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Evaluation of Functional Equivalence of Other Scientifically Relevant Information (OSRI) to EPA's Tier 1 Screening Battery for Evaluating the Potential Estrogen, Androgen or Thyroid (EAT) Effects of Pyriproxyfen

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STATEMENT OF GOOD LABORATORY PRACTICE COMPLIANCE

This document provides an evaluation of functional equivalence of Other Scientifically Relevant Information (OSRI) to EPA's Tier 1 screening battery for evaluating the potential estrogen, androgen, and thyroid (EAT) effects of pyriproxyfen. As such, compliance with Good Laboratory Practice standards is not applicable to this document.

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Evaluation of Functional Equivalence of Other Scientifically Relevant Information (OSRI) to EPA's Tier 1 Screening Battery for Evaluating the Potential Estrogen, Androgen or Thyroid (EAT) Effects of Pyriproxyfen

I. Executive Summary

The US Environmental Protection Agency (EPA) is required under the 1996 Food Quality Protection Act to require testing the potential endocrine-modulating effects of a variety of chemicals. EPA established the Endocrine Disruptor Screening Program (EDSP) to help fulfill this requirement. The objective of this program is to use appropriate validated tests to assess whether chemicals may modulate hormone function in humans or wildlife. EDSP has developed a Tier 1 screen of selected *in vitro* and *in vivo* tests which it believes meets these objectives for identifying the potential of compounds to affect estrogen, androgen or thyroid (EAT) endpoints. In some cases, "Other Scientifically Relevant Information" (OSRI) may preclude the need for some or all of the Tier 1 tests. This is particularly the case for food use pesticides, which undergo multiple toxicity studies prior to registration.

In this paper, we have evaluated the available toxicity database for pyriproxyfen to determine whether sufficient functionally equivalent information, comparable to that developed in EPA's proposed Tier 1 screening battery, is available for the purpose of assessing the endocrine-modulating potential of this chemical. Functionally equivalent, in this context, refers to data of a suitable nature and quality to provide the same essential predictive information on a chemical, even if different methods and procedures may be used for obtaining the data. The objective of defining functionally equivalent endpoints and studies is to avoid unnecessary use of testing resources, delays in the regulatory process, and sacrifice of animals while ensuring that sufficient data are available to evaluate both potential for endocrine activity and safety. Such data will be used to determine further testing needs, and if necessary, to develop protective risk assessments.

Studies conducted for regulatory purposes as well as studies published in the open literature were examined. An objective assessment of these studies was conducted to determine whether, as a body of data, they provide data functionally equivalent to that in EPA's Tier I screening battery and include sufficient information to characterize potential endocrine-modulating effects on estrogen, androgen or thyroid hormone pathways. Conclusions are based on a weight of evidence (WOE) analysis of the data.

The Pyriproxyfen Task Force is citing multiple pyriproxyfen studies from the regulatory data base as OSRI, which it believes is functionally equivalent to, and effectively substitutes for, the information that would be developed through many of the Tier 1 endocrine screening assays. The cited (previously submitted) regulatory toxicology studies tabulated in Appendix I, Table 1 are considered OSRI for assessment of the potential of pyriproxyfen to affect endocrine function. Copies of the title pages of these studies, as requested in the Test Order/Data Call-In Notice for Pyriproxyfen dated January 14, 2010 are included in Appendix VI. Comparisons of endpoints from the mammalian data from the regulatory data base to that required in the four *in vivo* mammalian Tier 1 screening assays

is provided in matrices in Appendix II. Ecotoxicological endpoints are summarized in the matrix in Appendix III.

Published studies cited as OSRI are tabulated in Appendix I, Table 2. The Pyriproxyfen Task Force has identified relatively few published studies on pyriproxyfen either in *vitro*, in mammals or in aquatic vertebrates. The Pyriproxyfen Task Force is citing several published *in vitro* studies that provide somewhat similar data to that developed in one of the Tier 1 *in vitro* assays (estrogen receptor (ER) transactivation). These studies are reviewed in Appendix IV and a comparison of two of these studies to the specific Tier 1 Guideline is also provided. We excluded one fish study of pyriproxyfen found in the published literature from OSRI citation because of its use of formulated test material and acute pulse exposure; this study is also reviewed in Appendix IV.

