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OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: 11/16/2016

SUBJECT: Florpyrauxifen-benzyl: Toxicology Disciplinary Chapter

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A set of required guideline toxicology studies on florpyrauxifen-benzyl were reviewed, and a toxicology chapter was written based on the information presented in the data evaluation records. The Toxicology Disciplinary Chapter for Florpyrauxifen-benzyl is attached.

FLORPYRAUXIFEN-BENZYL

(XDE-848 Benzyl Ester, Rinskor)

PC Code: 0390093

Toxicology Disciplinary Chapter

Date Completed
October 27, 2016

Prepared for:
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1.0 Hazard Characterization of

Mode of Action

XDE-848 benzyl ester is a synthetic auxin (plant hormone) belonging to arylpicolinate class of herbicides. It asserts its effect via binding to auxin receptor, causing the affected plants to essentially grow themselves to death. The mechanism of action with respect to mammalian toxicity is not available.

Absorption, Distribution, Metabolism, and Elimination

The ADME data demonstrate that orally administered XDE-848 benzyl ester was absorbed moderately at low dose level (10 mg/kg) (36-42% of the administered dose). In contrast, at the high dose (300 mg/kg), the absorption was low relative to the increase in dose levels (8-9% of the administered dose) (Note: this pattern of absorption will be explore further in this section). The maximum plasma concentration was reached within 2 hour of dosing. The tissue distribution data indicated the administered compound was mostly found in the portal of entry (GI tract), the blood, excretory organ (urinary bladder, plasma, kidneys), and liver. The data also suggest little potential for bioaccumulation.

The absorbed compound undergoes hydrolysis resulting in a major metabolite (X11438848 or XDE-848 acid) which is present in the blood in substantially higher quantity than the parent compound (Appendix A, Metabolic Pathway). In contrast the parent compound is essentially undetectable in the majority of blood samples, but it is found in large quantities in feces indicating much of the administered dose is not absorbed. In the blood, X11438848 concentration was considered to reflect the concentration of the parent compound. The majority of the administered dose was eliminated within the first 24 hours post-dosing.

Overall, the kinetic data indicate only ≈40% of the administered dose is absorbed. The absorption reaches a plateau between 200 and 300 mg/kg as indicated in Figure 1, which has been graphed with the data from the 90-day oral toxicity in rats only. Figure 2 is graphed from a composite data set composed of 90-day, 6-month, and 12-month data on the blood concentrations X11438848. The data set is derived from studies employed the same strain of rats, experimental procedures, and similar dose levels. This figure clearly shows that blood concentration of X1143848 reached an inflection point for absorption at approximately 200 mg/kg/day and a definite maximum level for absorption at 300 mg/kg/day. Based on these data, the rat reproduction and the chronic/carcinogenicity toxicity studies employed 300 mg/kg/day as the highest dose. The 300 mg/kg/day is considered as the kinetically derived maximum dose (KMD).

Figure 1: Blood AUC_{24h} of the Metabolite X11438848 in Rats After 90-days of Exposure to XR-848 Benzyl Ester via the Diet

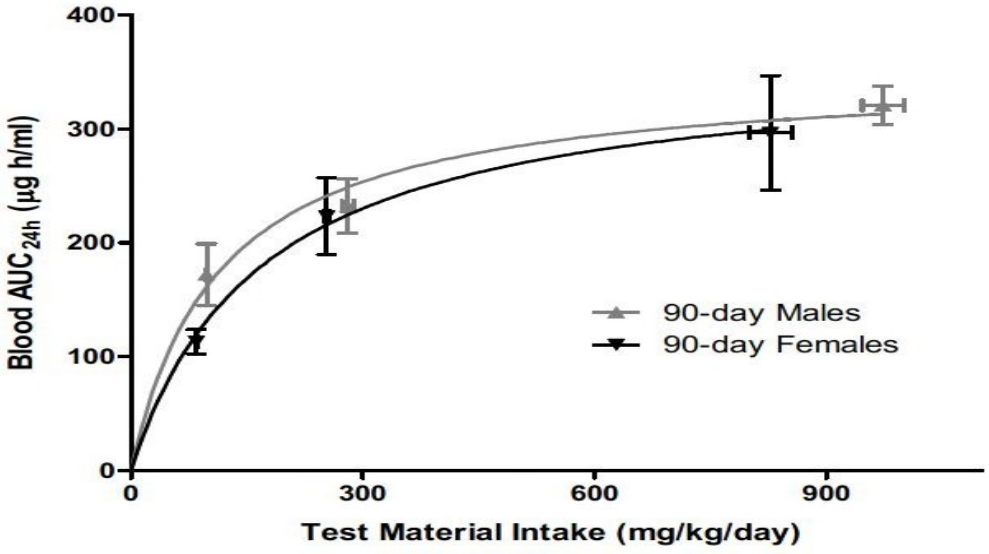
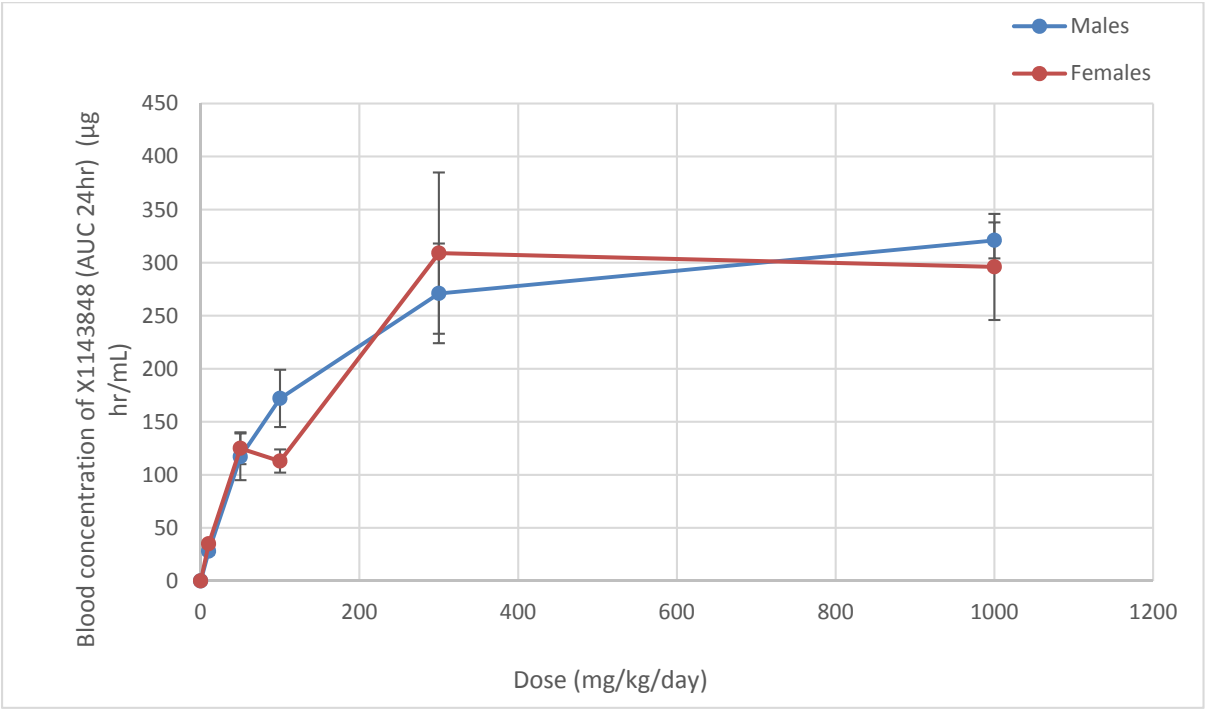


Figure 2. Blood concentrations of X11438848 from 90-day, 6-month and 12-month kinetics data (Composite data).



The kinetics data from the 2-generation reproduction and developmental toxicity studies in rats demonstrated that the major metabolite, X11438848, was found in milk and blood of dams, pups and fetuses. The concentrations were less in the milk and blood of pups and fetuses when compared to the concentrations in the blood of the maternal animals. Blood levels of X11438848 in pups ranged from approximately 6% to 17% of those in dams on PND 4. Milk levels of X11438848 were approximately 50% of blood levels for dams. Fetal blood concentration of X11438848 was approximate 56% of the dam blood concentration. **The data indicated that fetuses were exposed to XDE-848 benzyl ester *in utero*, and the pup were exposed during the *in utero* and postnatal life stages.**

Dermal Absorption

No dermal penetration study on technical grade is available. However, a 28-day dermal toxicity study in rats demonstrated no adverse effects at the limit dose (1000 mg/kg/day). In addition, the kinetics data from this dermal toxicity study indicated less than 1% of the applied dose was found in the blood.

Observed Toxicological Effects in the Submitted Studies

The submitted animal toxicity studies on XDE-848 benzyl ester demonstrate low toxicity in the required guideline studies. In subchronic oral toxicity studies in rats, mice and dogs, XDE-848 benzyl ester did not produce adverse effects at doses at or above the limit dose (1000 mg/kg/day). Similarly, developmental toxicity studies in rats and rabbits did not demonstrate adverse effects in maternal test animals or fetuses up to the limit dose. In the 2-generation reproduction study in rats, no adverse parental, reproductive, or offspring effects were found at the kinetically derived maximum dose (300 mg/kg/day) as described above.

The combined chronic/carcinogenicity in rats conducted at the kinetically derived maximum dose (300 mg/kg/day) showed no adverse effect and no compound-related increase in tumor incidence was found.

A carcinogenicity study in mice was conducted at the limit dose and found no adverse effect or any evidence of carcinogenic potential. There is no evidence of mutagenicity in *in vivo* or *in vitro* assays.

XDE-848 benzyl ester has a low acute toxicity (Toxicity Category IV) by the oral, dermal, or inhalation exposure routes, but it has a weak dermal sensitization potential.

2.0 TOXICOLOGY DATA REQUIREMENTS

The requirements (CFR 158.340) for food use for XDE-848 benzyl ester are in Table 1. Use of the new guideline numbers does not imply that the new (1998) guideline protocols were used.

Table 1. Toxicology Data Requirements

Test		Technical	
		Required	**Satisfied**
870.1100	Acute Oral Toxicity	yes	yes
870.1200	Acute Dermal Toxicity	yes	yes
870.1300	Acute Inhalation Toxicity	yes	yes
870.2400	Primary Eye Irritation	yes	yes
870.2500	Primary Dermal Irritation	yes	yes
870.2600	Dermal Sensitization	yes	yes
870.3050	28-Day Oral (rodent)	no	yes
870.3100	Oral Subchronic (rodent)	yes	yes
870.3150	Oral Subchronic (nonrodent)	yes	yes
870.3200	21-Day Dermal	yes	yes
870.3250	90-Day Dermal	CR	-
870.3465	90-Day Inhalation (28-day inhalation –rats)	yes	waived[a]
870.3700a	Developmental Toxicity (rodent)	yes	yes
870.3700b	Developmental Toxicity (nonrodent)	yes	yes
870.3800	Reproduction	yes	yes
870.4100b	Chronic Toxicity (dog)	no	yes
870.4200b	Carcinogenicity (mouse)	yes	yes
870.4300	Chronic toxicity (1year) (rats)	yes	yes
	Oncogenicity (rat)	yes	yes
870.5100	Mutagenicity—Gene Mutation - bacterial	yes	yes
870.5300	Mutagenicity—Gene Mutation - mammalian	yes	yes
870.5xxx	Mutagenicity—Structural Chromosomal Aberrations	yes	yes
870.5xxx	Mutagenicity—Other Genotoxic Effects	yes	yes
870.6100a	Acute Delayed Neurotox. (hen)	no	-
870.6100b	90-Day Neurotoxicity (hen)	no	-
870.6200a	Acute Neurotox. Screening Battery (rat)	yes	yes
870.6200b	90 Day Neurotox. Screening Battery (rat)	yes	waived[a]
870.6300	Developmental Neurotoxicity	CR	-
870.7485	General Metabolism	yes	yes
870.7600	Dermal Penetration	CR	-
870.7800	Immunotoxicity	yes	yes

[a]: HASPOC, TXR 0057486; 08/04/2016

3.0 TOXICOLOGY DATA GAP(S)

All the required studies are available.

