendocrine signalling to the arcuate nucleus in the hypothalamus. For the sedaxane one-year interim sacrifice of the 2-year rat study, leptin levels were reduced but not statistically significant by 15% for the 3600 ppm sedaxane group only (body weight reduction stated -13% at 1-year). The dossier submitter pointed out that also a decrease in adiponectin due to treatment with sedaxane would be expected, but was measured unchanged. The ratio of adiponectin:leptin remained fairly unchanged for sedaxane 3600 ppm (1.15-fold).

RAC concludes that leptin decrease in sedaxane-treated animals was weak with -15% and biological significance of this marginal change is questionable. In relation to the marked decrease in body weight gain, the leptin data are not plausible based on the known correlation of leptin with body fat content. It is further considered that the postulated decrease in leptin signalling to the hypothalamus is speculative. Leptin signalling to the hypothalamus has not been investigated, nor was a functional role proven.

Key event: Hypothalamus "Suppression of age-related decrease in hypothalamic signalling"; Associative event "Higher DOPAC levels in median eminence/increased dopaminergic signalling"

It was postulated that the decrease in adipose tissue and leptin-signalling to the hypothalamus would lead to a suppression of age-related increase in pituitary prolactin secretion caused by increased dopaminergic signalling due to maintained functional activity of tuberoinfundibular dopamine neurons (TIDA) neurons. TIDA function decreases with age in rats, and it was postulated by the pesticide applicant that age-related decrease in TIDA function and dopamine release by this pathway is supressed by sedaxane. In the 2-year rat sedaxane study, formalin-fixed paraffin-embedded brains from the 104-week sacrifice have been analysed for the relative abundance of tyrosine hydroxylase (TH), the enzyme hydroxylating tyrosine to DOPA (which is then converted to dopamine), in the TIDA region of the hypothalamus, in the arcuate nucleus (ARC) and medium eminence (ME) using in situ hybridisation (ISH) for mRNA expression and immunohistochemistry (IHC) to evaluate protein expression. In addition, age-related changes in these neurons as indicated by TH expression have been analysed in a control Wistar rat population after 90 days, 1 year, and 2 years.

For the latter experiment with a control population, the DS concluded that protein staining did not support age-related decrease in TIDA-TH (no difference 2y-vs-90d) in control rats and noted a lack of correlation between mRNA and protein staining. RAC agrees with the dossier submitter on this experiment, that age-related TIDA senescence has not been conclusively demonstrated. In the brain samples from the sedaxane 2-year study, 12 rats per group were quantified. TH mRNA was increased by approximately 2-fold for sedaxane 3600 ppm in the ARC and ARC+ME quantification (no increase for 1200 ppm). For the data as presented, in contrast the mid dose of 1200 ppm sedaxane increased protein levels in the ARC, ME and ARC+ME significantly and this to higher extend than the high dose of 3600 ppm, thus not dose-related (see Figure 6 of CLH report, section 3.2.4.1 on tyrosine hydroxylase mRNA and protein expression in brain samples from 2-year study). In the view of RAC, these results on sedaxane do not present a robust evidence. It is noted that protein expression by immunohistochemistry staining is a semi-quantitative methodology and quantified differences and statistical analysis need to be taken

with caution. Furthermore, TIDA neuron activity is sensitive upon a variety of stimuli such as stress, hormones, i.e. oestrogens in females, and even endogenous daily activity (Ben-Jonathan *et al.*, 2001). Hypothalamic TH protein is constitutively active to meet the high dopamine demand and TH activity might be influenced by post-translational modifications, importantly the functional activity has not been proven to be increased by sedaxane. Regarding biological plausibility of the proposed MoA, considering an increase in TH protein in the mid dose, it is difficult to establish an association to increased uterine tumours and decreased mammary gland tumours, since the incidence were unchanged for the mid dose and the tumour shift observed only for the high dose group.

To summarise, 1) For aging control rats, decrease in dopamine with time, TH protein staining, did not support age-related TIDA senescence. 2) For sedaxane-treated rats (2-yr-study), experiments on TH expression are inconclusive (no prove of dose-response in TH protein, no correlation in mRNA and protein levels for the mid dose, potentially related to methodological issues). 3) The functional TH activity has not been objectified and thus not proven to be increased by sedaxane. RAC agrees with the DS that this important key event in the postulated MoA, preservation of dopaminergic signalling, is not sufficiently supported.

Key event: pituitary "Suppression of age-related increase in pituitary prolactin secretion", Associated event "Lower plasma prolactin"

It has been postulated that maintenance of dopaminergic activity in the hypothalamus suppresses age-related increase in pituitary prolactin secretion into the blood and consequently decreases prolactin-mediated progesterone secretion from the corpora lutea from the ovaries. The lower levels of prolactin in sedaxane-treated aging rats should cause a change in transition into reproductive senescent. Prolactin is an established trophic drive for mammary tumour formation in rats. Indeed, a reduced incidence of mammary tumours was associated with sedaxane treatment: fibroadenoma 14/52 (27%), 9/50 (18%), 10/51 (20%), 0/52 (0%) for control, low, mid and high dose, respectively. Prolactin levels have been analysed in 1-year (frozen) serum samples from the 2-year carcinogenicity study with sedaxane. Due to the inherent level of variation in prolactin levels between individual animals at 52 weeks of age, it was not possible to determine any differences in prolactin concentrations between control and sedaxane-treated groups from the available serum samples. No further studies have been performed, such as prolactin measurements at later time points.

The dossier submitter correctly concluded that there is no direct experimental evidence for suppression of age-related prolactin suppression by sedaxane, and that the data for 52-weeks do not show a treatment-related effect. To summarise, prolactin alteration has not been proven for sedaxane.

Key event: Ovary "Delayed progression from persistent oestrus to persistent dioestrus"; Associated event: Vagina "Decreased mucification and related changes"

It has been postulated that the lack of rise in circulating prolactin levels in blood results in a delay in reproductive senescence of rats, which continue to experience more periodic oestrous cycles compared to control rats. The objective of the histology re-evaluation investigation (CLH report Annex I, 3.9.4.11) was to determine the cycle stage based on the microscopic examination of the vagina, uterus, and ovary of rats exposed to sedaxane in their diets for intervals ranging from 13 weeks in the available 90-day dietary study to 104 weeks in the 2-years carcinogenicity study. Based on the time period when necropsy occurred in the 90-day and 2-year studies, the following groups have been analysed for oestrous cyclicity and reproductive senescence: A = sacrificed at 13 weeks, B = died 0 - 52 weeks, C = sacrificed at 52 weeks, D = died 53 - 104 weeks, E = sacrificed at 104 weeks, for the mid (1200 ppm) and high dose (3000 ppm) as compared to the control group. Looking across all age groups, the results indicated that

regardless of treatment group, virtually all of the females in the 13-week study as well as the 52-week interim sacrifice subgroup were cycling at the time of death/sacrifice. In older rats (subgroups D and E combined) by 104 weeks, the vast majority of animals showed evidence of senescent stages (either in repetitive pseudopregnancy or persistent anoestrus) comparable for all treatment groups (44/50, 47/52, 47/51 for control, mid and high dose, respectively). The only difference was a lower incidence of repetitive pseudopregnancy (29/50 (control), 28/52 (1200 ppm), 19/51 (3600 ppm)) and a higher incidence of persistent anoestrus (15/50 (control), 18/52 (1200 ppm), 27/51 (3600 ppm)), the last stage of reproductive senescence, observed for the 3600 ppm animals compared to the control. Regardless of treatment group, persistent oestrus was virtually absent at all ages up through 104 weeks in the subchronic and chronic study with sedaxane. It needs however to be considered that oestrous cycling staging need to be performed in regular time intervals by vaginal lavage and therefore these data may not be sufficient to draw firm conclusions. No hormone levels (estrogen:progesterone) have been measured in any of the studies for sedaxane.

Therefore, RAC agrees with the conclusion of the dossier submitter, that a similar rate of reproductive senescence was noted in all dosing groups and a delay in reproductive senescence of rats by sedaxane cannot be concluded from these data. From these data, an increase in the total number of oestrous cycles and proliferation is not supported and a change in oestrogen:progesterone levels that would lead to a sustained stimulation of the uterine endometrium (key event, uterus, leading to higher incidence of uterine adenocarcinomas) has not been objectified. Overall, RAC concludes that the data do not evidence the postulated key event of a delayed progression into persistent oestrus and from there to persistent dioestrus. A higher estrogenic state with altered (=elevated) estrogen:progesterone levels for sedaxane remains hypothetical.

Key event: uterus "Sustained stimulation of uterine epithelium" (eventually leading to a higher incidence of uterine adenocarcinoma)

A sustained proliferative drive of the uterus epithelium has been postulated to result from an increased estrogenic state. It is noted that no data have been provided to substantiate this key event ultimately leading to uterine adenocarcinoma. For sedaxane, there were no treatment-related effects on the uterus indicative for sustained proliferation/hyperplasia of the endometrium at the 52-week interim sacrifice, and no non-neoplastic micropathology changes to the uterus in the 104-week carcinogenicity study.

