SUMMARY OF TOXICOLOGY DATA

Sedaxane

Chemical Code # 6116, Document Processing Number (DPN) # 53210
SB 950 # NA
12/4/13

DATA GAP STATUS

Chronic toxicity, rat:
No data gap, no adverse effect evident

Chronic toxicity, dog:
No data gap, no adverse effect evident

Oncogenicity, rat:
No data gap, possible adverse effect

Oncogenicity, mouse:
No data gap, no adverse effect evident

Reproduction, rat:
No data gap, no adverse effect evident

Developmental toxicity, rat:
No data gap, no adverse effect evident

Developmental toxicity, rabbit:
No data gap, no adverse effect evident

Gene mutation:
No data gap, no adverse effect evident

Chromosome effects:
No data gap, no adverse effect evident

DNA damage:
No data gap, no adverse effect evident

Neurotoxicity:
No data gap, possible adverse effect evident

Toxicology one-liners are attached.

All record numbers for the above study types through 272165 (Document No. 53210-0065) were examined. This includes all relevant studies indexed by DPR as of 12/4/13

In the 1-liners below:
- indicates an acceptable study.
- Bold face indicates a possible adverse effect.
- ## indicates a study on file but not yet reviewed.

File name: T131204
Revised by T. Moore, 12/4/13
NOTE: The following symbols may be used in the Table of Contents which follows:
* = data adequately address FIFRA requirement
† = study(ies) flagged as “possible adverse effect”
N/A = study type not currently required

This record contains summaries of studies. Individual worksheets may be useful for detailed assessment.

Table of Contents
METABOLISM AND PHARMACOKINETICS ................................................................. 3
Metabolism, Rat....................................................................................................... 3
GUIDELINE ACUTE STUDIES ON ACTIVE INGREDIENT ........................................ 6
Acute oral toxicity, rat ............................................................................................. 6
Acute dermal toxicity ............................................................................................. 6
Acute inhalation toxicity, rat ................................................................................... 6
Primary eye irritation, rabbit .................................................................................. 7
Primary dermal irritation ......................................................................................... 7
Dermal sensitization ............................................................................................... 7
SUBCHRONIC STUDIES (units of mg/kg/day unless specified) ............................ 7
Rat Subchronic Dietary Toxicity Studies ............................................................... 9
CHRONIC STUDIES ............................................................................................... 11
Chronic, rat ............................................................................................................. 11
Chronic, dog .......................................................................................................... 12
Oncogenicity, rat .................................................................................................... 12
Oncogenicity, mouse ............................................................................................ 12
GENOTOXICITY .................................................................................................... 13
Gene mutation ........................................................................................................ 13
Chromosome damage ............................................................................................ 13
DNA damage or miscellaneous effects .................................................................... 14
REPRODUCTIVE TOXICITY, RAT ...................................................................... 15
DEVELOPMENTAL TOXICITY ............................................................................. 16
Rat ........................................................................................................................ 16
Rabbit ..................................................................................................................... 16
NEUROTOXICITY ................................................................................................... 17
Acute neurotoxicity, rat ......................................................................................... 17
90-day neurotoxicity, rat ....................................................................................... 18
Developmental neurotoxicity, rat ........................................................................................................... 18
Delayed neurotoxicity, hen ..................................................................................................................... 18

IMMUNOTOXICITY ................................................................................................................................. 19
ENDOCRINE DISRUPTOR STUDIES ........................................................................................................ 19
SUPPLEMENTAL STUDIES ................................................................................................................... 19
STUDIES ON METABOLITES ............................................................................................................... 19

METABOLISM AND PHARMACOKINETICS

Metabolism, Rat
53210-0064; 272160, “SYN524464 – Pharmacokinetics in the Rat following a Single Oral Administration of 1 mg or 80 mg [Pyrazole-5-14C]-SYN524464/kg”; (J. Shaw; Charles River, Tranent, Edinburgh EH32 2NE, UK; Study No. 187591; 10/28/09); Nine Wistar rats/sex/group were dosed orally by gavage with 1.0 or 80 mg/kg of Pyrazole-5-14C]-SYN524464 (6:1 ratio of cis (SYN508210)- and trans (SYN508211)-isomers) (low dose: batch no. CL-LXII-15, specific activity: 135.2 uCi/mg, radiochemical purity: 99.2%; high dose: batch no. CL-LXII-14, specific activity: 1.7 uCi/mg, radiochemical purity: 97.0%). A pharmacokinetic study was performed in which blood was collected from the tail vein of 3 animals/sex/group at specified time points up to 72 hours post-dose. An excretion balance study was performed in which 3 animals/sex/group were maintained in metabolism cages and urine and feces samples were recovered up to 72 hour post-dose. An additional cohort (Metabolite Profiling) of two animals/sex/group was dosed with 1.0 or 80 mg/kg of the test material. The 1.0 mg/kg males and females were euthanized at 1 and 1.5 hours post-dose, respectively. Both sexes in the 80 mg/kg group were euthanized at 5 hours post-dose. The concentration of radiolabel in the plasma and whole blood was determined. In the pharmacokinetic study, the time to peak plasma and blood concentrations ranged from 1 to 1.5 hours post-dose for the 1.0 mg/kg treatment group and 4 to 6 hours post-dose for the 80 mg/kg treatment group. The t1/2 values for the plasma and blood of the males were 23 and 40 hours at 1.0 mg/kg and 29 and 32 hours at 80 mg/kg, respectively. For the females, the t1/2 values were 25 and 34 hours and 23 and 21 hours for the plasma and blood at 1.0 and 80 mg/kg, respectively. In the excretion balance study, 72 to 79% of the administered dose was recovered in the feces of the 1.0 mg/kg treatment group and 73 to 83% was recovered in the feces of the 80 mg/kg group. The percentage which was absorbed could not be determined. Study supplemental (only a portion of the data requirements for a rat metabolism study were fulfilled) (Moore, 11/15/13)

53210-0064; 272161, “SYN524464 – Excretion in Bile Duct Cannulated Rats following Single Oral Administration of 1 mg or 80 mg [Pyrazole-5-14C]-SYN524464/kg”; (J. Shaw; Charles River, Tranent, Edinburgh EH32 2NE, UK; Study No. 187628; 11/27/09); Four bile-duct catheterized Wistar rats/sex/group were dosed orally by gavage with 1 or 80 mg/kg of [Pyrazole-5-14C]-SYN524464 (6:1 ratio of cis (SYN508210)- and trans (SYN508211)-isomers), low dose: batch no. CL-LXII-15, specific activity: 135.2 uCi/mg, radiochemical purity: 99.2%; high dose: batch no. CL-LXII-14, specific activity: 1.7 uCi/mg, radiochemical purity: 97.0). Urine, feces and bile were collected at specified time intervals up to 48 hours post-dose. Seventy nine percent of the administered dose was recovered in the bile by 48 hours post-dose for the 1 mg/kg treatment
group. This percentage increased to 81 to 82% of the dose for the 80 mg/kg treatment group. The percentage recovered in the urine and feces ranged from 6.5 to 6.7% and from 4.7 to 6.6%, respectively for the 1 mg/kg treatment group and from 5.9 to 10.2% and from 3.3 to 7.1%, respectively for the 80 mg/kg treatment group. The percentage of the administered dose which was absorbed was 87 to 88% for the 1 mg/kg treatment group and 89.5 to 92.5% for the 80 mg/kg group. **Study supplemental.** (Moore, 11/18/13)

53210-0065; 272162; “SYN524464 – Tissue Depletion in the Rat following Single Oral Administration of 1 mg or 80 mg [Pyrazole-5-^{14}C]-SYN524464/kg”; (J. Shaw; Charles River, Tranent, Edinburgh EH32 2NE, UK; Study No. 187649; 12/8/09); Fifteen Wistar rats/sex/group were dosed orally by gavage with 1.0 or 80 mg/kg of [Pyrazole-5-^{14}C]-SYN524464 (6:1 ratio of cis (SYN508210)- and trans (SYN508211)-isomers) (**low dose**: batch no. CL-LXII-15, specific activity: 135.2 uCi/mg, radiochemical purity: 99.2%; **high dose**: batch no. CL-LXII-14, specific activity: 1.7 uCi/mg, radiochemical purity: 97.0%). Three animals/sex/group/time point were euthanized at various times up to 96 hours post-dose. The concentration of radiolabel in specified tissues/organs was determined over that time course. The highest concentrations of the radiolabel were in the gastrointestinal tract and the gastrointestinal tract. The liver was the predominant site of recovery among the various tissues/organs which were assayed. Otherwise, the adrenals, fat, kidneys, and pancreas were also noted for having higher levels of the radiolabel. Over the course of the 96-hour sample collection, radiolabelling in the tissues gradually declined. For most of the tissues, the concentrations were at barely detectable levels or below the detection limits. The gastrointestinal tract, gastrointestinal contents, liver, kidneys and blood and/or plasma had the higher levels by the end of the sampling period. The level of radiolabelling in the brain and bone was negligible. There was no apparent difference in the distribution and depletion of radiolabel in the tissues between sexes or the dosage levels. **Study supplemental.** (Moore,11/20/13)

53210-0065; 272163; “SYN524464 – Excretion and Tissue Distribution in the Rat following Single Oral Administration of 1 mg or 80 mg [Pyrazole-5-^{14}C]-SYN524464/kg”; (J. Shaw; Charles River, Tranent, Edinburgh EH32 2NE, UK; Study No. 187633; 9/30/09); Four Wistar rats/sex/group were dosed orally by gavage with 1.0 or 80 mg/kg of Pyrazole-5-^{14}C]-SYN524464 (6:1 ratio of cis (SYN508210)- and trans (SYN508211)-isomers) (**low dose**: batch no. CL-LXII-15, specific activity: 135.2 uCi/mg, radiochemical purity: 99.2%; **high dose**: batch no. CL-LXII-14, specific activity: 1.7 uCi/mg, radiochemical purity: 97.0%). An excretion balance study was performed in which urine and feces samples were recovered up to 168 hour post-dose. At 168 hours post-dose, the animals were euthanized and specified tissues/organs were assayed for residual radiolabel in the tissues. In the excretion balance study, 79 to 88% of the administered dose was recovered in the feces of the 1.0 mg/kg treatment group and 75 to 83% was recovered in the feces of the 80 mg/kg group. The total of the administered dose recovered in the urine ranged from 12 to 20% for both treatment groups. At 168 hours post-dose, the liver and kidneys were the primary tissues/organ where radiolabel was recovered. Quantifiable amounts of radiolabel were also recovered from GI tract, lungs, spleen, thyroid gland, or whole blood of one or more of the treatment groups. The percentage which was absorbed could not be determined. **Study supplemental** (only a portion of the data requirements for a rat metabolism study were fulfilled) (Moore, 11/18/13)