The Pyriproxyfen Task Force is also citing data from relevant *in vitro* studies similar to those required in the Tier 1 *in vitro* assay battery that were conducted under the auspices of the US EPA in the ToxCast™ screening program for prioritizing chemicals for testing. These studies, which include ER binding and transactivation, androgen receptor (AR) binding, and aromatase inhibition are summarized in Appendix V. We have also reviewed several other ToxCast™ assays including AR transactivation and thyroid receptor binding because of their relevance to the Tier 1 screening objectives.

The Pyriproxyfen Task Force will conduct several Tier 1 studies (as shown in Table 1 below) and believes that these studies, in addition to existing data cited as OSRI, will provide sufficient data for identification of potential endocrine effects in mammals and fish and will meet the Tier 1 screening goals. In conjunction, these studies provide a test battery that conserves animal usage, uses existing information appropriately, and will adequately identify any potential relevant EAT endocrine modulating effects of pyriproxyfen. Further, data from these studies will provide sufficient data to perform a weight-of-the-evidence evaluation of whether any Tier 2 endocrine testing of pyriproxyfen will be necessary.

The following table (Table 1) summarizes the assays that The Pyriproxyfen Task Force will conduct and those assays for which waivers are requested.

Table 1: Tier 1 Test Battery and Requested EPA Action for Pyriproxyfen Based on OSRI Weight of the Evidence

Endocrine Disruptor Screening Program Tier 1 Test Guideline	Requested Action
OPPTS 890.1250: Estrogen Receptor Binding Assay Using Rat Uterine Cytosol (ER-RUC). EPA 2009a	WAIVER requested based on Functional Equivalence of OSRI
OPPTS 890.1300: Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa-9903)). EPA 2009b	WAIVER requested based on Functional Equivalence of OSRI
OPPTS 890.1150: Androgen Receptor Binding (Rat Prostate Cytosol). EPA 2009c	WAIVER requested based on Functional Equivalence of OSRI
OPPTS 890.1200: Aromatase (Human Recombinant). EPA 2009d	The Pyriproxyfen Task Force will conduct this study.
OPPTS 890.1550: Steroidogenesis (Human Cell Line –H295R). EPA 2009e	The Pyriproxyfen Task Force will conduct this study.
OPPTS 890.1600: Uterotrophic Assay. EPA 2009f	WAIVER requested based on Uterotrophic assay in existing data set and Functional Equivalence of OSRI.
OPPTS 890.1450: Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Female Rats. EPA 2009g	WAIVER requested based on Functional Equivalence of OSRI
OPPTS 890.1400: Hershberger Bioassay.EPA 2009h	The Pyriproxyfen Task Force will conduct this study.
OPPTS 890.1500: Pubertal Development and Thyroid Function in Intact Juvenile/ Peripubertal Male Rats. EPA 2009i	WAIVER requested based on Functional Equivalence of OSRI
OPPTS 890.1100: Amphibian Metamorphosis (Frog) EPA 2009j	The Pyriproxyfen Task Force will conduct this study.
OPPTS 890.1350: Fish Short-Term Reproduction Assay EPA 2009k	WAIVER requested based on Functional Equivalence of OSRI

A. *In vitro* Tier 1 Assay Requirements

Justification for waiving the *in vitro* assays (OPPTS 890.1150; OPPTS 890.1300, and OPPTS 890.1250) is presented in this document in the context of the overall WOE for estrogenic, anti-estrogenic, androgenic or anti-androgenic effects, and then specifically in Appendix IV, which provides a review of published *in vitro* studies of pyriproxyfen. A discussion of the specific EPA ToxCast™ assays cited as OSRI is provided in Appendix V. The ToxCast™ assays provide information on the endpoints evaluated in all three of these *in vitro* assays using several different test systems.