RAC concludes that this key event, as assumed is plausible based on the literature (glandular endometrium hyperplasia is a pre-neoplastic lesion of uterine adenocarcinoma), but was not recorded in the data package.

RAC assessment of Initiating Key Event of the postulated MoA – further considerations

The applicant considered the mechanism of secondary nature due to reduced food utilisation and body fat tissue, this hypothesis should decrease the concern. In the view of RAC, this is not sufficiently supported:

It is noted, based on the toxicokinetic studies, that the substance (or metabolites) reach the target organ uterus (Annex 1 of the CLH report, table 2-18). Furthermore it should be noted, that in the two-generation reproduction toxicity study (see next section) in the top dose females (1500 ppm) of the P and F1 generation, effects on the reproductive organs (ovary weight reduction, number of corpora lutea and number of antral follicles reduced, uterus weights reduction) were recorded, in absence of marked toxicity or body weight gain reduction.

Generally, it is not considered very plausible, as not all body weight reductions lead to increased tumour formation in carcinogenicity studies, not all chemically induced alterations in reproductive

senescence lead to uterine tumour formation, and not all Wistar rat feed restriction studies show an increase in uterine adenocarcinoma. However, it is acknowledged that some studies have shown an association of diet / feeding status with uterine tumour incidences (Tucker *et al.,* 1979; Roe *et al.,* 1995), but the diet composition itself rather than caloric restriction could play a role. Also, an association of caloric/feeding status with (decrease) in mammary gland tumours is known. In the view of RAC it is true that reproduction and oestrous cycling is sensitive to feeding and weight loss (McShane and Wise, 1996; Frisch *et al.,* 1975; Tropp *et al.,* 2001). However, not all studies on caloric restriction that show a delay in reproductive senescence result in induction of uterine carcinoma (Keenan *et al.,* 1995). Then, in the literature it is not well demonstrated whether activity of TIDA neurons changes during different feeding states and inconsistent results have been reported. Recent findings in transgenic mice suggested that short-term fasting attenuated TIDA neuron activity and increased serum prolactin levels (Kubota *et al.,* 2018).

Ultimately, as the HPO-key events have not been robustly proven, the question of a primary or secondary effect on the hypothalamic dopamine system seem of less importance to RAC. Nevertheless, RAC notes that further data have been generated in order to exclude a direct effect on the dopamine system, such as those of the dopamine receptor agonist bromocriptine. The potential of sedaxane to bind to isolated dopamine D2S receptor was investigated:

- Dopamine receptor agonist activity

Sedaxane was tested in triplicate at a single concentration of 10 µM for its potential to bind the dopamine D2S receptor (Eurofins Cerep assay for binding potential to dopamine receptor D2S isoform, human recombinant, obtained from HEK-293 cells transfected at Eurofins Cerep with and stably expressing the human D2S gene). The assay evaluated binding by displacement of [3H]methyl-spiperone, a known binder of the dopamine receptor. When tested at a concentration of 10 µM, sedaxane did not trigger any significant reduction in control specific binding (<50%). A reference substance has been included as control in the assay and performed as expected. According to the CLH report, for the test substance, strict criteria for determination of a positive response for dopamine D2S receptor binding were not applied to the assay. However, a guideline value of $\geqslant$ 50% inhibition of control specific binding was used to indicate a positive response, in conjunction with other considerations, if applicable, such as increasing effect with increasing concentration. Under the conditions of the study control specific binding was > 93% and inhibition by sedaxane less than 7%. Therefore, sedaxane was not considered to bind to the dopamine D2S receptor *in vitro*. According to the CLH report, the concentration of 10 µM has been selected based on pharmacokinetic considerations, the maximum µM concentration was calculated based upon the Cmax at day 14 of dietary administration, which would represent the maximum concentration at steady state. RAC notes uncertainties in the results interpretation, i.e. only the result of one single concentration has been presented and no log competition curve of ligand binding, which is usually set up in such type of studies. This is considered important, as the dopamine receptor is a neuro-endocrine receptor and as such operates rather in the nM range (high concentrations could, for instance, result in steric hindrance). Then, sedaxane is extensively metabolised *in vivo* (see Annex 1 of the CLH report, table 2-26) and RAC wonders whether metabolic conversion of the parent compound would not be required for full evaluation of receptor binding. Finally, the experiments have been conducted to exclude direct effects of sedaxane on the dopamine system and consequent prolactin alteration. Apart from RAC's observation, that no direct evidence for prolactin alteration by sedaxane is available, the presented experiment does not exclude the possibility of effects on one or several downstream components of the different dopamine signal transduction pathways. Other factors than hypothalamic dopamine, within the brain, pituitary gland, and peripheral organs have been shown to inhibit or stimulate prolactin secretion as well (Freeman *et al.,* 2000). In the view of RAC, further specific robust mechanistic evidence on prolactin regulation would be required. This is in particular true for investigating

whether other effects than an indirect markedly reduced body weight gain would have contributed.

In conclusion, the result of this biochemical dopamine receptor-binding assay provides limited information in support of the postulated MoA.

RAC conclusion on the sedaxane mechanistic data

The uterus is an oestrogen-dependent organ and endometrial cells proliferate as a result of oestrogen stimulation and early neoplastic growth requires continuous presence of oestrogen, later becoming oestrogen-independent. Yoshida *et al.* (2015) described five mode of actions, among three the major pathways for uterine carcinogenesis considered relevant for humans and rodents: 1) oestrogens/chemicals with estrogenic activity, 2) continuous increase in E2:P4 ratio, and 3) modulation of oestrogen metabolism via CYP induction. For the other two MoA, i.e. 4) decreased E2 excretion/increased E2 levels in the blood or 5) increase *in situ* aromatase, little or no evidence so far in rodents has been reported. The authors also discussed other factors, i.e. the role of prolactin and dopamine modulating activity in uterine, pituitary and mammary carcinogenesis. This MoA however is less investigated and the key events are not sufficiently well described in the literature, so far.

Based on literature references, the applicant postulated the MoA involving sustained dopaminergic activity and prolactin-dependent alteration of reproductive senescence with an estrogenic state leading to sustained cell proliferation, the adverse outcome, typically observed with higher frequency in Wistar rats, an increase incidence in uterine adenocarcinoma and concomitant decrease in mammary gland and pituitary tumours. This pathway is discussed by Harleman *et al.* (2012). The authors point out that such a tumour shift is seen in dietary restriction studies in Wistar rats, but less frequent in SD rats. The prolactin-mediated effect in rats on increased oestradiol:progesterone ratio and resulting uterine tumour formation is considered as not relevant for humans, since prolactin is not luteotrophic in humans. Yoshida *et al.* (2015) concluded that for such a MoA extrapolation to humans, more clear evidence is needed.

RAC acknowledges that a dopaminergic-prolactin, i.e. disruption of the HPO-axis dependent mechanism, is discussed, rather recently, in the available literature as one possible mechanism that could lead to endometrium carcinogenesis, and that relevance for humans is uncertain. In particular, however, the regulatory role of hypothalamic dopamine in prolactin release from the pituitary and the role of prolactin in mammary gland carcinogenesis is settled (O´Connor *et al.*, 2000; Ben-Jonathan and Hnasko, 2001).

It is further considered that this Adverse Outcome Pathway (AOP) in Wistar rats is so far not robustly developed with its molecular initiating and key events, and thus is still in an uncertain stage. A limited amount of publications investigating/discussing this MoA is available (in mice: Gunin *et al.* 2002; in rats: Klaunig *et al.*, 2016; Harleman *et al.,* 2012; Yoshida *et al.,* 2009, 2015) for a limited number of substances, such as dopamine agonists bromocriptine and antagonist sulpiride. Up to know, only a putative AOP on an *increased dopaminergic activity leading to endometrial adenocarcinomas (in Wistar rat)* is under development (see AOP Wiki, for comparison, molecular initiating (MIE) and key events (KE)*: MIE: Increase, dopaminergic activity; KE: Decreased prolactin; KE: Increased oestrogen receptor (ER) activity; KE: Decreased progesterone from corpus luteum; KE: Increase hyperplasia of glandular epithelial cells of endometrium; AO: Increase endometrial adenocarcinomas*).

For RAC however, it is of outmost importance that key events are robustly established and measured. The toxicology and mechanistic data package for the substance in question should demonstrate the association of key events with the adverse outcome. Moreover, a causality of key events with the adverse outcome should be valid. Unless a MoA has not been demonstrated with sufficient certainty, human relevance assessment is not warranted, as the standard

assumption in toxicology is that effects observed in animals are relevant for humans. RAC further points out, that endometrial cancer is highly relevant in humans, and is both in humans and in rodents an oestrogen sensitive lesion.