53210-0065; 272164; “SYN524464 – Excretion in Bile Duct Cannulated Rats following Single Oral Administration of 1 mg or 80 mg [Phenyl-U-^{14}C]-SYN524464/kg”; (J. Shaw; Charles River, Tranent, Edinburgh EH32 2NE, UK; Study No. 188574; 11/26/09); Four bile-duct catheterized Wistar rats/sex/group were dosed orally by gavage with 1 or 80 mg/kg of [Phenyl-U-^{14}C]-
SYN524464 (6:1 ratio of cis (SYN508210) - and trans (SYN508211)-isomers) (low dose: batch no. RDR-III-25, specific activity: 122.3 uCi/mg, radiochemical purity: 99.1%; high dose: batch no. RDR-III-24, specific activity: 1.8 uCi/mg, radiochemical purity: 98.6%). Urine, feces and bile were collected at specified time intervals up to 48 hours post-dose. Seventy seven to 81% percent of the administered dose was recovered in the bile by 48 hours post-dose for the 1 mg/kg treatment group. This percentage increased to 81 to 85% of the dose for the 80 mg/kg treatment group. The percentage recovered in the urine and feces ranged from 6.5 to 8.1% and from 5.7 to 8.6%, respectively for the 1 mg/kg treatment group and from 5.3 to 6.7% and from 4.4 to 10.6%, respectively for the 80 mg/kg treatment group. The percentage of the administered dose which was absorbed was 86 to 89% for the 1 mg/kg treatment group and 87 to 94% for the 80 mg/kg group. Study supplemental. (Moore, 11/19/13)

53210-0065; 272165; “SYN524464 – Tissue Distribution and Elimination in the Rat following Repeated Daily Oral Administration of 1 mg [Pyrazole-5-14C]-SYN524464/kg”; (J. Shaw; Charles River, Tranent, Edinburgh EH32 2NE, UK; Study No. 187654; 11/12/09); Thirty three male Wistar rats were dosed orally by gavage with 1.0 mg/kg/day of [Pyrazole-5-14C]-SYN524464 (6:1 ratio of cis (SYN508210) - and trans (SYN508211)-isomers) (batch no. RDR-II-75, specific activity: 27.4 uCi/mg, radiochemical purity: 98.6%) for 14 days. Three animals/time point were euthanized on study days 4, 8, 11, 15, 21, 24, 28, 35, 42, and 56. Specified tissues/organs were assayed for radiolabel content. In addition, urine and feces were recovered from 3 designated animals over the 24-hour period after the 1st and 14th treatments. The highest levels of radiolabel were recovered from the gastrointestinal tract and the gastrointestinal tract contents during the 14-day dosing period. Thereafter, the radiolabel gradually declined to a non-detectable level. Among the organs which were assayed, the kidneys and liver demonstrated the highest levels. There was a gradual increase in the concentrations of radiolabel in all of the tissues/organs assayed up to the end of the treatment period. During the 4-week recovery period, the radiolabel declined in all of the tissues to non-detectable levels except for the liver, kidneys and spleen. The radiolabel had the longest half-life in the spleen of 33 days. The concentrations of radiolabel in the urine and feces excreted after the 1st and 14th treatment also demonstrated an increase over that time period. Overall, there was an apparent sequestration of the radiolabel in all of the tissues/organs over the course of the treatment period. Study supplemental (only a portion of the data requirements for a rat metabolism study were fulfilled) (Moore, 11/19/13)

53210-0064; 272159; “SYN524464 – Investigation of the Nature and Identity of Radiolabelled Metabolites Present in the Plasma, Urine, Feces and Bile Collected from Rats following Oral Administration of [14C]-SYN524464/kg”; (M. Green; Charles River, Tranent, Edinburgh EH32 2NE, UK; Study No. 213097; 11/26/09); The urine, feces, plasma and bile samples derived from five studies were analyzed for the presence of metabolites. The protocols for these studies entailed the collection of the excretion products according to a single dose regimen for up to 7 days or a 14-day multiple dose regimen at various time points during that dosing period. The studies from which these samples were derived were study nos. 187591 (rec. no. 272160), 187628 (rec. no. 272161), 187633 (rec. no. 272163), 188574 (rec. no. 272164) and 187654 (rec. no. 272165). The two test materials were [Pyrazole-5-14C] SYN524464 and [Phenyl-U-14C] SYN524464. Data derived from the urine, feces and bile samples indicated that SYN524464 is readily hydroxylated on its phenyl or cyclopropane rings and demethylated on the pyrazole ring. Once the hydroxylation occurs, glucuronide, sulfate and glutathione conjugates are formed. The cleavage of the terminal cyclopropane ring resulted in the formation of β-hydroxycarbonyl metabolites. Cleavage of the amide linkage between the pyrazole and phenyl rings was a very minor factor in the metabolism of SYN524464. Study supplemental. (Moore, 11/22/13)
GUIDELINE ACUTE STUDIES ON ACTIVE INGREDIENT

Acute oral toxicity, rat
53210-0006, 271810, “SYN524464 - Acute Oral Toxicity in the Rat (Up-and-Down Procedure)", Acute oral toxicity; 811; Rat; RCC Ltd., Switzerland, Laboratory Study # B35537, 1/14/2008; Arcelin, G.; SYN524464, Batch ID SMU6LP006/MILLED, 95.3% (83.0% trans, 12.3% cis) SYN524464, solid white powder, administered by gavage; One healthy female rat was used in a limit test at 5000 mg/kg test item by oral gavage. Due to mortality of the rat within 3 hours of administration, nine additional healthy female rats were tested sequentially, one at a time, at 175, 550, 1750, 5000, 5000, 1750, 5000, 5000, and 5000 mg/kg. All rats dosed at 175, 550 and 1750 mg/kg survived, ruffled fur, hunched posture in all, slight to moderate sedation, poor coordination and ventral recumbency in some were observed during the post dose observation period, mostly within 5 days after dosing. All animals gained weight during the observation period and no gross abnormalities were noted upon necropsy. Four animals treated with 5000 mg/kg test substance were killed for ethical reasons, one was found dead and two survived. Clinical signs of ruffled fur, hunched posture, slight to moderate sedation, poor coordination and ventral recumbency, deep respiration, rales, salivation and bradypnea were observed before death. The surviving animals had slight to moderate ruffled fur, hunched posture, poor coordination and deep respiration. The body weights were within normal range. A yellowish discolorated jejunum in one animal killed at extremis, discolored, pale lungs in another killed at extremis was noted at necropsy. No macroscopic findings were recorded in the surviving animals. Reported LD50 (F): 5000 mg/kg with a 95% Confidence interval of 2513 mg/kg to 9210 mg/kg. **Toxicity Category III; Acceptable** (Pan & Leung, 8/21/13).

Acute dermal toxicity
53210-0006, 271811, “SYN524464 - Acute Dermal Toxicity in the Rat”, Acute dermal toxicity; 812; Rat; RCC Ltd., Switzerland, Laboratory Study # B35548, 12/28/2007; Arcelin, G.; SYN524464, Batch ID SMU6LP006/MILLED, 95.3% (83.0% trans, 12.3% cis) SYN524464, solid white powder, moistened with 1mL of purified water, administered by dermal application for 24 hours to 5 male and 5 female healthy rats at 5000 mg/kg. Slight erythema in the application sites was observed in 8 animals (3M5F) at day 2. Test substance residues were present at the application sites on day 2 in 5 animals(3M2F) and up to day 4 in 1 among them. All animals gained weight during the study. No macroscopic finds were observed at necropsy. LD50 (M&F): > 5000 mg/kg. **Toxicity Category IV; Acceptable** (Pan & Leung, 8/22/13).

Acute inhalation toxicity, rat
53210-0006, 271812, “SYN524464 – 4-hour Acute Inhalation Toxicity in Rats”, Acute inhalation toxicity; 813; Rat; RCC Ltd., Switzerland, Laboratory Study # 36235, 1/22/2008; Decker, U.; SYN524464, Batch ID SMU6LP006/MILLED, 95.3% (83.0% trans, 12.3% cis) SYN524464, solid white powder. Five male and five female healthy rats were administered with 5.244 mg/L (GSD: 2.85, MMAD: 3.00 um) test atmosphere by nose-only inhalation for 4 hours. Animals were observed for mortality, signs of gross toxicity, and behavioral changes during the first several hours post dosing and once daily thereafter for a total of 15-day observation period; body weights were recorded prior to dosing and on days 1, 4, 8 and 15. No mortality. Bradypnea, and hunched posture in all from 3 or 4 hours to 5 hours post exposure, breath sounds (rales), decreased spontaneous activity and ruffled fur in all from 4 hours to day 2 post exposure was observed. Body weight gain was observed in all during the 15-day observation period. No gross abnormalities were noted at final necropsy. LC50 (M&F): > 5.244 mg/L. **Toxicity Category IV; Acceptable** (Pan, 8/22/13).
Primary eye irritation, rabbit
53210-0006, 271814, “SYN524464 - Primary Eye Irritation Study in the Rabbit”, Primary Skin Irritation; 814; Rabbit; RCC Ltd., Switzerland, Laboratory Study # B35561, 12/18/2007; Arcelin, G.; SYN524464, Batch ID SMU6LP006/MILLED, 95.3% (83.0% trans, 12.3% cis) SYN524464, solid off-white powder, 0.1 g of pulverized test substance was applied to left eyes of three young adult New Zealand White rabbits. Eye irritation was scored at 1, 24, 48 and 72 hours after test item instillation. The following eye irritation was observed: score 1 conjunctivae redness in all at 1 and 24 hours, in 1/3 at 48 hours, with clearance in all at 72 hours; score 1 chemosis in 1/3 at 1 hour, with clearance in all at 24 hours; score 1 sclera in 2/3 at 1 and 24 hours with clearance in all at 48 hours. Body weights of all rabbits were within the normal range. Toxicity Category IV; Acceptable (Pan & Leung, 8/23/13).

