Response to Comments Pertaining to the Notice of Intent to List Sedaxane as Causing Cancer under Proposition 65

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency
July 2016

The Office of Environmental Health Hazard Assessment (OEHHA) has determined that sedaxane meets the criteria for listing under Proposition 65[1] via the authoritative body mechanism based on conclusions by the US Environmental Protection Agency (US EPA) that sedaxane causes cancer, and on the scientific evidence relied on by US EPA[2]. US EPA is designated as an authoritative body for purposes of listing chemicals as causing cancer pursuant to Title 27, Cal. Code of Regulations, section 25306[3]. Sedaxane will therefore be added to the Proposition 65 list as a chemical known to cause cancer on July 1, 2016. This document responds to public comments received on the Notice of Intent to List sedaxane under Proposition 65.

Background

On June 26, 2015, OEHHA issued a Notice of Intent to List (NOIL)[4] sedaxane under Proposition 65 as a chemical known to the state to cause cancer. The action was based on Proposition 65 statutory requirements[5] and on the authoritative bodies provision of the Proposition 65 implementing regulations, Section 25306.

Under Section 25306, a chemical has been "formally identified" as causing cancer by an authoritative body if: (1) the chemical has been included in a list of chemicals causing cancer published by the authoritative body; is the subject of a report which is published by the authoritative body and which concludes that the chemical causes cancer; or has been "otherwise identified" as causing cancer by the authoritative body in a document that indicates that the identification is a final action; and (2) if the list, report, or document meets specified criteria in Section 25306(d)(2).

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[1] The Safe Drinking Water and Toxic Enforcement Act of 1986 (codified at Health and Safety Code section 25249.5 *et seq*.) hereinafter referred to as Proposition 65 or the Act.
[2] US EPA (2011). Cancer Assessment Document, Evaluation of the Carcinogenic Potential of Sedaxane. Final Report. Health Effects Division, Office of Pesticide Programs. May 18, 2011.
[3] Title 27, Cal. Code of Regulations, section 25306; all further references are to sections of Title 27 of the California Code of Regulations, unless otherwise indicated.
[4] Notice of Intent to List: CMNP (Pyrazachlor) and Sedaxane. Available at http://oehha.ca.gov/proposition-65/crnr/intent-list-cmnp-pyrazachlor-and-sedaxane
[5] Health and Safety Code section 25249.8(b)

OEHHA has reviewed the conclusions and statements in the US EPA 2011 report entitled *Cancer Assessment Document, Evaluation of the Carcinogenic Potential of Sedaxane*[6]. OEHHA has determined that these conclusions and statements satisfy the Section 25306(d)(1) requirement because sedaxane is the subject of a report published by the authoritative body that concludes that sedaxane causes cancer; *and* the US EPA 2011 report indicates this identification is a final action. Further, OEHHA has determined that the report meets the Section 25306(d)(2) requirements, thus the US EPA 2011 report satisfies the formal identification criteria in the Proposition 65 regulations for sedaxane. In the 2011 report, US EPA concludes that sedaxane is "Likely to be Carcinogenic to Humans"[7]. OEHHA is relying on US EPA's discussion of data and conclusions in the report that sedaxane causes cancer. Evidence described in the report includes studies showing that sedaxane increased the incidence of uterine adenocarcinomas and combined adenocarcinomas and adenomas in female rats, and combined hepatocellular carcinomas and adenomas in male mice.

The evidence cited by US EPA in support of these conclusions was reviewed by OEHHA with regard to the sufficiency of evidence criteria in Section 25306(e)(2). Based on US EPA's conclusions and the data relied on by US EPA in reaching those conclusions, OEHHA has determined that sedaxane meets the sufficiency of evidence criteria in Section 25306.

Summary of Comments and Responses

The June 26, 2015 notice initiated a 30-day public comment period. Comments on the NOIL were submitted on July 27, 2015 by Arthur Lawyer on behalf of Syngenta Crop Protection, LLC ("Syngenta"). Syngenta requested that "the listing of sedaxane under Proposition 65 be stayed until October, 2015, at which time" they would submit additional reports related to the mode of action of sedaxane that were not considered by US EPA's Cancer Assessment Review Committee in 2011[8]. OEHHA received four subsequent submissions from Syngenta on October 21, 2015, November 2, 2015, November 24, 2015, and February 8, 2016. These submissions consisted of reports (studies and assessments) related to the four tumor responses observed in the sedaxane animal studies that US EPA[9] concluded are treatment-related (i.e., male mouse liver tumors, female rat uterine tumors, male rat liver tumors, male rat thyroid tumors). As indicated in OEHHA's NOIL for sedaxane, two of the tumor responses identified by US EPA meet the sufficiency of evidence criteria in Section 25306, namely

[6] US EPA (2011). Cancer Assessment Document, Evaluation of the Carcinogenic Potential of Sedaxane. Final Report. Health Effects Division, Office of Pesticide Programs. May 18, 2011.
[7] *Ibid.*
[8] *Ibid.*
[9] *Ibid.*

the liver tumor response in male mice and the uterine tumor response in female rats. Tables 1 and 2 list the reports submitted by Syngenta related to the observed liver tumors in male mice and uterine tumors in female rats, respectively. Reports submitted by Syngenta related to the rat liver and thyroid tumor responses (see Table 3) are not relevant to OEHHA's determination that the sufficiency of evidence criteria in Section 25306(e)(2) have been met for sedaxane, and thus will not be discussed further here.

Comments in these submissions relevant to the listing are summarized, grouped and numbered by topic, and responses follow below.

Table 1. Reports submitted by Syngenta on liver tumors in male mice

Citation	**Submission Date**	**Study number**
Lake, B. (2013). Sedaxane – Hepatic Enzyme Activities after 28 and 90 Days of Dietary Administration to Male CD-1 Mice – Final Report. Unpublished study conducted by Leatherhead Food Research (LFR), Surrey, UK. Pages: 27.	October 21, 2015	TK0172602
Omiecinski, C. (2014). Sedaxane - CAR3 Transactivation Assay with Mouse, Rat and Human CAR. Unpublished study conducted by Dept of Vet & Biomedical Sciences, University Pk, PA, USA. Pages: 25.	October 21, 2015	TK0212217
Peffer, R.C. and Minnema, D. (2016). Sedaxane – Mode of action and human relevance assessment of liver tumor incidences in rats and mice. Final Report. Unpublished assessment by Syngenta Crop Protection, LLC. Pages: 58.	February 9, 2016	TK0285153
Draft: Peffer, R.C. and Minnema, D. (2015). Sedaxane – Mode of action and human relevance assessment of liver tumor incidences in rats and mice. Draft Report. Unpublished assessment by Syngenta Crop Protection, LLC. Pages: 58.	*November 24, 2015*	*TK0285153*
Perry, C.J. (2010a). SYN524464 – 80 Week Mouse Dietary Carcinogenicity Study. Final Report. Unpublished study conducted by Charles River Laboratories, Edinburgh, UK. Pages: 1451.	November 2, 2015	T012103-05
Strepka, C. (2016). Sedaxane – A 21 Day Dietary Liver Mode of Action Study in Male CD-1 Mice – Final Report. Unpublished study conducted by Charles River Laboratories, Edinburgh, UK. Pages: 471.	February 9, 2016	TK0172605

Table 1 (cont'd). Reports submitted by Syngenta on liver tumors in male mice

Citation	Submission Date	Study number
Draft: Strepka, C. (2015). Sedaxane – A 21 Day Dietary Liver Mode of Action Study in Male CD-1 Mice – Draft Report V1.0. Unpublished study conducted by Charles River Laboratories, Edinburgh, UK. Pages: 448.	*October 21, 2015*	*TK0172605*
Strepka, C. and Robertson, A.G. (2015). Sedaxane – A 14 Day Range Finding Study by Oral (Dietary) Administration in Male CD-1 Mice – Final Report Amendment 1. Unpublished study conducted by Charles River Laboratories, Edinburgh, UK. Pages: 85.	October 21, 2015	TK0172604
Toyokawa, K. and Sherf, B. (2014). SYN524464 – Pregnane X Receptor (PXR) Trans-activation Assays with Rat, Mouse and Human PXR – Final Report. Unpublished study conducted by Indigo Biosciences, Inc., State College, PA, USA. Pages: 36.	October 21, 2015	TK0212218

Table 2. Reports submitted by Syngenta on uterine tumors in female rats

Citation	Submission Date	Study number
Jolas, T. (2015). Sedaxane – *In Vitro* Dopamine D_{2S} Receptor Binding Assay – Final Report. Unpublished study conducted by Eurofins Cerep, Celle l'Evescault, France. Pages: 22.	October 21, 2015	TK0176307
Kappeler, C.J. (2014). Sedaxane – Uterotrophic Assay in Ovariectomized Wistar Han Rats – Final Report. Unpublished study conducted by WIL Research, Asland, OH. Pages: 257.	October 21, 2015	TK0176306
Peffer, R.C. and Yi, K.D. (2016). Sedaxane – Mode of action and human relevance assessment of uterine tumors in female Han Wistar rats. Unpublished assessment by Syngenta Crop Protection, LLC. Pages: 63.	February 9, 2016	TK0285154
Perry, C.J. (2010b). SYN524464 – 104 Week Rat Dietary Carcinogenicity Study with Combined 52 Week Toxicity Study. Final Report. Unpublished study conducted by Charles River Laboratories, Edinburgh, UK. Pages: 2310.	November 2, 2015	T012104-05