RAC summarises the following deficiencies in the sedaxane data package. Key events in the disruption of the HPO-axis are not supported and/or only assumptions have been made*:*

- The dopaminergic activity has not been conclusively proven, this is considered a major deficiency as it represents a key event in the hypothesis,
- Prolactin secretion was unchanged for sedaxane, respectively has been insufficiently investigated (not measured after 52 weeks), this is considered a major deficiency as it represents a key event in the hypothesis (data on a structural analogue is not considered sufficient), and is the basis for the postulate that the sedaxane-associated uterine tumours are not relevant for humans, as prolactin is not luteotrophic in humans,
- Important key events have not been measured/objectified with robust methodologies, in particular oestrous cycling and alteration of (cycle-sensitive) oestrogen:progesterone levels,
- Sustained proliferative stimulation is postulated, but endometrial hyperplasia due to an estrogenic-mediated stimulation was not apparent in the 2-year carcinogenicity study with sedaxane, nor in any other repeated dose studies of the available data package.

Therefore, an association of the key events with the adverse outcome has not been demonstrated.

To further substantiate some key events, additional supportive data have been generated with the structural analogue isopyrazam. This included key serial events as postulated (table 13 of the position paper submitted in the PC). However, isopyrazam carcinogenicity has not been assessed so far by RAC and is not subject to this CLH proposal. A read across to isopyrazam would require prior assessment of toxicokinetics and toxicity of this substance on its own based on the substance's data package, including conclusions on carcinogenicity and underlying MoA, furthermore a robust justification for read across from isopyrazam to sedaxane. In absence of such assessment, RAC has reservations to consider the proposed read across from isopyrazam and related conclusions as robust evidence for the sedaxane MoA. According to the EFSA Peer Review conclusion (EFSA, 2012), long-term exposure to isopyrazam produced liver hepatocellular adenomas and uterine endometrial adenocarcinomas in rats. Apart from the similar tumour profile, that may indeed by related to the structural similarity of the two compounds, RAC takes note of a distinct classification proposal in the EFSA conclusion for isopyrazam regarding human health compared to sedaxane (Carc. 2; H351, Repr. 2; H361d, Acute Tox. 4; H302, Skin Sens. 1; H317).

During public consultation, the pesticide applicant concluded on sedaxane mainly based on an 18-months MoA-study conducted with isopyrazam, which has been submitted in PC. RAC cannot consider these conclusions due to the aforementioned reasons. It is further noted that the dossier submitter considered these data as not sufficient to establish the underlying MoA for uterine carcinogenesis.

RAC assessment of alternative MoA for the induction of uterine tumours

Data in relation to alternative MoA have been submitted and assessed:

- Direct genotoxicity

It was considered that this MoA can be excluded as sedaxane has been demonstrated to be negative for genotoxicity in a comprehensive package of *in vitro* and *in vivo* assays. RAC agrees that a direct genotoxic MoA appears unlikely. Based on the toxicokinetic studies it is noted that the substance (or metabolites) reach the target organ uterus (Annex 1, table 2-18). However, sedaxane has been tested negative in series of *in vitro* and *in vivo* genotoxicity assays and it is concluded that the substance is unlikely to be genotoxic (see section of germ cell mutagenicity).

RAC concludes that the data do not support a (direct) genotoxic MoA for uterine adenocarcinoma.

- Direct endocrine/estrogenic activity

Sedaxane was investigated in the uterotrophic assay according to OECD TG 440. The substance was administered orally, by gavage, to one group of six young adult female ovariectomized Crl:WI (Han) rats once daily for three consecutive days at a dose level of 375 mg/kg bw/d. A positive control group of 6 ovariectomized rats received the oestrogenic positive control agent (17α-ethynylestradiol) in corn oil at a dose level of 0.3 mg/kg bw/day and a control group of 6 ovariectomized rats received the vehicle on a comparable regimen. As a result, mean body weight in this group was slightly (4.0%) lower than the control group on study day 3. Mean wet and blotted uterus weights in the 375 mg/kg bw/day group were similar to the control group values. The absence of effects on uterus weights demonstrated a lack of oestrogenicity for the test substance at the dose level evaluated. For the positive control, increases in mean wet and blotted uterus weights (7.1 and 3.9-fold, respectively) were noted compared to the control group. In conclusion, sedaxane was considered to be negative for oestrogenicity in the uterotrophic assay. RAC agrees to this conclusion. Although no oestrogen-receptor-binding studies have been conducted, the uterotrophic assay is highly sensitive assay for detection of *in vivo* estrogenic activity of an oestrogen/chemical (either parent or metabolite). Sedaxane has been tested with 375 mg/kg bw/d, which exceeds the carcinogenic dose level of the 2-year study (3600 ppm: 218/261 mg/kg bw/d males/females). It is also noted by RAC, that for an oestrogenic mechanism, focal glandular endometrial hyperplasia is an obligatory pre-neoplastic precursor lesions. For sedaxane, no such hyperplasia is reported. Then, for sufficiently high treatment levels, leading to oestrus cycle disruption, vaginal cytology and morphology show persistent oestrus and cornification. No increase in cornification and no increase in persistent oestrous is reported for sedaxane in the 2-years study (notable insufficient data is available on oestrous cycling).

RAC concludes that direct oestrogen-activity as a MoA for uterine adenocarcinoma is not supported by the data.

- Modulation of oestrogen metabolism via induction of CYP and related oxidative stress

In the liver and other tissues, oestrogens are converted to 2- or 4-hydroxylestradiol (2-HE, 4-HE), catechol oestrogens, by oxidative drug metabolising enzymes such as CYP1A1 and CYP1B1. CYP induction therefore can modulate oestrogen metabolism and 4-HE has a suspected role in carcinogenesis. In a 28-day rat study (Annex I, 3.12.1), sedaxane has been demonstrated to increase liver weights and induce liver centrilobular hypertrophy (of adaptive type). However, liver hypertrophy is a commonly observed event and not specific for this MoA and as such not a sufficient indication. The substance also increased the metabolic capacity of the liver, being a potent CYP2B inducer based on hepatic PROD activity and increased 16β hydroxylation of testosterone, immunoblotting showed increased levels of CYP2B and CYP3A, and 2- and 6β hydroxylation of testosterone also supported CYP3A activity. However, only a weakly increased EROD activity indicated that CYP1A/1B induction was unlikely. *In vitro*, treatment of isolated male Han Wistar rat hepatocyte cultures with sedaxane resulted in increases in PROD and BROD activities, again, mainly representative of CYP2B and CYP2B/3A induction (Annex I of the CLH report, 3.9.4.5). In isolated male human hepatocyte cultures from one donor, treatment with sedaxane BROD activity was induced, and PROD activity was unaffected by treatment (Annex I, 3.9.4.6). Analysis of increased 4-HE in the blood would be a direct evidence for this mechanism, but such data is not available.

RAC concludes that the data provide no evidence for CYP-induction mediated modulation of oestrogen metabolism and ROS formation.

RAC considers furthermore the following alternative mechanisms:

- Increase in oestrogen:progesterone ratio

Indirect senescent or chemically-induced imbalance in sex steroid hormones in the ovary leads to decrease of both oestrogen and progesterone and a status similar to a high oestrogen status may manifests by persistent oestrous in vaginal cytology, an atrophic ovary with cystic atretic follicles, lack/few corpora lutea, cornification of the cervix/vagina mucosa, and/or squamous metaplasia of endometrial epithelial cells. Atypical precancerous hyperplasia may be increased (Yoshida *et al.,* 2015; Cruz *et al.,* 2017). For sedaxane no oestrous cycle staging study with vaginal lavage at regular time intervals is available, only the 2-years study where for the animals dying between 52 and 104 weeks in the histopathology re-evaluation persistent oestrous was virtually absent (Annex I, 3.9.4.11). In this study, as compared to the control group, no increase in cornification, metaplasia, precancerous hyperplasia, was recorded, and no decrease in corpora lutea and no ovary atrophy was reported, but an increase in atretic follicles was apparent for the animals dying from 52-104 weeks (30/63, 49/63, 56/61 for control, 1200 ppm, 3600 ppm, respectively). In the 2-generation reproduction study, ovarian atrophy and decrease in corpora lutea was noted. Importantly for clarification, no oestrous-cycle-sensitive hormone measurements on oestrogen:progesterone ratio are available.

RAC considers that the data available do not allow a firm conclusion on the role of this MoA due to insufficient data.

- Modulation of oestrogen excretion

A decrease in oestrogen excretion and related increase in oestrogen blood levels can be related to test substance ADME (Yoshida *et al*., 2015; Sanders *et al.,* 2016; Mungenast *et al.,* 2016) and oestrogen metabolism and excretion might be modulated *in situ*. Sedaxane does reach the uterus as seen in the toxicokinetic studies, but no specific data are available, such as oestrogen levels and phase 2 enzyme induction and activity.

Therefore, no conclusions are possible on this MoA.

- Increased *in situ* aromatase

According to Yoshida *et al.* (2015), this is a mechanism with no evidence so far in rodents, but human relevance has been demonstrated with increased protein and mRNA expression for aromatase (and related *in situ* oestrogen production) in epithelial stromal cells in endometrial carcinomas (Watanabe *et al.* 1995). Aromatase is a key factor for mammary carcinogenesis.