Primary dermal irritation
53210-0006, 271813, “SYN524464 - Primary Skin Irritation in the Rabbit (4 hour semi-occlusive application)”, Primary Skin Irritation; 815; Rabbit; RCC Ltd., Switzerland, Laboratory Study # B35550, 12/18/2007; Arcelin, G.; SYN524464, Batch ID SMU6LP006/MILLED, 95.3% (83.0% trans, 12.3% cis) SYN524464, solid off-white powder, 0.5 g of test substance was moistened with 0.5mL of purified water, administered by dermal application for 4 hours to 1 male and 2 female healthy rabbits. No skin irritation was observed at 1 to 72 hours post patch removal. No clinical signs of systemic toxicity were observed in the animals during the study. Body weights of all rabbits were within the normal range. Toxicity Category IV; Acceptable (Pan & Leung, 8/22/13).

Dermal sensitization
53210-0006, 271815, “SYN524464 - Local Lymph Node Assay in the Mouse”, skin sensitization; 816; mouse; Safepharm Laboratories Limited, Derbyshire DE72 2GD, UK, Laboratory Study # 2364/0054, 12/20/2007; Pooles, A.; SYN524464, Batch ID SMU6LP006/MILLED, 95.3% (83.0% trans, 12.3% cis) SYN524464, off white powder. Groups of 5 female mice received three consecutive daily topical applications of 25 ul of test substance at 0, 10, 25 and 50% w/w in acetone/olive oil 4:1, or positive control material, α-Hexycinnamaldehyde at the concentration of 15% v/v in acetone/olive oil 4:1, on the dorsal surface of both ears. Five days following the first topical application of the test material, all mice were injected with 250ul of phosphate buffered saline (PBS) containing ³H-methyl thymidine via the tail vein. Five hours following the injection all mice were killed by CO₂ asphyxiation, and lymph nodes were excised and processed for single cell preparation. The radiolabelled material incorporated into cellular components was precipitated by resuspending the lymph node cells in 5% Trichloroacetic acid (TCA) and was measured by β-scintillation counting. The ratio of ³HtdR incorporation into lymph node cells of test nodes relative to that recorded for the control nodes was Simulation Index (SI). No mortality. No signs of systemic toxicity were noted in the test animals, or the vehicle and positive control animals. Mild redness to the base of the ears, head and neck was noted in all positive control animals. Body weight changes were normal for the test animals. Simulation indices of 1.12, 0.96 and 0.71 were noted in the 10, 25 and 50 % w/w test material treated group, simulation index of 5.67 was noted in the positive control treated group. Therefore, the test material is not a dermal sensitizer. Acceptable (Pan & Leung, 8/23/13).

SUBCHRONIC STUDIES  (units of mg/kg/day unless specified)

Rat 28-Day Dietary Toxicity Studies
53210-0029; 271863; “SYN508210, SYN508211 and SYN524464 – 28-Day Comparative Study in the Rat”; (R. Peffer, J.P. Noakes; Central Toxicology Laboratory, Alderley Park,
Macclesfield, Cheshire SK10 4TJ, UK; Study No. KR1595; 2/26/10); Five Wistar rats/sex/group received 0, 500, 2000 or 5000 of SYN508210 (trans isomer) (batch no. KI 7193/5, purity: not reported), SYN508211 (cis isomer) (batch no. KI-7245/5, purity: not reported) or SYN524464 (1:1 mixture of the cis and trans isomers) in the diet for 28 days ((M) SYN508210: 0, 47.0, 187.4, 438.2 mg/kg/day, SYN508211: 0, 45.9, 182.7, 438.2 mg/kg/day, SYN524464: 0, 47.5, 181.2, 444.6 mg/kg/day, (F) SYN508210: 0, 48.4, 177.1, 384.3 mg/kg/day, SYN508211: 0, 47.6, 179.6, 435.8 mg/kg/day, SYN524464: 0, 46.7, 181.1, 428.1 mg/kg/day). An additional 3 animals/sex/group/test material received the treatment for 1 or 14 days and were euthanized after blood collection was performed at 17:00, 21:00, 01:00, 05:00, 09:00, and 13:00, beginning 8 hours after the introduction of the test material in the diet on day 1 or 14. These animals were the toxicokinetics satellite cohort. No deaths resulted from the treatment. The mean body weights of both sexes in the 5000 ppm groups and the 2000 ppm females treated with all three test materials were less than those of the control group over the course of the study (NS, p<0.01). The 2000 ppm males treated with SYN508211 and SYN524464 also demonstrated lower mean body weights over the course of the study (NS). Food consumption was affected for both sexes in the 2000 and 5000 ppm groups treated with all three test material and for the females treated with 500 ppm of all three test materials. Although specific parameters demonstrated statistical significance in the hematology evaluation, there was no consistent pattern of effect which indicated a treatment-related etiology. In the clinical chemistry evaluation, serum total protein, cholesterol, triglyceride and total bilirubin were increased for both sexes in the 5000 ppm groups treated with all three compounds. Serum gamma glutamyl transferase activity was increased for both sexes in the 5000 ppm groups treated with SYN508211 and SYN524464 and for the females in the 5000 ppm group treated with SYN508210 (NS, p<0.05 or 0.01). Triglyceride levels were also increased in the 500 and 2000 ppm group females in a treatment-related manner (NS, p<0.05 or 0.01). The females in the 2000 and 5000 ppm groups treated with SYN508210 and SYN508211 also demonstrated increased concentrations of Na and Ca levels in the serum in a treatment-related manner. In the liver enzyme assay, the pentoxyresorufin-O-dealkylase (PROD) activity of both sexes in the 2000 and 5000 ppm groups treated with the three compounds and the males in the 5000 ppm groups treated with SYN508210 and SYN524464 was greater than that of the control group (p<0.01). For ethoxyresorufin-O-dealkylase (EROD), the females in the 5000 ppm groups treated with SYN508211 and SYN524464 and the females in the 2000 ppm group treated with SYN524464 demonstrated an increased level of activity in a treatment-related manner (p<0.05 or 0.01). Testosterone hydroxylation in the liver was measured at 6 sites, 7α, 6β, 16α, 16β, 2α and 2β. There was a significant treatment-related increase in hydroxylation at the 6β site for females in the SYN508211 and the SYN524464 treatment groups. At the 16α site, an increase was noted for the females in all three treatment groups. A treatment-related increase in hydroxylation at the 16β site was evident for both sexes in all of the treatment groups. For the 2α site, there was a treatment-related decrease in activity for the males in the 3 treatment groups. There was a treatment-related increase at the 2β site for the females in the SYN508211 and the SYN524464 treatment groups. At the 7α site, there was no apparent effect upon the hydroxylation activity for either sex. Immunoblotting revealed an overall increase in CYP 2B and CYP 3A protein in the liver for all of the treatment groups. The overall protein content in the liver increased marginally up to 145% of the control value (mg/g of liver) (SYN524464, females, 5000 ppm). In the necropsy examination, the mean liver weights adjusted for the body weight of both sexes in the 2000 and 5000 ppm groups treated with the three test materials were greater than the control values (p<0.01). In the histopathological examination, hepatocellular centrilobular hypertrophy was noted in the livers of both sexes in the 5000 ppm groups treated with the 3 chemicals and the males in the 2000 ppm groups treated with SYN508210 and SYN 524464 (p<0.01). The toxicokinetic data for each of the study groups demonstrated a biphasic response apparently reflecting the feeding patterns of the animals.
During the early evening the animals came off their day long fast and vigorously ingested the diet. Later after midnight, the feeding commenced again. By midday, the serum concentrations had returned to a level which was below the limits of quantification. The Tmax values ranged from 12 to 20 hours post dosing initiation. There was no apparent residual build-up of the test material in the serum between day 1 and day 14. **No adverse effect indicated.**

**Rat 28-Day Dietary Toxicity NOEL:** (M/F) < 500 ppm for all three test materials ((M) < 45.9 mg/kg/day, (F) < 46.7 mg/kg/day) (based upon the increased PROD activity noted in the livers of the 500 ppm males treated with all 3 test materials and the increased serum triglyceride levels noted for the 500 ppm females treated with all 3 test materials). **Study supplemental.** (Moore, 9/10/13)

### Rat Subchronic Dietary Toxicity Studies

53210-0012; 271842-SUP; "SYN524464 - 90 day dietary toxicity study in rats"; (Noakes, J.P., Central Toxicology Laboratory, Cheshire SK10 4TJ, UK, 7/23/07, Laboratories Study #PR1327); SYN524464 (6:1 ratio of trans:cis), Batch ID S01F002249U, 98.2% w/w, white powder, stored in refrigerator. Groups of 12/sex HsdRccHan:WIST rats were exposed by diet to 0 (blank diet), 250, 1000 or 4000 ppm test substance for 90 days of duration. Mean achieved dose levels: 0, 18.6, 72.9 and 299.6 mg SYN524464/kg/day for males and 0, 21.4, 85.7 and 315.3 mg/kg/day for females, corresponding to dietary inclusion levels of 0, 250, 1000 or 4000 ppm respectively. No test substance treatment related effects on clinical signs, clinical pathology, hematology, blood clinical chemistry, urinalysis, ophthalmology, as well as Functional observation battery and motor activity assessments were observed. Statistically significantly reduction of body weights, food consumption, food utilization, increased liver weight in 4000 ppm group male and female rats were observed. Increased hepatocyte hypertrophy in 4000 ppm group male and female rats corresponded with increased liver weights. **NOEL (No Observed Effect Level): 1000 ppm in male and female rats (72.9 mg/kg/day for males, 85.7 mg/kg/day for females) due to body weight, food consumption, liver weights and microscopic findings at 4000 ppm group animals. Acceptable** (Pan& Leung, 9/6/13).