1. **OPPTS 890.1250 (Estrogen Receptor Binding Assay Using Rat Uterine Cytosol [ER-RUC]):**

This assay provides information on the ability of the test compound to bind to the estrogen receptor (ER). This has been tested in several different test systems for pyriproxyfen.

- The ToxCast™ assays developed under the auspices of the US EPA cited as OSRI and reviewed in Appendix V showed no evidence for pyriproxyfen binding to ERα in a cell-free system.

- Further, the reproductive and developmental toxicity, uterotrophic and subchronic and chronic toxicity and/or oncogenicity data studies for pyriproxyfen cited as OSRI do not show any pattern of effects suggesting that pyriproxyfen binds to the ER. Additionally, no exposure-related effects were seen on male vitellogenin levels in a fish lifecycle study with pyriproxyfen, demonstrating an absence of estrogenic potential.

Based on the extensive data available and cited as OSRI, The Pyriproxyfen Task Force requests a waiver from an additional *in vitro* assay of ER binding.

2. **OPPTS 890.1300 (Estrogen Receptor Transcriptional Activation [Human Cell Line - HeLa-9903]):**

This assay evaluates the potential of the test compound to transactivate the ER. This potential has been tested in several assay systems for pyriproxyfen.

- The ToxCast™ assays developed under the auspices of the US EPA cited as OSRI and reviewed in Appendix V showed no evidence for pyriproxyfen interacting with ERα in a cell-based system, with the exception of weak estrogenic activity in the Multiplex assay (see below).

- Three estrogen receptor transactivation assays reported in the published literature showed pyriproxyfen caused transactivation of the estrogen receptor. It is considered that the concentrations showing effects in the ToxCast™Multiplex assay and in the published assays were higher than those reasonably anticipated to be achievable *in vivo*.

- Key to interpretation of the *in vitro* assay results is the negative uterotrophic assay of pyriproxyfen. The lack of findings in this assay, which showed a positive response to estradiol, should alleviate concerns regarding the potential estrogenicity of pyriproxyfen.

- As noted above, the reproductive and developmental toxicity, and subchronic and chronic toxicity and/or oncogenicity data from regulatory studies for pyriproxyfen cited as OSRI do not show any consistent patterns of effects suggesting interaction with the estrogen receptor. Additionally, no effects were seen on male vitellogenin levels in a fish lifecycle study with pyriproxyfen.

Based on the lack of estrogenicity in the *in vivo* uterotrophic assay of pyriproxyfen, the very weak estrogenic response in some *in vitro* assays (at concentrations not anticipated to be achievable *in vivo* (Yoshino 1993, Yoshitake 1988)), as well as the extensive data available and cited as OSRI, The Pyriproxyfen Task Force requests a waiver from an additional *in vitro* assay of ER transactivation.

3. OPPTS 890.1150 (Androgen Receptor Binding [Rat Prostate Cytosol]):

This assay provides information on the ability of the test compound to bind to the androgen receptor (AR).

- The ToxCast™ assays developed under the auspices of the US EPA cited as OSRI and reviewed in Appendix V showed no evidence of interacting with the androgen receptor in a cell-based or cell-free system.

- The reproductive and developmental toxicity and subchronic and chronic toxicity and/or oncogenicity data from regulatory studies for pyriproxyfen cited as OSRI do not show any consistent patterns of effects suggesting binding of pyriproxyfen to the androgen receptor. Note also The Pyriproxyfen Task Force is planning to conduct a Hershberger assay of pyriproxyfen which will provide additional data for evaluating anti-androgenic and androgenic potential.

Based on the extensive data available and cited as OSRI, The Pyriproxyfen Task Force requests a waiver from an *in vitro* assay of AR binding.