Based on the tumour profile of sedaxane (tumour shift with decrease in mammary tumours) such pathway is unlikely.

- Cytotoxicity / regenerative cell proliferation

Chronic cytotoxicity with subsequent regenerative cell proliferation is considered a mode of action by which tumour development can be enhanced. For sedaxane no persistent inflammation/cytotoxicity and regenerative hyperplasia is evident in the repeated dose and carcinogenicity studies. Carcinogenesis due to excessive toxicity and necrosis that would trigger regenerative proliferation is unlikely as there is no histopathological hint for this mechanism. Sedaxane is not irritating and apart from body weight reduction, no clinical signs indicative of excess toxicity was evident in the 2-years study.

RAC concludes that the data do not support a cytotoxicity-mediated MoA.

- Inhibition of Succinate Dehydrogenase Inhibition

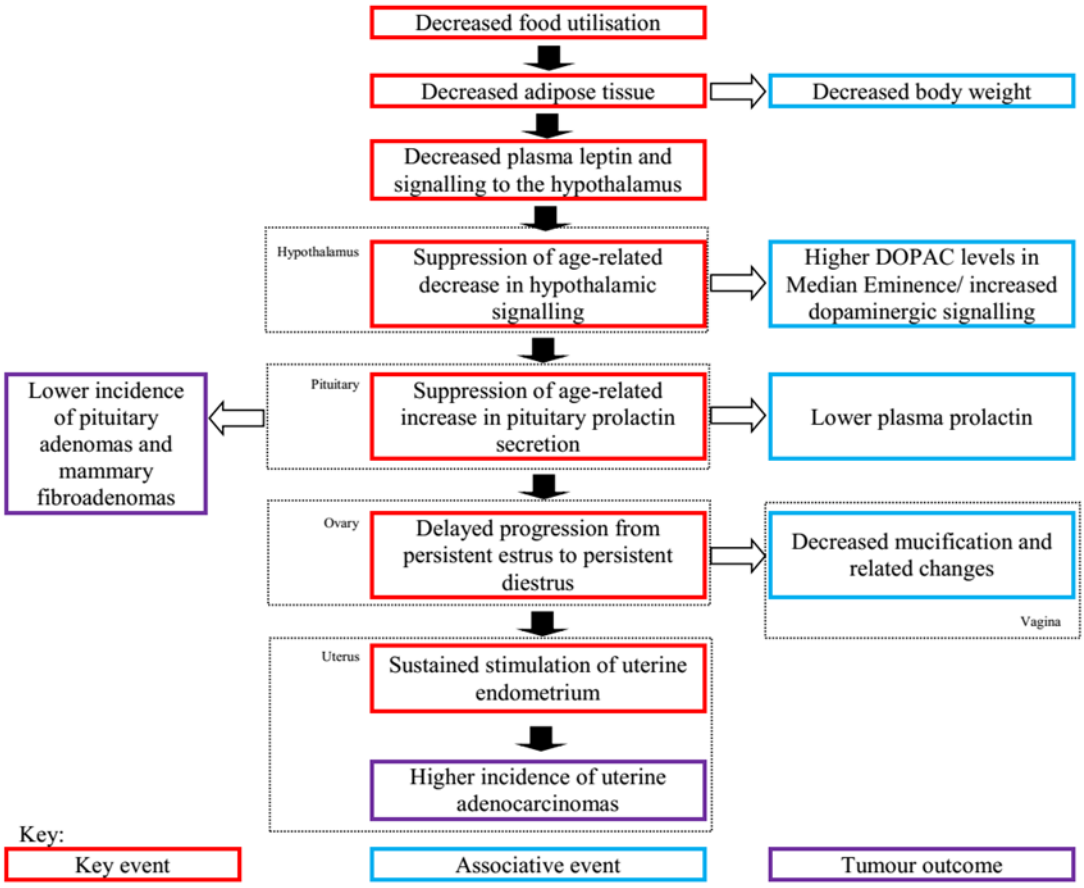
Concerning alternative MoA, the dossier submitter raised the possibility that the molecular mechanism of tumour formation might be SDH inhibition. This idea was based on the observation, that the SDHI isopyrazam also induced uterine adenocarcinoma at a comparable dose level and this was also associated with significant loss of body weight. Recently, Bénit *et al.* (2018) published that SDHIs readily inhibited the human enzyme of respiratory chain complex II. This

is seen as a concern because human germline mutations in one of the four genes encoding SDH subunits have been observed to result in different types of cancer, and epigenetic modifications due to long-term succinate accumulation rather than random mutations seem associated with this. RAC notes that the carcinogenicity study on isopyrazam is not subject of the current assessment, which hampers the conclusion on such association. RAC further notes that other SDHI have been assessed by RAC in the past. While apparently the liver and thyroid is a common target of these substances (in general liver is a frequent target of toxic compounds and the observation therefore might be of unspecific nature), uterine tumours as observed for sedaxane and isopyrazam are not a common effect. No specific data are available to analyse a relevance of succinate accumulation acting as an oncometabolite.

RAC concludes that SDHI as the relevant MoA cannot be assessed.

In summary, a prediction of an alternative MoA is not possible and the MoA remains undefined (see decision tree in Yoshida *et al.,* 2015).

Figure: *Proposed MoA for uterine tumours in rats*



In conclusion on the MoA for uterine adenocarcinoma*, RAC considers that the available data on the MoA of uterine adenocarcinoma are insufficient to support the by the applicant-postulated MoA. The remaining uncertainties are considerably high, such that no human relevance assessment seem warranted. This conclusion is in line with the dossier submitter. Regarding alternative MoA, a prediction is not possible and the MoA remains undefined.*

Comparison with the criteria

Carcinogenic potential of sedaxane has been observed with increased incidences of three types of tumours in two species: malignant uterine tumours and benign thyroid tumours in rats, higher

incidences of hepatocellular adenomas in male rats and in male mice and hepatocellular carcinomas in male mice.

The dossier submitter considered that liver tumours observed in male rats and male mice at high dose levels as well thyroid adenomas observed in male rats at high dose levels do not trigger classification for carcinogenicity taking into account that an underlying CAR-mediated MoA is substantiated by the available data, the tumour outcome considered of limited relevance to humans. As regards to uterine tumours, in the absence of an established MoA, classification for carcinogenicity is warranted. While these tumours are malignant and also observed in one structurally similar compound, the DS considered classification as Carc. 2; H351 as appropriate since the uterine tumours were limited to a very high dose level in a single species.

According to Regulation (EC) No 1272/2008 a substance is classified for carcinogenicity, *Category 1 - Known or presumed human carcinogens on the basis of epidemiological and/or animal data.*

- *Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or*
- *Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.*

Category 2 - Suspected human carcinogens on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations.

RAC is of the opinion that classification in category **Carc. 1A is not warranted**. According to the CLP criteria for carcinogenicity Category 1A, *known to have carcinogenic potential for humans*, classification is largely based on human evidence. For sedaxane, no information on carcinogenicity in humans is available.

According to the CLP criteria (Annex 3.6.2.2.3) for Category 1B *"sufficient evidence of carcinogenicity", a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence […]".*

Placing of a substance in category 2 is done on the "*basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies".*

Based solely on a combination of benign and malignant neoplasms in two species, a classification as Category 1B could be argued. However, there were several additional factors that were considered by RAC when assessing the overall level of concern:

- Background incidences

For malignant uterine tumours in rats, control incidences were zero and although the top dose tumour incidence with 17% was just inside the HCD range (0-19%), the distribution of HCD (mean of 7%) indicate that the effects are treatment-related.

The higher incidences of hepatocellular adenomas (10% vs 2% in control), thyroid follicular cell adenomas (15% vs 6% in control) and thyroid adenoma/carcinoma combined (17% vs 6% in control) in males at the top dose in rats clearly exceeded the concurrent control incidences and also the HCD from the testing laboratory.

Considering the background variability, the incidence of liver tumours in male mice are of minor concern. But for carcinoma, the incidence in the high dose (20%) exceeded the concurrent (10%) and historical control data (6-10%).

- Multi-site responses

In male rats increased incidences of both liver and thyroid adenoma were observed. While in female rats and male mice only one organ was affected, uterus and liver respectively.

- Progression to malignancy

Malignant tumours, uterine adenocarcinomas, were increased at the top dose in rats. There was no increase of adenoma incidence or pre-neoplastic lesions. In male rats, liver tumour were limited to adenoma with no progression to malignancy, however in male mice both hepatocellular adenomas and hepatocellular carcinomas incidences were increased at the top dose. For the thyroid only benign adenoma were increased in male rats at the top dose.

- Single or both species

Uterine tumours were only observed in rats. No uterine lesions, including pre-neoplastic lesions or non-neoplastic lesion were observed in any other tested species in the repeated dose studies (mice, dogs), which decreases the concern. Liver tumours were observed in two species, rats and mice. For thyroid tumours only male rats were affected.

- Single or both sex

Tumour types were only observed in one sex. Uterine tumours are obviously limited to females. Liver and thyroid tumours were only observed in males.