Table 2 (cont'd). Reports submitted by Syngenta on uterine tumors in female rats

Citation	Submission Date	Study number
Plummer, S. (2015a). Isopyrazam – Evaluation of Hypothalamic Tyrosine Hydroxylase in Control Female Wistar Rats at 3, 12 or 24 Months by Immunohistochemistry and *In-situ* Hybridization – Final Report. Unpublished study conducted by MicroMatrices Associated Ltd, Dundee, UK. Pages: 32.	October 21, 2015	TK0115807
Plummer, S. (2015b). Sedaxane – Analysis of Stored Tissue from 2-Year Rat Study for Hypothalamic Tyrosine Hydroxylase via Immunohistochemistry and In Situ Hybridization – Final Report. Unpublished study conducted by MicroMatrices Associated Ltd, Dundee, UK. Pages: 41.	October 21, 2015	TK0176309
Seely, J.C. (2016). SYN524464 – Microscopic Evaluation of Vagina, Uterus, and Ovary from Subchronic and Chronic Rat Dietary Studies to Determine Cycle Stage – Final Report. Unpublished study conducted by Experimental Pathology Laboratories, Inc., Durham, NC. Pages: 49.	February 9, 2016	TK0280945
Draft: Seely 2015. Protocol. SYN524464 – Pathology Evaluation to Determine Cycle State of the Vagina, Uterus and Ovary of Female Rats Exposed to SYN524464 in the Diet for 13 to 104 Weeks. Study Director: John Curtis Seely. Performing Laboratory: EPL, Inc.. Study Sponsor: Syngenta Crop Protection. Lab. Study No.: 140-130. Pages: 11.	*November 2, 2015*	*TK0280945*
Study plan amendment: Mullins, P. (2015). Study plan amendment 2 for "SYN524464 – An evaluation of cycle stage based on microscopic evaluation of ovary, uterus, and vagina tissues from 13, 52 and 104 week rat dietary studies." Unpublished report by Sequani Limited, Herefordshire, UK. Pages: 33.	*November 2, 2015*	*TK0261027*

Table 3. Reports submitted by Syngenta on rat liver and thyroid tumor responses*

Citation	Submission Date	Study number
Chubb, D. (2015). Sedaxane – 28 Day Oral (Dietary) Mechanistic Study to Evaluate Effects on the Liver and Thyroid in the Male Rat. Final Report. Unpublished study conducted by Sequani Limited, Herfordshire, UK; Ani Lytics, Inc., Gaithersburg, MD; Leatherhead Food Research, Surrey, UK. Pages: 849.	November 24, 2015 and February 9, 2016	TK0172606
Draft: Chubb, D. (2015). Sedaxane – 28 Day Oral (Dietary) Mechanistic Study to Evaluate Effects on the Liver and Thyroid in the Male Rat. Draft Report. Unpublished study conducted by Sequani Limited, Herfordshire, UK; Ani Lytics, Inc., Gaithersburg, MD; Leatherhead Food Research, Surrey, UK. Pages: 836	*October 21, 2015*	*TK0172606*
Lake, B.G. (2014). Sedaxane – Effect on Rat Thyroid Peroxidase Activity *In Vitro.* Final Report Amendment 1. Unpublished study conducted by Leatherhead Food Research, Surrey, UK. Pages: 18.	October 21, 2015	TK0172608
Peffer, R.C. and Cowie, D. (2015). Sedaxane – Mode of Action and Human Relevance Assessment of Thyroid Follicular Cell Tumors in Male Rats. Draft. Unpublished assessment by Syngenta Crop Protection, LLC, Greensboro, NC. Pages: 39.	November 24, 2015	TK0285152
Peffer, R.C. and Noakes, J.P. (2010). SYN508210, SYN508211 and SYN524464. 28 Day Comparative Study in the Rat. Final Report Amendment 1. Unpublished study conducted by Central Toxicology Laboratory, Cheshire, UK. Pages: 780.	October 21, 2015	T022693-04
Polivy, S. (2015). Sedaxane – Method Validation of Radioimmunoassay Analysis of Control Rat Serum for TSH. Final Report. Unpublished study conducted by Ani Lytics, Inc., Gaithersburg, MD. Pages: 27.	October 21, 2015	TK0172606-001

***** Tumor responses not relevant to the NOIL (see text)

1. **Mode of action data not considered by US EPA is predicted to ultimately result in US EPA changing the cancer classification.**

Comment:
"We believe that, based on the new mode of action data that have been provided to the OEHHA, the mode of action data, and the associated human relevance framework documents, none of which were considered by the Authoritative Body as part of the process which resulted in the 2011 classification of sedaxane as "Likely to be Carcinogenic to Humans", the classification by the Authoritative Body will ultimately be changed to "Not Likely to be Carcinogenic to Humans", and, in the interim, OEHHA will have sufficient evidence to make an analogous determination under Section 25306 of Title 27 of the California Code of Regulations." (p. 7 of letter dated Feb. 8, 2016; similar statements appear on pp. 3-4 of letter dated Nov. 24, 2015, p. 3 of letter dated Nov. 2, 2015, p. 1 of letter dated Oct. 21, 2015, and pp.1-2 of letter dated July 27, 2015)

Response:
Health and Safety Code section 25249.8(b) and OEHHA's implementing regulations require chemicals to be listed via the authoritative bodies listing mechanism as known to cause cancer where they meet the criteria set out in the regulation. As detailed above, OEHHA has determined that the US EPA 2011 report[10] meets the Section 25306 criteria, thus satisfying the formal identification and sufficiency of evidence criteria in the Proposition 65 regulations.

OEHHA has also evaluated the mode of action and human relevance information provided by the commenter on sedaxane-induced liver tumors in mice and uterine tumors in rats (see OEHHA's responses under topics 2 and 3 below) in light of Section 25306(f):

> "The lead agency shall find that a chemical does not satisfy the definition of 'as causing cancer' if scientifically valid data which were not considered by the authoritative body clearly establish that the chemical does not satisfy the criteria of subsection (e), paragraph (1) or subsection (e), paragraph (2)."

For the reasons discussed below (see responses to topics 2 and 3), OEHHA finds that the evidence provided by the commenters does not "clearly establish" that sedaxane does not "cause cancer" according to the criteria of 25306(e)(2).

[10] US EPA (2011). Cancer Assessment Document, Evaluation of the Carcinogenic Potential of Sedaxane. Final Report. Health Effects Division, Office of Pesticide Programs. May 18, 2011.

If the authoritative body changes its classification of a chemical, the Proposition 65 regulations provide a mechanism for delisting[11]. In the event US EPA changes its determination and no longer formally identifies sedaxane as causing cancer, OEHHA would refer this chemical to the Carcinogen Identification Committee for reconsideration, pursuant to Section 25306(j).

As noted earlier, information submitted on male rat liver and thyroid tumor responses are not part of the basis for listing this chemical and will therefore not be addressed in these responses.

2. Mode of action and human relevance of liver tumors in male mice

Background on sedaxane-induced mouse liver tumors. As shown in Table 4 below, statistically significant increases in liver adenomas and combined adenomas and carcinomas were observed in male mice in an 80-week study[12].

Table 4. Liver tumors in male CD-1 mice administered sedaxane in feed for 80 weeks[13]

Tumor type	Dose (ppm)			
	0	200	1250	7000
Hepatocellular adenomas	7/48* (15%)	9/45 (20%)	10/45 (22%)	15/48* (31%)
Hepatocellular carcinomas	5/48* (10%)	5/45 (11%)	3/45 (7%)	10/48 (21%)
Hepatocellular adenomas and carcinomas combined	9/48* (19%)	13/45 (29%)	12/45 (27%)	19/48* (40%)

*p < 0.05. Significance of trend denoted at control. Significance of pairwise comparison with control denoted at dose level.

Summary of studies submitted by the commenter. Syngenta submitted a report titled "Mode of action and human relevance assessment of liver tumor incidences in rats and mice,"[14] which reviews data from multiple short-term and mechanistic studies,

[11] Title 27, Cal. Code of Regs., section 25306(j)
[12] Perry, C.J. (2010a). SYN524464 – 80 Week Mouse Dietary Carcinogenicity Study. Final Report. Unpublished study conducted by Charles River Laboratories, Edinburgh, UK. Pages: 1451.
[13] As reported in US EPA (2011). Cancer Assessment Document, Evaluation of the Carcinogenic Potential of Sedaxane. Final Report. Health Effects Division, Office of Pesticide Programs. May 18, 2011.
[14] Peffer, R.C. and Minnema, D. (2016). Sedaxane – Mode of action and human relevance assessment of liver tumor incidences in rats and mice. Task Number: TK0285153. Unpublished assessment by Syngenta Crop Protection, LLC.

including each of the studies submitted to OEHHA related to the induction of mouse liver tumors by sedaxane. Brief descriptions of each of the studies reviewed in the Syngenta report are as follows:

In vitro studies:

(a) *Toyokawa and Sherf (2014)*

Sedaxane was tested for its potential to activate the pregnane X receptor (PXR) in a reporter assay system. The ligand-binding domain of mouse, rat, or human PXR was fused to the DNA binding domain of a transcription factor in human embryonic kidney cells. Cells were incubated with sedaxane concentrations of 14 nM to 30,000 nM, and emission of light was quantified as a marker of PXR activity[15].