4. OPPTS 890.1200: (Aromatase [Human Recombinant]):

This assay focuses on whether the test compound inhibits aromatase activity in an *in vitro* system.

- The ToxCast™ assay developed under the auspices of the US EPA cited as OSRI and reviewed in Appendix V showed no evidence of aromatase inhibition; however information on the methodology for this assay is limited.
- Further, a review of the *in vivo* mammalian and ecotoxicological regulatory toxicity data, discussed in detail below, shows no evidence of anti-estrogenicity (which would be predicted if this enzyme were inhibited).

However, based on limitations with the available *in vitro* data, The Pyriproxyfen Task Force will conduct an additional aromatase inhibition assay.

5. OPPTS 890.1550 (Steroidogenesis [Human Cell Line –H295R]):

This assay evaluates the potential of a compound to impact steroidogenesis.

- No *in vitro* steroidogenesis assays of pyriproxyfen were identified
- A review of the *in vivo* mammalian regulatory toxicity data, discussed in detail below, shows no clear pattern of androgenic effects, anti-androgenic effects, estrogenic effects or anti-estrogenic effects that would be anticipated if steroidogenesis were either increased or inhibited. A uterotrophic assay of pyriproxyfen was negative. The Pyriproxyfen Task Force will conduct a Hershberger assay, which will more definitely evaluate androgenic/antiandrogenic effects.

However, to provide the redundancy desired in the Tier 1 assay battery, The Pyriproxyfen Task Force is planning to conduct the steroidogenesis assay under the current Tier 1 Guideline.

B. *In vivo* mammalian Tier 1 assay requirements

A direct comparison of the endpoints evaluated in the Uterotrophic, Female Pubertal, Hershberger and Male Pubertal assays with the endpoints evaluated in the regulatory studies available on pyriproxyfen, including those cited as OSRI, is provided in the mammalian study matrices in Appendix II. Note: specific matching endpoints are provided first in each matrix (in the left hand column); below these are endpoints that provide supporting information to address the assay's objectives. These matrices also present other supplementary data from the regulatory toxicology studies that assist in EAT endocrine endpoint evaluation and address the objectives of the Tier 1 assays.

1. OPPTS 890.1600 (Uterotrophic Assay):

The primary purpose of the uterotrophic assay is to identify estrogenic compounds. It looks for evidence of uterine stimulation (uterine engorgement) in either immature or ovariectomized females.

- A uterotrophic assay in immature female rats by the oral gavage route of administration has been conducted for pyriproxyfen. There was no evidence of estrogenicity at the HDT of 1000 mg/kg/day, which caused decreased body weight and increased liver weight. The positive control response in this study was as expected.

- As noted above pyriproxyfen showed no evidence of binding to the ER (including ERα or ERβ) in *in vitro* studies, including data developed for ToxCast™ under the auspices of the US EPA, with the exception of a weak positive response for pyriproxyfen in the Multiplex assay, and similar weak responses in three published ER transactivation assays. In all cases the concentration of pyriproxyfen resulting in a positive response was greater than a reasonably anticipated *in vivo* concentration.

- There were no findings in the regulatory toxicology data base for pyriproxyfen suggesting an estrogenic effect. As shown in the Uterotrophic Assay Matrix in Appendix II and discussed in the text, pyriproxyfen was evaluated in multiple reproductive or perinatal toxicity studies including a two-generation reproductive toxicity study. These evaluations included estrous cyclicity assessment, which provided sufficient data to conclude that there is no evidence of estrogenic activity; time to vaginal opening (assessed in a perinatal exposure study and a developmental exposure with post-natal component study), reproductive organ weight evaluations and detailed histopathology of female reproductive organs. (Note a slight high dose delayed vaginal opening seen in one study was attributable to growth retardation, including decreased body weight.)

- High dose effects were seen on pup growth and mortality; there is no indication these findings occurred below maternally systemically toxic doses, and no indication they were estrogen-mediated.