- Confounding effects of excessive toxicity

The malignant uterine tumours, and thyroid adenoma, and liver adenoma in rats occurred at doses with markedly reduced body weight (gains). Animals treated at the tumorigenic high dose of 3600 ppm showed a consistent and lower body weight and weight gain compared to their respective controls throughout the treatment period. The reduced cumulative body weight gain throughout the study in the high dose represented a maximum of 23.5% decrease in males and 49.6% decrease in females at termination (reduction of terminal body weight in females by 37%). However, RAC considers the MTD not exceeded and the findings relevant for classification and labelling. No other signs of severe toxicity were apparent and there is no general (causal) association of body weight reduction with higher tumour incidences. According to the CLH dossier, in addition it is considered that the structural analogue isopyrazam also induced increased incidences of uterine adenocarcinoma in rats (EFSA, 2012). This may indicate a specific effect.

For the liver adenoma and carcinoma in mice, no excessive toxicity was apparent.

- Reduced tumour latency

There was no evidence of reduced latency for any kinds of tumours. For uterine tumours, control incidence was zero.

- Mode of action and its relevance for humans

According to the EU specialised experts (1999), classification for thyroid tumours in rodents was not recommended for non-genotoxic substances causing thyroid tumours mediated by UDP glucuronyltransferase (UGT) induction. For thyroid tumours, RAC concludes that the sequence of events for the MoA proposed by the DS, i.e. an induction of the UDP glucuronyltransferase

(UDPGT) leading to decrease in serum T4 and T3 levels and a compensatory increase in TSH that would in turn result in thyroid hyperplasia and tumours, is overall supported, however uncertainties remain as not all events were fully demonstrated. Some alternative MoAs have been ruled out, but not all. However, tumours were benign and only observed in rats in the high dose level, and RAC concludes that these thyroid adenomas do not warrant classification for carcinogenicity taking into account that a CAR-mediated MoA via UGT induction is likely based on the available data. Such MoA is considered less relevant for humans.

Concerning liver tumours in rats and mice, RAC concludes that the liver adenomas in rats and mice together with the carcinoma in mice most likely are caused by a CAR-mediated MoA. However, still significant uncertainties remain, as the data not sufficiently support this hypothesis, mainly as no assay in CAR-Knock-Out mice has been performed, and in the human hepatocyte assay on cell proliferation, only one human donor has been used in order to demonstrate that the adverse outcome would not be relevant for humans. The liver tumours therefore are considered in the overall weight-of-evidence assessment.

As regards to the uterine tumours, in the absence of an established MoA, RAC concludes that sedaxane warrants classification for carcinogenicity. These tumours are malignant and endometrial cancer is considered highly relevant for women, both in humans and in rodents this type of cancer is an oestrogen sensitive lesion. The mode of action of sedaxane has not been conclusively investigated.

RAC considers the uterine neoplastic lesions together with the remaining uncertainty related to the missing or insufficient mechanistic data package for the liver carcinoma in mice. Uterine adenocarcinoma were reported only in the top dose of one species, accompanied by marked decrease in body weight gain. In addition, sedaxane is unlikely to be genotoxic. RAC considers therefore that classification in Category 1B is not justified and that the overall pattern of effects justifies downgrading classification.

In a weight-of-evidence approach, RAC agrees with the dossier submitter's proposal and recommends **classification of sedaxane as Carc. 2; H351 (suspected of causing cancer).** As it has not been proven that no other routes of exposure cause the hazard, the route of exposure should not be stated.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Sedaxane was tested in HanWistar rats in an oral two-generation reproductive toxicity study according to OECD TG 416, and GLP compliant, at 200, 500 or 1500 ppm, equal to 16, 41 or 120 mg/kg bw/d. The test included an investigation of endocrine disrupting properties. Fertility and reproductive performance were not affected by treatment up to the highest dose that induced parental toxicity. No adverse effects were observed that would warrant classification for fertility, and this position of the dossier submitter is in line with the EFSA conclusion. In the F1 and F2 offspring, body weights from PND 14 were reduced, which could reflect toxicity of the test substance by direct intake by the pups, and suggesting a similar sensitivity for the offspring and adults. Similar to parental animals, adjusted liver weights were increased in the F1 offspring at the high dose.

Two developmental toxicity studies according to OECD TG 414, and GLP compliant, are available, one in rats and one in rabbits, both at concentrations of 25, 100, and 200 mg/kg bw/d administered by oral gavage. The results showed no potential for sedaxane to induce foetal death or teratogenic effects. In rabbits, a 13th full rib, considered a foetal variation, and 9% decrease

in foetal weights as compared to controls was observed in the presence of maternal toxicity. The dossier submitter did not propose classification for developmental toxicity due to the nature of the effects observed in high-dose rabbit foetuses and the concurrent maternal toxicity.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

Sexual function and fertility

In the two-generation reproduction toxicity study, the mid dose of 41 mg/kg bw/d gave the NOAEL for parental toxicity. The high dose of 120 mg/kg bw/d was the NOAEL for toxicity for sexual function and fertility and 41 mg/kg bw/d was the NOAEL for developmental toxicity.

The main effects observed in the parental generations in the high dose included reductions in body weights as compared to controls with changes of generally less than 10% and rarely statistically significant for males, but often significant for females of both generations throughout the study. As compared to controls, the body weight gain reduction in females during the pre-mating period was 15% in P generation, but less than 5% in F1 parental females. Reduced food consumption in females in both P and F1 generations and in P males, increased liver weights and centrilobular hypertrophy in P and F1 animals of both sexes, as well as increased thyroid weights and thyroid follicular hypertrophy in P and F1 males were apparent. In the mid dose, liver weights were only slightly increased by 9% and 6% for males in P and F1 generation, respectively, without histopathological correlates.

Reproductive performance was not affected by treatment at any dose level as parameters such as mating index, fertility indexes, number of pups at birth, or litter size were not different between the treated and control groups.

In the high dose females of the P and F1 generation, effects on the reproductive organs were observed. Absolute (P: -14%; F1: -19.5%) and adjusted ovary weights (F1, -15.5%) were reduced and the reduction was apparent at both ovary sites. The number of corpora lutea and the number of antral follicles were significantly reduced for P and F1 generations. Absolute and adjusted uterus weights were reduced (statistically significant for F1) and the number of females in the lactational dioestrus at the time of termination was increased in P and F1 generations.

Effects on the female reproductive organs may be due to a direct organ-toxic effect or due to primary or secondary – stress-related - endocrine disturbance. The latter could be a result of reduced food consumption and decreased body weight (Everds *et al.,* 2013). The DS suggested that the effects of sedaxane on ovary weights and the decreased number of ovarian follicles could be secondary to decreased body weights, and the delay in returning to oestrous cycling could be related to the prolonged nursing stimulus by the pups. To assess the hypothesis by the DS, a careful analysis of the individual animal data, which is not available to RAC, would be required. RAC notes, however, that the body weight changes were rather mild indicating only mild stress that was unlikely to be the cause of the reduction in uterus and ovary weights and of the decrease in the number of ovarian follicles and corpora lutea. For example, in female rats, a dietary restriction leading to a > 16% decrease in mean body weight compared to controls has been associated with persistent dioestrus, decreased number of corpora lutea, and decreased fertility (likely due to the decreased corpora lutea; Terry *et al.,* 2005). On the basis of minor changes seen in the oestrous cyclicity, decreases of 10 to 15% in body weight gain were considered not to cause adverse effects on sexual function and fertility in the female rat. Studies in rats evaluating the effects of feed restriction have also demonstrated that female body weight must

be reduced to approximately 70% of that in controls before the ovary weights will decrease (Chapin *et al*., 1993; Seki *et al.,* 1997). The effects on female body weight observed in the available 2-generation study on sedaxane were not of similar magnitude. In the repeated dose toxicity studies, no findings were recorded that would suggest specific toxicity on reproductive organs. Moreover, the changes observed in the 2-generation study were rather moderate and not associated with compromises in sexual function and fertility.

The mean time to vaginal patency (VP) was increased from day 32.5 to day 34.2 in the F1 offspring at the top dose. In F2 female offspring the mean anogenital distance was statistically significantly increased by 8% as compared to control. No adverse effects on the offspring were observed at the lower doses. In general, a delay in puberty could be related to a general growth retardation as the VP, the primary sexual development landmark for females, can positively correlate with the body weight. However, in the absence of body weight changes, differences of 2.0 days or more is a general indicator for test substance-related toxicity. For sedaxane, the delay of less than 2 days was statistically significant in the F1 pups. The body weights of pups were reduced as compared to controls, but only between PND 14 to 21, and at the time of sexual maturation the body weights of pups were similar compared to control as stated in the CLH dossier (no body weight data at the time of vaginal opening is available in the CLH dossier). No data on VP were recorded for F2 pups, thus it cannot be judged whether both generations would be consistently affected. In addition, only the high dose in F1 showed the delay in VP. Difference in the mean litter anogenital distance (AGD) of 5% or greater is an indicator of toxicity and the primary landmark for sexual development (the most frequent effects on AGD are observed in response to antiandrogenic agents or 5a-reductase inhibitors) in addition to the balanopreputial separation in males. The 8% sedaxane-associated increase in AGD in F2 female pups is of unclear biological significance. No data were recorded for F1 offspring, no effects were observed in F2 male pups, and the increase in F2 females is only slight.