(b) *Omiecinski (2014)*

Sedaxane was tested in a reporter assay for its ability to directly activate the constitutive androstane receptor (CAR). CAR3 variants of mouse, rat, and human CAR were transfected into COS-1 cells, incubated with sedaxane at concentrations of 1, 3, 10, and 30 µM, and the extent of CAR activation was quantified[16].

In vivo studies:

(c) *Perry (2010a)*

In an 80-week carcinogenicity study, groups of 50 male and 50 female CD-1 mice were fed diets containing 0, 200, 1250 and 7000 ppm sedaxane[17].

(d) *Lake (2013)*

Frozen mouse liver samples from 90-day (Shearer and Foster, 2008)[18] and 28-day studies (Shearer and Robertson, 2008)[19] were analyzed for protein and CYP450 content and enzyme activity[20].

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[15] Toyokawa, K. and Sherf, B. (2014). SYN524464 - Pregnane X Receptor (PXR) Trans-activation Assays with Rat, Mouse and Human PXR. Task number TK0212218. Unpublished study conducted by Indigo Biosciences, Inc., State College, PA, USA.

[16] Omiecinski, C. (2014). Sedaxane - CAR3 Transactivation Assay with Mouse, Rat and Human CAR. Task number TK0212217. Unpublished study conducted by Dept of Vet & Biomedical Sciences, University Pk, PA, USA.

[17] Perry, C.J. (2010a). SYN524464 – 80 Week Mouse Dietary Carcinogenicity Study. Final Report. Unpublished study conducted by Charles River Laboratories, Edinburgh, UK. Pages: 1451.

[18] CD-1 male and female mice were treated for 90 days with 0, 500, 3500, and 7000 ppm sedaxane. No effects were observed on liver histopathology (Shearer, J. and Foster, B. (2008). SYN524464 - 90 Day Mouse Preliminary Carcinogenicity Study. Task number T012102-05. Unpublished study conducted by Charles River Laboratories, Edinburgh, United Kingdom. As cited in Peffer and Minnema (2016).)

[19] CD-1 male and female mice were treated for 28 days with 0, 1000, 5000, and 7000 ppm sedaxane. No effects were observed on body weight, liver weight, or liver histopathology (Shearer, J. and Robertson, B. (2008). SYN524464 - 4 Week Mouse Dietary Preliminary Study. Task number T022781-04. Unpublished

(e) *Strepka and Robertson (2015)*
In a preliminary 14-day dietary study, groups of five male CD-1 mice were fed 0, 7000, 10000, and 14000 ppm sedaxane[21].

(f) *Strepka (2016)*
Male CD-1 mice (6 mice/group/time point) were fed 0, 1250, 7000, and 14000 ppm in the diet for 1, 3, 7, or 21 days before termination (Study Days 2, 4, 8, and 22). Livers were collected for toxicogenomic analysis, standard histopathology examination and hepatocellular proliferation assessments (BrdU and Ki67 analyses), and liver tissue biochemistry analysis[22].

(g) *Peffer and Minnema (2016)*
Reports the findings of 90-day studies in CD-1 male and female mice treated with 0, 500, 3500, and 7000 ppm sedaxane[23], and the findings of 28-day studies in CD-1 male and female mice treated with 0, 1000, 5000, and 7000 ppm sedaxane[24].

2.1 Comment:
The mode of action and human relevance assessment report proposes that the "non-genotoxic MOA [for induction of liver tumors] is initiated by activation of the constitutive androstane receptor (CAR) and/or pregnane X receptor (PXR)"[25]. The report hypothesizes that sedaxane is a direct activator of CAR, and that the proposed MOA is not relevant to humans:

> "The available data also demonstrates that this MOA is not relevant for humans due to the established qualitative differences in response to CAR/PXR activation between rodents (rats and mice) and humans. Experimental data demonstrate

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study conducted by Charles River Laboratories, Edinburgh, United Kingdom. As cited in Peffer and Minnema (2016).)

[20] Lake, B. (2013). Sedaxane – Hepatic Enzyme Activities after 28 and 90 Days of Dietary Administration to Male CD-1 Mice. Task number TK0172602. Unpublished study conducted by Leatherhead Food Research (LFR), Surrey, UK.

[21] Strepka, C. and Robertson, A.G. (2015). Sedaxane – A 14 Day Range Finding Study by Oral (Dietary) Administration in Male CD-1 Mice – Final Report Amendment 1. Task number TK0172604. Unpublished study conducted by Charles River Laboratories, Edinburgh, UK.

[22] Strepka, C. (2016). Sedaxane - 21 Day Dietary Liver Mode of Action Study in Male CD-1 Mice. Task number TK0172605. Unpublished study conducted by Charles River Laboratories, Edinburgh, UK.

[23] Shearer, J. and Foster, B. (2008). SYN524464 - 90 Day Mouse Preliminary Carcinogenicity Study.Task number T012102-05. Unpublished study conducted by Charles River Laboratories, Edinburgh, United Kingdom. As cited in Peffer and Minnema (2016).

[24] Shearer, J. and Robertson, B. (2008). SYN524464 - 4 Week Mouse Dietary Preliminary Study. Task number T022781-04. Unpublished study conducted by Charles River Laboratories, Edinburgh, United Kingdom. As cited in Peffer and Minnema (2016).

[25] Peffer, R.C. and Minnema, D. (2016). Sedaxane – Mode of action and human relevance assessment of liver tumor incidences in rats and mice. Task Number: TK0285153. Unpublished assessment by Syngenta Crop Protection, LLC, p. 12.

that sedaxane does not produce the key event of cell proliferation in human liver cells *in vitro.* This pattern of effects matches the known species differences that have been demonstrated for other CAR activators, and the weight of evidence indicates that it represents a qualitative difference in the established MOA for sedaxane between rodents (rats and mice) and humans. In summary, the data support the conclusion that sedaxane does not pose a carcinogenic hazard to humans"[26].

The key and associative events proposed by the commenter for the hypothesized MOA are summarized below, followed by OEHHA's summary of the submitted data relevant to each proposed event (page numbers are for the report by Peffer and Minnema, 2016).

Proposed key events:

(1) CAR/PXR activation;
(2) Altered expression of CAR-responsive genes;
(3) Altered expression of pro-proliferative genes/anti-apoptotic genes (e.g., *gadd45β, gadd45γ*);
(4) Transiently increased hepatocellular proliferation and decreased apoptosis; and
(5) Clonal expansion and development of altered hepatic foci.

Proposed associative events:

(1) Increased expression of genes encoding cytochrome P450 isozymes, particularly CYP2b and CYP3a families;
(2) Hepatocellular hypertrophy; and
(3) Increased liver weight.

Submitted data relevant to proposed events:

<u>Key event #1: CAR and PXR activation</u> (p. 13-15)

Sedaxane's ability to activate CAR and PXR in rats, mice, and humans was evaluated in *in vitro* reporter assay systems. Activation of mouse CAR3 was observed, as demonstrated by statistically significantly increased responses at 3 – 30 μM sedaxane. The rat CAR3 response to sedaxane was statistically significantly increased at 10 and

[26] Peffer, R.C. and Minnema, D. (2016). Sedaxane – Mode of action and human relevance assessment of liver tumor incidences in rats and mice. Task Number: TK0285153. Unpublished assessment by Syngenta Crop Protection, LLC, p. 12.

30 µM, and the human CAR3 response was statistically significantly increased at 30 µM[27].

Sedaxane was shown to activate human and rat PXR at concentrations of 3.33 to 30 µM. Sedaxane showed no agonist activity in the mouse PXR assay[28]. The report concluded that sedaxane is a direct activator of both CAR and PXR in rats and humans, and an activator of CAR, but not PXR, in mice[29].