- There were no exposure-related changes in mammary tumors or in estrogen responsive pituitary (either increases or decreases) in the chronic toxicity or oncogenicity studies of pyriproxyfen suggesting either an estrogenic or anti-estrogenic effect.

In toto, a uterotrophic assay has been conducted for pyriproxyfen. Based on the extensive data available and submitted as OSRI, The Pyriproxyfen Task Force requests a waiver from a repeat uterotrophic assay.

2. OPPTS 890.1450 (Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Female Rats):

The female pubertal assay is intended to characterize estrogenic or anti-estrogenic effects of the test compound. Androgenic effects may also be observed in this assay. In addition, it evaluates parameters relevant to thyroid, adrenal, renal, hepatic and pituitary structure or function.

- As noted above pyriproxyfen showed no evidence of binding to or interacting with the ER (including ERα or ERβ) in data developed for ToxCast™ under the auspices of the US EPA, except for a weak positive response in the Multiplex assay and similar weak responses in three published ER transactivation assays. In all cases the concentration of pyriproxyfen resulting in a positive response was greater than a reasonably anticipated *in vivo* concentration.

- Pyriproxyfen was also characterized as not binding to the human thyroid hormone receptor in data developed for ToxCast™ under the auspices of the US EPA.

- There were no findings in the regulatory toxicology data base for pyriproxyfen suggesting an estrogenic or anti-estrogenic effect. As discussed in the text and summarized in the matrix for the female pubertal assay in Appendix II, pyriproxyfen was evaluated in multiple reproductive and perinatal toxicity studies including a two-generation reproductive toxicity study, as well as in a recent uterotrophic assay. These evaluations overlapped the exposure time frame for the female pubertal assay, and arguably the reproductive study design tests a more sensitive time frame, due to the perinatal exposure in this study paradigm. Parameters evaluated included estrous cyclicity, time to vaginal opening (assessed in two studies), reproductive organ weight evaluations and detailed histopathology of female reproductive organs. These endpoints are sensitive to estrogenic and anti-estrogenic modes of action (MOA) and showed no evidence of exposure-related effects. (Note a slight high dose delayed vaginal opening seen in one study was attributable to growth retardation including decreased body weight.)

- High-dose effects were seen on pup growth and mortality; however, there is no indication these findings occurred below maternally systemically toxic doses or were related to anti-estrogenicity.

- There were no exposure-related changes in estrogen responsive tumors, including mammary tumors (either increases or decreases) or pituitary chromophobe adenomas in the chronic toxicity or oncogenicity studies of pyriproxyfen; further pyriproxyfen showed no oncogenic effects on the ovaries, thyroid, pituitary, or adrenal. Adrenal

weight increases seen at high doses of pyriproxyfen were considered stress-related and not due to EAT modulation.

- Thyroid function was assessed by evaluation of several parameters measured in the pyriproxyfen regulatory studies. These include thyroid weights and thyroid tissue histopathology. Increased thyroid weights were observed in a chronic oral study in beagle dogs; no gross or histopathological correlates were observed. This finding was not considered exposure related. None of the rodent toxicity studies in which the thyroid was evaluated histopathologically showed evidence of thyroid follicular cell hypertrophy, hyperplasia or neoplasia. Although thyroid hormones have not been measured in these studies, thyroid follicular cell changes are sensitive to thyroid hormone deficits in rats and subsequent thyroid stimulating hormone (TSH) increases (Yamada et al., 2004; Jahnke et al., 2004).

- Adrenal weights and histopathology were characterized in multiple studies. Adrenal weight increases were seen at very high and toxic doses and no correlating histopathological changes were noted. The adrenal weight increases are attributed to stress related to systemic toxicity including decreased body weight.

- No exposure-related histopathological findings in the pituitary were observed in sub-chronic or chronic studies in three species (except for congestion in the 90-day mouse study at an extremely high dose causing lethality).