53210-0013; 271843-SUP; "SYN524464 - 13 week rat dietary toxicity study"; (Shearer, J. and Foster, B., Charles River, Edinburgh EH32 2NE, UK, 10/22/09, Laboratories Study #458299); SYN524464 (83.0% trans isomer, 12.3% cis isomer), Batch ID SMU6LP006/Milled, 95.3% w/w, off white powder, stored at ambient temperature in the dark. Groups of 10/sex HsdRccHan:WIST rats were exposed by diet to 0 (blank diet), 300, 2000 or 4000 ppm test substance for 90 days of duration. Mean achieved dose levels: 0, 24.8, 168.0 and 325.1 mg SYN524464/kg/day for males and 0, 28.3, 186.0 and 349.8 mg/kg/day for females, corresponding to dietary inclusion levels of 0, 300, 2000 or 4000 ppm respectively. No mortality. No test substance treatment related effects on clinical signs, clinical pathology, hematology, blood clinical chemistry, urinalysis, ophthalmology, as well as Functional observation battery and motor activity assessments were observed. The following treatment related effects were observed: Reduction of body weight, food consumption, cumulative body weight change in 4000 ppm group males, in 2000 and 4000 ppm group females; Increased liver weights (absolute and relative) in 2000 and 4000 ppm group males and females (statistically significant only in 4000 ppm groups); Increased hepatocellular hypertrophy in 4000 ppm group males and females in microscopic examinations. **NOEL (No Observed Effect Level): 300ppm in male and female rats (24.8 mg/kg/day for males, 28.3 mg/kg/day for females) due to body weight, food consumption, liver weights and microscopic findings at 2000 ppm and 4000 ppm group animals. Acceptable** (Pan& Leung, 9/12/13).
Rat 28-Day Repeated Dosing Dermal Toxicity Study
53210-0017; 271847 822; "SYN524464 - 28-day dermal toxicity (semi-occlusive) study in the Wistar Rat"; (Sommer, E.W., Harlan Laboratories Ltd., Switzerland, 12/11/09, Laboratories Study C21075); SYN524464 (83.0% trans isomer, 12.3% cis isomer), Batch ID SMU6LP006/Milled, 95.3% w/w, off white powder, stored at ambient temperature in the dark. Groups of 10/sex Wistar Rats were exposed by dermal application to 0 (control), 100, 300, or 1000 mg/kg/day test substance for 28 days, 6 hours a day, 5 days a week. No mortality. No test substance treatment related effects on clinical signs, body weights and food consumption, hematology, clinical biochemistry, functional observational battery, urinalysis, ophthalmology and pathology were observed. NOEL (No Observed Effect Level): 1000 mg/kg/day for male and female rats due to no treatment related effects at HDT. Acceptable (Pan & Leung, 9/20/13).

Mouse 4-Week Dietary Toxicity Study
53210-0010; 271840-SUP; "SYN524464 - 4-week mouse dietary preliminary study "; (Shearer, J., and Robertson, B., Charles River, Edinburgh EH32 2NE, UK, 12/16/08, Laboratories Study # 458168); SYN524464, Batch ID S01F002249U MILLED, 98.2% (83.4% trans, 14.8% cis) SY524464, solid white powder, groups of 5/sex CD-1 mice (Crl:CD-1(ICR)) were exposed by diet to 0 (blank diet), 1000, 5000 or 7000 ppm test substance for at least 28 days of duration. Mean achieved dose levels: 0, 178, 920 and 1268 mg SYN524464/kg/day for males and 0, 248, 1150 and 1800 mg/kg/day for females, corresponding to dietary inclusion levels of 0, 1000, 5000 and 7000 ppm respectively. Once a week all animals received a detailed clinical examination, body weights were recorded twice weekly during pretrial, daily during the first week, and then weekly thereafter until the completion of treatment. Food consumptions were recorded twice weekly. Clinical pathology was conducted from all animals prior to terminal kill after 28 days of treatment. Necropsy was conducted for all animals. Additional liver samples were taken from all animals during necropsy for possible RT-PCR(reverse transcription – polymerase chain reaction) and gene expression profiling and metabolomics (LC/MS, GC/MS and NMR analysis of liver extract). Abnormal vocalization in hand in 1 of 7000 ppm group male, agitation, hunched body and piloerection among 2 females at 1000 ppm group, damages left ear in 1 female at 7000 ppm were observed. No test substance related effects were observed in body weight, body weight gains, food consumption, clinical pathology and histologic findings. NOEL(No Observed Effect Level): 7000 ppm (1268 mg/kg/day for males, and 1800 mg/kg/day for female mice in the 4 week dietary preliminary study. Supplemen tal (Pan & Leung, 8/29/2013).

Mouse Subchronic Dietary Toxicity Study
53210-0014; 271844-SUP; "SYN524464 - 90 day mouse preliminary carcinogenesis study "; (Shearer, B., Foster, B., Laboratories Study # 458278); SYN524464, Batch ID SMU6LP006/Milled, 95.3% (83.0% trans, 12.3% cis) SYN524464, off white powder, groups of 10/sex CD-1 mice were exposed by diet to 0 (diet only), 500, 3500 or 7000 ppm test substance for at least 90 days. Mean test substance intake: 0, 80, 567 and 1167 mg SYN524464/kg for males and 0, 112, 810 and 1455 mg SYN524464/kg for females, corresponding to dietary inclusion levels of 0, 500, 3500 and 7000 ppm, respectively. Animals were observed for viability twice daily, for clinical examinations, body weight and food consumption once weekly. Blood samples were collected from all animals prior to terminal kill. Necropsies on all animals were conducted at the end of the treatment period. All animals survived the scheduled treatment period. No mortality or clinical signs related to treatment. Decreased body weight, body weight gain and food utilisation was observed in the male mice at 7000 ppm group. NOEL(No
**Observed Effect Levels**: 3500 ppm (567 mg/kg/day) in male mice due to body weight and food utilization changes, 7000 ppm (1455 mg/kg/day) for female mice due to no effects at HDT. **Supplemental** (Pan & Leung, 9/4/2013).

**Dog 4-Week Oral Toxicity Study**
53210-0011; 271841-SUP; "SYN524464 - 4-week Oral (capsule) toxicity study in the Beagle Dog"; (Jackson, A. M., 12/09/08, Laboratories Study # B09325); SYN524464, Batch ID SO1F002249U MILLED, 98.2% (83.4% trans, 14.8% cis) SYN524464, solid white powder, groups of 1/sex Beagle Dog were exposed by capsule to 0 (blank capsule), 50, 100 or 300 mg/kg/day test substance for 4 weeks. Animals were observed for viability and clinical signs twice daily, food consumption at daily, body weight once weekly. Ophthalmoscopic and veterinary examinations were conducted at pretest and after week 4. Blood and urine samples were collected from all animals at pretest, week 2 and week 4. Necropsies on all animals were conducted at the end of the treatment period. All animals survived the scheduled treatment period. Medication during two days of the treatment of Betadine was prescribed by the study veterinarian to the control male dog for the injury of the foot pad on the right side of hind limb. Loose and watery feces were observed in all dogs during the treatment. Vomiting of feed was observed in the male dog at 300 mg/kg on week 4. Decreased food consumption and body weight was observed in the female dog at 300 mg/kg throughout the study. An enlarged liver in the male dog at 300 mg/kg was observed. This finding corresponds to microscopical findings of slight hepatocellular hypertrophy with minimal hepatocellular vacuolation. Slight hepatocellular vacuolation was recorded in female dog at 300 mg/kg also. No Observed Effect Level: (NOEL): 100 mg/kg due to body weight and food consumption changes in female dog, pathological findings in male and female dogs at 300 mg/kg. **Supplemental** (Pan & Leung, 9/5/2013).

**Dog Subchronic Oral Toxicity Study**
53210-0015; 271845; "SYN524464 - 13 week oral (capsule) toxicity study in the Beagle Dog"; (Jackson, A. M., Harlan Laboratories Ltd., Switzerland, 12/9/08, Laboratories Study B18911); SYN524464 (83.0% trans isomer, 12.3% cis isomer), Batch ID SMU6LP006/Milled, 95.3% w/w, off white powder, stored at ambient temperature in the dark. Groups of 4/sex Beagle Dogs were exposed by capsule to 0 (blank capsule), 50, 150 or 400 mg/kg/day test substance for 90 days of duration. No mortality. No test substance treatment related effects on clinical signs, hematology, urinalysis, and ophthalmology were observed. The following treatment related effects were observed: Reduction of body weights in 150 and 400 mg/kg/day group females compared with those of the control group dogs throughout the study, reduction of cumulative body weight changes throughout the study in all treated males, from day 43 in all treated females, reduced food consumption throughout the study in 400 mg/kg/day males and all treated females, reduction of total cholesterol in 150 and 400 mg/kg/day males; grade 1 thyroid follicular cell hypertrophy in 1, 2, and 1 males of 50, 150 and 400 mg/kg/day groups, respectively, and in 2 high dose group females. NOEL (No Observed Effect Level): < 50 mg/kg/day for males due to reduced cumulative body weight changes and microscopic findings in the thyroid gland, in females due to cumulative body weight changes and reduced food consumption. **Acceptable** (Pan & Leung, 9/16/13).