Key event #2: Altered expression of CAR-responsive genes (p. 18-21)
Expression of 5 CAR-responsive genes (Cyp2b10, Cyp2c65, Gadd45β, Cdc20, and Fos) in the livers of male mice was evaluated by RT-PCR (reverse transcription polymerase chain reaction, a technique commonly used to detect RNA expression) on days 2, 4, 8, and 22 in a 21-day dietary study in response to treatment with sedaxane. TCPOBOP, a known CAR activator, used as a positive control; expression was measured on days 2 and 4. Upregulation of hepatic Cyp2b10, Cyp2c65, and Gadd45β expression (measured as mRNA) was observed in sedaxane treated animals. Upregulation of Cyp2b10 was greatest on day 4 of the study then decreased on days 8 and 22, and was similar to the response observed in mice treated with TCPOBOP. With the exception of the low dose on day 22, upregulation of Cyp2c65 was observed at all dose levels and time points. Upregulation of Gadd45β was transient; no significant differences in fold-change were observed at Day 22 of treatment. Sedaxane had no treatment-related effects on Cdc20 or Fos mRNA expression levels at any dose level or time point. This was in contrast to the response observed with TCPOBOP, in which significant increases in Cdc20 and Fos were observed on day 4[30].

Altered gene expression was also measured using microarrays from liver samples from Days 2, 4, and 22 of treatment in the 21-day study. Following analysis of patterns of differentially expressed genes, changes were observed in genes involved in the CAR and PXR pathways, including Cyp2b10 and Cyp3a11 in the mid- and high-dose groups[31].

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[27] Omiecinski, C. (2014). Sedaxane - CAR3 Transactivation Assay with Mouse, Rat and Human CAR. Task number TK0212217. Unpublished study conducted by Dept of Vet & Biomedical Sciences, University Pk, PA, USA.
[28] Toyokawa, K. and Sherf, B. (2014). SYN524464 - Pregnane X Receptor (PXR) Trans-activation Assays with Rat, Mouse and Human PXR. Task number TK0212218. Unpublished study conducted by Indigo Biosciences, Inc., State College, PA, USA.
[29] Peffer, R.C. and Minnema, D. (2016). Sedaxane – Mode of action and human relevance assessment of liver tumor incidences in rats and mice. Task Number: TK0285153. Unpublished assessment by Syngenta Crop Protection, LLC.
[30] Strepka, C. (2016). Sedaxane - 21 Day Dietary Liver Mode of Action Study in Male CD-1 Mice. Task number TK0172605. Unpublished study conducted by Charles River Laboratories, Edinburgh, UK.
[31] *Ibid*.

Key event #3: Altered expression of pro-proliferative genes/anti-apoptotic genes (p. 19, 21)
In the 21-day study, changes in genes involved in cell proliferation and apoptosis were observed, including up-regulation of Gadd45β in the high-dose group and down-regulation of Gadd45γ only in the mid-dose group. Gadd45β and Gadd45γ are involved in cell proliferation and apoptosis. The study authors suggest this may be consistent with a weak or mild proliferative effect in the liver[32].

Key event #4: Transiently increased hepatocellular proliferation and decreased apoptosis (p. 24-26)
Cell proliferation was evaluated via distribution of Ki67 in the liver in the 21-day study. A slight increase in proliferation relative to controls was observed on day 8 in the mid- and high-dose groups. This effect was not present in the low-dose group, and did not persist until Day 22. BrdU-labelling was also conducted, but no statistically significant increases in proliferation were observed[33].

Key event #5: Clonal expansion and development of altered hepatic foci (p. 39-40)
Altered hepatic foci were not observed in the 21-day study in male mice[34]. In the 80-week study, eosinophilic and basophilic foci were observed, but were not statistically significantly increased and were not associated with sedaxane treatment [35].

Associative event #1: Increased expression of genes encoding cytochrome P450 isozymes, particularly CYP2b and CYP3a families (p. 22, 32-33)
In the 21-day study, liver enzyme activity was measured in Day 8 samples. Significant increases in 7-pentoxyresorufin O-depentylase (PROD) activity (indicative of Cyp2b induction), and testosterone 6β-hydroxylase activity (indicative of Cyp3a induction) were observed. The increase in testosterone 6β-hydroxylase activity was significant only in the high-dose group, while the increase in PROD activity was significant in all treated groups. Microsomal protein levels were also measured, but were not significantly increased in any of the dose groups[36].

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[32] Strepka, C. (2016). Sedaxane - 21 Day Dietary Liver Mode of Action Study in Male CD-1 Mice. Task number TK0172605. Unpublished study conducted by Charles River Laboratories, Edinburgh, UK.
[33] *Ibid.*
[34] *Ibid.*
[35] Perry, C. (2010a). SYN524464 - 80 Week Mouse Dietary Carcinogenicity Study. Task number T012103-05. Unpublished study conducted by Charles River Laboratories, Edinburgh, United Kingdom.
[36] Strepka, C. (2016). Sedaxane - 21 Day Dietary Liver Mode of Action Study in Male CD-1 Mice. Task number TK0172605. Unpublished study conducted by Charles River Laboratories, Edinburgh, UK.

Frozen samples from the 28-day mouse study (low- and high-dose groups) and 90-day mouse study (high-dose group) were analyzed for enzyme activity. Significantly increased cytochrome P450 content was observed in the high-dose groups of both studies. Increased PROD activity was observed in the low- and high-dose groups of the 28-day study and in the high dose group of the 90-day study. Increased testosterone 6β-hydroxylase activity was observed in the high dose group of the 90-day study. Significant increases of whole homogenate protein and microsomal protein were not observed[37].

Associative event #2: Hepatocellular hypertrophy (p. 29, 31)
In the 21-day study, mild diffuse hepatocellular hypertrophy was observed on day 8 in the high-dose group, and mild centrilobular hypertrophy was observed on day 22 of the high-dose group[38]. Hepatocellular hypertrophy was not observed in the 28-day mouse study[39], the 90-day mouse study[40], or the 80-week mouse study[41].

Associative event #3: Increased liver weights (p. 28, 31)
In the 21-day study, mean relative liver weights were significantly increased in the mid-dose group on days 8 and 22 and in the high-dose group on days 4, 8, and 22[42]. No effects were observed on liver weights in the 28-day study[43]. In the 90-day study, significantly increased mean relative liver weights were observed in the high-dose male mice[44]. In the 80-week study, significantly increased mean relative liver weights were observed in the high dose group only[45].

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[37] Lake, B. (2013). Sedaxane – Hepatic Enzyme Activities after 28 and 90 Days of Dietary Administration to Male CD-1 Mice. Task number TK0172602. Unpublished study conducted by Leatherhead Food Research (LFR), Surrey, UK.
[38] Strepka, C. (2016). Sedaxane - 21 Day Dietary Liver Mode of Action Study in Male CD-1 Mice. Task number TK0172605. Unpublished study conducted by Charles River Laboratories, Edinburgh, UK.
[39] Shearer, J. and Robertson, B. (2008). SYN524464 - 4 Week Mouse Dietary Preliminary Study. Task number T022781-04. Unpublished study conducted by Charles River Laboratories, Edinburgh, United Kingdom. As cited in Peffer and Minnema (2016).
[40] Shearer, J. and Foster, B. (2008). SYN524464 - 90 Day Mouse Preliminary Carcinogenicity Study. Task number T012102-05. Unpublished study conducted by Charles River Laboratories, Edinburgh, United Kingdom. As cited in Peffer and Minnema (2016).
[41] Perry, C. (2010a). SYN524464 - 80 Week Mouse Dietary Carcinogenicity Study. Task number T012103-05. Unpublished study conducted by Charles River Laboratories, Edinburgh, United Kingdom.
[42] Strepka, C. (2016). Sedaxane - 21 Day Dietary Liver Mode of Action Study in Male CD-1 Mice. Task number TK0172605. Unpublished study conducted by Charles River Laboratories, Edinburgh, UK.
[43] Shearer, J. and Robertson, B. (2008). SYN524464 - 4 Week Mouse Dietary Preliminary Study. Task number T022781-04. Unpublished study conducted by Charles River Laboratories, Edinburgh, United Kingdom. As cited in Peffer and Minnema (2016).
[44] Shearer, J. and Foster, B. (2008). SYN524464 - 90 Day Mouse Preliminary Carcinogenicity Study. Task number T012102-05. Unpublished study conducted by Charles River Laboratories, Edinburgh, United Kingdom. As cited in Peffer and Minnema (2016).
[45] Perry, C. (2010a). SYN524464 - 80 Week Mouse Dietary Carcinogenicity Study. Task number T012103-05. Unpublished study conducted by Charles River Laboratories, Edinburgh, United Kingdom.

Response:
The information submitted by the commenter, including the studies listed in Table 1, does not provide substantial evidence supporting the hypothesis that sedaxane induces liver tumors via the commenter's proposed MOA involving activation of CAR. The mechanisms by which sedaxane induces mouse liver tumors remain unknown. Moreover, it has not been scientifically established that CAR activation does not contribute to liver tumors in humans. Thus, there is no reason to dismiss the relevance to humans of the mouse liver tumors induced by sedaxane. For the reasons discussed below, OEHHA finds that the mode of action studies presented by Syngenta do not clearly establish that sedaxane does not satisfy the criteria for listing in Section 25306(e)(2)[46].

A brief discussion of the relevant information submitted by the commenter in the context of the proposed key and associative events in the proposed CAR MOA is presented below, followed by additional discussion regarding data on the possible mechanisms of action of sedaxane.