- Kidney and liver weights have been evaluated in the pyriproxyfen reproductive toxicity study, three subchronic toxicity studies, and three chronic toxicity studies. Kidney and liver histopathology were evaluated all of these studies. Exposure-related renal and hepatic toxicity were seen at high doses of pyriproxyfen; these findings are not considered endocrine-mediated.

In toto, no effects were seen in females that were likely to be related to disruption of EAT parameters, and no consistent pattern of effects was seen in evaluations of the ovaries, uterus, mammary gland, thyroid, adrenal, pituitary, liver or kidney suggesting EAT-mediated effects. The potential for thyroid effects from exposure to pyriproxyfen will be further evaluated in the amphibian metamorphosis assay, which The Pyriproxyfen Task Force is planning to conduct.

In view of the extensive evaluation of pyriproxyfen and its potential influence on the female hormonal system and reproductive toxicity which showed no reliable or consistent indicators of any adverse endocrine-mediated effects, we consider conduct of a female pubertal assay redundant and wasteful of both animal lives and testing resources. Based on the extensive data available and cited as OSRI, The Pyriproxyfen Task Force requests a waiver from a female pubertal assay.

3. OPPTS 890.1400 (Hershberger Assay):

The Hershberger assay primary objective is to assess potential androgenicity or anti-androgenicity.

- *In vitro* ToxCast™ studies conducted under the auspices of the US EPA and cited as OSRI showed no evidence of interaction with the androgen receptor.

- As discussed in the text and summarized in the matrix for the Hershberger assay in Appendix II, review of the reproductive toxicity and subchronic and chronic toxicity regulatory toxicity data for pyriproxyfen cited as OSRI, as well as other supplementary studies, do not show any patterns of effects that could be related to androgenicity or anti-androgenicity at any dose. Pyriproxyfen was evaluated in a two-generation reproductive toxicity study and several perinatal reproductive toxicity studies, as well as in a complete set of sub-chronic and chronic oral exposure studies. There is no consistent evidence of findings caused by an endocrine mechanism. This is supported by the absence of effects or lack of exposure relationship of pyriproxyfen on parameters sensitive to androgenicity and anti-androgenicity including testicular descent, fetal and pup sex ratio, urogenital malformations in offspring, organ weights and testicular and accessory sex organ histopathology. Note there were non-dose related changes in testicular descent; in one study there was accelerated descent, but the control mean event day was unusually high; in the other slight delayed descent. Neither of these findings is considered likely to represent a treatment-related effect.

However, it is noted that evaluations conducted did not include some of the parameters most sensitive to anti-androgenic effects such as anogenital distance or nipple retention, as well as certain male accessory sex organ weights. To provide the redundancy desired in the Tier 1 assay battery, and to provide assurance that androgenic or anti-androgenic effects are adequately addressed, The Pyriproxyfen Task Force will conduct a Hershberger assay under the Tier 1 Guideline.

4. OPPTS 890.1500 (Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats):

The male pubertal assay is intended to characterize androgenic or anti-androgenic effects of the test compound. Estrogenic effects may also be observed in this assay. In addition, it evaluates parameters relevant to thyroid, adrenal, renal, hepatic and pituitary structure or function.

- *In vitro* ToxCast™ studies conducted under the auspices of the US EPA and cited as OSRI showed no evidence of interaction with the androgen or thyroid receptors.

- As discussed in the text and summarized in the matrix for the Male Pubertal assay in Appendix II, review of the reproductive toxicity and subchronic and chronic toxicity regulatory toxicity data for pyriproxyfen cited as OSRI, as well as other supplementary studies, do not show any consistent patterns of effects that could be related to androgenicity or anti-androgenicity at any dose. Pyriproxyfen was evaluated in a two-generation reproductive toxicity study and several perinatal reproductive toxicity studies, as well as in a complete set of sub-chronic and chronic oral exposure studies. There is no consistent evidence of findings caused by an endocrine mechanism. This is supported by the absence of effects or lack of exposure relationship of pyriproxyfen on parameters sensitive to androgenicity and anti-androgenicity including testicular descent, fetal and pup sex ratio, urogenital malformations in offspring, organ weights and testicular and accessory sex organ histopathology. Note there were non-dose related changes in testicular descent; in one study there was accelerated descent, but the control mean event day was unusually high; in the other slight delayed descent. Neither of these findings is considered likely to represent a treatment-related effect.