Overall, it can be stated that the effects on the female sexual maturation and offspring development were only apparent at the top dose without any impact at lower doses where no general systemic toxicity or growth retardation in the offspring was observed. Reproductive performance was not affected at any of the tested dose level. However, RAC notes that in this study a top dose level of 1500 ppm (corresponding to 120 mg/kg bw/d) was administered, which induced only limited parental toxicity. According to OECD TG 416 the highest dose level should be chosen with the aim to induce toxicity but not death or severe suffering, taking into account any existing toxicity data. According to the CLH report, Annex I, 3.10.1, dietary concentrations were based on the results of a single generation study (not included in the CLH report) and long term feeding studies in rats. To compare, in the 2-year chronic study a dietary level of 3600 ppm was applied as the top dose, not inducing severe toxicity, suffering or death. Further, RAC takes note of the study summary of a single-generation dose-range finder reproductive toxicity study[1], with nominal dose levels of 500, 1500, and 3600 ppm for 10 weeks before pairing until weaning of the F1 generation. It was concluded that, based on the results of this single-generation range finder and the 2-year chronic study, the top dose chosen in the two-generation study is too low. This leaves uncertainties on the informative value of the chosen dosing regimen. With reference to the criteria set out in Annex I, 3.7.2.2 of CLP, RAC therefore concludes that **no classification is warranted for sexual function and fertility** based on inconclusive data.

[1] SEDAXANE 769–839 JMPR 2012, *who.int/pesticide-residues-jmpr-database/Document/58*

Developmental toxicity

According to the CLH report, Annex I, 3.10.1.3, the dosing regimen of the rabbit developmental toxicity studies was selected based on a range finder tolerability study in non-pregnant (day 0 to 9; 250, 500, 750, 1000 mg/kg bw/d) and in pregnant rabbits (day 7 to 28; 100, 300, 500 mg/kg bw/d). Administration to pregnant rabbits resulted in moribund condition at 500 mg/kg bw/d, body weight loss and reduced food consumption at 300 and 500 mg/kg bw/d, and a trend for increased liver weights was noted at 100, 300, 500 mg/kg bw/d. There was no evidence of developmental toxicity at any dose level.

Based on the range finder results, dose levels of 25, 100, 200 mg/kg bw/d were selected for the main study.

In rabbits, the NOAELs for maternal and developmental toxicity were the mid dose of 100 mg/kg bw/d. Maternal toxicity encompassed defaecation and increased (adjusted) liver weights. Body weights were not different between the groups, but the weight gain was statistically significantly reduced in the first week of treatment. In the offspring, foetal examination revealed 8-9% lower mean foetal weights at 200 mg/kg bw/d in males, females and combined sexes, being statistically significant in females, and considered treatment-related. On the other hand, such rather slight reductions < 10% in intrauterine growth in the presence of maternal toxicity are of minor concern and do not warrant classification. No effects on intrauterine growth and development were observed at 25 and 100 mg/kg bw/d. At 200 mg/kg bw/d, a decrease in the foetal incidence of unossified #5 and/or #6 sternebrae was within the historical control range and is considered by RAC a normal variation in this species. Statistically significant increase in the foetal incidence of skeletal variations included the 13^{th} full ribs at 200 mg/kg bw/d. RAC considers that the increased incidence of full 13^{th} ribs is a developmental variation and presents no toxicological or teratogenic concern that would warrant classification. In rabbits this variation is common. In the sedaxane-treated groups, there was no major or minor malformations, foetal deaths or functional impairment.

For rats, the dose levels were also selected based on a dose range-finding toxicity study in Han Wistar rats. A dose of 200 mg/kg bw/d resulted in decreased maternal body weight gain and food consumption and was therefore expected to produce some effects on maternal body weight and food consumption without excessive toxicity in the main study (CLH report, Annex I, 3.10.1.2).

Based on the range finder results, dose levels of 25, 100, 200 mg/kg bw/d were selected for the main study.

In the rat study, the NOAEL for maternal toxicity was the low dose of 25 mg/kg bw/d and the developmental NOAEL was the high dose of 200 mg/kg bw/d. Maternal toxicity at 100 mg/kg bw/d constituted of moderate reduction of weight gain (statistically significant only on days 6-13 with 12.5%) and food consumption. Foetal weights of the female offspring were reduced by 4% at the high dose. No developmental toxicity was overt up to the highest dose level.

With reference to the CLP criteria, RAC concludes that no adverse effects on the developing organism, including death, structural abnormalities, altered growth or functional deficiency were associated with the exposure to sedaxane during pregnancy or as a result of parental exposure in developing rats or rabbits that would warrant classification. Therefore, RAC agrees with the dossier submitter that **classification for developmental toxicity is not warranted.**

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

Liquid substances and mixtures which contain hydrocarbons ≥ 10% and which show kinematic viscosity < 20.5 cSt (mm^2/s) should be classified. Sedaxane is a solid, therefore the classification criteria are not met.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC agrees with the dossier submitter that **sedaxane does not require classification with regards to aspiration toxicity.**

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS proposal was to classify sedaxane as Aquatic Acute 1 – H400 (Very toxic to aquatic life) with M-factor = 1 and Aquatic Chronic 2 – H411 (Toxic to aquatic life with long lasting effects).

Degradation

The DS's summary of relevant information on degradability:

Table: *Summary of studies on degradation*

Method	Results	Remarks	Reference
Ready biodegradability 28 days, 22°C, pH 7.2-7.6 Test substance: sedaxane (purity 95.3%) Test concentration = 101 mg/L OECD TG 301F GLP	No biodegradation in 28 days; Not readily biodegradable	43% degradation observed in the toxicity control, therefore no significant inhibitory effect	Seyfried, 2007
Hydrolysis, pH 4, 5, 7 and 9, 25°C, 30 days, dark. Test substance: [Phenyl-U-^{14}C] - sedaxane (Radiochemical purity 99.1%, chemical purity 99.6%) Nominal concentration = 0.0017 mg/mL OECD TG 111 EPA subdivision N-161-1 GLP	Prelim study at 50°C; < 10% degradation at pH 4, 5, 7 and 9 after 5 days. After 30 days at 25°C, sedaxane accounted for 95.9, 102.8 and 101.3% of applied radioactivity at pH 5, 7 and 9, respectively. $DegT_{50}$ > 1 year		Nicollier, 2007a
Direct and indirect photolysis pH 7, up to 34 days, 25 ± 2°C, sterile and natural water, Test substance: ^{14}C-phenyl and ^{14}C-pyrazole sedaxane (purity > 99%) Test concentration = *c.a.* 2.0 mg/L OECD draft guideline Aug 2000, JMAFF 12 Nousan no. 8147, 2001	Direct photolysis in sterile buffer; $DegT_{50}$ = 42, 52 and 71 days at 30, 40 and 50°N	Sedaxane level 57.3% AR after 34 days continuous irradiation (95.2% in dark controls) Total ^{14}C recoveries 98.1–101.4% (phenyl) and 91.5-99.2% (pyrazole)	Hand and Flemming, 2007

Method	Results	Remarks	Reference
EPA 540/9-82-021 GLP		Multiple degradates and minimal volatiles (max 1.8%)	
	Indirect photolysis in natural water; $DegT_{50}$ = 16.3, 16.5 and 17.1 days at 30, 40 and 50°N	Sedaxane level 23.9% AR after 28 days continuous irradiation (97.7% in dark controls) Total ^{14}C recoveries 94.5–107.6% (phenyl) and 98.2-105.4% (pyrazole) Multiple degradates and minimal volatiles (max 11.1% (phenyl only))	
Water-sediment degradation, aerobic (179 days) and anaerobic (360 days), 20 ± 1°C, dark, pond & river systems Test substance: ^{14}C-phenyl sedaxane (purity > 96%) Nominal concentration = 0.03 µg/mL OECD TG 308 EPA subdivision N-162-3 GLP	Total system $DegT_{50}$ Aerobic: Pond; >> 1 year River; >> 1 year Anaerobic: Pond; >> 1 year River; >> 1 year	For aerobic and anaerobic systems; CO_2 evolution ≤ 2.0% AR Total mean recoveries 93.9-105.2%	Stoll and Nicollier, 2008
Soil adsorption/desorption, 6 soils Test substance: ^{14}C-phenyl sedaxane (purity > 99%) OECD TG 106 EPA subdivision N-163-1 GLP	Mean $K_{FOC(ads)}$ = 534L/kg for all soils (range 262-666 L/kg) Mean $K_{FOC(des)}$ = 704L/kg for all soils (range 367-907 L/kg)	Total ^{14}C recovery 90-110% in all soils	Nicollier, 2008

Sedaxane is not readily biodegradable (Seyfried, 2007) and is hydrolytically stable (Nicollier, 2007a).