4.0 AVAILABLE TOXICOLOGY DATA

Essentially all the required toxicological studies are available; the requirement for a subchronic inhalation toxicity study and a 90-day neurotoxicity study has been waived (HASPOC, TXR 0057486; 08/04/2016). The available studies for assessing the health risk are the following:

- subchronic oral toxicity studies in rats, dogs, and mice,
- combined chronic/carcinogenicity study in rats,
- carcinogenicity study in mice,
- developmental toxicity studies in rats, rabbits,
- reproduction study in rats,
- subchronic neurotoxicity studies in rats,
- battery of mutagenicity studies,
- metabolism study in rats, and
- immunotoxicity study in rats

4.1 Acute Toxicity

The data base for acute toxicity is considered complete. No additional studies are required at this time. The acute toxicity data on the XDE-848 benzyl ester technical is summarized in the table below. XDE-848 benzyl ester is not acutely toxic via the oral, dermal or inhalation routes of exposure. The LD_{50} for both oral and dermal acute exposure is > 5000 mg/kg/day (Tox Cat. VI), and the LC_{50} for acute inhalation exposure is > 5.23 mg/L (Tox Cat. IV). XDE-848 benzyl ester is not an eye or skin irritant. It has, however, weak dermal sensitization potential. The acute inhalation study did not report any portal of entry effects or acute irritation.

Summary of acute toxicity data for XDE-Benzyl Ester (Technical ai)

Type of study	MRID	Results	Toxicity Category
Acute oral LD_{50}-rat	49677703	LD_{50} > 5000 mg/kg	IV
Acute dermal LD_{50}-rat	49677704	LD_{50} >5000 mg/kg body weight	IV
Acute inhalation LC_{50} (4 h)-rat	49677705	LC_{50} > 5.23 mg/L	IV
Skin irritation-rabbit	49677707	Not a skin irritant	IV
Eye irritation-rabbit	49677706	Not an irritant	IV
Skin sensitisation-mouse (local lymph node assay)	49677708	EC_3 at 19.1% of the applied concentration:	weak dermal sensitization potential

4.2 Subchronic Toxicity

The data base for subchronic toxicity is considered complete with a subchronic inhalation toxicity study waived (HASPOC, TXR 0057486; 08/04/2016). No additional studies are required at this time.

In general, XDE-848 benzyl ester administration by oral and dermal routes for 28-day or 90-days produced no adverse effects at dose levels as high as or above the limit dose (1000 mg/kg/day) in rats, mice, and dogs.

870.3050 28-Day Oral Toxicity Studies

28-Day feeding study in rats (MRID 49677845)

In a 28-day oral toxicity study (MRID 49677845), groups of F344/DuCrl rats (5/sex/dose) were administered XR-848 benzyl ester (94.5%; Lot No.: 201102376-16) in the diet at target dose levels of 0, 250, 500, or 1000 mg/kg/day for at least 28 days. These targeted doses corresponded to time-weighted average doses of 0, 268, 541, or 1065 mg/kg/day for males and 0, 262, 517, or 1043 mg/kg/day for females, respectively. Parameters evaluated were cage-side observations, weekly detailed clinical observations, ophthalmic examinations, body weights, body weight gains, feed consumption, hematology, prothrombin time, clinical chemistry, urinalysis, toxicokinetics of XR-848 benzyl ester and its major metabolite X11438848 (XR-848 acid) in blood and urine, selected organ weights and gross and histopathologic examinations.

Under the conditions of the study, there were no treatment-related effects on clinical sign, body weight, body weight gain, feed consumption, ophthalmology, hematology, prothrombin time, clinical chemistry parameters, urinalysis, organ weights, and gross or histopathologic observations.

The kinetic analysis data showed that XR-848 benzyl ester was present at detectable levels in only three out of 120 whole blood samples from male and female rats. However, the major metabolite, X11438848 (XR-848 acid), was present in all blood samples from treated animals. It should be noted that the metabolism data appeared to show that the absorbed parent compound was rapidly and essentially completely metabolized to the X11438848 by hydroxylation at the benzyl ester moiety of the molecule. As such, this metabolite was considered as a surrogate for the parent compound in the blood. Both XR-848 benzyl ester and X11438848 were present in the urine of male and female rats collected over 24 hours at all doses. On average, only 0.04% of the dose was excreted as XR-848 benzyl ester in the 24 hour urine while 13% of the dose was excreted as the major metabolite X11438848.

Based on the daily measurements (AUC_{24h}) for X11438848, it exhibited sublinear kinetics at the targeted dose levels of 500 and 1000 mg/kg/day. Similarly, the total amount of X11438848 excreted over 24 hours in urine was sublinear when plotted against dose at the targeted dose levels of 500 mg/kg/day in females and 1000 mg/kg/day in males. Urine levels of XR-848 benzyl ester

appeared to exhibit a sublinear relationship with dose at 1000 mg/kg/day (target dose) in males.

Under the conditions of this study, the no-observed-effect level (NOAEL) for male and female rats was the targeted dose of 1000 mg/kg/day (highest dose tested) (time weighted average doses were 1065 and 1043 mg/kg/day for males and females, respectively).

This subchronic toxicity study in the rats is reliable (acceptable/guideline) and satisfies the guideline requirement (OECD Guideline 407 (2008), EEC, Part B.7 (2008); US EPA, PPTS 870.3050 (2000)) for a repeat-dose oral study; in rats.

28-Day feeding study in mice (MRID 49677846)

In a 28-day oral toxicity study (MRID 49677846), groups of Crl:CD1(ICR) mice (5/sex/dose) were administered XR-848 benzyl ester (94.5%; Lot #: 201102376-16) in the diet at target dose levels of 0, 250, 500, or 1000 mg/kg/day for 28 days. Parameters evaluated were daily cage-side observations, weekly detailed clinical observations, ophthalmic examinations, body weights, feed consumption, body temperatures, hematology, clinical chemistry, toxicokinetics of XR-848 benzyl ester and the major metabolite X11438848 (XR-848 acid) in blood and urine, selected organ weights and gross, and histopathologic examinations.

There were no treatment-related effects on mortalities, clinical signs, body weights, feed consumption, body temperatures, ophthalmic, hematology, or clinical chemistry parameters. There were no treatment-related organ weight effects, and no adverse gross or histopathologic changes were found in any dose groups relative to the controls.

The parent compound, XR-848 benzyl ester, was not present at detectable levels in most of the blood samples from treated male and female mice after 28 days of exposure. XR-848 benzyl ester was present at low levels in a majority of the urine samples from male mice at all doses, and in female mice only at the high dose. The major metabolite, X11438848, was present in all blood and urine samples from treated animals. Levels of XR-848 benzyl ester in the blood (when present) were approximately 2% of those of the metabolite in males and 0.4 percent in females. Levels of XR-848 benzyl ester in urine were less than 0.01 percent of the metabolite levels in both males and females. The high concentration of the major metabolite reflects the finding from the rat metabolism study, which showed that the absorbed XDE-848 benzyl ester was rapidly and efficiently metabolized to X1143848 by hydroxylation at the benzyl moiety. Blood levels of X11438848 were sublinear (with respect to dose) at the high dose in males and females. Urine levels of parent and metabolite were linear with respect to dose.

Under the conditions of this study, no effects were seen in any parameters examined. The no-observed-adverse-effect level (NOAEL) was 1000 mg/kg/day (highest tested dose; limit dose). No increase in tumor incidence was seen at the highest dose tested.

This study is reliable (acceptable/guideline) and satisfies the data requirements for a 28-day oral toxicity study [OECD Guideline 407 (2008); USEPA, OPPTS 870.3050 (2000)].

28-Day feeding study in dogs (MRID 49677867)

This study was conducted to evaluate the palatability and potential toxicity of the test material; it employed only 2 females in the low dose group (15,000 ppm) and 2 males and 2 females in the 30,000 ppm group. The most serious flaw was that a control group was not included. In addition, the compound intake during the duration of the study was determined to be in a range from approximately 286 to 718 mg/kg/day for 15,000 ppm group (2 female dogs) and from 656.9 to 1343.6 mg/kg/day and 344.6 to 1282.8 mg/kg/day in males and females, respectively, for 30,000 ppm groups.

Without the controls, it would be rather impossible to determine any possible effect or lack of any effects the test compound might have on the test animals. The compound intake data demonstrated such a wide range of dose levels and presented so much uncertainties as to the real dose the test animal were receiving. This study is considered scientifically unacceptable, and it has not been evaluated by the primary reviewer. The following is the registrant's review as presented in the Tier II Summary.

In a 28-day oral toxicity study (MRID 49677867) in beagle dogs, XR-848 benzyl ester (92.9%; Lot/batch no.: E3536-60-1) was administered in the diet to 2 females at 15,000 ppm and 2 male and 2 females at 30,000 ppm for 28 days. Dose levels were calculated to be ranging from 285.9 to 718.0 mg/kg/day for 15,000 ppm group (2 female fogs) and for 30,000 ppm groups, from 656.9 to 1343.6 mg/kg/day and 344.6 to 1282.8 mg/kg/day in males and females, respectively. No control group was employed. The study was conducted to evaluate the palatability and potential toxicity of the test material.

Observations for morbidity, mortality, injury, and the availability of food and water were conducted twice daily for all animals. Toxicity was assessed by clinical observations, body weight and food consumption measurements, test material intake, and clinical pathology evaluations. At study termination, necropsy examinations were performed and tissues were microscopically examined.

On Day 18, one male given XR-848 benzyl ester at 30,000 ppm in the diet was found prostrate with pale gums, slow capillary refill time, skin cold to touch and frothy white vomitus in the cage. Veterinary findings included laterally recumbent, eyes straight forward and nonresponsive to touch, body tense but not rigid, and abdomen doughy upon palpation. The dog was euthanized at veterinary recommendation. Clinical pathology findings suggested an inflammatory response with evidence of dehydration, and changes that were potentially secondary to a hemolytic process and typical of hepatobiliary and skeletal muscle injury. At necropsy, a moderate depletion of body fat, small thymus, irregular surface of the right kidney, and a urinary bladder mildly distended with red urine were observed. The moribund condition of this dog was interpreted to be associated with prolonged contraction and subsequent degeneration of skeletal muscles throughout the body following histopathologic evaluation and the death was considered unrelated to treatment and unique to this individual animal.

All remaining animals survived to the scheduled necropsy. Although food consumptions were generally reduced in all study animals only during the first one to three days of exposure to diet containing XR-848 benzyl ester, body weights were reduced (4 to 5%) in dogs (both sexes) at 30,000 ppm during the 21 days of dosing. No effect on body weights were observed in females at 15,000 ppm. No clinical signs of toxicity were observed in animals that survived to the scheduled necropsy, and no treatment-related effects were noted in clinical pathology parameters or at necropsy. There were no treatment-related histopathologic effects in males or females at any dose level.

In conclusion, body weight was slight reduced (4 to 5%) in animals at 30,000 ppm during the course of the study. No treatment-related clinical signs of toxicity or effects on clinical pathology parameters were observed, and no target organs were identified at either 15,000 or 30,000 ppm. Therefore, in a 90-day dietary study in dogs, the highest concentration of 30,000 ppm would be acceptable

870.3100 90-Day Oral Toxicity — Rat

90-day feeding study in rats. (MRID 49677848).

In a 90-day toxicity study (MRID 49677848), groups of F344/DuCrl rats (10/sex/dose) were administered XDE-848 benzyl ester (94.5%; lot/batch no. 201102376-16) in the diet at targeted doses of 0, 100, 300, or 1000 mg/kg/day for 91 days (males) or 92 days (females). The parameters evaluated were daily cage-side observations, weekly detailed clinical observations, ophthalmic examinations, body weights, feed consumption, hematology, prothrombin time, urinalysis, clinical chemistry, selected organ weights, gross and histopathologic examinations, and toxicokinetics. This study included evaluation of functional observational battery parameters, a detailed histologic evaluation of the nervous system.