- A number of parameters related to thyroid function were measured in the pyriproxyfen studies conducted for pesticide registration purposes. These include thyroid weights and histopathology. There are no findings suggesting an effect on the thyroid. It should be noted that the potential for thyroid effects will be further evaluated in the amphibian metamorphosis assay (AMA), which The Pyriproxyfen Task Force is planning to conduct. This AMA also provides the redundancy appropriate to a screening battery.

- Adrenal weights and histopathology were characterized in multiple studies. Adrenal weight increases were seen at very high and toxic doses and no correlating histopathological changes were noted. The adrenal weight increases are attributed to stress related to systemic toxicity including decreased body weights.

- No exposure-related histopathological findings in the pituitary were observed in sub-chronic or chronic studies in three species (except for congestion in the 90-day mouse study at an extremely high dose causing lethality).

- Kidney and liver weights have been evaluated in the pyriproxyfen reproductive toxicity study, three subchronic toxicity studies, and three chronic toxicity studies. Kidney and liver histopathology were evaluated all of these studies. Exposure-related renal and hepatic toxicity were seen at high doses of pyriproxyfen; these findings are not considered endocrine-mediated.

In toto, no effects were seen in males that were likely to be related to disruption of EAT parameters, and no consistent pattern of effects was seen in evaluations of the thyroid, adrenal, pituitary, liver or kidney suggesting EAT-mediated effects. The potential for thyroid effects from exposure to pyriproxyfen will be further evaluated in the amphibian metamorphosis assay, which The Pyriproxyfen Task Force is planning to conduct.

In view of the extensive evaluations of pyriproxyfen and its potential influence on the male hormonal system and reproductive toxicity, which showed no reliable or consistent indicators of any adverse endocrine-mediated effects, and the plans to conduct a Hershberger assay of pyriproxyfen to supplement the existing data base, we consider conduct of a male pubertal assay redundant and wasteful of both animal lives and testing resources. Based on the extensive data available and cited as OSRI, The Pyriproxyfen Task Force requests a waiver from a male pubertal assay.

C. Ecotoxicity Tier 1 Studies

1. OPPTS 890.1100: Amphibian Metamorphosis (Frog):

This study evaluates growth, development and thyroid function in the African clawed frog (*Xenopus laevis).* The Pyriproxyfen Task Force did not find sufficient information in the regulatory data base or published literature to establish functional equivalence of existing data for the endpoints evaluated in this assay. Therefore, The Pyriproxyfen Task Force is planning to conduct this assay. This assay will also provide the redundancy for evaluation of potential thyroid effects, appropriate to a screening battery, to the data from the existing regulatory studies in mammals.

2. OPPTS 890.1350: Fish Short-Term Reproduction Assay :

This study evaluates reproductive performance in the fathead minnow. Pyriproxyfen has been evaluated in a life cycle study in Japanese Medaka (a species proposed for Tier 2 endocrine testing), and showed no evidence of endocrine modulation up to the highest concentration tested. This study included all of the parameters required in a fish short term reproduction assay. Although there were statistically significant differences in some potentially endocrine-related parameters, there was no evidence of dose response. Most changes were seen in the F0 generation at post-hatch day 60, and were not replicated at post-hatch day 114; or for parameters evaluated in both generations, were not replicated in the F1 generation at post-hatch day 60. Based on this study, the Pyriproxyfen Task Force is requesting a waiver from a fish short term reproduction study.