Proposed key event #1. CAR activation is the molecular initiating event of the proposed MOA. Compounds activate CAR either directly or indirectly, and the downstream consequences of either direct or indirect activation are similar, including alteration of specific genes and increases in hepatocyte proliferation[47]. It appears that sedaxane is capable of activating CAR. Based on the reporter assays, it is possible that sedaxane is a direct activator of CAR in mice, rats, and humans and of PXR in rats and humans.

Gene knockout animal models are useful tools for investigating the role of specific genes and gene products, such as CAR, in biological disease processes, such as liver tumor formation. However, CAR knockout mouse studies were not conducted with sedaxane. Thus, it is unclear whether CAR activation is a required event for the induction of liver tumors in male mice exposed to sedaxane.

Proposed key event #2. Cyp2b10, Cyp2c65, Gadd45β, Cdc20, and Fos are all known targets of CAR[48, 49]. Cyp2c65 and Gadd45β are CAR-dependent genes that were upregulated by sedaxane. Cyp2b10, which can be induced by either CAR or PXR, was

[46] Per Section 25306(f), such a finding would result in OEHHA not proceeding with the listing.
[47] Oshida, K., Vasani, N., Jones, C., *et al*. (2015). Identification of chemical modulators of the constitutive activated receptor (CAR) in a gene expression compendium. Nuclear Receptor Signaling, 13, e002.
[48] *Ibid.*
[49] Tojima, H., Kakizaki, S., Yamazaki, Y., *et al*. (2012). Ligand dependent hepatic gene expression profiles of nuclear receptors CAR and PXR. Toxicology Letters 212:288-297.

also upregulated following exposure to sedaxane. Cdc20 and Fos, which are induced and repressed, respectively, by both CAR and PXR[50], were not affected by sedaxane. Taken together, these data suggest that activation of CAR by sedaxane in mouse liver results in induction of Cyp2b subfamily genes and gene expression changes in some but not all of the CAR-marker genes evaluated.

Proposed key events #3, 4, and 5. Hepatocellular proliferation, decreased apoptosis, and altered hepatic foci are hepatic changes typically observed with chemicals that activate CAR, and key events in the hypothesized CAR MOA[51, 52]. In the 21-day study, Ki67 labeling demonstrated mild hepatocellular proliferation in the mid- and high-dose groups on day 8, but not days 2, 4, or 22. BrdU labeling did not reveal any changes in proliferation. Slight changes were observed in expression of genes involved in cell proliferation and apoptosis (Gadd45β and Gadd45γ), but the biological consequences of these gene expression changes are unclear. Treatment-related increases in altered hepatic foci were not observed in the 21-day or the 80-week studies in male mice.

Proposed associative event #1. PROD liver enzyme activity is used as a functional measure of the CYP2b10 enzyme, and increased activity is characteristic of CAR activation (Lubet *et al.*, 1985[53]). Testosterone 6β-hydroxylase activity is a functional measure of Cyp3a activity[54], which is associated with PXR response. It appears that sedaxane is capable of increasing both PROD and testosterone 6β-hydroxylase activity in mouse liver *in vivo*, yet the *in vitro* reporter assay results showed that sedaxane activates CAR but not PXR in mice. This suggests that either sedaxane is a weak activator of PXR or crosstalk between CAR and PXR accounts for the Cyp3a induction.

Proposed associative events #2 and 3. Hepatocellular hypertrophy with the CAR inducer phenobarbital usually occurs in the centrilobular region of the liver lobule and is presumed to be secondary to induction of microsomal enzymes and cell replication

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[50] Tojima, H., Kakizaki, S., Yamazaki, Y., *et al*. (2012). Ligand dependent hepatic gene expression profiles of nuclear receptors CAR and PXR. Toxicology Letters 212:288-297.
[51] Elcombe, C.R., Peffer, R.C., Wolf, D.C., *et al*. (2014). Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: A case study with phenobarbital as a model constitutive androstane receptor (CAR) activator. Crit Rev Toxicol 44(1):64–82.
[52] Oshida, K., Vasani, N., Jones, C., *et al*. (2015). Identification of chemical modulators of the constitutive activated receptor (CAR) in a gene expression compendium. Nuclear Receptor Signaling, 13, e002.
[53] Lubet RA, Mayer RT, Cameron JW, *et al.* (1985). Dealkylation of pentoxyresorufin: a rapid and sensitive assay for measuring induction of cytochrome(s) P-450 by phenobarbital and other xenobiotics in the rat. *Arch Biochem Biophys* 238(1):43-48.
[54] Peffer, R.C. and Minnema, D. (2016). Sedaxane – Mode of action and human relevance assessment of liver tumor incidences in rats and mice. Task Number: TK0285153. Unpublished assessment by Syngenta Crop Protection, LLC.

(Whysner *et al.*, 1996[55]). Hypertrophy generally occurs rapidly and persists for the duration of exposure to chemicals that induce cytochrome P450 enzymes[56]. Increased liver weight accompanies hepatocellular hypertrophy, and is expected in the high doses after longer exposure times. Mild liver hypertrophy and increased relative liver weights (adjusted for body weight) were observed in the 21-day study in the high-dose group on days 2 and 4 and in the mid- and high-dose groups on days 8 and 22. However, in the 80-week carcinogenicity study, liver hypertrophy was not observed, and relative liver weight was increased only in the high-dose group. Thus, there is no evidence that sedaxane induces persistent hepatocellular hypertrophy in male mice, and increased relative liver weight was observed at 80 weeks only in the high-dose animals.

In summary, sedaxane has been shown to activate CAR in male mice, to induce changes in expression of some but not all CAR-responsive genes assessed, and to induce changes in enzyme activity characteristic of activation of CAR and PXR. However, several of the other key and associative events in the proposed CAR MOA have not been consistently demonstrated in sedaxane-treated mice, including the following non-neoplastic liver changes: hepatocellular proliferation, altered hepatic foci, and persistent hepatocellular hypertrophy.

Alternative mechanisms must be considered before concluding that a single mechanism is operative for a particular tumor type[57]. Peffer and Minnema (2016) list a number of possible modes of action for induction of liver tumors in rodents, and conclude that sedaxane was negative in all genotoxicity studies reported to date, that there was not strong evidence for either peroxisome proliferator-activated receptor alpha (PPARα) or aryl hydrocarbon receptor (AhR) activation, and that sedaxane did not show estrogenic activity in a rat uterotrophic assay[58]. These data address only a limited set of possible mechanisms of carcinogenesis, and do not adequately investigate the range of key characteristics associated with carcinogens[59].

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[55] Whysner J, Ross PM and Williams GM (1996). Phenobarbital mechanistic data and risk assessment: enzyme induction, enhanced cell proliferation, and tumor promotion. *Pharmacol Ther* **71**(1-2):153-191.
[56] Ross, J., Plummer, S.M., Rode, A., *et al*. (2010). Human Constitutive Androstane Receptor (CAR) and Pregnane X Receptor (PXR) support the hypertrophic but not the hyperplastic response to the murine nongenotoxic hepatocarcinogens phenobarbital and chlordane *in vivo*. *Toxicological Sciences* 116(2): 452-466.
[57] Smith, M.T., Guyton, K.Z., Gibbons, C.F., *et al*. (2015). Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. *Env Health Perspectives* [Epub ahead of print].
[58] Kappeler, C.J. (2014). Sedaxane – Uterotrophic Assay in Ovariectomized Wistar Han Rats – Final Report. Unpublished study conducted by WIL Research, Asland, OH.
[59] Smith MT, Guyton KZ, Gibbons, CF, Fritz JM, Portier CJ, Rusyn I, DeMarini DM, Caldwell JC, Kavlock RJ, Lambert P, Hecht SS, Bucher JR, Stewart BW, Baan R, Cogliano VJ, Straif K. (2015). Key Characteristics of Carcinogens as a Basis for Organizing Data on Mechanisms of Carcinogenesis. Environ Hlth Perspect DOI: 10.1289/ehp.1509912

A broader approach is necessary to identify other potential pathways of tumor induction. This can be accomplished, for example, through the use of genome-wide association studies, chromosome-wide association studies, and transcriptome-wide association studies[60]. One example of such an approach was applied by Nesnow *et al*. (2009)[61] to three CAR activators, namely phenobarbital, triadimefon, and propiconazole. These investigators looked at transcriptional profiles in animals treated with these compounds, and found the profiles differed significantly across the three CAR activators. This work led Nesnow *et al*. (2009) to conclude that the mechanisms of tumorigenic action were likely to differ across the three CAR activators, and to investigate novel MOAs for propiconazole, based on transcriptomics and metabolomics data[62].