There were no adverse effects observed in clinical signs, body weights, feed consumption, ophthalmic examinations, hematology, prothrombin time, or clinical chemistry parameters. There were no treatment-related effects on organ weights or gross pathological observations.

Treated females had a slightly higher incidence of increased urine pH (≥9.0) (2/10, 5/10, 5/10, and 8/18 for 0, 100, 300 or 1000 mg/kg/day groups, respectively). This finding was interpreted to be not adverse as there were no treatment-related changes in any of the urinalysis parameters of males at any dose level and no correlated histopathological changes. In addition, similar changes were not seen in the 28-day oral toxicity study with similar dose levels and in the chronic toxicity study tested up to 300 mg/kg/day. The changes in urinary pH values could also be influenced by the diet; it was not considered as adverse.

Treatment-related histopathological changes were limited to the kidneys of females. Females given 100, 300 or 1000 mg/kg/day had a marginal increase in the severity of background medullary tubular mineralization as compared to that observed in the controls. The severity of mineralization in all female treatment-groups was only a marginal increase over that observed in the controls and

was interpreted to be far below a threshold which would relate to any biological significance. Moreover, there were no corresponding treatment-related alterations in serum clinical chemistry parameters or signs of any systemic toxicity. There were no treatment-related histopathological changes in the kidneys of males at any dose-level. Hence, this finding was considered to be not adverse. Additional details for determining the non-adversity of this finding are presented in the "Discussion and Conclusion" section of this review.

Toxicokinetic analyses revealed no quantifiable levels of XDE-848 benzyl ester in blood samples from treated male and female rats. However, the metabolite, X11438848 (XDE-848 acid), was present in all blood samples from treated animals. It should be noted that the metabolism data appeared to show that the absorbed parent compound was rapidly and essentially completely metabolized to the X11438848 by hydroxylation at the benzyl ester moiety of the molecule. As such, this metabolite was considered as a surrogate for the parent compound. Both XDE-848 benzyl ester and X11438848 were present in the urine of male and female rats collected over 24 hours at all doses. On average, <0.05% of the administered dose was excreted as XDE-848 benzyl ester in the 24 hour urine, while 5-33% of the administered dose was excreted as the metabolite X11438848. Considering the concentrations of the major metabolite in the blood over the duration of treatment, the concentrations appeared to reach a plateau at approximately 200 mg/kg/day dose level for both males and females, and this set of data suggested a saturation of absorption at or above this level.

Daily systemic exposures measured in the blood (AUC_{24h}) for X11438848 exhibited sublinear kinetics at the targeted dose of 300 mg/kg/day in both male and female rats. The total amount of X11438848 excreted over 24 hours in urine was also sublinear at 300 mg/kg/day in female rats and at 1000 mg/kg/day in male rats. The trace levels of XDE-848 benzyl ester present in urine exhibited a linear relationship with dose in both male and female rats.
Under the conditions of this study, the no-observed-adverse effect level (NOAEL) was 1000 mg/kg/day (highest dose tested; also limit dose).

This study is fully reliable (acceptable/guideline) and meets the requirements for a 90-day oral toxicity study (OECD, Guideline 408 (1998); EEC, Part B.26 (2008); JMAFF (2000); US EPA OPPTS 870.3100 (1998).

<u>90-day oral toxicity study in mice. (MRID</u> 49677847<u>)</u>

In a 90- oral toxicity study (MRID 49677847), groups of Crl:CD1(ICR) mice (10/sex/dose) were administered XDE-848 benzyl ester (94.5%; Lot #: 201102376-16) in the diet at targeted doses of 0, 100, 300, or 1000 mg/kg/day for at least 90 days. Parameters evaluated were daily cage-side observations, weekly detailed clinical observations, ophthalmic examinations, body weights/body weight gains, feed consumption, hematology, clinical chemistry, toxicokinetics of XDE-848 benzyl ester and its major metabolite X11438848 (XDE-848 acid) in blood and urine, selected organ weights, and gross and histopathologic examinations.

There were no treatment-related effects on mortality, clinical signs, feed consumption, ophthalmic

examinations, hematology, clinical chemistry parameters, and gross or histopathologic observations. Decreases in the body weights (≈10%) were seen in 1000 mg/kg/day female mice when compared to the controls, but the body weight reduction was not seen in males of any dose group. The body weight reduction was not considered to be adverse because similar effect was not seen in the 18-month carcinogenicity study where the same strain of mice were fed at similar dose levels with larger number of mice/dose group (n=50), substantially longer duration of treatment, and magnitude of reduction barely approached the border of adversity.

Female mice given 1000 mg/kg/day had decreases in mean absolute (34.2%) and relative (27.6%) ovary weights when compared to those of the controls. These ovary weight changes were interpreted to be secondary to the lower final body weights at this dose level, and not indicative of a primary toxicological effect. Again similar effects were not seen in the carcinogenicity study.

In the blood, XDE-848 benzyl ester was not present at detectable levels in the samples from treated male and female mice after 90 days of exposure, but it was present at low levels in some of the urine samples, primarily from the higher dose groups. In contrast, the major metabolite, X11438848, was present in all blood and urine samples from treated animals at levels more than 10,000-fold higher than the XDE-848 benzyl ester in urine. This finding reflected the results seen in the rat metabolism study, where the absorbed XDE-848 benzyl ester was rapidly and efficiently metabolized to X1143848 by hydroxylation at the benzyl moiety. No significant kinetic nonlinearity was apparent in the blood levels of X11438848. Urine levels of parent and metabolite were also mostly linear with respect to dose. The only exception was for the trace levels of XDE-848 benzyl ester in the urine of male mice at the top dose, which were less than dose proportional.

Under the conditions of this study, the test compound did not produce adverse toxicity at any dose level tested; the no-observed-adverse-effect level (NOAEL) was 1000 m/kg/day (the limit dose). No increase in tumor incidence was seen at the limit dose.

This study is considered fully reliable (acceptable/guideline) and meets the requirements for a subchronic oral toxicity study [OECD, Guideline 408 (1998), EEC, Part B.26 (2008); JMAFF (2000); USEPA, OPPTS 870.3100 (1998)].

870.3150 90-Day Oral Toxicity — Dog

90-Day oral toxicity study in dogs (MRID 49677849)

In a 90-day oral toxicity study (MRID 49677849), groups of beagle dogs (4/sex/dose) were administered XDE-848 benzyl ester (96.4%, Lot no.: ENBK-121853-013B) in diet at concentrations of 0, 3,000, 10,000, and 30,000 ppm. (0, 100, 333, and 1000 mg/kg/day) for 90 days. Mortality, clinical observations, body weight, food consumption, test material intake; ophthalmoscopic examinations; and clinical chemistry were evaluated. Blood and urine samples for determination of the concentration of test material and metabolites were collected from all animals at designated time points during Week 13. Gross and histopathology were conducted on all test animals.

The results showed no deaths occurred during dosing. No treatment-related effects on body weights, food consumption, ophthalmology, or clinical pathology parameters were observed. No effects on organ weights and no macroscopic necropsy findings related to treatment were observed. There were no treatment-related histopathologic effects in male or female dogs at any dose levels.

Kinetics analysis: Both XDE-848 benzyl ester and the metabolite, X11438848 ('XDE-848 acid'), were present at quantifiable levels in nearly all blood and urine samples from treated animals. Daily systemic exposures (AUC_{24h}) for XDE-848 benzyl ester exhibited sublinear kinetics by the 10,000 ppm (333 mg/kg/day) in both male and female dogs. Blood levels of the metabolite, X11438848, were approximately 10-fold higher than those of the parent molecule. The metabolism data derived from the rat study indicated that the absorbed XDE-848 benzyl ester was rapidly metabolized to X11438848 by hydrolysis at the benzyl ester moiety. Similar to the parent compound, daily blood concentration for X11438848 exhibited sublinear kinetics by the 10,000 ppm (333 mg/kg/day) in both male and female dogs.

Less than 0.3% of the dose was excreted as XDE848 benzyl ester in the 24-hour urine, while a maximum 24% of the dose was excreted as the metabolite X11438848. The levels of XDE-848 benzyl ester in urine exhibited a sublinear relationship with the 30,000 ppm (1000 mg/kg/day) in males and the 10,000 ppm in females. The total amount of X11438848 excreted over 24 hours in urine was sublinear by 10,000 ppm in both sexes.

Overall, the kinetics data demonstrate that the blood level of the parent compound, as reflected by the major metabolite, X11438848, reached the maximum blood concentration and a maximum level of absorption around 10000 ppm (333 mg/kg/day).

The results indicated that XDE-848 benzyl ester was well-tolerated when given to beagle dogs in the diet for 90 days at concentrations up to 30,000 ppm (1000 mg/kg/day). No adverse findings were observed and no target organs were identified. Therefore, the no-observed adverse effect level (NOAEL) was 30,000 ppm (1000 mg/kg/day), the limit dose for a subchronic toxicity study in dogs.

This study is fully reliable (acceptable/guideline) and meets the guideline requirements for a subchronic oral toxicity study in dogs [OECD Guideline 409 (1998), EC Part B.27 (2008), EC Directive 2004/10 EC (2004), JMAFF (2000); OPPTS 870.3150 (1998)].

870.3250 Subchronic Dermal Toxicity — Rat

28-Day dermal toxicity study in rat (MRID 49677850)

In a 28-day dermal toxicity study (MRID 49677850), groups F344/DuCrl rats (5/sex/dose) were dermally applied XDE-848 benzyl ester (95%; Lot no. ENBK-135600-003, TSN306037) in 0.5% carboxymethyl cellulose, at a dose of 1000 mg/kg/day, for 6 hours/day and for 28 consecutive days. Parameters evaluated were daily cage-side observations, weekly detailed clinical observations, weekly dermal observations, ophthalmic examinations, body weights, body weight gains, feed consumption, hematology, prothrombin time, urinalysis, clinical chemistry,

toxicokinetic analysis of whole blood and urine, selected organ weights, and gross and histopathologic examinations.

The results showed no treatment-related effects on cage-side and detailed clinical observations. Ophthalmic and dermal examinations showed no adverse changes. No adverse effects were seen in body weight, body weight gains, feed consumption, hematology, prothrombin time, urinalysis, clinical chemistry, organ weight or gross pathology examinations in treated males or females relative to the controls.

Kinetics data showed that XDE-848 benzyl ester was not present in most blood samples during the fourth week of dermal exposure. In contrast, the major metabolite, X11438848 was consistently present in most blood samples of all treated animals. On average, less than 0.01% of the dose was excreted as XDE-848 benzyl ester while 0.3% of the dose was excreted as the major metabolite, X11438848, in the 24 hour urine of both males and females. Under the conditions of the study, the data indicated a small percentage (<1%) of the applied dose was absorbed via the skin.

Relative to controls, very slight hyperplasia and hyperkeratosis of the epidermis at the dermal test site occurred in the majority of males and females dermally exposed to 1000 mg/kg/day. The hyperplasia and hyperkeratosis were minimal in severity and consisted of very slight thickening of epidermis with no accompanying inflammation, degeneration, or necrosis. The epidermal hyperplasia and hyperkeratosis were attributed to possible mechanical effects during application and/or removal of the test material. This minimal alteration of the skin at the dermal test site was considered to be not adverse. There was no evidence of systemic toxicity noted in the treated animals.

Under the conditions of this study, the no-observed-adverse-effect level (NOAEL) for male and female rats following repeated dermal exposure was 1000 mg/kg/day (limit dose for a dermal toxicity study).

The study is considered reliable (acceptable/guideline) and meets the data requirement for a subchronic dermal toxicity study [OECD, Guideline 410 (1981), EEC, Part B.9 (2008); JMAFF (2000); USEPA, OPPTS 870.3200 (1998)].