One relevant study on the degradation of sedaxane in aquatic water-sediment systems (Stoll and Nicollier, 2008) shows primary degradation half-lives >> 1 year and negligible (< 2%) CO_2 evolution.

Overall, based on the data available, sedaxane is considered not to be rapidly degradable for classification purposes.

Bioaccumulation

Table: *Summary of relevant information on bioaccumulation*

Substance	Species	Test guidelines	Endpoint	Value	Condition	Reference
^{14}C-sedaxane purity 95.2%, radiochemical purity 99.1%	Rainbow trout (*Oncorhynchus mykiss*)	OECD TG 305	BCF (whole fish)	97	Flow-through, 14 day uptake and 14 day depuration 0.5 µg/L + solvent (DMF) control. pH 7.24-7.69 15.1-15.3°C GLP	Anonymous, 2010 *Annex I. 4.2.1.1*

The experimentally derived Log Kow of sedaxane is 3.3. For classification and labelling purposes, a substance with Log Kow < 4 may be considered unlikely to bioaccumulate in aquatic organisms. This is the case for sedaxane (Log Kow = 3.3). This is also supported by the measured BCF value of 97 L/kg which is below the trigger value of 500 L/kg according to CLP criteria. A measured

BCF ≥ 500 indicates a potential for bioaccumulation. Since the BCF for sedaxane is < 500, it is considered not to be bioaccumulative for the purpose of classification and labelling.

Acute aquatic hazard

The summary of acute aquatic toxicity, as presented by the DS is presented below.

Table: *summary of the acute aquatic toxicity studies*

Substance	Species	Test guidelines	Endpoint	Toxicity value (mg/L)	Conditions	Reference
Fish						
Sedaxane (purity 95.3%)	*Cyprinus carpio* (Common carp)	OECD TG 203 OPPTS 850.1074	96h LC_{50}	0.62 mg/L (mm)	96 h static test. Dilution water control. pH 8.3 – 8.5 21.5 – 22.1°C GLP	Anonymous, 2008a ***SYN524464_11104***
Sedaxane (purity 95.3%)	*Oncorhynchus mykiss* (Rainbow trout)	OECD TG 203 OPPTS 850.1074	96h LC_{50}	1.1 mg/L (mm)	96 h static test. Dilution water control. pH 8.2 – 8.4 13°C GLP	Anonymous, 2008 ***SYN524464/0067***
Sedaxane (purity 98.2%)	*Pimephales promelas* (Fathead minnow)	OECD TG 203 OPPTS 850.1075	96h LC_{50}	0.98 mg/L (mm)	96 h static test. Dilution water control. pH 7.59 – 8.29 24.1 – 24.5°C GLP	Anonymous, 2006 ***SYN524464/0012***
Sedaxane (purity 95.3%)	*Cyprinodon variegatus* (Sheepshead minnow)	OPPTS 850.1075	96h LC_{50}	4.2 mg/L (mm)	96 h static test. Dilution water control. pH 8.0 – 8.3 21.8 – 22.7°C GLP	Anonymous, 2008b ***SYN524464/0062***
Aquatic Invertebrates						
Sedaxane (purity 95.3%)	*Americamysis bahia* (saltwater mysid)	OPPTS 850.1035	96h LC_{50}	1.5 mg/L (mm)	96 hour static test Dilution water control. pH 8.1 – 8.2 24.9– 25.4°C (start) 25.2 – 25.3°C (end) GLP	Gallagher *et al.*, 2008c ***SYN524464/0059***
Sedaxane (purity 98.2%)	*Daphnia magna* (Cladoceran)	OECD TG 202	48h EC_{50}	6.10 mg/L (mm)	48 hour static test Dilution water control. pH 7.50–7.59 20.7 – 21.2°C GLP	Ricketts and Paddick, 2006 ***SYN524464/0011***
Algae and aquatic plants						
Sedaxane (purity 95.3%)	*Pseudokirchneriella subcapitata* (freshwater green alga)	OECD TG 201	72h E_bC_{50} 72h E_rC_{50} 72h E_yC_{50}	1.9 mg/L 2.8 mg/L 1.6 mg/L (mm)	96 hour static Culture medium control pH 8.2 (start) 8.1 - 9.2 (end) 22 - 23°C GLP	Bätscher, 2007a ***SYN524464/0037***
			96h E_bC_{50} 96h E_rC_{50} 96h E_yC_{50}	1.9 mg/L 3.0 mg/L 1.8 mg/L (mm)		

Sedaxane (purity 95.3%)	*Navicula pellicola* (Freshwater diatom)	OECD TG 201	72h E_bC_{50} 72h E_rC_{50} 72h E_yC_{50}	4.8 mg/L 8.7 mg/L 4.8 mg/L (mm)	96 hour static Culture medium & filtrate control. pH: 7.4 (start) 7.7 – 9.2 (end) 23ºC GLP	Büche, 2007a ***SYN524464/0044***
			96h E_bC_{50} 96h E_rC_{50} 96h E_yC_{50}	5.3 mg/L 10 mg/L 5.7 mg/L (mm)		
Sedaxane (purity 95.3%)	*Anabaena flos-aquae* (freshwater Cyanobacteria)	OECD TG 201	72h E_bC_{50} 72h E_rC_{50} 72h E_yC_{50}	> 6.5 mg/L > 6.5 mg/L > 6.5 mg/L (mm)	96 hour static Culture medium & filtrate control. pH: 8.5 (start) 9.0-9.1 (end) 22 - 23°C GLP	Büche, 2007b ***SYN524464/0045***
			96h E_bC_{50} 96h E_rC_{50} 96h E_yC_{50}	> 6.5 mg/L > 6.5 mg/L > 6.5 mg/L (mm)		
Sedaxane (purity 95.3%)	*Skeletonema costatum* (Marine diatom)	OECD TG 201	72h E_bC_{50} 72h E_rC_{50} 72h E_yC_{50}	> 6.0 mg/L > 6.0 mg/L > 6.0 mg/L (mm)	96 hour static Culture medium control. pH: 8.1-8.1 (start) 8.4-8.5 (end) 19.8 – 20.5ºC GLP	Minderhout *et al.*, 2007 ***SYN524464/0058***
			96h E_bC_{50} 96h E_rC_{50} 96h E_yC_{50}	> 6.0 mg/L > 6.0 mg/L > 6.0 mg/L (mm)		
Sedaxane (purity 95.3%)	*Lemna gibba* (duckweed)	OECD TG 221	<u>Frond No</u> 7d E_rC_{50} 7d E_yC_{50}	6.5 mg/L 3.6 mg/L (mm)	7 day semi-static Dilution water control. pH: 7.3–7.4 (start) 8.6 - 9.0 (end) 23°C GLP	Bätscher, 2007b ***SYN524464/0039 ****
			<u>Dry Weight</u> 7d E_rC_{50} 7d E_yC_{50}	4.8 mg/L 2.7 mg/L (mm)		

Based on the results from the four available and reliable experimental studies on fish, the lowest acute toxicity value has been derived for *Cyprinus carpio* (Common carp), with an LC_{50} value of 0.62 mg/L).

The 48 hour EC_{50} value in daphnia was 6.10 mg/L. The lowest EC_{50} of 1.5 mg/L in the saltwater mysid shrimp (Gallagher *et al.*, 2008c) is considered appropriate to use for classification of acute toxicity to aquatic invertebrates.

The lowest EC_{50} value (72h ErC_{50}) in freshwater green algae (*Pseudokirchneriella subcapitata*) of 2.8 mg/L (Bätscher, 2007a) is considered appropriate to use for classification of acute toxicity to algae and aquatic plants.

On this basis, the following acute classification and labelling of sedaxane is proposed by the dossier submitter:

Aquatic Acute 1 – H400 (Very toxic to aquatic life); as the lowest L(E)C_{50} (= 0.62 mg/L) is between 0.1 and 1.0 mg/L, the associated M-factor is 1.