In conclusion, chemicals are listed as causing cancer under the authoritative bodies provision using the criteria in Section 25306(e)(1) or (e)(2). Section 25306(f) provides that a chemical does not "satisfy the definition of 'as causing cancer' if scientifically valid data which were not considered by the authoritative body clearly establish that the chemical does not satisfy the criteria of subsection (e)(1) or (e)(2). OEHHA solicits comments in order to determine if there are any data that clearly establish that a chemical does not satisfy the sufficiency of evidence requirement. According to Section 25306(i), interested parties may object to the addition of chemicals to the Proposition 65 list on the basis that there is no "substantial evidence" that the criteria identified in subsection (e) have been satisfied. "Substantial evidence" in Section 25306(i) means that sufficient evidence of carcinogenicity is not refuted by comments or data provided by the interested party. The question here is whether any data clearly establish that sedaxane does not cause cancer. For the reasons discussed above, taken together with OEHHA's findings related to rat uterine tumors (see topic 3 below), OEHHA finds that the information submitted by the commenter does not "clearly establish" that sedaxane does not "cause cancer" according to the criteria of 25306(e)(2).

2.2 Comment:

The commenter states that "the incidences of adenomas and the incidences of carcinomas in male mice at 7000 ppm reflected normal background variability in these

[60] Shen, H., McHale, C.M., Smith, M.T., and Zhang, L. (2015). Functional Genomic Screening Approaches in Mechanistic Toxicology and Potential Future Applications of CRISPR-Cas9. *Mutat Res Rev Mutat Res* 764: 31–42.

[61] Nesnow S., Ward W., Moore T., Ren H. and Hester S.D. (2009). Discrimination of tumorigenic triazole conazoles from phenobarbital by transcriptional analyses of mouse liver gene expression. *Toxicol Sci* 110(1): 68-83.

[62] Nesnow S. (2013). Integration of toxicological approaches with "omic" and related technologies to elucidate mechanisms of carcinogenic action: propiconazole, an example. Cancer Lett 334(1):20-27.

relatively common histopathologic findings in older male CD-1 mice, and were not related to treatment"[63].

"Compared to the HCD [Historic Control Data], the incidences in 7000 ppm male mice of hepatocellular adenomas alone (30%) or hepatocellular carcinomas alone (20%) were within one of the two HCD ranges or close to the top of a range; however, the combined incidence of adenomas + carcinomas at 7000 ppm (38%) was outside of both the laboratory HCD range and the RITA *[Registry of Industrial Toxicology Animal data]* HCD range"[64].

Response:
The Statement of Reasons for Section 25306 states: "It is not the intention of the Agency to substitute its scientific judgment for that of the authoritative body. The Agency's inquiry will be limited to whether the authoritative body relied upon scientific data in an amount sufficient to conclude that the chemical causes cancer." In this case, US EPA considered the liver tumors to be treatment-related in male mice at the high dose (7000 ppm):

> "Increased incidences of hepatocellular adenomas and/or carcinomas were seen in male mice at the high dose compared to concurrent controls. This finding was considered treatment-related, but at a dose level that approached a limit dose (900 mg/kg/day)… Liver adenomas and carcinomas in males were dose-responsive and above the rates in concurrent control CD-1 mice"[65].

US EPA (2011) also compared the liver tumor incidences observed in the sedaxane male mouse study (conducted from 2007 to 2009) to control data from male mouse studies conducted in the same laboratory (Charles River Edinburgh) in 2007. These data were compiled from three 80-week studies conducted in CD-1 mice, each of which began in 2007[66]. These data are reported in US EPA (2011) as follows:

- "Laboratory Control Adenomas: Range is 10-28%, Mean is 20.0%, SD is 9.2% (N=3 Studies)

[63] Peffer, R.C. and Minnema, D. (2016). Sedaxane – Mode of action and human relevance assessment of liver tumor incidences in rats and mice. Task Number: TK0285153. Unpublished assessment by Syngenta Crop Protection, LLC, p. 10.
[64] *Ibid.*
[65] US EPA (2011). Cancer Assessment Document, Evaluation of the Carcinogenic Potential of Sedaxane. Final Report. Health Effects Division, Office of Pesticide Programs. May 18, 2011.
[66] Perry, C. (2010a). SYN524464 - 80 Week Mouse Dietary Carcinogenicity Study. Task number T012103-05. Unpublished study conducted by Charles River Laboratories, Edinburgh, United Kingdom.

- Laboratory Control Carcinomas: Range is 6-10%, Mean is 7.3%, SD is 2.3% (N=3 Studies)
- Laboratory Control Total Tumors: Range is 22-28%, Mean is 23.3%, SD is 4.2% (N=3 Studies) [67]".

US EPA found that the incidences of hepatocellular adenomas, hepatocellular carcinomas, and combined hepatocellular adenomas and carcinomas in the high-dose (7000 ppm) sedaxane group exceeded the laboratory historical control ranges.

US EPA went on to state:

> "The concurrent control incidences of hepatocellular adenomas (15%) and the combined tumors (adenomas+carcinomas) (19%) were comparable to the historical control mean (20%) and range (10-28%) for adenomas, but slightly lower than the mean (23.3%) and range (22-28%) range for the combined tumors.[68]"

The range of combined hepatocellular adenomas and carcinomas from the Registry of Industrial Toxicology Animal (RITA) database is not appropriate for comparison with the incidence seen in the sedaxane-treated male mice because the RITA database studies were not conducted in the same laboratory as the sedaxane study and because the RITA database studies were conducted outside the appropriate timeframe for comparison with the sedaxane study (studies in the RITA database were conducted in 1994, 1996, 1997, and 1998). Thus, use of information from the RITA database is inconsistent with accepted guidance regarding historical control data:

> "The most relevant historical data come from the same laboratory and the same supplier and are gathered within 2 or 3 years one way or the other of the study under review; other data should be used only with extreme caution"[69].

[67] US EPA (2011). Cancer Assessment Document, Evaluation of the Carcinogenic Potential of Sedaxane. Final Report. Health Effects Division, Office of Pesticide Programs. May 18, 2011.
[68] *Ibid.*
[69] US EPA (2005). United States Environmental Protection Agency. Guidelines for carcinogen risk assessment. Washington, DC. Document number EPA/630/P-03/001F.

2.3 Comment:
The commenter states, "Clear thresholds exist for the key events in this MOA in rodents, and therefore a margin of exposure approach for cancer risk assessment is appropriate for sedaxane"[70].

Response:
As discussed in response to comment 2.1, the proposed MOA for the induction of liver tumors hypothesized by the commenter has not been clearly established and the mechanisms through which sedaxane induces liver tumors are not known. As indicated in Table 4 above, there was a significant, dose-related trend for hepatocellular adenomas, carcinomas, and combined adenomas and carcinomas. Of note, US EPA stated, "A linear low-dose extrapolation model (Q_1*) will be used for quantification of cancer risk to humans"[71].

The listing of a chemical under Proposition 65 involves only identification that the chemical can cause cancer as provided in Section 25306(e)(2). Specific dose response issues, such as whether sufficient evidence exists to indicate the presence of a dose threshold, below which there is no cancer risk, are addressed once a chemical has been placed on the Proposition 65 list, during the development of a "No Significant Risk Level"[72].

3. Uterine tumors in female rats

Background on sedaxane-induced rat uterine tumors. As shown in Table 5 below, statistically significant increases in uterine adenocarcinomas and combined adenomas and adenocarcinomas were observed in female rats in a 104-week study[73].

[70] Peffer, R.C. and Minnema, D. (2016). Sedaxane – Mode of action and human relevance assessment of liver tumor incidences in rats and mice. Task Number: TK0285153. Unpublished assessment by Syngenta Crop Protection, LLC, p. 51.
[71] US EPA (2011). Cancer Assessment Document, Evaluation of the Carcinogenic Potential of Sedaxane. Final Report. Health Effects Division, Office of Pesticide Programs. May 18, 2011.
[72] Title 27, Cal. Code of Regs., section 25701 *et seq*., *Exxon Mobil Corp. v. Office of Environmental Health Hazard Assessment*, (2009) 169 Cal.App.4th 1264
[73] Perry, C.J. (2010b). SYN524464 – 104 Week Rat Dietary Carcinogenicity Study with Combined 52 Week Toxicity Study. Final Report. Task number T012104-05. Unpublished study conducted by Charles River Laboratories, Edinburgh, UK.

Table 5. Uterine tumors in female Han Wistar rats administered sedaxane in feed for 104 weeks[74]

Tumor type	Dose (ppm)			
	0	200	1200	3600
Adenomas	0/44 (0%)	0/35 (0%)	1/38 (3%)	0/44 (0%)
Adenocarcinomas	0/50** (0%)	3/43* (7%)	2/44 (5%)	9/49** (18%)
Adenomas and adenocarcinomas combined	0/50** (0%)	3/43* (7%)	3/44* (7%)	9/49** (18%)

* p < 0.05; ** p < 0.01. Significance of trend denoted at control. Significance of pairwise comparison with control denoted at dose level.

Mean body weights at 52 and 104 weeks for control and treated groups of female rats are shown in Table 6.