**CHRONIC STUDIES**

**Chronic, rat**
** 53210-0019; 271853 835; "SYN524464 - 104 week rat dietary carcinogenicity study with combined 52 week toxicity study"; (Perry, C.J., Charles River Laboratories, Edinburgh, EH33 2NE, UK, Laboratories Study 458304); SYN524464 (83.0% trans isomer, 12.3% cis isomer),
Batch ID SMU6LP006/Milled, 95.3% w/w, off white powder, stored at ambient temperature in the dark. Groups of 52/sex Wistar Rats were exposed by diet to 0 (control), 200, 1200, or 3600 ppm test substance for at least 104 weeks. In addition, groups of 12/sex animals were included and dosed in an identical fashion for a period of 52 consecutive weeks for a toxicity study. Mean achieved test substance uptake: 11, 67 and 218 mg/kg/day for males, and 14, 86 and 261 mg/kg/day for females, corresponding to dietary inclusion levels of 200, 1200 and 3600 ppm respectively. No test substance treatment related mortality. No test substance treatment related effects on clinical signs, hematology, urinalysis, and ophthalmology were observed. Reductions of body weights, body weight changes and food consumption in high dose males and high and mid-dose females, changes in clinical biochemistry including decreased phosphate, increased glucose and total protein in high and mid dose males, decreased alanine aminotransferase and aspartate aminotransferase, increased cholesterol in high and mid dose females at selected time points were observed. Increased liver weight in high and mid dose males was observed. In summary of histological findings for Toxicity and Carcinogenicity study animals, increased thyroid follicular cell hyperplasia in high dose males, increased kidney inflammation and thymus tubular epithelial hyperplasia in high dose females, increased liver hepatocyte pigment, hepatocyte hypertrophy, eosinophilic cell focus and thyroid colloid basophilia in high dose male and females, were observed when analyzed by Mann-Whitney U test. Increased thyroid follicular cell desquamation in high dose females were observed by pairwise Fisher's Exact test. Increased liver hepatocyte hypertrophy was observed in 1200ppm males. Increased uterus adenocarcinoma in in carcinogenicity and all high dose females was observed in the Fisher’s test and the Peto test. NOEL (No Observed Effect Level): 200 ppm (11 and 14 mg/kg/day for male and female rats, respectively) due to body weight, body weight changes, food consumption, clinical chemistry, and non neoplastic and neoplastic histological findings. Acceptable (Pan& Leung, 10/1/13).

Chronic, dog
** 53210-0016; 271846 831; "SYN524464 - 52 week oral (capsule) toxicity study in the Dog"; (Braun, L., Harlan Laboratories Ltd., Switzerland, 11/25/09, Laboratories Study B18900); SYN524464 (83.0% trans isomer, 12.3% cis isomer), Batch ID SMU6LP006/Milled, 95.3% w/w, off white powder, stored at ambient temperature in the dark. Groups of 4/sex Beagle Dogs were exposed by capsule to 0 (blank capsule), 15, 50, or 200 mg/kg/day test substance for 52 weeks of duration. No mortality. No test substance treatment related effects on clinical signs, hematology, urinalysis, and ophthalmology were observed. The following treatment related effects were observed: lower body weights, cumulative body weight changes and food consumption in 200 mg/kg/day group males and females, increased plasma alkaline phosphatase and increased liver weight in high dose group males. NOEL (No Observed Effect Level): 50 mg/kg/day for males and females due to reduced food consumption, body weight and cumulative body weight changes, clinical biochemistry and higher liver weights in high dose group males and females. Acceptable (Pan& Leung, 9/19/13).

Oncogenicity, rat
See Chronic Toxicity, rat above.

Oncogenicity, mouse
** 53210-0020; 271854 835; "SYN524464 - 80 week mouse dietary carcinogenicity study"; (Perry, C.J., Charles River Laboratories, Edinburgh, EH33 2NE, UK, Laboratories Study 458283); SYN524464 (83.0% trans isomer, 12.3% cis isomer), Batch ID SMU6LP006/Milled, 95.3% w/w, off white powder, stored at ambient temperature in the dark. Groups of 50/sex CD-1 mice were exposed by diet to 0 (control), 200, 1250, or 7000 ppm test substance for at least 80 weeks. Mean achieved test substance uptake: 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. No test substance treatment related mortality. No test substance treatment related effects on clinical signs, hematology, organ weights and microscopic findings were observed. Reductions of body weights, body weight changes and food consumption in high dose males and high dose females were observed. No treatment related neoplastic findings were observed. NOEL (No Observed Effect Level): 1250ppm (157 mg/kg for males, 185 mg/kg for females) for males and females due to reduced body weight, body weight change and food consumption in the 7000 ppm dose level. Acceptable (Pan & Leung, 10/11/13).

GENOTOXICITY

Gene mutation
** 53210-0018 271848 “SYN524464 - Salmonella Typhimurium and the Escherichia Coli reverse mutation assay”, 842, Bacteria reverse mutation assay. Sokolowski, A., Harlan Cytotest Cell Research GmbH (Harlan CCR), Germany. 11/5/09. Laboratory Project Study no.1074301. SYN524464 (83.0% trans isomer, 12.3% cis isomer), Batch ID SMU6LP006/Milled, 95.3% w/w, off white powder, stored at ambient temperature in the dark. Test substance up to 5000 ug/plate in DMSO was tested in Salmonella Typhimurium TA 1535, TA1537, TA100 and TA98; Escherichia Coli WP2uvrA (pKM 101) and WP2(pKM 101) for potential mutagenicity with or without metabolic activation of rat liver S9-mix employing plate incorporation (experiment I) or preincubation assay (experiment II) for 60 minutes prior to incubating for 48 hours at 37°C. Strain specific positive control materials induced expected responses in both assays with or without metabolic activation. Test substance showed negative responses over the dose range tested in all tester strains, with or without metabolic activation. The test substance is not mutagenic. Acceptable. (Pan & Leung, 10/2/2013)

** 53210-0018 271850 “SYN524464 - Cell mutation assay at the Thymidine Kinase Locus (TK+)/­ in mouse lymphoma L5178Y cells”, 842, In vitro Mammalian cell gene mutation test. Wollny, H-E., Harlan Cytotest Cell Research GmbH (Harlan CCR), Germany. 11/18/09. Laboratory Project Study no.1074303. SYN524464 (83.0% trans isomer, 12.3% cis isomer), Batch ID SMU6LP006/Milled, 95.3% w/w, off white powder, stored at ambient temperature in the dark. Test substance at concentrations up to 110 ug/ml (in DMSO) was tested in the mouse lymphoma L5178Y cells with or without rat liver S9-mix for 4 hours. A two-day recovery period followed before 10-15 days selection time with TFT (Trifluorothymidine, 5 ug/mL). Cloning efficiencies were determined for survival and viability before and after the recovery period. Positive control materials demonstrated increases in mutant colony numbers after selection of forward mutation on the Thymidine Kinase Locus (TK+/­), in conditions with or without metabolic activation. Test substance treatment did not induce changes in forward mutation mutant colony numbers with or without rat liver S9-mix. Not mutagenic effect in mouse lymphoma L5178Y cells was observed under conditions tested. Acceptable. (Pan & Leung, 10/10/13)

Chromosome damage
** 53210-0018 271849 “SYN524464 - Chromosome aberration test in Human lymphocytes In vitro”, 843, In vitro Chromosome aberration test. Bohnenberger, S., Harlan Cytotest Cell Research GmbH (Harlan CCR), Germany. 10/22/09. Laboratory Project Study no.1074302. SYN524464 (83.0% trans isomer, 12.3% cis isomer), Batch ID SMU6LP006/Milled, 95.3% w/w, off white powder, stored at ambient temperature in the dark. Test substance up to 5000 ug/plate in DMSO was tested in Salmonella Typhimurium TA 1535, TA1537, TA100 and TA98; Escherichia Coli WP2uvrA (pKM 101) and WP2(pKM 101) for potential mutagenicity with or
without metabolic activation of rat liver S9-mix. Strain specific positive control materials induced expected responses in both assays with or without metabolic activation. Test substance showed negative responses over the dose range tested in all tester strains, with or without metabolic activation. The test substance is not mutagenic. **Acceptable.** (Pan & Leung, 10/2/2013)

** 53210-0018 271851 “SYN524464 - Micronucleus assay in bone marrow cells of the mouse”, 843, mammalian erythrocyte micronucleus test. Reichenbach, M., Harlan Cytotest Cell Research GmbH (Harlan CCR), Germany. 2/15/2010. Laboratory Project Study no.1074304. SYN524464 (83.0% trans isomer, 12.3% cis isomer), Batch ID SMU6LP006/Milled, 95.3% w/w, off white powder, stored at ambient temperature in the dark. Test substance at concentrations of 0, 500, 1000 or 2000 mg/kg (in 0.5% carboxymethylcellulose (CMC)) was given to 6 male mice per group by a single oral administration, followed by collection of bone marrow cells at 24 or 48 hours later. Bone marrow smears were prepared, evaluated for polychromatic erythrocytes (PCE), and at least 2000 polychromatic erythrocytes (PCE) were analysed per animal for micronuclei. Positive control cyclophosphamide at 40 mg/kg produced statistically significant increase in micronuclei as expected. There was no test substance treatment related, biologically significant changes in numbers of PCEs with micronuclei in treated mice. **Acceptable.** (Pan & Leung, 10/10/13)