870.3465 90-Day Inhalation — Rat

The requirement of for a subchronic inhalation toxicity study has been waived (HASPOC, TXR 0057486; 08/04/2016). The reason is that XDE-848 benzyl ester has low acute inhalation toxicity and no potential for portal of entry effects.

4.3 Prenatal Developmental Toxicity

Adequacy of data base for prenatal developmental toxicity: The data base for prenatal developmental toxicity is complete. No additional studies are required at this time.

XDE-848 benzyl ester did not produce maternal or developmental toxicity in either rats or rabbits tested up to the limit dose (1000 mg/kg/day).

870.3700a Prenatal Developmental Toxicity Study — Rat

Developmental toxicity study in rats. MRID 49577854

In a rat developmental toxicity study (MRID 49577854), groups of 24 time-mated female Crl:CD(SD) rats were administered XDE-848 benzyl ester (96.4%. Lot/batch No.: ENKK-12153-013B/ TSN304217) in the diet at concentrations of 0 and 14000 ppm on gestation day (GD) 6–21. The concentrations corresponded to timeweighted average doses of 0 and 975 mg/kg/day, respectively. Based upon the absence of toxicity at the limit dose (1000 mg/kg/day) in the dose range-finding rat developmental study (MRID 49677868), this study was conducted as a "limit test" as defined by the OECD 414 and US EPA OPPTS 870.3700 Prenatal Developmental Toxicity Study guidelines. This 'limit test' provides a significant reduction in animal use by eliminating the low- and mid-dose.

Test parameters included clinical observations, body weight, body weight gain, and feed consumption. On GD 21, all rats were euthanized and examined for gross pathologic alterations. Liver, kidneys and gravid uterine weights were recorded, along with the number of corpora lutea, uterine implantations, resorptions and live/dead fetuses. All fetuses were weighed, sexed and examined for external alterations. Approximately one half of the fetuses were examined for visceral and craniofacial alterations, while skeletal examinations were conducted on the remaining fetuses.

Blood concentrations of XDE-848 benzyl ester and the metabolite X11438848 were analyzed in samples collected from 4 dams/group and their respective litters at necropsy. The results showed that administration of XDE-848 benzyl ester in the diet at 14000 ppm (limit dose), produced no treatment-related maternal toxicity and no indication of developmental toxicity in the fetuses. Analytical data on the blood samples indicated that XDE-848 benzyl ester was not detected in blood samples collected from the dams or their fetuses on GD 21. However, the metabolite, X11438848, was present in all samples collected on GD 21 from treated dams and their litters. Fetal blood concentrations of X11438848 were on average approximately 56% of the dam blood concentrations, and these results indicated that fetuses of the dams treated with XDE-848 benzyl ester were exposed to the test compound *in utero*.

Therefore, under the conditions of this "limit test" study, the no-observed adverse-effect level (NOAEL) for maternal toxicity and developmental toxicity was the limit dose of 14000 ppm (975 mg/kg/day). This study is considered reliable (acceptable/guideline) and meet the requirements for a developmental toxicity study with a "limit test" as defined by OECD Guideline 414 (2001); US EPA: OPPTS 870.3700 (1998).

It should be noted that the results derived from the main study were consistent with those seen in the dose-range finding study (MRID 49677868) where the highest dose tested was similar to that

in the main study and no adverse effects were found at the highest dose tested (898 mg/kg/day).

870.3700b Prenatal Developmental Toxicity Study — Rabbit

Developmental toxicity study in rabbits. (MRID 49677853)

In a rabbit developmental toxicity study (MRID 49677853), groups of 24 time-mated female NZW rabbits were administered XDE-848 benzyl ester (96.4%; lot/batch no. ENBK 121853-013B) at dietary concentrations 0 or 27000 ppm on gestation days (GD) 7-28, which corresponded to time-weighted average doses of 0 and 1042 mg/kg body weight/day (mg/kg/day). Based upon the absence of toxicity at the limit dose (1000 mg/kg/day) in the range-finding rabbit developmental study (MRID 49677869), this study was conducted as a "limit test" as defined by OECD and US EPA guidelines. This 'limit test' approach provides a significant reduction in animal use by eliminating the low- and mid-dose.

Parameters evaluated for both the control and the treated group included cage-side/clinical observations, body weight, body weight gain, and feed consumption. On GD 28, all surviving rabbits were euthanized and examined for gross pathologic alterations. Liver, kidneys, and gravid uterine weights were recorded, along with the number of *corpora lutea*, uterine implantations, resorptions, and live/dead fetuses. All fetuses were weighed, sexed, and examined for external and visceral alterations. The heads were examined for craniofacial alterations by serial sectioning in approximately one half of the fetuses in each litter, while skeletal examinations were performed on all fetuses. Blood concentrations of XDE-848 benzyl ester and its major metabolite X11438848 were measured in pregnant does and their respective litters on GD 28 to evaluate dose proportionality and systemic exposure.

Administration of XDE-848 benzyl ester in the diet at 27000 ppm produced no treatment-related maternal and developmental toxicity. However, an increase in the incidence of paraovarian cysts was seen in the 27,000 ppm litters (8/24; 33%) relative to the concurrent controls (4/24; 17%). This finding was not considered compound-related because (1) the incidence of paraovarian cysts had a high back ground rate and could occur spontaneously arising from the mesonephric or paramesonephric duct, (2) the incidence was within the historical control range (11-50%), and (3) it did not impact any reproductive or developmental parameters examined.

The kinetics data showed that XDE-848 benzyl ester was not detected in any of the blood samples collected from does or their respective litters (fetuses) on GD 28. However, the major metabolite, X11438848, which was formed by hydrolysis of the benzyl ester moiety of the parent compound, was present at concentrations above the analytical lower limit of quantitation (LLQ) in all samples collected on GD 28 from treated animals and their litters. The concentrations of X11438848 in the fetal blood were approximately 10% of the doe blood concentrations. The data indicate that the fetuses of the treated does were exposed to the test chemical.
Therefore, under the conditions of this study, the no-observed adverse effect level (NOAEL) for maternal toxicity and developmental toxicity was 27000 ppm (1042 mg/kg/day) (limit dose).

This study is reliable (acceptable/guideline) and meets the guideline requirement for rabbit

developmental toxicity study OECD Guideline 414 (2001); EEC, No. L 142, 37 (2008); JMAFF, OPPTS 870.3700 (1998)

It should be noted that, in the dose range-finding study (MRID 49677869), conducted by the same testing laboratory at comparable highest dose level, no maternal or developmental effects were seen at the highest dose tested (1116 mg/kg bw/day).

4.4 Reproductive Toxicity

Adequacy of data base for reproductive toxicity: The data base for reproductive toxicity is complete. No additional studies are required at this time.

XDE-848 benzyl ester produced no parental, reproductive, and offspring toxicity at tested doses; the highest tested dose was kinetically derived maximum dose (300 mg/kg/day). This finding was also supported by range finding study (one generation) (MRID 49677852) which tested up to the limit dose (1000 mg/kg/day), and no adverse effects was found on any parameters examined in any dose groups.

870.3800 Reproduction and Fertility Effects in rats.

2-generation reproduction study (MRID 49677855)

In a 2-gneration reproductive toxicity study (MRID 49677855), groups of 25 male and 25 female Crl:CD(SD) rats were fed diets containing XDE-848 benzyl ester (95%; lot No. ENBK-135600-003) at targeted doses of 0, 10, 50, and 300 mg /kg/day for approximately ten weeks prior to breeding, and continuing through breeding, gestation and lactation for two generations (P1 and P2). The time weighted-average dose level during premating period for P1 generation were 0, 11 53, & 317 mg/kg/day for males and 10, 52, and 309 mg/kg/day for females. A high-dose level of 300 mg/kg/day was chosen for this study based upon toxicokinetic non-linearity in blood levels of the XDE-848 benzyl ester at dose levels above 100 mg/kg/day. For toxicokinetic analysis, blood samples were collected from 4 dams/dose group and from culled pups at PND 4. Milk samples were collected from the dams on lactation day 4 (LD 4).

Most of the blood samples were found to contain non-quantifiable level of XDE-848 benzyl ester; in contrast, all the samples contained high levels of the major metabolite, X11438848, which resulted from hydrolysis of the benzyl ester moiety of the absorbed compound. Hence, it was employed as a surrogate for measuring the XDE-848 benzyl ester in the blood samples of parental animals and pups, and in the milk of the P1 and P2 dams.

In-life parameters examined included clinical observations, feed consumption, body weights, estrous cyclicity, reproductive performance, pup survival, pup body weights, and puberty onset. In addition, post-mortem evaluations included gross pathology, histopathology, organ weights, oocyte quantitation and sperm count, motility and morphology in adults, and gross pathology and organ weights in pups.

Toxicokinetic analysis in the current study revealed that the parent XDE-848 benzyl ester was present in only a few blood samples from adult male and female rats, lactation day (LD) 4 dams and postnatal day (PND) 4 offspring of both the P1 and P2 generations. Low levels of XDE-848 benzyl ester were present in most milk samples, especially in the higher dose groups, with corresponding PND 4 offspring blood levels of XDE-848 benzyl ester similar to LD 4 dams. In contrast, essentially all samples contained high levels of the major metabolite, X11438848, which resulted from hydrolysis of the benzyl ester moiety of the absorbed compound. Hence, it was employed as a surrogate for measuring the XDE-848 benzyl ester in the blood samples of parental animals and pups, and in the milk of the P1 and P2 dams. The major metabolite, X11438848, was present in blood and milk at relatively high levels at all doses and time points (approximately 53-1000x higher than parent XDE-848 benzyl ester). Blood levels of X11438848 in pups were in the range of 6% to 17% of those in dams, while the milk levels of X11438848 were approximately 50% of blood levels in the dams.

The systemic exposure to parent XDE-848 benzyl ester, based on blood and milk levels of X11438848, was less than dose proportional (sublinear) at the top dose (300 mg/kg/day) in adult males and females, and in dams and pups for the majority of analyses from both generations. These sublinear toxicokinetic data were consistent with results from previous rat oral XDE-848 benzyl ester repeat dose studies showing sublinear toxicokinetics of X11438848 in blood at administered XDE-848 benzyl ester dose levels ≥100 mg/kg/day.

The results showed that treatment of rats with XDE-848 benzyl ester for two generations did not result in adversed effects on any parameters for systemic toxicity in parental or offspring animals, reproductive function, or developmental parameters of the offspring. The kinetics data clearly showed that the offsprings were exposed to the test chemical in utero and during the lactation period.

Under the conditions of the study, based on lack of any treatment-related effects on all the parameters examined, the no-observed-adverse effect level (NOAEL) for parental, reproductive, and offspring toxicity was 300 mg/kg/day (highest dose level tested). This finding was also supported by range finding study (one generation) (MRID 49677852) which tested up to the limit dose (1000 mg/kg/day), and no adverse effects was found on any parameters examined in any dose groups.

This study is considered fully reliable (acceptable/guideline) and meets the requirements for a two generation reproduction study in rats [OECD, Guideline 416 (2001), EEC Part B.35 Directive 2008/440/EC (2008); USEPA, OPPTS 870.3800 (1998); JMAFF, Guideline 2-1-17, Reproduction Study (2000)].

4.5 Chronic Toxicity and Carcinogenicity

Adequacy of data base for chronic toxicity: The data base for chronic toxicity is considered complete. No additional studies are required at this time.

In rats. XDE-848 benzyl ester was tested at the kinetically-derived maximum dose (300 mg/kg/day), and no adverse toxicity was observed in treated males and females.

Adequacy of data base for carcinogenicity: The databases for carcinogenicity in rats and mice are complete. No additional studies are required at this time.

In both rats and mice, no compound-related increase in tumor incidence was found at the kinetically-derived maximum dose in rats (300 mg/kg/day) and the limit dose in mice (1000 mg/kg/day).