Table 6. Mean body weights (grams) in female Han Wistar rats administered sedaxane in feed for 104 weeks[75]

Weeks		Dose (ppm)		
	0	200	1200	3600
52	292.9	295.5	281.1 (-4.1%)	240.2** (-19.7%)
104	392.5	389.9	362.2* (-7.5%)	264.1** (-33.1%)

* p < 0.05; ** p < 0.01 (Percent difference vs. control in parentheses)

Summary of studies submitted by the commenter. Syngenta submitted a report titled "Sedaxane - Mode of action and human relevance assessment of uterine tumors in female Han Wistar rats,"[76] which reviews data from multiple short-term and mechanistic studies, including each of the studies submitted to OEHHA related to the induction of rat uterine tumors by sedaxane. Brief descriptions of each of the studies reviewed in the Syngenta report are as follows:

[74] As reported in US EPA (2011). Cancer Assessment Document, Evaluation of the Carcinogenic Potential of Sedaxane. Final Report. Health Effects Division, Office of Pesticide Programs. May 18, 2011.
[75] *Ibid.*
[76] Peffer, R.C. and Yi, K.D. (2016). Sedaxane – Mode of Action and Human Relevance Assessment of Uterine Tumors in Female Han Wistar Rats. Unpublished Assessment by Syngenta Crop Protection, LLC. Pages: 63.

In vitro studies:

(a) *Jolas (2015)*

Sedaxane, at a concentration of 10 µM, was tested for its potential to bind to the dopamine D_{2S} receptor *in vitro* (the human recombinant D_{2S} isoform was obtained from HEK-293 cells transfected with the human D_{2S} gene). Binding was evaluated by measuring displacement of a known ligand for the dopamine D_{2S} receptor, [3H]methyl-spiperone[77].

In vivo studies:

(b) *Kappeler (2014)*

A uterotrophic assay was conducted in which sedaxane (in the vehicle, carboxymethylcellulose) was administered via oral gavage to a group of 6 ovariectomized female Crl:WI(Han) rats once daily for 3 days at a dose of 375 mg/kg/day. A positive control group of 6 ovariectomized female rats was administered 0.3 mg/kg/day 17α-ethynylestradiol and a vehicle control group of 6 ovariectomized females was administered carboxymethylcellulose. Ovariectomies were performed at 42 days of age and test substance administration began at 56 days of age. Animals were euthanized on study day 3. Uterine weights were measured to evaluate estrogenicity[78].

(c) *Peffer and Yi (2016)*

This report discusses the findings of the 104-week study in female Han Wistar rats treated with 0, 200, 1200, and 3600 ppm sedaxane[79]. It proposes a mode of action for the induction of uterine tumors observed in this study[80].

(d) *Perry (2010b)*

Four groups of 52 male and 52 female Han Wistar rats were dosed with diets containing 0, 200, 1200, or 2600 ppm sedaxane for at least 104 weeks. Additional toxicity studies were conducted in which 4 groups of 12 male and 12 female rats were given the same doses for 52 weeks[81].

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[77] Jolas, T. (2015). Sedaxane – *In Vitro* Dopamine D_{2S} Receptor Binding Assay – Final Report. Unpublished study conducted by Eurofins Cerep, Celle l'Evescault, France. Pages: 22.

[78] Kappeler, C.J. (2014). Sedaxane – Uterotrophic Assay in Ovariectomized Wistar Han Rats – Final Report. Unpublished study conducted by WIL Research, Asland, OH. Pages: 257.

[79] Perry, C.J. (2010b). SYN524464 – 104 Week Rat Dietary Carcinogenicity Study with Combined 52 Week Toxicity Study. Final Report. Unpublished study conducted by Charles River Laboratories, Edinburgh, UK. Pages: 2310.

[80] Peffer, R.C. and Yi, K.D. (2016). Sedaxane – Mode of action and human relevance assessment of uterine tumors in female Han Wistar rats. Unpublished assessment by Syngenta Crop Protection, LLC. Pages: 63.

[81] Perry, C.J. (2010b). SYN524464 – 104 Week Rat Dietary Carcinogenicity Study with Combined 52 Week Toxicity Study. Final Report. Unpublished study conducted by Charles River Laboratories, Edinburgh, UK. Pages: 2310

(e) *Plummer (2015a)*
Fixed tissue sections from the hypothalamus of control female Wistar rats were examined to determine changes over time in the number of dopaminergic neurons. Tissue sections were obtained from 90-day and 24-month dietary studies of isopyrazam in rats. Brain sections of the arcuate nucleus and median eminence areas of the tuberoinfundibular dopaminergic region were stained with tyrosine hydroxylase (TH), and immunohistochemistry was used to detect TH protein and *in situ* hybridization was used to detect TH mRNA[82].

(f) *Plummer (2015b)*
Fixed tissue sections from the hypothalamus of female Han Wistar rats of the 104-week sedaxane bioassay were examined. Immunohistochemistry and *in situ* hybridization followed by quantitative image analysis was used to measure TH in tuberoinfundibular dopaminergic neurons to investigate senescence of dopaminergic neurons in the hypothalamus[83].

(g) *Seely (2016)*
Hematoxylin and eosin stained sections of the vagina, uterus, and ovaries of 212 female Wistar rats from sedaxane dietary toxicity studies were examined to determine reproductive status or estrous cyclicity[84].

3.1 Comment:

In the comments submitted to OEHHA on July 27, 2015, Syngenta asserts that "…the rat uterus tumors were generated through delayed reproductive senescence that is the consequence of the large deficits in body weight that were observed at the highest dosing level in female rats, a mechanism that is not relevant to humans. A strong scientific precedence for the postulated Mode of Action for these high dose rat uterine tumors exists (see references by Tucker et al., 1979; Roe et al., 1995; and Harleman et al., 2012), because the same shift in the female rat tumor profile as seen with sedaxane can be obtained via calorie restriction and resulting body weight deficits."

The mode of action and human relevance assessment report[85] proposes a mode of action for sedaxane induced rat uterine tumors in which decreased body weight

[82] Plummer, S. (2015a). Isopyrazam – Evaluation of Hypothalamic Tyrosine Hydroxylase in Control Female Wistar Rats at 3, 12 or 24 Months by Immunohistochemistry and *In-situ* Hybridization – Final Report. Unpublished study conducted by MicroMatrices Associated Ltd, Dundee, UK. Pages: 32.
[83] Plummer, S. (2015b). Sedaxane – Analysis of Stored Tissue from 2-Year Rat Study for Hypothalamic Tyrosine Hydroxylase via Immunohistochemistry and In Situ Hybridization – Final Report. Unpublished study conducted by MicroMatrices Associated Ltd, Dundee, UK. Pages: 41.
[84] Seely, J.C. (2016). SYN524464 – Microscopic Evaluation of Vagina, Uterus, and Ovary from Subchronic and Chronic Rat Dietary Studies to Determine Cycle Stage – Final Report. Unpublished study conducted by Experimental Pathology Laboratories, Inc., Durham, NC. Pages: 49.
[85] Peffer, R.C. and Yi, K.D. (2016) Sedaxane – Mode of action and human relevance assessment of uterine tumors in female Han Wistar rats. Unpublished assessment by Syngenta Crop Protection, LLC.

suppresses age-related decreases in dopaminergic activity, which results in lower levels of prolactin secretion, and decreased prolactin-mediated progesterone secretion from the corpora lutea of the ovaries. The lower levels of prolactin delay the onset of reproductive senescence, resulting in prolonged exposure of the uterus to a higher estrogen: progesterone ratio. Elevated exposure of the uterus to estrogen is associated with an increase in the incidence of uterine adenocarcinomas.

The report proposes the following key and associative events for the hypothesized MOA:

Proposed key events:

(1) Decrease in body weight gain
(2) Decreased adipose tissue after 1-2 years = decrease in signals to hypothalamus
(3) Hypothalamus: increased dopaminergic activity in tuberoinfundibular dopaminergic (TIDA) neurons after 2 years; increased TH mRNA levels
(4) Hypothalamus: increased TH protein levels
(5) Increased age at reproductive senescence = increased total number of estrus cycles + uterine endometrial proliferation

Proposed associative events:

(1) Marker of increased dopamine from TIDA: decreased proliferation in anterior pituitary
(2) Marker of decreased blood prolactin levels: decreased mammary gland hyperplasia and fibroadenoma
(3) Decreased senescent mucification of the vagina, plus related changes observed at 2 years

Outcome: Increased incidence of uterine adenocarcinomas[86]

"The control of the female reproductive cycles and the drivers for reproductive senescence in humans are fundamentally different than in rats, and therefore, this MOA for uterine tumors in rats is not relevant to human risk assessment due to qualitative differences between the species"[87].

[86] *Ibid*.
[87] Peffer, R.C. and Yi, K.D. (2016) Sedaxane – Mode of action and human relevance assessment of uterine tumors in female Han Wistar rats. Unpublished assessment by Syngenta Crop Protection, LLC.

Response:
The initial key event in the commenter's proposed MOA for the induction of rat uterine tumors by sedaxane is decreased body weight gain. However, the body weight and uterine tumor data in Tables 5 and 6 do not support, and in fact provide direct evidence against this proposed MOA. Statistically significant increases in the incidence of uterine adenocarcinomas were observed in the low- and high-dose sedaxane-treated groups, and statistically significant increases in combined uterine adenomas and adenocarcinomas were observed in the low-, mid-, and high-dose groups (see Table 5). Thus a statistically significant tumorigenic response was observed in all three treatment groups. As indicated in Table 6 above, there were no differences in body weight in low-dose females compared to untreated controls. Therefore, the evidence on carcinogenicity is inconsistent with the proposed MOA.