DNA damage or miscellaneous effects

** 53210-0018 271852 “SYN524464 - unscheduled DNA synthesis in mammalian cells in culture”, 844, unscheduled DNA synthesis in mammalian cells in culture. Durward, R., Harlan Laboratories Ltd., Derbyshire DE72 2GD, UK. 11/25/2009. Laboratory Project Study no. 2364/0089. SYN524464 (83.0% trans isomer, 12.3% cis isomer), Batch ID SMU6LP006/Milled, 95.3% w/w, off white powder, stored at ambient temperature in the dark. Test substance at concentrations of 0 (5 animals), 667 or 2000 mg/kg (in 0.5% carboxymethylcellulose (CMC)) was given to 4 male mice per group by a single oral administration, followed by perfusion of livers at 16 or 2 hours later. Additional groups of 3 male rats were given oral administration of positive control materials 2-Acetylaminofluorine (2AAF, 50 mg/kg) in Experiment 1 or Dimethylhydrazine dihydrochloride (NDHC, 40 mg/kg) in Experiment 2. Hunched posture, lethargy and pilo-erection were observed in the 2000 mg/kg group rats in both Experiments. The hepatocytes were isolated using a two stage in situ method immediately after termination of the animal. The cell suspension was filtered, centrifuged and washed, suspended in attachment medium, seeded onto cover slips in 6 well plates to allow cell attachment. After cell attachment, cells were washed and incubated with serum free medium containing 10 uCi/ml (370 kBq/ml) of [methyl-3H] thymidine for 3 hours at 37°C. Following the radiolabelling of the cells, the cultures were washed, fixed, the coverslips were air-dried and were mounted onto glass slides, coated with autoradiographic emulsion and incubated at 4°C for 7 to 14 days in a sealed light proof container. After the exposure period the slides were processed, the cells were stained with H&E and were assessed for signs of toxicity, reduced numbers and poor labeling. Three slides per animal were scored when possible with a maximum of 50 cells per slide. The number of silver grains within the nucleus (N), mean cytoplasmic grain count from three cytoplasmic areas (C) were used to calculate a net Nuclear Grain Count (N-C), and the percentage cells in repair (%R) were calculated, “Cells in repair” were those with net Nuclear Grain Count (N-C) greater than or equal to 5. The positive control materials induced statistically significantly increased net Nuclear Grain Count (N-C) in both experiments, there were no changes in net Nuclear Grain Count (N-C) in the slides from treated animals compared with those from vehicle control treated animals. Therefore the test substance was deemed negative in genotoxicity under the conditions of this study. **Acceptable.** (Pan & Leung, 10/10/13)
REPRODUCTIVE TOXICITY, RAT
** 53210-0022; 271856; “SYN524464 – Two-Generation Reproduction Toxicity Study in the Han Wistar Rat”; (S. Whitlow; Harlan Laboratories Ltd., 4414 Fullinsdorf, Switzerland; Study No. C18904; 1/26/10); Twenty five HanRcc:WIST rats/sex/group were treated in the diet with 0, 200, 500, or 1500 ppm of SYN524464 (Sedaxane Technical) (batch no. SMU6LP006/Milled, purity: 95.3% (SYN508210 (trans isomer): 83.0%, SYN508211 (cis isomer): 12.3%) for two generations. The treatment included 10 weeks prior to mating, mating, 3 weeks of gestation and 3 weeks of lactation for the P generation. At that time, 25 F1 animals/sex/group were selected as parents and treated for 13 weeks prior to mating, followed by mating and 3 weeks each of gestation and lactation of the F2 generation. No treatment-related deaths occurred during the study. The P generation females in the 1500 ppm group had lower mean body weight gain than those of the controls at the end of the premating and lactation periods (p<0.01). The mean food consumption of these animals during these periods was lower than that of the control animals as well. The mean adjusted liver weights of both sexes in the 1500 ppm group for both generations and for the 500 ppm males for both generations were greater than those of the control group (p=0.01 or 0.05). In the histopathological evaluation, centrilobular hypertrophy was noted in the livers of both sexes in the 1500 ppm group of both generations. There was no treatment-related effect upon the estrous cycling or sperm motility, morphology or sperm head count in either generation. There was no treatment-related effect upon sexual maturation for either generation. The mean time to sexual maturation was delayed by approximately 1.5 days for the 1500 ppm F1 generation. Evaluation of ovarian staging revealed a reduction in the number of corpora lutea in the 1500 ppm females of both generations. There were no treatment-related effects upon the fertility or gestation indices. The mean litter sizes were not affected by the treatment. There was no treatment-related effect upon pup viability. Mean pup weights for the 1500 ppm group of both generations were lower than those of the control group on days 14 and 21 of lactation (p<0.05 or 0.01). No adverse effect indicated. Parental NOEL: (M) 200 ppm (16 to 17 mg/kg/day) (based upon the increased adjusted liver weights of the males in the 500 ppm group) (F) 500 ppm (40 to 93 mg/kg/day) (based upon increased adjusted liver weight and/or reduced body weight gain of the adults females of the 1500 ppm group), Reproduction NOEL: 1500 ppm (M:120 to 134 mg/kg/day, F: 117 to 282 mg/kg/day) (based upon the lack of a treatment-related effect at the highest treatment level), Developmental NOEL: 500 ppm (F: 87 to 93 mg/kg/day) (based upon lower mean pup weights during lactation of the 1500 ppm group of both generations); Study acceptable. (Moore, 8/23/13)

53210-0021; 271855; “SYN524464 – Oral (Dietary) Multigeneration Range Finding Study in the Rat”; (E.S. Richmond; Sequani Limited, Ledbury, Herefordshire HR8 1 LH, UK; Study No. BFI0005; 11/20/09); In the F0 generation, ten Wistar rats/sex/group received 0, 500, 1500 or 3600 ppm of SYN524464 (Sedaxane Technical) (batch no. SMU6LP006, purity: 95.3% (SYN508210 (trans isomer): 83.0%, SYN508211 (cis isomer): 12.3%) in the diet for 10 weeks prior to mating, during the mating period, and during the 3-week gestation and 4-week lactation periods. The F1 generation received the test material in the diet up to post-natal day 35. No deaths resulted from the treatment. The mean body weight gain and food consumption of both sexes in the 3600 ppm treatment groups were less than the control values during the premating period (p<0.001). The mean body weight gains of the females in the 3600 ppm group were also less than those of the control group during the gestation and lactation periods (p<0.01 or 0.001). The reproductive parameters were not apparently affected by the treatment. The viability indices for the treated groups were reduced in a treatment-related manner. The mean body weights of the pups in both sexes of the 3600 ppm treatment group of the F1 generation were less than those of the control group by 21 days post-natal (p<0.05 or 0.001). In the necropsy of both the adults in the F0 generation and the 21-day old pups of the F1 generation, the mean absolute and relative liver weights of both sexes in the 1500 and 3600 ppm groups
were greater than the control group values (p<0.001). **No adverse effects evident.** No NOELs were established for the various parameters due to the limited scope of the study. **Study supplemental.**  (Moore, 8/21/13)

**DEVELOPMENTAL TOXICITY**

**Rat**

** 53210-0024; 271858; “SYN524464 – Prenatal Developmental Toxicity Study in the Han Wistar Rat, Final Report, Amendment 1”; (C. Senn; Harlan Laboratories Ltd., 4414 Fullinsdorf, Switzerland; Study No. C23955; 12/28/09); Twenty four mated female Han Wistar rats/group were dosed orally by gavage with 0 (aqueous 0.5% carboxymethyl cellulose), 25, 100 or 200 mg/kg/day of SYN524464 (Sedaxane Technical) (batch no. SMU6LP006/Milled, purity: 95.3% (SYN508210 (trans isomer): 83.0%, SYN508211 (cis isomer): 12.3%) from gestation day 6 through gestation day 20. No unscheduled deaths occurred during the study. Reduced body weight gain was noted for the dams in the 200 mg/kg group. The mean food consumption of the dams in the 100 and 200 mg/kg groups was reduced during the course of the treatment (p<0.01 or 0.05). The mean body weight of the fetuses in the 200 mg/kg group was less than that of control group ((males, NS), (females, p<0.05)). There were no other treatment-related effects upon the fetuses. **No adverse effect evident. Maternal NOEL:** 25 mg/kg/day (based upon the reduced food consumption noted for the dams in the 100 mg/kg treatment group); **Developmental NOEL:** 100 mg/kg/day (based upon the reduced weights of the fetuses in the 200 mg/kg treatment group); **Study acceptable.**  (Moore, 8/27/13)

** 53210-0023; 271857; “SYN524464 – Dose Range-Finding Prenatal Developmental Toxicity Study in the Han Wistar Rat, Final Report Amendment 1”; (S. Whitlow; Harlan Laboratories Ltd., 4414 Fullinsdorf, Switzerland; Study No. C23944; 12/22/09); Ten mated Wistar female rats/group were dosed orally by gavage with 0 (vehicle: aqueous 0.5% carboxymethyl cellulose), 200, 500 or 750 mg/kg/day of SYN524464 (Sedaxane Technical) (batch no. SMU6LP006/Milled, purity: 95.3% (SYN508210 (trans isomer): 83.0%, SYN508211 (cis isomer): 12.3%) from gestation day 6 through day 20. The test material was sufficiently toxic that all of the animals in the 500 and 750 mg/kg groups died or were euthanized in extremis by the termination of the study. The dams in the 200 mg/kg group did not demonstrate any apparent treatment-related effects. Fetal development was not affected by the treatment. **No adverse effect indicated. Study supplemental.**  (Moore, 8/23/13)

**Rabbit**

** 53210-0026; 271860; “SYN524464 – A Prenatal Developmental Toxicity Study in New Zealand White Rabbits”; (P. Sawhney Coder; WIL Research Laboratories, LLC, Ashland, OH; Study No. WIL-639037; 1/26/10); Twenty five time-mated female New Zealand White rabbits/group were dosed orally by gavage with 0 (aqueous 0.5% (w/v) methylcellulose), 25, 100, or 200 mg/kg/day of SYN524464 (Sedaxane Technical) (batch no. SMU6LP006/Milled, purity: 95.3% (SYN508210 (trans isomer): 83.0%, SYN508211 (cis isomer): 12.3%) from day 7 through day 28 of gestation. No unscheduled deaths resulted from the treatment. One doe in both the 25 and 100 mg/kg groups and two does in the 200 mg/kg group aborted by day 28. The mean body weight gain of the 200 mg/kg does was less than that of the control group over the course of the treatment. The mean food consumption for this group was also less than that of the control group.. The mean fetal weights of both sexes in the 200 mg/kg group were less than the control values (males, NS, females, p<0.05). There were no other treatment-related effects upon the development of the fetuses. **No adverse effect indicated. Study supplemental.**  (Moore, 8/23/13)
treatment group); **Developmental NOEL**: 100 mg/kg/day (based upon the reduced weight of the fetuses in the 200 mg/kg group); **Study acceptable.** (Moore, 8/28/13)