870.4100a (870.4300) Combined Chronic Toxicity /Carcinogenicity — Rat

Combined Chronic toxicity/carcinogenicity study in rats. MRID 49677857

In a 2-year combined chronic toxicity/carcinogenicity study (MRID 49677857), groups F344/ DuCrl rats (60/sex/dose) were administered XDE-848 benzyl ester (96.4%; Lot/batch no. ENBK-121853-013B) in the diet at targeted doses of 0, 10, 50 or 300 mg/kg/day for up to 24 months in evaluating the potential for systemic toxicity and carcinogenicity. The targeted dose levels were similar to time-weighted average doses calculated based on the treated diet consumptions. Of the 60 animals/sex in each dose group, 10 rats/sex/dose were treated for 12 months for chronic toxicity phase of the study, while the remaining 50 rats/sex/ dose were treated for 24 months for carcinogenicity portion of the study.

Animals were evaluated by cage-side examinations, detailed clinical observations, body weight, feed consumption, and feed efficiency. Ophthalmic examinations were conducted on all rats, pre-treatment and all surviving rats prior to the scheduled necropsies. Clinical pathology evaluations (hematology, clinical chemistry, electrolytes, and urinalysis) were conducted at 3, 6, 12, 18, and 24 months. Toxicokinetic analyses were conducted on blood and urine samples from 4 rats/sex/dose at 6 months post-dosing and 10 rats/sex/dose at 12 months post-dosing. All rats had a complete necropsy with weights of selected organs recorded at the scheduled necropsies. A complete histopathological examination was performed on all control and high-dose groups and all rats that died spontaneously or were euthanized due to their moribund condition. Histopathological examination of rats from the low- and intermediate-dose levels of the 12-month chronic toxicity group and 24-month carcinogenicity groups was limited to relevant gross lesions.

The results showed no treatment-related or adverse changes in mortality rates, clinical observations, body weights, body weight gains, feed consumption, food efficiency, ophthalmologic observations, clinical pathology evaluations (hematology, clinical chemistry, electrolytes, and urinalysis) for males and females at any dose level after 12 or 24 months of dosing. There were no treatment- or compound related changes in organ weight, gross or histopathological changes (non-neoplastic and neoplastic changes) in any of the organs/tissues examined at 12 or 24 months.

Toxicokinetic analysis revealed that XDE-848 benzyl ester was not present in most blood samples after six or twelve months of exposure. In contrast, the metabolite X11438848 was present in blood at relatively high levels in all dosed groups and time points. The metabolism data appeared to show that the absorbed parent compound was rapidly and essentially completely metabolized to the X11438848 by hydroxylation at the benzyl ester moiety of the molecule. As such, this metabolite was considered as a surrogate for the parent compound. Elimination half-life values based on X11438848 blood levels were approximately 4 hours on average. Both XDE-848 benzyl ester and X11438848 were present in most urine samples. On average, 0.06% of the dose was excreted as XDE-848 benzyl ester in 24 hour urine. In males, approximately 20% of the dose was excreted as X11438848, on average; and in females, approximately 40% of the dose was excreted as X11438848, on average, in 24h urine.

Blood and urine levels of X11438848 were less than dose proportional (sublinear) at the top dose (300 mg/kg/day target dose) in both males and females, at both 6 and 12 months of exposure. Blood levels were also sublinear at the middle dose (50 mg/kg/day target dose) at twelve months. The low levels of XDE-848 benzyl ester in 24 hour urine were dose-proportional through all tested doses. Due to the clear nonlinear kinetics between 50 and 300 mg/kg/day, the high dose of 300 mg/kg/day selected for this study met the requirements for a kinetically derived maximum dose according to the OECD Guidance Document 116.

It should be noted that in treated male rats only, there were minimally higher incidence of mammary gland adenocarcinomas, skin keratoacanthomas, testicular interstitial cell adenomas, and epididymides measotheliomas relative to the controls. Most of these incidences either showed no dose related response or were within the historical control range. For testicular interstitial cell adenomas, only unilateral incidence showed an increase in the 300 mg/kg/day group; the combined unilateral and bilateral incidences showed no increase relative to the controls. The details of the tumor incidences and the rationale for considering un-related to compound treatment were discussed in the Results section. Overall, the data indicate that XDE-848 benzyl ester was not carcinogenic to F344/DuCrl rats under the conditions of this study; a battery of both *in vitro* and *in vivo* genotoxicity studies also demonstrated that XDE-848 benzyl ester was not genotoxic.

As the highest dose tested in this study was 300 mg/kg/day, the issue of whether or not the highest dose tested (HDT) was sufficiently high for a carcinogenicity study should be evaluated. In considering this issue, the toxicokinetics data of the 90-day oral study and those derived from this study should be examined. The results of both studies showed that the parent compound, XDE-848 benzyl ester was not present in practically all the blood samples after 90-day, 6-months, or 12 months of exposure. In contrast, the metabolite X11438848 was present in every blood samples at relatively high levels at all dosed groups and time points. As discussed before, the blood concentration of X11438848 reflected the parent compound concentration. The blood concentrations of X1143848 reached an inflection point of absorption at approximately 200 mg/kg/day and a definite maximum absorption level at 300 mg/kg/day in both males and females. Dosing beyond this dose level would not yield any higher exposure for the parent compound as reflected by the blood concentration of the surrogate metabolite.

Therefore, the 300 mg/kg/day is considered appropriate for the highest dose for the chronic and carcinogenicity phases of the study.

Under the conditions of this study, the no-observed-effect level (NOAEL) was 300 mg (highest dose tested), and this dose was considered as the kinetically derived maximum dose. No compound-related increase in tumor incidence was found

A subgroup of the members of the Cancer Assessment Review Committee met and considered the tumor incidences of this study (06/29/2016). The group concluded that (1) overall, none of the tumor incidences was considered to be treatment-related due to one or more of the following reasons: weak or no dose-response, no statistical significance, tumor incidence was within historical control range, or no supporting non-neoplastic lesions found in the study. (2) The highest dose tested (300 mg/kg/day) was adequate for a carcinogenicity study as supported by the kinetics data (described above).

This study was fully reliable (acceptable /guideline) and meet the requirement of a chronic and carcinogenicity study in rats according to guidelines of OECD 453 (2009), EEC Part B.33 (2008); JMAFF Combined Chronic Toxicity/Oncogenicity Study (2000) ; OPPTS 870.4300 (1998).

870.4200b Carcinogenicity (feeding) — Mouse

Mouse carcinogenicity study. MRID 49677856

In a mouse carcinogenicity study (MRID 49677856) groups of 50 male and 50 female Crl:CD1(ICR) mice were fed diets containing XDE-848 benzyl ester (96.4%; Lot/bach no.: ENBK-121853-013B, TSN304217) at target dose levels of 0, 50, 200, 800 (females) or 1000 (males) mg/kg/day for up to 18 months; the time-weighted average doses were 0, 50, 200, or 1001 mg/kg/day for males, and 0, 50, 201, or 803 mg/kg/day for females. The test animals were evaluated by cage side observations, detailed clinical examinations, body weights, feed consumption, and feed efficiency. Ophthalmic examinations were conducted pre-exposure and prior to the scheduled necropsy. Toxicokinetic analysis of blood and urine was performed at various intervals throughout the study. All mice had a complete necropsy examination. Total white blood cell (WBC) counts, differential WBC counts and weights of selected organs were evaluated from all animals that survived to the terminal necropsy at the end of 18 months. Histopathology was conducted on control and high-dose groups, as well as all mice that died or were euthanized in moribund condition. For low- and intermediate-dose groups, histopathologic examination was also conducted on selected major organs (liver, kidneys and lungs) and all gross lesions identifies at the terminal necropsy.

There were no treatment-related effects in mortality rates, clinical observations, body weights, body weight gains, feed consumption, feed efficiency, ophthalmologic observations, total and differential WBC counts, organ weights, gross pathological or histopathological observations in any of the XDE-848 benzyl ester treated groups as compared to the respective controls.

Toxicokinetic analysis showed that no quantifiable XDE-848 benzyl ester was present in most blood or urine samples collected after six or twelve months of XDE-848 benzyl ester exposure. In contrast, the major metabolite, X11438848, was present in the blood at relatively high levels at all doses and time points. At six months of exposure, blood levels of X11438848 were less than dose proportional (sublinear) at the top dose (800 and 1000 mg/kg/day target dose in females and males, respectively). Urine levels of X11438848 were less than dose proportional (sublinear) at the top dose (1000 mg/kg/day) in males but dose proportional at the top dose (800 mg/kg/day) in females. At twelve months, blood and urine levels of X11438848 were linear in females; however, in males, blood level of X11438848 was less than dose proportional (sublinear) at, and above 200 mg/kg/day. The urine level of X11438848 was less than dose proportional (sublinear) at the top dose of 1000 mg/kg/day. The trace levels of XDE-848 benzyl ester detected in male urine were linear through all tested doses at twelve months. The concentration of the major metabolite was substantially higher in urine than in the blood suggesting that rapid elimination of metabolite via urine as the parent compound was metabolized to X1143848 by hydroxylation of benzyl moiety.

No treatment-related or statistically identified increase in tumor incidence was observed in either male or female mice at any dose level, indicating that XDE-848 benzyl ester did not have carcinogenic potential in CD-1 mice under the conditions of this study.

The results demonstrated the no-observed-adverse-effect level (NOEL) was 1000 mg/kg/day in males and 800 mg/kg/day in females based on the lack of any treatment-related effects in any dose group. The highest dose tested was at (1000 mg/kg for males) or approaching the limit dose (800 mg/kg/day for femless).

This study is reliable (acceptable/guideline) and meets the data requirements for a carcinogenicity study [OECD, Guideline 451 (2009), EEC, Part B.32 (2008), JMAFF, Oncogenicity Study (2000) OPPTS 870.4200 (1998)].

870.4100b Chronic Toxicity — Dog (Not Required)

4.7 Mutagenicity (Genotoxicity Studies)

Adequacy of data base for Mutagenicity: The data base for mutagenicity is considered complete. No additional studies are required at this time.

The results of the required genotoxicity studies are summarized. XDE-848 benzyl ester is not mutagenic in bacteria (Salmonella typhimurium or Escherichia coli) or in mammalian cells (mouse lymphoma cells). Similarly, it did not induce chromosome aberrations in cultured Chinese hamster CHL/IU cells, and it was ***negative*** *in an in-vivo micronucleus assay in mice.*

All studies are acceptable and satisfy the guideline requirements for mutagenicity testing. According to the currently available data, there is no mutagenic concern for XDE-848 benzyl ester.

Bacteria reverse mutation assay

Guideline #,870.5100 Bacteria reverse gene mutation assay MRID 49677859 Acceptable/guideline	XDE-848 benzyl ester was evaluated for mutagenicity in Salmonella typhimurium strains TA98, TA100, TA102 TA1535, and TA1537, and with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9) in two phases using the plate incorporation method. XDE-848 benzyl ester was administered to the test system as a solution in dimethyl sulfoxide (DMSO) at a concentration of approximately 50 mg/mL. For Trial I, XDE-848 benzyl ester at concentrations of 156.25, 312.5, 625, 1250, 2500 and 5000 µg/plate, while for Trail II, the confirmatory test, 51.2, 128, 320, 800, 2000 and 5000 µg/plate were used. The results showed XDE-848 benzyl ester was **negative** in bacteria reverse mutation assay in presence or absence of the S9 activation system.

In vitro gene mutation assay in mammalian cell

Guideline #,870.5300 In vitro forward gene mutation assay with Chinese Hamster ovary cell (CHO/HGPRT) MRID 49677860 Acceptable /guideline	In an *in vitro* Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward gene mutation assay, XDE-848 benzyl ester was tested in two independent trials in the absence and presence of an externally supplied metabolic activation (S9) system. The concentrations ranged from 0 (solvent control) to 75 µg/ml with and without S9. The highest concentration selected was based on the solubility limitations of the test material in the treatment the positive controls were ethyl methanesulfonate (EMS) for assays in the absence of S9 and 20-methylcholanthrene (MCA) for assays in the presence of S9. Solvent control cultures were treated with 1% dimethyl sulfoxide (DMSO). Under the conditions of this study, XDE-848 benzyl ester was **negative** in CHO/HGPRT forward gene mutation assay.