Moreover, the information submitted by the commenter, including the studies listed in Table 2, does not provide substantial evidence that sedaxane induces uterine tumors via the commenter's hypothesized MOA involving decreased body weight. Since effects on the mammary gland and vagina were not observed in the low- and mid-dose groups, but uterine tumors were observed in these groups, it is unclear if the mammary and vaginal effects reported in the high dose group provide any insight into sedaxane's mechanism of action. Thus, the mechanisms through which sedaxane induces uterine tumors remain unknown. For these reasons, taken together with the findings related to mouse liver tumors discussed above (see topic 2), OEHHA finds the information submitted by the commenter does not clearly establish that sedaxane does not satisfy the criteria of Section 25306(e)(2)[88].

3.2 Comment:
"Clear thresholds exist for the key events in this MOA"[89].

Response:
As discussed in response to comment 3.1, the mechanisms through which sedaxane induces uterine tumors remain unknown, and the information submitted by the commenter does not provide substantial evidence that sedaxane induces uterine tumors via the proposed MOA involving decreased body weight. In fact, the data on uterine tumor incidence and body weight from the female rat carcinogenicity study of sedaxane provides direct evidence against the commenter's proposed MOA. As indicated in Tables 5 and 6 above, there was a significant, dose-related trend for uterine

[88] Per Section 25306(f), such a finding would result in OEHHA not proceeding with the listing.
[89] Peffer, R.C. and Yi, K.D. (2016) Sedaxane – Mode of action and human relevance assessment of uterine tumors in female Han Wistar rats. Unpublished assessment by Syngenta Crop Protection, LLC, p. 52.

adenocarcinomas, and combined adenomas and carcinomas. Statistically significant increases in uterine adenocarcinomas and combined adenomas and carcinomas were observed in the low-dose group, as compared to controls. Of note, US EPA stated "A linear low-dose extrapolation model (Q_1*) will be used for quantification of cancer risk to humans"[90].

The listing of a chemical under Proposition 65 involves only identification that the chemical can cause cancer as provided in Section 25306(e)(2). Specific dose response issues, such as whether sufficient evidence exists to indicate the presence of a dose threshold, below which there is no cancer risk, are addressed once a chemical has been placed on the Proposition 65 list, during the development of a "No Significant Risk Level."

3.3 Comment:
"The original 2-year rat study considered the incidences of uterine adenocarcinomas in the 3600 ppm group within biological variability and not an effect of treatment based on Historic Control Data (HCD) from the performing laboratory and the RITA database that were summarized in the study report."

"Compared to the HCD ranges (Table 1), the incidence of uterine adenocarcinomas (17%) in female rats at 3600 ppm was within the range of values from the test laboratory and the RITA database. In addition, the incidence of uterine adenocarcinomas and combined uterine tumors in the concurrent control group of the sedaxane rat study (0%) was somewhat lower than a typical study in this strain of rat. Of the 10 studies where HCD values were available from the test laboratory, only two had an incidence of 0% for adenocarcinomas, and one had a combined uterine tumor incidence of 0%. Two studies in the RITA database out of 22 studies had 0% for adenocarcinomas as well as combined tumors. Therefore, the incidences of uterine adenocarcinomas in all treatment groups are within normal range of HCD for both the performing laboratory as well as the RITA database[91]".

Response:
As discussed in Comment 2.2, the Statement of Reasons for Section 25306 states: "It is not the intention of the Agency to substitute its scientific judgment for that of the authoritative body. The Agency's inquiry will be limited to whether the authoritative

[90] US EPA (2011). Cancer Assessment Document, Evaluation of the Carcinogenic Potential of Sedaxane. Final Report. Health Effects Division, Office of Pesticide Programs. May 18, 2011.
[91] Peffer, R.C. and Yi, K.D. (2016) Sedaxane – Mode of action and human relevance assessment of uterine tumors in female Han Wistar rats. Unpublished assessment by Syngenta Crop Protection, LLC, pp. 9-10.

body relied upon scientific data in an amount sufficient to conclude that the chemical causes cancer." US EPA (2011) states:

> "No statistically significant increases were seen for uterine adenomas. Female rats had a statistically significant trend ($p<0.01$), and significant pair-wise comparisons of the 200 ppm ($p<0.05$) and 3600 ppm dose groups ($p<0.01$) with the controls, for uterine adenocarcinomas. There was a statistically significant trend ($p<0.01$) and significant pair-wise comparisons of the 200 ppm ($p<0.05$), 1200 ppm ($p<0.05$) and 3600 ppm ($p<0.01$) dose groups with the controls for combined tumors[92]".

US EPA (2011) compared uterine tumor incidences to control data from female rat studies conducted in the same laboratory. These data are reported in US EPA (2011) as follows:

- "Laboratory Control Adenoma: Range is 0-4%, Mean is 2.8%, SD is 1.8% (N=5 Studies)
- Laboratory Control Adeno. Carc. *[Adenocarcinoma]*: Range is 0-19%, Mean is 10.4%, SD is 7.0% (N=5 Studies)
- Laboratory Control Total Tumors: Range is 2-22%, Mean is 12.6%, SD is 7.2% (N=5 Studies)"[93].

US EPA considered the historical control data, and stated:

> "It should be noted that while no adenocarcinomas were seen in the concurrent controls in this study, the historical control data from 5 studies shows a mean of 10.4% (with a range was 0-19%) for this tumor type. The incidences of the combined tumors (adenomas + adenocarcinomas) were significantly increased at all dose levels. No combined tumors were seen in the concurrent controls, whereas the historical control data for the combined tumors ranged from 2-22% (with a mean of 12.6%)"[94].

US EPA went on to state that the agency "considered the uterine tumors to be treatment-related in female rats"[95].

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[92] US EPA (2011). Cancer Assessment Document, Evaluation of the Carcinogenic Potential of Sedaxane. Final Report. Health Effects Division, Office of Pesticide Programs. May 18, 2011.
[93] *Ibid*.
[94] *Ibid*.
[95] US EPA (2011). Cancer Assessment Document, Evaluation of the Carcinogenic Potential of Sedaxane. Final Report. Health Effects Division, Office of Pesticide Programs. May 18, 2011.

OEHHA notes that US EPA's evaluation of the historical control data for uterine tumors observed in the sedaxane female rat study is consistent with guidance from the International Agency for Research on Cancer:

> "It is generally not appropriate to discount a tumour response that is significantly increased compared with concurrent controls by arguing that it falls within the range of historical controls, particularly when historical controls show high between-study variability and are, thus, of little relevance to the current experiment"[96].

As stated in Comment 2.2, US EPA considers the most relevant historical data to come from the same laboratory and the same supplier. The RITA database studies were not conducted in the same laboratory as the sedaxane study, thus the use of information from the RITA database is inconsistent with accepted guidance regarding historical control data.

3.4 Comment:
"There was no evidence for a treatment-related effect on the incidence of uterine tumors in an 80 week study in CD-1 mice (Perry, 2010a). In this study, male and female CD-1 mice were treated with sedaxane at 25, 157 and 900 mg/kg/day for males, and 29, 185 and 1001 mg/kg/day for females, corresponding to dietary inclusion levels of 200, 1250 and 7000 ppm respectively for both sexes[97]".

Response:
The absence of any increase in uterine tumors in sedaxane-treated mice does not call into question the validity of the rat uterine tumor findings. Tumor site concordance between rats and mice is neither predicted for chemical carcinogens, nor required in order to meet the sufficiency of evidence criteria in Section 25306(e)(2), which reads as follows:

> "Sufficient evidence of carcinogenicity exists from studies in experimental animals. For purposes of this paragraph, "sufficient evidence" means studies in experimental animals indicate that there is an increased incidence of malignant tumors or combined malignant and benign tumors in multiple species or strains,

[96] IARC (2006). International Agency for Research on Cancer. IARC monographs on the evaluation of carcinogenic risks to humans: Preamble. World Health Organization. Lyon, France. Available from http://monographs.iarc.fr/ENG/Preamble/index.php
[97] Peffer, R.C. and Yi, K.D. (2016) Sedaxane – Mode of action and human relevance assessment of uterine tumors in female Han Wistar rats. Unpublished assessment by Syngenta Crop Protection, LLC, p. 10.

> in multiple experiments (e.g., with different routes of administration or using different dose levels), or, to an unusual degree, in a single experiment with regard to high incidence, site or type of tumor, or age at onset."

US EPA considered uterine tumors to be treatment-related in female rats and liver tumors to be treatment-related in male mice. Thus, these findings by US EPA that sedaxane increased the incidences of malignant and combined malignant and benign uterine tumors in female rats, and combined malignant and benign liver tumors in male mice meet the sufficiency of evidence criteria in Section 25306(e)(2).