53210-0025; 271859; “SYN524464 – Dose Range-Finding Prenatal Developmental Toxicity Study in the New Zealand White Rabbit”; (P. Sawhney Coder; WIL Research Laboratories, LLC, Ashland, OH; Study No. WIL-639036; 1/26/10); Ten time-mated female New Zealand white rabbits/group were dosed orally by gavage with 0 (vehicle: aqueous 0.5% (w/v) carboxymethyl cellulose), 100, 300 or 500 mg/kg/day of SYN524464 (Sedaxane Technical) (batch no. SMU6LP006/Milled, purity: 95.3% (SYN508210 (trans isomer): 83.0%, SYN508211 (cis isomer)): 12.3%) from gestation day 7 through 29. One doe in the control group died as a consequence of an intubation error. Two does in the 500 mg/kg group were euthanized *in extremis* on day 16. Another doe delivered on day 29. The mean body weight gain and food consumption of the does in the 300 and 500 mg/kg groups were less than the control values over the course of the treatment period. The mean absolute and relative liver weights of the does in these two groups were greater than that of the control group. There was no apparent treatment-related effect upon the development of the fetuses. **No adverse effect indicated.** Study supplemental. (Moore, 8/27/13)

---

**NEUROTOXICITY**

**Acute neurotoxicity, rat**

53210-0028; 271862; “SYN524464 – Acute Oral (Gavage) Neurotoxicity Study in the Rat”; (E.W. Sommer; Harlan Laboratories Ltd., 4452 Itingen, Switzerland; Study No. B86591; 10/8/09); Ten Wistar rats/sex/group were dosed orally by gavage with 0 (vehicle: aqueous 0.5% (w/v) carboxymethylcellulose), 30, 250 or 2000 mg/kg of SYN524464 (Sedaxane Technical); batch no. SMU6LP006/Milled, purity: 95.3% (SYN508210 (trans isomer): 83.0%, SYN508211 (cis isomer): 12.3%). At 6 hours post-dose, 4 males and 3 females in the 2000 mg/kg group were euthanized *in extremis* due to the severity of clinical signs. The mean body weight gains of both sexes in the 2000 mg/kg group and the males in the 250 mg/kg group were less than those of the control group during the 1st week post-dose. The mean food consumption of both sexes in the 250 and 2000 mg/kg groups was less than that of the control group during the 1st 24 hours post-dose (p<0.01). In the Functional Observational Battery, at 2 to 4 hours post-dose, recumbency, reduced muscle tone, decreased activity, decreased rearing, piloerection, bradypnea, initial inactivity, reduced body temperature and/or diminished fore- and hindlimb grip strength were noted for both sexes in the 250 and 2000 mg/kg groups. The signs were indicative of CNS depression. The session totals for total distance and number of rearings in the motor activity assessment were lower for both sexes in the 250 and 2000 mg/kg groups on the 1st day at 2 to 4 hours post-dose (NS, p<0.01). There was no treatment related effect upon brain weight and no treatment-related lesions were noted in the nervous tissues of the study animals. **Possible adverse effect**: Clinical signs indicative of CNS depression. **Rat Acute Neurotoxicity NOEL**: (M/F) 30 mg/kg (based upon the treatment-related effects noted in the FOB for the animals in the 250 mg/kg treatment level). **Study acceptable.** (Moore, 8/30/13)

53210-0027; 271861; “SYN524464 – Preliminary Acute Oral (Gavage) Neurotoxicity Study in the Rat”; (E.W. Sommer; RCC, Ltd., 4452 Itingen, Switzerland; Study No. B67408; 8/19/08); Three Wistar rats/sex/group were dosed orally by gavage with 0 (vehicle: aqueous 0.5% (w/v) carboxymethyl cellulose), 80, 1000 or 2000 mg/kg of SYN524464 (Sedaxane Technical) (batch no. SMU6LP006/Milled, purity: 95.3% (SYN508210 (trans isomer): 83.0%, SYN508211 (cis isomer): 12.3%). In a modified Functional Observational Battery assessment, reduced activity, recumbency, spasms and piloerection, were noted for animals at all of the treatment levels.
Uncoordinated movements were also exhibited by animals in the 1000 and 2000 mg/kg treatment groups. The time to peak effect was 3 hours. **Possible adverse effect:** uncoordinated movements. **Study supplemental.** (Moore, 8/28/13)

90-day neurotoxicity, rat

53210-0031, -0038; 271865, 271908; “SYN524464: 90-Day Neurotoxicity (Dietary) Study in the Rat”; (E.W. Sommer; Harlan Laboratories Ltd., 4452 Itingen, Switzerland; Study No. B67432; 10/29/09); Ten Wistar rats rats/sex/group received 0, 300, 1000, or 4000 ppm of SYN524464 (Sedaxane Technical) (batch no. SMU6LP006/Milled, purity: 95.3% (SYN508210 (trans isomer): 83.0%, SYN508211 (cis isomer): 12.3%) in diet for 13 weeks ((M) 0, 19.7, 66.0, 260.0 mg/kg/day, (F) 0, 24.3, 79.7, 302.9 mg/kg/day). All study animals survived to the termination of the study. The mean body weight gains of both sexes in the 4000 ppm group were less than those of the control group over the course of the study (p<0.01). The mean food consumption of these animals was less than that of the control group as well. There were no treatment-related effects upon any of the FOB parameters over the course of the study. The motor activity of both sexes in the 4000 ppm group was generally less than that of the control group over the treatment period (NS, p<0.05). No treatment-related effect was noted in the ophthalmological examination. **No adverse effect indicated.** **Rat Subchronic Neurotoxicity NOEL:** (M/F) 4000 ppm ((M) 260.0 mg/kg/day, (F) 302.9 mg/kg/day) (based upon the lack of neurotoxic effects noted for the 4000 ppm treatment group); **Rat Non-Neurotoxic Subchronic Dietary Toxicity NOEL:** (M/F) 1000 ppm ((M) 66.0 mg/kg/day, (F) 79.7 mg/kg/day) (based upon lower mean body weights and body weight gains for both sexes in the 4000 ppm group); **Study acceptable.** (Moore, 9/11/13)

Rat 28-Day Dietary Toxicity Study Preliminary to Subchronic Neurotoxicity Study

53210-0030; 271864; “SYN524464 – 28-Day Dietary Toxicity Study in the Rat (Preliminary to a 90-Day Neurotoxicity Study)”; (E.W. Sommer; Harlan Laboratories Ltd., 4452 Itingen, Switzerland; Study No. B67421; 7/2/09); Eight Wistar rats/sex/group received 0, 500, 2000 or 5000 ppm of SYN524464 (Sedaxane Technical); batch no. SMU6LP006/Milled, purity: 95.3% (SYN508210 (trans isomer): 83.0%, SYN508211 (cis isomer): 12.3%) for 28 days ((M) 0, 37.8, 153.5, 360.1 mg/kg/day, (F) 0, 40.1, 156.4, 338.8 mg/kg/day). No deaths resulted from the treatment. No treatment-related clinical signs were noted. The mean body weight gains of both sexes in the 5000 ppm group and the females in the 2000 ppm group were less than those of the control group over the course of the study (p<0.05 or 0.01). The mean food consumption of these groups was also less than that of the control group (NS, p<0.05 or 0.01). No macroscopic lesions were noted in any of the treatment groups. **No adverse effect indicated.** These data indicated that the maximal treatment level for the rat subchronic neurotoxicity study should be between 2000 and 5000 ppm. **Study supplemental.** (Moore, 9/10/13)

Developmental neurotoxicity, rat

Not submitted.

Delayed neurotoxicity, hen

Not submitted.
IMMUNOTOXICITY

53210-0033; 271886; “SYN524464: A 28-Day Dietary Immunotoxicity Study in CD-1 Male Mice”; (P.L. Crittenden; WIL Research Laboratories, LLC, Ashland, OH; Study No. WIL-639053; 4/7/10). Ten CD-1 male mice/group received 0, 500, 2000 or 5500 ppm of SYN524464 (Sedaxane Technical) (batch no. SMU6LP006/Milled, purity: 95.3% (SYN508210 (trans isomer): 83.0%, SYN508211 (cis isomer)): 12.3%) in the diet for 28 days (0, 93.0, 367.3, 1080.1 mg/kg/day). Another 10 males were dosed by intraperitoneal injection with 50 mg/kg of cyclophosphamide on study days 24 through 27 as the positive control group. On day 24, each animal received an iv injection of 1x10^8 sheep red blood cells (SRBC) (0.2 ml volume). SRBC specific IgM plaques were determined for each animal by incubating a spleen cell suspension preparation with guinea pig complement and SRBC. No deaths occurred during the treatment period. There was no apparent treatment-related effect upon the mean body weights or food consumption. There were no treatment-related lesions noted in the necropsy examination. There was no treatment-related effect upon the thymus or spleen weights. The body weight-adjusted mean liver weight of the 5500 ppm animals was greater than that of the control group (p<0.05). There was no treatment-related effect evident in the plaque-forming cell assay. No adverse effect indicated. The positive control was functional. Study acceptable. (Moore, 9/12/13)

ENDOCRINE DISRUPTOR STUDIES

Study not submitted.