In vitro mammalian cell chromosome aberration assay (test for clastrogenicity)

Guideline #,870.5375 In vitro mammalian chromosome aberration assay with rat lymphocytes MRID 49677862 Acceptable /guideline	In an *in-vitro* chromosomal aberration assay, XR-848 benzyl ester was evaluated utilizing rat lymphocytes. Approximately 48 hours after the initiation of whole blood cultures, cells were treated either in the absence or presence of S9 activation with up to eight concentrations ranging from 0 (solvent control) to 75 µg/ml of XR-848 benzyl ester. The highest concentration was based on the limit of solubility of the test material in the treatment medium. The duration of treatment was 4 or 24 hours without S9 and 4 hours with S9 (metabolic activation). Based upon the mitotic indices, cultures treated with targeted concentrations of 0 (solvent control), 9.4, 18.8, and 75 µg/ml in the absence (4 and 24 hour treatment) and presence (4 hour treatment) of S9 activation were selected for determining the incidence of chromosomal aberration. The clastogenic potential of XDE-848 benzyl ester was **negative.**.

In vivo mammalian cell assay

Guideline #,870 5395 In vivo cytogenetics-Erythrocyte micronucleus assay in mice MRID 49677846 Classification: acceptable/ guideline	This *in-vivo* micronucleus assay was integrated into the 28-day mouse oral toxicity study (MRID 49677846). In this study, groups of Crl:CD1(ICR) mice (5/sex/dose) were administered XDE-848 benzyl ester at 0, 250, 500, or 1000 mg/kg/day for 28 days, which corresponded to time-weighted average doses of 0, 244, 506, or 1025 mg/kg/day for males and 0, 256, 516, or 979 mg/kg/day for females, respectively. Groups of 5 mice/sex was treated with a single dose of cyclophosphamide (CP) (40 mg /kg) to serve as positive controls for the micronucleated peripheral blood reticulocytes (MN-RET) evaluation. Parameters evaluated were daily cage-side observations, weekly detailed clinical observations, ophthalmic examinations, body weights, feed consumption, body temperatures and MN-RET. There were no treatment-related mortalities or effects in clinical signs, body weights, feed consumption, body temperatures, or ophthalmic parameters. There were no significant differences in MN-RET frequencies or percent RET values between the groups treated with XR-848 benzyl ester and the 0 mg/kg/day controls. There was a significant increase in the frequency of MN-RET and a decrease in the percentage of RET in the positive control CP group compared to the 0 mg/kg/day controls. Therefore, under the conditions of this study, XR-848 benzyl ester was considered to be negative in the mouse peripheral blood micronucleus test.

4.8 Neurotoxicity

Adequacy of data base for Neurotoxicity: The subchronic neurotoxicity study is available, but the requirement for an acute neurotoxicity is waived (HASPOC, TXR 0057486; 08/04/2016). No additional neurotoxicity data are required at this time.

The subchronic neurotoxicity study in rats was conducted with doses at the limit dose (1000 mg/kg/day), no treatment-related effect was seen in any of the parameters examined.

870.6200a Acute Neurotoxicity Screening Battery

The requirement for an acute neurotoxicity study has been waived as no neurotoxicity was seen in a subchronic neurotoxicity or other studies in the XDE-848 benzyl ester database (HASPOC, TXR 0057486; 08/04/2016).

870.6200b Subchronic Neurotoxicity Screening Battery

Subchronic oral neurotoxicity study in rats. MRID 49677848

In a subchronic neurotoxicity portion of the 90-oral toxicity study in rats (MRID 49677848), groups of F344/DuCrl rats (10/sex/dose group) were administered XDE-848 benzyl ester (94.5%; Lot/batch no.: 201102376-16) via diet at doses of 0, 100, 300, and 1000 mg/kg/day for 91 days.

Parameters evaluated were daily cage-side observations, weekly detailed clinical observations, ophthalmic examinations, functional observational battery, body weights, feed consumption, hematology, prothrombin time, urinalysis, clinical chemistry, organ weights, gross and histopathologic examinations, including a detailed histologic evaluation of the nervous system. In addition, a toxicokinetic evaluation was also conducted.

The results showed no treatment-related effects on any of the parameters for functional observation battery, neuropathological examinations, and other parameters examined. Under the conditions of this study, the no-adverse observed-effect level (NOAEL) was 1000 mg/kg/day.

The results of this study are considered reliable (acceptable/non-guideline), but the tissues from at least 5 animals/sex/dose were not fixed in situ, as specified in both OECD and US EPA neurotoxicity study guidelines (OECD, Guideline 424 (1997); US EPA: OPPTS 870.6200 (1998), OPPTS 870.7800 (1998). Although this is a shortcoming of the neurotoxicity portion of the study, it does not seriously impact the interpretation of the neurotoxicity aspect of the results because no neurohistopathological change were found in the chronic oral toxicity study in rats and mice and 90-day oral toxicity studies in dogs and mice.

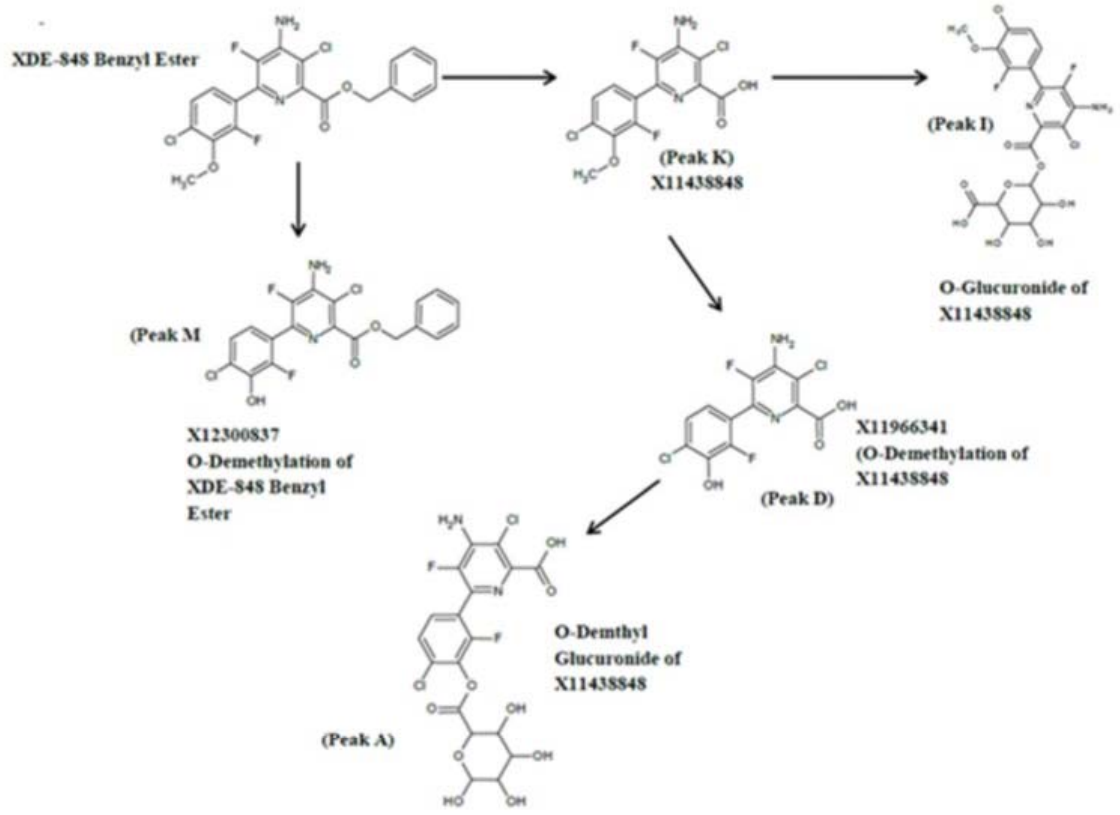
4.9 Metabolism and Pharmacokinetics

Adequacy of data base for metabolism: The data base for metabolism is complete at this time. No additional studies are required at this time.

The ADME data show that with oral dosing, XDE-848 benzyl ester is absorbed rapidly at low dose (10 mg/kg), and the absorption occurred within 12 hours post-dosing, accounting for ≈91% of the absorbed activity. The total percent of the administered dose absorbed in the low dose group was as ≈ 40% of the administered dose; in contrast in the high dose (300 mg/kg) group, the absorption is ≈6%.

The absorbed XDE-848 benzyl ester under goes mainly o-demethylation at 3-methoxyphenyl moiety, hydroxylation at the benzyl ester moiety, and subsequent glucuronidation at the hydroxyl and carboxylic acid moieties leading to the various metabolites (proposed metabolic pathway and metabolites are shown in the figure below). A total of 13 radiochemical peaks are detected in the acidified urine and/or fecal samples across the profiles of all 3 groups. X11438848 (XDE-848 acid, a hydrolysis product) is most abundant metabolite in the blood and urine, accounting for up to ~39 % of the administered dose, and it is used as a surrogate for measuring the XDE-848 benzyl ester in the blood. The remaining metabolites account for < 5% of the administered dose. ***The parent compound is not detected in the urine samples. In contrast, it is the most abundant in the feces, accounting for ~35% to ~92% of the administered dose depending on the administered dose level.*** *The metabolite X11438848 is also found in the feces in substantially less amount relative to the urine, accounting for ~3 % to ~6 % of the administered dose. X12300837 has also been identified and accounted for ~2% to ~11 % of the administered dose. The remaining metabolites observed in the radiochemical profiles of rat fecal samples account for < 5% of the administered dose. Overall the data indicate approximately 40% of the orally administered dose is absorbed and metabolized.*

Proposed Metabolic Pathway in Rats for XDE-848 Benzyl Ester



One of the unique feature for XDE-848 benzyl ester is that with oral administration, the maximum absorption occurs at approximately 300 mg/kg, above which the blood level of the test material remains constant. Hence, 300 mg/kg is considered as the kinetically-derived maximum dose.

870.7485 Metabolism — Rat

Absorption, distribution, metabolism, and elimination study in rats. MRID 49677864.

In a pharmacokinetic and metabolism study (MRID 49677864) ^{14}C- or non-labeled XDE-848 Benzyl (94.5%, Lot No. ENBK-121853-013B) was administered (gavage) to F344/DuCrl rats. Non-labeled-XDE-848 Benzyl ester was administered for 14 daily dose in the repeat-dosing studies. All the studies were conducted for 7 days (168 hours) post dosing to determine absorption, distribution, metabolism, and excretion (ADME). The studies employed single or multiple dose of 10 mg/kg (low dose), single dose of 300 mg/kg (high dose) in males and females, and repeated dose of 300 mg/kg in males.

Orally administered ^{14}C-XDE-848 Benzyl ester was absorbed rapidly without any apparent lag time. The absorption occurred within 12 hours post dosing, accounting for ≈ 91% of the absorbed

radioactivity for the low dose males and females; 73% and 59% for high dose males and females, respectively. In the low dose group, the percent absorption was as high as 42% and 41% of the administered dose in males and females, respectively. The percent absorption in the high dose group was approximately 6% of the administered dose in males and 9% in females; the percent absorption for the high dose group is substantially less that than that for the low group by at least 3 folds; the data appear to suggest there is a saturation of absorption at 300 mg/kg.