Long-term aquatic hazard (including bioaccumulation potential and degradation

The endpoints for chronic aquatic toxicity endpoints relevant for classification of sedaxane are summarised in the table below:

Table

Substance (purity)	Species	Test guidelines	Endpoint	Toxicity value	Conditions	Reference
Fish						
Sedaxane (95.3%)	*Pimephales promelas* (Fathead minnow)	OECD TG 210 OPPTS 850.1400	21 days LC_{50} NOEC	0.469 mg/L 0.165 mg/L (mm)	33 days flow-through (28 days post hatch) dilution water and solvent control. pH 8.1 - 8.3 23.9 – 25.4°C GLP	Anonymous, 2008d ***SYN524464/0065*** *Annex I. 4.4.1.1*
Aquatic Invertebrates						
Sedaxane (95.3%)	*Daphnia magna*	OECD TG 211	21 days EC_{50} (reproduction) 21 days NOEC (survival and reproduction)	1.5 mg/L 0.82 mg/L (nom)	21 days semi-static culture medium and solvent control. pH 7.6 - 8.1 20°C GLP	Bätscher, 2007c SYN524464/0038
Algae and aquatic plants						
Sedaxane (purity 95.3%)	*Pseudokirchneriella subcapitata* (freshwater green alga)	OECD TG 201	72h NOE_rC 96h NOE_rC	1.0 mg/L (mm) 1.0 mg/L (mm)	96 hours static Culture medium control pH 8.2 (start) 8.1 - 9.2 (end) 22 - 23°C GLP	Bätscher, 2007a ***SYN524464/0037***
Sedaxane (purity 95.3%)	*Navicula pellicola* (Freshwater diatom)	OECD TG 201	72h NOE_rC 72h E_rC_{10} 96h NOE_rC 96h E_rC_{10}	2.4 mg/L 4.3 mg/L (mm) 2.4 mg/L 5.3 mg/L (mm)	96 hours static culture medium & filtrate control. pH: 7.4 (start) 7.7 – 9.2 (end) 23ºC GLP	Büche, 2007a ***SYN524464/0044***
Sedaxane (purity 95.3%)	*Anabaena flos-aquae* (freshwater Cyanobacteria)	OECD TG 201	72h NOE_rC 96h NOE_rC	4.3 mg/L (mm) 4.3 mg/L (mm)	96 hours static culture medium & filtrate control. pH: 8.5 (start) 9.0 - 9.1 (end) 22 - 23°C GLP	Büche, 2007b ***SYN524464/0045***
Sedaxane (purity 95.3%)	*Skeletonema costatum* (Marine diatom)	OECD TG 201	72h NOE_rC 96h NOE_rC	6.0 mg/L (mm) 6.0 mg/L (mm)	96 hours static culture medium control. pH: 8.1 - 8.1 (start) 8.4 - 8.5 (end) 19.8 – 20.5ºC GLP	Minderhout *et al.*, 2007 ***SYN524464/0058***

Sedaxane (purity 95.3%)	*Lemna gibba* (duckweed)	OECD TG 221	<u>Frond No</u> 7d NOEC 7d E_rC_{10}	0.59 mg/L 2.4 mg/L (mm)	7 days semi-static Dilution water control. pH: 7.3 – 7.4 (start) 8.6 - 9.0 (end) 23°C GLP	Bätscher, 2007b ***SYN524464/0039***
			<u>Dry Weight</u> 7d NOE_rC 7d E_rC_{10}	1.2 mg/L 1.5 mg/L (mm)		

One study is available on the long-term toxicity of sedaxane to fish referring to a toxicity study to the early life-stages of fathead minnow (*Pimephales promelas*), which derived a NOEC value of 0.165 mg/L. There were no statistically significant treatment-related effects on hatching success at any of the concentrations tested. There were also no statistically significant treatment-related effects on survival or growth at the 6.1, 18, 56 and 165 µg/L test concentrations. There was a statistically significant reduction in survival at the 469 µg/L test concentration that resulted in 100% mortality for this treatment group. Based on mean measured concentrations, the 33 days NOEC for sedaxane to early life-stages of fathead minnow (*Pimephales promelas*) was 165 µg/L, resulting from effects on fry survival.

Effects of sedaxane on Pimephales promelas:

Table

Mean measured concentration (µg/L)	**Quantal responses**		**Non quantal responses**		
	Hatching success (%)[1]	**Fry survival to test end (%)[2]**	**Mean length (mm) ± SD**	**Mean wet weight (mg) ± SD**	**Mean dry weight (mg) ± SD**
Control	100	86	19.8 ± 0.59	53.1 ± 4.71	9.6 ± 0.89
Solvent control	96	83	20.5 ± 0.65	63.8 ± 7.74	11.1 ± 1.41
Pooled control	98	85	20.2 ± 0.70	58.4 ± 8.23	10.3 ± 1.34
6.1	96	91	19.8 ± 0.10	55.4 ± 2.65	9.7 ± 0.37
18	99	89	19.7 ± 0.40	55.9 ± 3.03	9.6 ± 0.64
56	98	94	20.0 ± 0.18	59.0 ± 2.79	10.3 ± 0.59
165	98	88	20.4 ± 0.32	65.5 ± 3.84	11.3 ± 0.89
469	100	0*	-	-	-
NOEC	469 µg/L	165 µg/L	165 µg/L	165 µg/L	165 µg/L

Concerning aquatic invertebrates, a long-term study on *Daphnia magna* performed according to OECD TG 211 derived a 21 days NOEC value of 0.82 mg/L based on survival and reproduction.

Concerning algae and aquatic plants, the lowest relevant endpoint for classification purposes was considered to be the 72 hours NOE_rC value of 1.0 mg/L for *Pseudokirchneriella subcapitata*.

Overall, based on all long-term results, the lowest NOEC for aquatic organisms was that of *Pimephales promelas* (NOEC = 0.165 mg/L). On this basis, the following classification and labelling of sedaxane was proposed by the dossier submitter:

Aquatic Chronic 2 – H411 (Toxic to aquatic life with long lasting effects).

Comments received during public consultation

There was general support for the Aquatic Acute 1 – H400 classification from the commenting MSCAs.

Regarding the aquatic chronic classification, one MSCA pointed out that the key chronic toxicity test was not performed with the most sensitive species like as in the acute toxicity test: *Pimephales promelas* NOEC 0.165 mg/L (mm). This test species was not the most acutely sensitive, as the lowest 96h LC_{50} of 0.62 mg/L (mm) was for *Cyprinus carpio,* while the *Pimephales promelas* 96h LC_{50} was 0.98 mg/L (mm). According to the commenting MSCA, considering the surrogate approach using the lowest acute effects endpoint would result in Aquatic Chronic 1 (M-factor = 1) for a non-rapidly degradable substance. An additional argument for following this approach was the fact that, although both fish species exhibited acute endpoints in the 0.1 - 1.0 mg/L range, the chronic NOEC for fish is close to the regulatory threshold value of 0.1 mg/L.

On this basis, the MSCA wondered whether Aquatic Chronic 1 (M-factor 1) should be considered and commented that it might be useful to also consider acute:chronic ratios and if EC_{10} endpoints were available.

On their response, the DS stated that there is only slight difference in sensitivity between the two fish species from the acute tests and considered their sensitivity to sedaxane as similar. Furthermore, they considered the NOEC of 165 µg/L robust, as it corresponds to the highest tested concentration without significant effects while significant effects were observed at the highest tested concentration in the study. No reliable EC_{10} value could be derived from the reported results of the study.

Assessment and comparison with the classification criteria

The substance sedaxane is not readily biodegradable and is hydrolytically stable. The experimentally derived Log Kow of sedaxane is 3.3 and may be considered unlikely to bioaccumulate in aquatic organisms. However, sedaxane may have surface-active properties that introduce uncertainty to the results of the experimental bioconcentration study. The BCF value of 97 L/kg is below the trigger value of 500 L/kg in CLP, although the BCF has not been growth-corrected. This will not have any influence on the classification.

Based on the LC_{50} = 0.62 mg/L for *Cyprinus carpio,* RAC agrees with the proposal by the dossier submitter that the substance should be classified as **Aquatic Acute 1 – H400 (very toxic to aquatic life) with an M-factor of 1**.

RAC notes that the acute toxicity dataset for fish seems to indicate that *Cyprinus carpio* (LC_{50} = 0.62 mg/L) may be slightly more sensitive to sedaxane than *Pimephales promelas* (LC_{50} = 0.98 mg/L). However, both acute toxicity values are within the same order of magnitude, with this small difference probably falling within the test variability range.

Furthermore, RAC considers that the substance is not a data poor one, there is reliable chronic toxicity data for all three trophic levels and the chronic toxicity study for *Pimephales promelas* should not be discarded. As such, the aquatic chronic classification should be based on the *Pimephales promelas* chronic study that derived a NOEC value of 0.165 mg/L.

Thus, RAC agrees with the proposal by the dossier submitter that the substance should be classified as **Aquatic Chronic 2 – H411 (Toxic to aquatic life with log lasting effects)**.

EVALUATION OF ADDITIONAL HAZARDS

Hazardous to the ozone layer

Summary of the Dossier Submitter's proposal

Transport of sedaxane in air is considered to be negligible due to its very low vapour pressure (6.5 × 10-8 Pa at 20°C and 1.7 × 10-7 Pa at 25°C) and Henry's constant (4.0 × 10-6 Pa m3 mol-1 at 25°C). Furthermore, the photochemical oxidative degradation of sedaxane in air is expected to be rapid. The estimated half-life is 5.1 hours, calculated using Atkinson method. Therefore, long-range transport is not considered to be of relevance.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

Transport of sedaxane in air is considered to be negligible due to its very low vapour pressure and Henry's constant, whilst its photochemical oxidative degradation in air is expected to be rapid. Therefore, local and global effects are expected to be negligible.

Thus, RAC agrees with the DS' proposal that **no classification** is warranted for this hazard class.

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ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).