SUPPLEMENTAL STUDIES

STUDIES ON METABOLITES

CSCD465008

** Rat Acute Oral Toxicity  
53210-0036; 271903; “C. Simon; RCC, Ltd., 4414 Fullinsdorf, Switzerland; Study No. B56362; 6/27/08); Five female RccHan: WIST rats were dosed orally by gavage with 2000 mg/kg of CSCD465008 (Sedaxane Metabolite) (batch no. MES-103/1; purity: 94%) in the Up and Down Procedure. No deaths resulted from the treatment. Clinical signs included slight sedation and hunched posture, clearing by day 2. No treatment-related lesions were evident in the necropsy examination. LD50 (F) > 2000 mg/kg; Toxicity Category III; Study acceptable. (Moore, 9/18/13)

Mutagenicity  
** 53210-0036; 271904; “CSCD465008-Salmonella typhimurium and Escherichia coli Reverse Mutation Assay”; (A. Sokolowski; RCC, Cytotest Cell Research GmbH, 64380 Rossdorf, Germany; Study No. 1129601; 6/12/08); S. typhimurium strains TA98, TA100, TA1535 and TA1537 and E. coli strains WP2 uvrA (pKM 101) and WP2 (pKM 101) were exposed for 48 hours at 37°C to CSCD465008 (Sedaxane Metabolite) (batch no. MES-103/1; purity: 94%) at concentrations ranging from 3 to 5000 ug/plate with and w/o activation in the first experiment, using plate incorporation as the exposure procedure. In the second experiment, cells were exposed to concentrations of the test material ranging from 33 to 5000 ug/plate with and w/o activation, using the pre-incubation procedure in which cells were exposed to the test material for 60 minutes prior to plating and incubated for another 48 hours. Each treatment level was plated in triplicate. A phenobarbital and beta-naphthoflavone- induced rat liver S9 fraction was used to metabolize the test material. There was no treatment-related increase in reverse
mutations with or w/o activation. **No adverse effect indicated.** The positive controls were functional. **Study acceptable.** (Moore, 9/18/13)

** 53210-0036; 271905; “CSCD465008-Cell Mutation Assay at the Thymidine Kinase Locus (TK+/-) in Mouse Lymphoma L5178Y Cells”; (H-E. Wollny; RCC, Cytotest Cell Research GmbH, 64380 Rossdorf, Germany; Study No. 1129603; 4/25/08); Mouse lymphoma L5178Y cells were treated with CSCD465008 (Sedaxane Metabolite) (batch no. MES-103/1; purity: 94%) at concentrations ranging from 55.0 to 1760 ug/ml under conditions of both non-activation under conditions of activation for 4 hours at 37° C in two experiments. Duplicate cultures/treatment level were included in the study. A phenobarbital/beta-naphthoflavone-induced rat liver S9 fraction was used to metabolize the test material. Cell survival and viability and mutation frequency for each treatment level were determined and compared to those of the solvent and/or negative control. There was no dose-related increase in mutation frequency under conditions of either non-activation or activation. Positive controls were functional. **No adverse effect indicated. Study acceptable.** (Moore, 9/18/13)

**Chromosome Aberration**

** 53210-0036; 271906; “CSCD465008-Chromosome Aberration Test in Human Lymphocytes In Vitro”; (S. Bohnenberger; RCC, Cytotest Cell Research GmbH, 64380 Rossdorf, Germany; Study No. 1129602; 6/26/08); Primary human lymphocyte cultures in whole blood (stimulated with PHA for 50 to 80 hours), procured from healthy female volunteers, were treated with CSCD465008 (Sedaxane Metabolite) (batch no. MES-103/1; purity: 94%). In trial no. 1, the cells were exposed to concentrations of the test material ranging from 11.4 to 1760 ug/ml with and w/o activation for 4 hours and incubated for an additional 18 hours. In trial no. 2, the non-activated samples were exposed to concentrations of the test material ranging from 35.0 to 1760 ug/ml for 22 hours. The activated preparations were exposed for 4 hours to concentrations of the test material ranging from 35.0 to 1760 ug/ml followed by an additional 18 hours of incubation. A phenobarbital/beta-naphthoflavone-induced rat liver S9 fraction was used to metabolize the test material. Two hundred metaphases/treatment level were examined for structural abnormalities. No treatment-related increase in chromosomal aberrations was evident under conditions of non-activation or activation. The positive controls were functional. **No adverse effect indicated. Study acceptable.** (Moore, 9/18/13)

**Rat 28-Day Dietary Toxicity Study**

53210-0037; 271907; “CSCD465008-A 28-Day Oral (Dietary) Toxicity Study in Wistar Rats”; (J.M. Walraven; WIL Research Laboratories, LLC, Ashland, OH; Study No. WIL-639008; 9/19/08); Five Wistar rats/sex/group received 0, 2000, 6000 or 12000 ppm of CSCD465008 (Sedaxane Metabolite) (batch no. MES-103/1; purity: 94%) in the diet for 28 days ((M) 0, 175, 497, 1018 mg/kg/day, (F) 0, 176, 525, 1107 mg/kg/day). There were no unscheduled deaths. The mean body weights and food consumption were not affected by the treatment. The functional observational battery and motor activity assessment did not identify any treatment-related effects. There was no treatment-related effect upon the urinalysis or ophthalmological examination. Although certain parameters in the hematology and clinical chemistry evaluations of the males in the 2000 and 6000 ppm treatment groups demonstrated a statistical significance from the control group, these results were isolated with no effect being noted for other physiologically-related parameters. The absolute mean kidney weight of the 12000 ppm males was less than that of the control group (p<0.05). However, the relative mean kidney weight for these animals was not statistically significant from the control value. The histopathological examination did not reveal any treatment-related lesions. **No adverse effect indicated. Rat 28-Day Dietary Toxicity NOEL:** (M/F) 12000 ppm ((M) 1018 mg/kg/day, (F) 1107 mg/kg/day)
(based upon the lack of treatment-related effects noted for both sexes in the 12000 ppm treatment group); Study supplemental. (Moore, 9/20/13)

DF-Pyrazole Acid

Mutagenicity
** 53210-0032; 271879; “Salmonella typhimurium and Escherichia coli Reverse Mutation Assay with DF-Pyrazole Acid (CA4312)”; (A. Solokowski; RCC, Cytotest Cell Research GmbH, 64380 Rossdorf, Germany; Study No. 1077403; 5/24/07); S. typhimurium strains TA98, TA100, TA1535 and TA1537 and E. coli strains WP2 uvrA (pKM 101) and WP2 (pKM 101) were exposed for 48 hours at 37°C to CA4312 (Sedaxane Metabolite) (batch no. AMS1234/1; purity: 99.6%) at concentrations ranging from 3 to 5000 ug/plate with and w/o activation in the first experiment, using plate incorporation as the exposure procedure. The first experiment was repeated for the WP2 uvrA (pKM 101) strain, non-activated, due to equivocal results in the first assay. In the second experiment, cells were exposed to concentrations of the test material ranging from 33 to 5000 ug/plate with and w/o activation, using the pre-incubation procedure in which cells were exposed to the test material for 60 minutes prior to plating and incubated for another 48 hours. Each treatment level was plated in triplicate. A phenobarbital and beta-naphthoflavone- induced rat liver S9 fraction was used to metabolize the test material. There was no treatment-related increase in reverse mutations with or w/o activation. No adverse effect indicated. The positive controls were functional. Study acceptable. (Moore, 9/13/13)

SYN545339

Mutagenicity
** 53210-0032; 271884; “SYN545339-Salmonella typhimurium and Escherichia coli Reverse Mutation Assay”; (A. Sokolowski; Harlan, Cytotest Cell Research GmbH, 64380 Rossdorf, Germany; Study No. 1309700; 3/9/10); S. typhimurium strains TA98, TA100, TA1535 and TA1537 and E. coli strains WP2 uvrA (pKM 101) and WP2 (pKM 101) were exposed for 48 hours at 37°C to SYN545339 (sedaxane metabolite) (batch no. AMS1290/1; purity: 97.7%) at concentrations ranging from 3 to 5000 ug/plate with and w/o activation in the first experiment, using plate incorporation as the exposure procedure. In the second experiment, cells were exposed to concentrations of the test material ranging from 1 to 2000 ug/plate with and w/o activation, using the pre-incubation procedure in which cells were exposed to the test material for 60 minutes prior to plating and incubated for another 48 hours. Each treatment level was plated in triplicate. A phenobarbital and beta-naphthoflavone- induced rat liver S9 fraction was used to metabolize the test material. There was no treatment-related increase in reverse mutations with or w/o activation. No adverse effect indicated. The positive controls were functional. Study acceptable. (Moore, 9/16/13)

SYN545783

Mutagenicity
** 53210-0034; 271888; “SYN545783-Salmonella typhimurium and Escherichia coli Reverse Mutation Assay”; (A. Sokolowski; Harlan, Cytotest Cell Research GmbH, 64380 Rossdorf, Germany; Study No. 1200100; 4/2/09); S. typhimurium strains TA98, TA100, TA1535 and TA1537 and E. coli strains WP2 uvrA (pKM 101) and WP2 (pKM 101) were exposed for 48 hours at 37°C to SYN545783 (sedaxane metabolite) (batch no. BPS 1287/1; purity: 97% (cis isomer: 13%, trans isomer: 84%)) at concentrations ranging from 3 to 5000 ug/plate with and w/o activation in the first experiment, using plate incorporation as the exposure procedure. In
the second experiment, cells were exposed to concentrations of the test material ranging from 10 to 5000 ug/plate with and w/o activation, using the pre-incubation procedure in which cells were exposed to the test material for 60 minutes prior to plating and incubated for another 48 hours. Each treatment level was plated in triplicate. A phenobarbital and beta-naphthoflavone-induced rat liver S9 fraction was used to metabolize the test material. There was no treatment-related increase in reverse mutations with or w/o activation. **No adverse effect indicated.** The positive controls were functional. **Study acceptable.** (Moore, 9/17/13)

**SYN545751**

**Mutagenicity**

** 53210-0034; 271891; “SYN545751-Salmonella typhimurium and Escherichia coli Reverse Mutation Assay”; (A. Sokolowski; Harlan, Cytotest Cell Research GmbH, 64380 Rossdorf, Germany; Study No. 1286900; 12/7/09); S. typhimurium strains TA98, TA100, TA1535 and TA1537 and E. coli strains WP2 uvrA (pKM 101) and WP2 (pKM 101) were exposed for 48 hours at 37°C to SYN545751 (Sedaxane Metabolite) (batch no. BPS 1302/1; purity: 95%) at concentrations ranging from 3 to 5000 ug/plate with and w/o activation in the first experiment, using plate incorporation as the exposure procedure. In the second experiment, cells were exposed to concentrations of the test material ranging from 10 to 5000 ug/plate with and w/o activation, using the pre-incubation procedure in which cells were exposed to the test material for 60 minutes prior to plating and incubated for another 48 hours. Each treatment level was plated in triplicate. A phenobarbital and beta-naphthoflavone-induced rat liver S9 fraction was used to metabolize the test material. There was no treatment-related increase in reverse mutations with or w/o activation. **No adverse effect indicated.** The positive controls were functional. **Study acceptable.** (Moore, 9/18/13)