The peak plasma concentration was reached at approximately 2 hours post-dosing in both males and females irrespective of dose levels. The time-course of ^{14}C-XDE-848 Benzyl ester-derived radioactivity in plasma exhibited a biphasic decline after reaching C_{max} and was, therefore, fit to a two-compartment pharmacokinetic model. Plasma radioactivity declined rapidly during α phase ($t_{½\alpha}$ = ~2 hours) after C_{max}, followed by relatively slow decline during the terminal β phase ($t_{½\beta}$ = 2751 hours). The XDE-848 Benzyl ester C_{max} and the total systemic exposure (as represented by the AUCs of radioactivity in plasma) in the high dose groups were well below dose proportionality with only a 3-4-fold difference compared to the 30-fold difference in dose level between the low and high single oral dose groups.

Less than 0.02% of the orally administered ^{14}C-XDE-848 Benzyl ester remained in the tissues after 168 hours (7 days) post-dosing in all of the groups suggesting negligible bioaccumulation. A portion (≈ 42% in low dose and ≈ 8% in high dose) of the administered ^{14}C-XDE-848 Benzyl ester-derived radioactivity was rapidly excreted in urine without any difference between the sexes. The majority of the urinary elimination (51-92%) occurred within the first 12 hours post-dosing. Most of the remaining oral dose (51-101%) was eliminated in feces, with the majority of the fecal elimination (88-97%) occurring within the first 24 hours post-dosing. It should be noted that in a separate study with bile duct cannulation, there was approximately 6.6% of the administered dose seen in the bile (MRID 49677873). Therefore, majority of the radioactivity represents unabsorbed parental compound.

The results from the metabolite analysis indicated that XDE-848-Benzyl ester under goes mainly o-demethylation at 3-methoxyphenyl moiety, hydroxylation at the benzyl ester moiety, and subsequent glucuronidation at the hydroxyl and carboxylic acid moieties leading to the various metabolites.

A total of 13 radiochemical peaks were detected in the acidified urine and/or fecal samples across the profiles of all 3 groups. **In the urine,** no parent XDE-848-Benzyl ester was observed, and X11438848 was most abundant metabolite in the urine, accounting for ~6 % to ~39 % of the administered dose in low and high groups. The remaining metabolites accounted for < 5% of the administered dose. The parent compound was not detected in the urine samples.

In the **feces**, parent XDE-848-Benzyl ester was the most abundant peak observed, accounting for ~35% to ~92% of the dose. Two additional metabolites were observed in the feces, accounting for > 5% of the dose. The metabolite X11438848 was also found in the feces in substantially less amount relative to the urine, accounting for ~3 % to ~6 %. o the administered dose. X12300837 also had been identifies and accounted for ~2% to ~11 % of the administered dose. The remaining metabolites observed in the radiochemical profiles of rat fecal samples accounted for < 5% of the

administered dose.

This study is fully reliable (acceptable/guideline) and fulfils the guideline requirements for a metabolism study [OECD, Guideline 417 (2010); EEC, Guideline B.36 (2008); JMAFF (2000); OPPTS 870.7485 (1998)].

Tissue Distribution in F344/NTac Rats MRID 49677865

In a tissue distribution study (MRID 49677865), groups of F344/NTac rats (4/sex/dose) were administered (by gavage) a single dose of ^{14}CXDE-848 Benzyl ester (96.4%, Lot No. ENBK-121853) at dose levels of 10 or 300 mg/kg to determine the tissue distribution of ^{14}C-XDE-848 Benzyl ester at the time of maximum (T_{max}) or half of maximum ($½T_{max}$) plasma concentrations (C_{max} and $½C_{max}$). T_{max} and $½T_{max}$ were determined previously to be 1-3 and 6 hours post-dosing, respectively.
The results indicated the total recovery of radioactivity across all groups was approximately 94% of administered dose. The tissue distribution data indicated that the concentration radioactivity (µg eq/g of tissue) at C_{max} was highest in the GI tract (ranging from 70% to 90% of administered dose), followed by urinary bladder, plasma, kidney and liver in males and females. These data indicated that, with oral dosing, the test material primarily found in the portal of entry and excretory tissues. The radioactivity in the majority of other tissues (other than portal of entry and excretory) were similar to, or lower than, that of systemic blood indicating low potential for accumulation. There were no major sex differences in the distribution for XDE-848 Benzyl ester under the conditions of this study.
This study is fully reliable (acceptable/guideline) and satisfies the guideline requirements for a tissue distribution study in rats (OECD Guideline 417 (2010), EEC, Guideline B.36 (2008), JMAFF (2000) US EPA Guideline(s):US EPA, OPPTS 870.7485 (1998)

A Probe Study of the Disposition of ^{14}C-XR-848 Benzyl Ester in Bile Duct Cannulated Rats MRID 49677873

In a bile duct cannulation study (MRID 49677873), [14C]-labeled XR-848 Benzyl Ester (98.7%), was formulated as a suspension in an aqueous vehicle containing 0.5% (w/v) carboxymethyl cellulose (CMC) and administered to 2 male and 2 female Fischer rats by oral gavage at a target dose level of 100 mg/kg (approximately 120 µCi/kg). Urine was collected on wet ice prior to dosing and on salted wet ice over the intervals of 0 to 12, 12 to 24, and 24 to 48 hours post-dosing. Cages were rinsed after each urine collection and washed after the 48-hour collections. Bile was collected on wet ice prior to dosing and on salted wet ice over the intervals of 0 to 4, 4 to 8, 8 to 12, 12 to 24, and 24 to 48 hours post-dosing. Urine and bile samples were stored at approximately 70°C; cage rinse and cage wash samples were stored at approximately -20°C. Aliquots of the urine, bile, cage-rinse, and cage-wash samples, which were collected after dosing were analyzed for ^{14}C concentration using liquid scintillation counting (LSC) techniques. Aliquots of bile, approximately 250 µL, were placed into vials containing approximately 250 µL of acidified acetonitrile. Acidified bile aliquots were stored at approximately 70°C until shipped to the Sponsor for possible further analysis.

Under the conditions of the study, over 48-hours post doing for **males**, approximately 13% of the dose was eliminated in the urine and 6.6% of the dose was found in the bile. For **females** approximately 9.1% of the dose was excreted in the urine and approximately 0.5% of the dose was found in the bile for the females. However, not all test animals produced bile at all intervals. One female did not produce bile from 0-24 hours, while another female also produced no bile at 12-24 hour period. As a result, no bile sample was collected from 12-24 hour period for female rats. One male did not produce bile at 8-12 hours post-dosing period. In addition, bile remaining in the tubing at the completion of the collection period was not retained. In total, approximately 10% and 20% of the dose was recovered within 48 hours post-dosing for females and males, respectively.

The reliability of the percentage of the administered dose found in the bile is questionable because the study only employed two test animals/sex and some animals did not produce bile at some collection intervals. It could only be concluded that a small percentage of the administered dose found in the feces could be attributed to that eliminated in the bile.

Under the conditions of this study, the results derived from this study can only be considered as qualitative information, and the study is considered not fully reliable (unacceptable/non-guideline) due to the deficiencies discussed in the previous paragraph.

4.10 Dermal Absorption (Penetration)

No dermal absorption study is available; however, a 28-day toxicity study is available and indicates that XDE-848 benzyl ester produces no adverse effect at the limit dose (1000 mg/kg/day)

4.11 Immunotoxicity

*An integrated immunotoxicity study within a 90-day feeding study in rats is available, and database for Immunotoxicity is complete. The rat immunotoxicity study was tested at a limit dose (1000 mg/kg/day), and no immunotoxicity was found***.**

870.7800 28-Day Immunotoxicity studies in mice

90-Day Immunotoxicity Study in rats Feeding MRID. 49677848

An integrated immunotoxicity component was incorporated in a 90-day oral toxicity in rats (MRID 49677848), groups of F344/DuCrl rats (10/sex/dose) were administered XDE-848 Benzyl Ester (94.5%; Lot/batch no. 201102376-16) in the diet at doses of 0, 100, 300, and 1000 mg/kg/day for 90 days. Five days prior to sacrifice (Day 87 males, Day 88 females), rats were immunized with a single, (0.5 ml) intravenous (*i.v.*) injection of sheep red blood cell (SRBC) at a concentration of 4 x 10^8 cells/ml. For the positive control, animals were injected intraperitoneally (i.p.) with cyclophosphamide (CP) at a dose of 20 mg/kg on days 87-91 (males) and 88-92 (females). After 90-day in study, blood samples from rats designated for assessment of anti-SRBC IgM were collected, serum separated from blood cells, and serum samples were analyzed for anti-SRBC IgM concentration using a commercially available enzyme-linked immunosorbent assay (ELISA) kit.

Under the conditions of this study, the systemic toxicity portion of the study demonstrated no adverse effects in any dose groups. For the immunotoxicity portion of the study, XDE-848 benzyl ester exhibited no evidence of immunotoxicity as it did not result in a treatment-related effect on the primary antibody response to SRBCs in male and female rats at any tested dose levels. Therefore, the NOAEL for Immunotoxicity was 1000 mg/kg/day, which was the highest dose tested and a limit dose.

This integrated immunotoxicity component of this study is considered fully reliable (acceptable/ guideline) and meets the requirements for an immunotoxicity study (OECD, Guideline 408 (1998) EEC, Part B.26 (2008) JMAFF (2000); US EPA 870.7800.

5.0 Classification of Carcinogenic Potential

There was no compound-related increase in tumor incidence in either rat or mouse carcinogenicity studies where the highest dose was tested up to the limit dose in mice and at the kinetically derive maximum dose in rats.

6.0 Summary of Points of Departure and Toxicity Endpoints for Risk Assessment.

Since there is no adverse toxicity found in any of the toxicity studies, no toxicity endpoints and points of departure are established for risk assessment.

7.0 Appendices

Toxicity Profile for XDE-848 Benzyl Ester

Summary of acute toxicity data for XDE-Benzyl Ester (Technical ai)			
Type of study	**MRID**	**Results**	**Toxicity Category**
Acute oral LD_{50}-rat	49677703	LD_{50} > 5000 mg/kg	IV
Acute dermal LD_{50}-rat	49677704	LD_{50} >5000 mg/kg body weight	IV
Acute inhalation LC_{50} (4 h)-rat	49677705	LC_{50} > 5.23 mg/L	IV
Skin irritation-rabbit	49677707	Not a skin irritant	IV
Eye irritation-rabbit	49677706	Not an irritant	IV
Skin sensitisation-mouse (local lymph node assay)	49677708	EC_3 at 19.1% of the applied concentration:	weak dermal sensitization potential

Subchronic, Chronic and Other Toxicity Studies on XDE-848 Benzyl Ester			
Guideline No	Study Type	MRID No. (Year)/ Classification /Doses	Results
Subchronic Toxicity Studies			
Non-guideline	Palatability-rat (F344) & mice	49677866 0, 250, 500 or 1000 mg/kg/day	No effect was seen at highest dose tested (HDT) Only 3 animals/sex/dose
870.3050	28-Day feeding study –rats (F344)	49677845 (2012) Acceptable/guideline 0, 250, 500, or 1000 mg/kg/day	NOAEL = 1000 mg/kg/day (HDT)
	28-Day Oral -mice	49677846 (2012) Acceptable/guideline 0, 250, 500 or 1000 mg/kg/day	NOAEL = 1000 mg/kg/day (HDT)
	28-Day oral -dogs	49677867 (2013) Unacceptable/non-guideline 15,000 ppm (286 to 718 mg/kg/day) for females only. 30000 ppm (≈ 657 to 1344 mg/kg/day for males and ≈345.6 to 1283 mg/kg/day for females)	NOAEL = 1344/1283 (M/F) (HDT) (**This study did not have a control group)**. 1500 ppm group employed only 2 female dogs, while 30,000 ppm employed 2 dogs/sex)
870.3100	90-day feeding study –rats (F344)	49677848 (2013) Acceptable/guideline 0, 100, 300, or 1000 mg/kg/day	NOAEL = 1000 mg/kg/day (HDT)

Subchronic, Chronic and Other Toxicity Studies on XDE-848 Benzyl Ester			
Guideline No	Study Type	MRID No. (Year)/ Classification /Doses	Results
870.3100	90-day feeding study –mice	49677847 (2015) Acceptable/guideline 0, 100, 300 and 1000 mg/kg/day	NOAEL = 1000 mg/kg/day (HDT)
870.3150	90-day oral toxicity in dogs (dietary)	49677849 (2014) Acceptable/guideline 0; 3000; 10000 and 30000 ppm (M: 0, 106, 366, & 1008 mg/kg/day; F: 0, 115, 329, & 1216 mg/kg/day)	NOAEL = 1008/1216; M/F) (HDT)
870.3200	28-Day Dermal in Rats	49677850 (2015) Acceptable/guideline 0, 1000 mg/kg/day	NOAEL = 1000 mg/kg/day (HDT) The kinetics data showed a small percentage of the applied dose was absorbed (<1%).
870.3465	28-Day inhalation toxicity	Waived on 8/4/16 meeting; the final is being reviewed by the HASPOC Chairs.	
Developmental and Reproductive Toxicity Studies			
870.3700a	Prenatal Developmental Toxicity Study in Rats (dietary) Probe (range finding) study	49677854 (2015) Acceptable/guideline 0, 14000 ppm (0, 975 m/kg/day) (limit test) 49677868 (2012) 0, 6750, & 13,500 ppm (0, 445, 898 mg/kg/day)	Maternal and developmental NOAEL = 975 mg/kg/day (HDT) *Kinetics data indicated fetuses were exposed to the test compound in-utero.* No effect was seen at 898 mg/kg/day
870.3700b	Prenatal Developmental Toxicity Study in Rabbits (dietary) Probe (range-finding) study	49677853 (2014) Acceptable/guideline 0 and 27000 ppm (0, 1042 mg/kg/day) (limit rest) 49677869 (2014) 0, 8000, 14000, 20000, & 27000 ppm (0, 304, 353, 752, & 116 mg/kg/day)	Maternal and developmental NOAEL =1042 mg/kg/day (HDT) *Kinetics data indicated fetuses were exposed to the test compound in-utero.* No maternal or developmental effects were seen.
870.3800	2- Generation Reproduction - rats (diet) Probe (range-finding) study	49677855 (2015) Acceptable/guideline 0, 10, 50, & 300 mg/kg/day *(300 mg/kg/day is the kinetically derived maximum dose)* 49677852 (2013) 0, 100, 300, 1000 mg/kg/day	Parental, reproductive, and offspring NOAEL = 300 mg/kg/day (HDT) *Kinetics data indicated pups were exposed to the test compound in-utero.* No parental, reproductive, and offspring effects were seen at any dose levels.
Chronic toxicity studies			

Subchronic, Chronic and Other Toxicity Studies on XDE-848 Benzyl Ester			
Guideline No	Study Type	MRID No. (Year)/ Classification /Doses	Results
870.4100 & 870.4200	Combined Chronic/Carcino-genicity (2-yrs)-rat	49677857 (2015) Acceptable/guideline 0, 10, 50, 300 mg/kg/day *(300 mg/kg/day is the kinetically derived maximum dose)*	No adverse effect was seen at the highest dose tested (HDT) NOAEL = 300 (HDT) No increase in compound-related tumor incidence was found.
870.4200b	Carcinogenicity study-mice (78 weeks)	49677856 (2015) Acceptable/guideline Males 0,5,0, 200,1000 mg/kg/day Females: 0,5,0, 200,1000 mg/kg/day	No adverse was seen at the highest dose levels tested (1000 and 800 mg/kg/day for males and females respectively); NOAEL = 1000/800 mg/kg/day (M/F) (HDT). No increase in compound-related tumor incidence was found.
Genotoxicity studies			
870.5100	Bacterial Reverse Mutation Test (*S. typhimurium* and *E. coli)*	49677859 (2012) Acceptable/guideline 51.2 to 5000 µg/plate +/- S9	Negative
870.5300	*In Vitro* Mammalian Cell Gene Mutation Test (CHO/HGPRT)	49677860 (2012) Acceptable/guideline <u>Initial Assay:</u> 0, 2.3, 4.7, 9.4, 18.8, 37.5, and 75 µg/ml +/- S9 (solubility limitation) <u>Confirmatory Assay:</u> 2.5 to 60 µg/ml in - S9; 5.0 to 80 µg/ml in the + S9	Negative
870.5375	*In Vitro* Mammalian Chromosome Aberration Test (rat lymphocytes)	49677862 (2012) Acceptable/guideline <u>4 hr treatment:</u> 0, 1.2, 2.3, 4.7, 9.4, 18.8, 37.5, 75.0 µg/ml +/- S9; <u>24 hr treatment:</u> 0, 0.6, 1.2, 2.3, 4.7, 9.4, 18.8, 37.5, 75.0 µg/ml - S9. (solubility limitation)	Negative
870.5395	(Other Genotoxic Effects) *In vivo* Mouse bone marrow micronucleus *(dietary administration))*	49677846 (2012) Acceptable/guideline 0, 250, 500 or 1000 mg/kg/day via diet for 28 days *(Integrated in the 28-day oral toxicity study in mice)*	Negative
Neurotoxicity Studies			

Subchronic, Chronic and Other Toxicity Studies on XDE-848 Benzyl Ester			
Guideline No	Study Type	MRID No. (Year)/ Classification /Doses	Results
870.6200a	Acute Neurotoxicity- rats (gavage)	Waived on 8/4/16 meeting; the final is being reviewed by the HASPOC Chairs.	
870.6200b	90-Day Dietary Neurotoxicity Study – rats	49677848 (2013) Acceptable/non-guideline 0, 100, 300, & 1000 mg/kg/day *(Integrated into the 90-day oral toxicity study in rats)*	NOAEL=1000 mg/kg/day (HTD)
Metabolism Studies			

870.7485	Metabolism Study –Rat ADME	49677864 (2014) Acceptable/guideline 10 mg/kg (low dose), single dose of 300 mg/kg (high dose) (gavage) With repeated dosing at 10 mg/kg/day	Orally administered ^{14}C-XDE-848 Benzyl ester was absorbed rapidly without any apparent lag time. The absorption occurred within 12 hours post dosing, accounting for ≈ 91% of the absorbed radioactivity for the low dose males and females; 73% and 59% for high dose males and females, respectively. In the low dose group, the percent absorption was as high as 42% and 41% of the administered dose in males and females, respectively. The percent absorption in the high dose group was approximately 6% of the administered dose in males and 9% in females. The percent absorption for the high dose group is substantially less that than that for the low group by at least 3 folds; the data appear to suggest there is a saturation of absorption at 300 mg/kg. The peak plasma concentration was reached at approximately 2 hours post-dosing in both males and females irrespective of dose levels. Less than 0.02% of the orally administered ^{14}C-XDE-848 Benzyl ester remained in the tissues after 168 hours (7 days) post-dosing in all of the groups suggesting negligible bioaccumulation. A portion (≈ 42% in low dose and ≈ 8% in high dose) of the administered ^{14}C-XDE-848 Benzyl ester-derived radioactivity was rapidly excreted in urine without any difference between the sexes. The majority of the urinary elimination (51-92%) occurred within the first 12 hours post-dosing. Most of the remaining oral dose (51-101%) was eliminated in feces, with the majority of the fecal elimination (88-97%) occurring within the first 24 hours post-dosing. It should be noted that in a separate study with bile duct cannulation, there was approximately 6.6% of the administered dose seen in the bile (MRID 49677873). Therefore, majority of the radioactivity in the feces represents unabsorbed parental compound. The results from the metabolite analysis indicated that XDE-848-Benzyl ester under goes mainly o-demethylation at 3-methoxyphenyl moiety, hydrolysis at the benzyl ester moiety, and subsequent glucuronidation at the hydroxyl and carboxylic acid moieties leading to the various metabolites. **In the urine,** no detectable amount of parent XDE-848-Benzyl ester was found, and X11438848 was most abundant metabolite in the urine, accounting for ~6 % to ~39 % of the administered dose. In the **feces**, parent XDE-848-Benzyl ester was most abundant, accounting for ~35 % to ~92 % of the dose. Two additional metabolites were observed in the feces, accounting for > 5% of the dose. The metabolite X11438848 was also found in the feces in substantially less amount relative to the urine, accounting for ~3 % to ~6 %. o the administered dose. X12300837 also had been identifies and accounted for ~2% to ~11 % of the administered dose. The remaining metabolites observed in the radiochemical profiles of rat fecal samples accounted for < 5% of the administered dose.

Subchronic, Chronic and Other Toxicity Studies on XDE-848 Benzyl Ester			
Guideline No	Study Type	MRID No. (Year)/ Classification /Doses	Results
	Tissue distribution study in rats	49677865 (2014) Acceptable/non-guideline 10 & 300 mg/kg	Under the conditions of this study, total radioactivity recovery cross all groups was ≈ 94% of administered dose. The radioactivity concentration at C_{max} was highest in the GI tract (ranging from 70% to 90% of administered dose), followed by urinary bladder, plasma, kidney and liver in males and females. Th data indicated that, with oral dosing, the test material primarily found in the portal of entry and excretory tissues. The radioactivity in the majority of the other tissues (other than portal of entry and excretory) were similar to, or lower than, that of systemic blood indicating low potential for accumulation. There were no major sex differences in the distribution for XDE-848 Benzyl ester.
	Bile duct cannulation study in rats	49677873 (2014) Acceptable/non-guideline This study only provides qualitative information at best because it employed only 2 rats/sex at 100 mg/kg.	Over 48-hours post doing for **males**, approximately 13% of the dose was eliminated in the urine and 6.6% of the dose was found in the bile. For **females** approximately 9.1% of the dose was excreted in the urine and approximately 0.5% of the dose was found in the bile. However, not all test animals produced bile at all intervals. One female did not produce bile from 0-24 hours, while another female also produced no bile at 12-24 hour period. As a result, no bile sample was collected from 12-24 hour period for female rats. One male did not produce bile at 8-12 hours post-dosing period. Approximately 10% and 20% of the dose was recovered within 48 hours post-dosing for females and males, respectively.
Non-guideline	ADME study –dog (a probe study)	49677872 (2013) Unacceptable/non-guideline 100 mg/kg (Single dose by gavage) The study used only 2 dogs/sex.	Male and female animals excreted XDE-848 benzyl ester primarily in the feces (about 60% to 80% of the dose). Males and females excreted approximately 7 to 12% and 0.4 to 5%, respectively, of the radioactivity in the urine. Radioprofiling of select urine and feces samples indicated that XDE-848 benzyl ester is metabolized primarily to a single metabolite that was identified as XDE-848 acid (X11438848). In plasma, the concentration of total radioactivity was insufficient for metabolite profiling analysis.
Non-guideline	Probe ADME in rats, mice, & rabbits (F344, Crl:CD1, & NZW)	49677871 (2012) Unacceptable/non-guideline 100 mg/kg (gavage) (Only 1 or 2 animals/sex/study)	The orally absorbed dose was rapidly excreted in urine. Total urinary elimination accounted for 11-15% of the dose in rats, 37-48% of the dose in mice and 67% in rabbits. Fecal elimination accounted for 59-77% in rats, 39-46% in mice and 25% in rabbits. No parent XDE-848 benzyl ester was detected in the urine, blood or liver samples. In the urine profiles, the most abundant urinary metabolite in all species was positively identified as XDE-848 acid (X11438848)
Immunotoxicity Study			

Subchronic, Chronic and Other Toxicity Studies on XDE-848 Benzyl Ester			
Guideline No	Study Type	MRID No. (Year)/ Classification /Doses	Results
870.7800	90-Day Immunotoxicity Study in rats (diet) (primary humoral response to SRBC)	49677848 (2013) Acceptable/guideline 0, 100, 300, or 1000 mg/kg/day *(Integrated in the 90-day oral toxicity study in rats)*	NOAEL = 1000 mg/kg/day (limit dose) No treatment-related effect on the primary antibody response to SRBCs in male and female rats.