II. Introduction

The US Environmental Protection Agency (EPA) is required under the 1996 Food Quality Protection Act to require testing the potential endocrine-modulating effects of a variety of chemicals. EPA established the Endocrine Disruptor Screening Program (EDSP) to help fulfill this requirement. EDSP has developed a Tier 1 screen of selected *in vitro* and *in vivo* tests which it believes meets these objectives for estrogen, androgen or thyroid (EAT) effects of compounds. The purpose of Tier 1 screening tests is to identify chemicals with the potential to interact with the endocrine system. The specific Tier 1 tests include a battery of *in vitro* tests (including estrogen receptor [ER] binding and transactivation assays, an androgen receptor [AR] binding assay, and tests of aromatase activity and steroidogenesis); certain *in vivo* mammalian tests (including male and female pubertal assays, the uterotrophic test and Hershberger assay); and two ecotoxicological assays (the fish lifecycle test and the amphibian metamorphosis assay).

The Tier I screening tests provide potential hazard characterization and mechanistic information regarding potential endocrine modulation; however, this information is not useful for risk assessment purposes because it does not characterize the dose-response or establish a no observable adverse effect level (NOAEL). Therefore, chemicals that test positive for possible endocrine modulation in the Tier I screening assays must be further evaluated in Tier II tests to determine what functionally relevant endocrine-related changes are associated with exposure *in vivo*, if any, and to provide information regarding dose-response. To date, Tier II tests have not been defined, but are proposed to include apical tests of reproduction and development in mammalian as well as other species, including Medaka.

In some cases, "Other Scientifically Relevant Information" (OSRI) may preclude the need for some or all of the Tier 1 screening battery assays. This is particularly the case for food use pesticides, which undergo multiple toxicity studies prior to registration. To meet the registration requirements of the

Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), pesticide manufacturers must conduct guideline-compliant mammalian and ecological toxicity tests that are designed to characterize the potential hazards associated with exposure to a chemical via a variety of different routes. These tests include apical studies of development and reproduction, oncogenicity, subchronic and chronic toxicity. The studies conducted in fulfillment of FIFRA requirements include measurement of a wide-range of toxicity endpoints, many of which assess the potential for endocrine modulation either through evaluation of impacts on hormone-producing tissues or endocrine-target organs, or by assessing secondary functional endpoints such as reproduction and fertility.

Although these regulatory studies do not measure all of the specific parameters of each assay in the Tier I battery, they often provide functionally equivalent information to address the objectives of the Tier 1 screening battery. Further, the regulatory studies are designed to provide information that may be used to determine NOAELs and may be incorporated into a risk assessment, which the Tier 1 screen is not designed to do. In particular, the two-generation reproductive toxicity study—especially when evaluated in conjunction with the current guideline subchronic and chronic toxicity studies—is similar in scope and breadth to what has been discussed as a proposed mammalian Tier 2 test, and provides sufficient information to assess whether endocrine targets are, in fact, altered with *in vivo* exposure. Conducting Tier 1 screening assays when Tier 2 equivalent data are available is wasteful of animal lives as well as both industry and EPA resources. Existing studies need to be considered carefully when deciding whether all or some of the Tier 1 screening is necessary. Indeed, EPA is specifically directed by statute to minimize duplicative testing under the EDSP. *See* Section 408(p)(5)(B) of the Federal Food, Drug, and Cosmetic Act (FFDCA); 21 U.S.C. § 346a(p)(5)(B).

Additional studies published in the scientific literature that investigated the potential of a chemical to induce endocrine-modulating effects may provide further functionally equivalent data, although such studies need to be carefully screened both for methodology and reporting detail. Findings from these studies should be considered in a weight-of-evidence framework, along with the body of unpublished guideline study data, both for consistency of findings and appropriate dose-selection. Thus, the availability of such information for a chemical from both guideline-compliant and other studies may negate the need to conduct some or all of the tests included in the EDSP Tier 1 screening battery.

III. Review of Pyriproxyfen Regulatory Toxicology Studies Conducted for Registration Purposes

A. Mammalian Studies

A total of 14 regulatory mammalian toxicity studies of pyriproxyfen conducted for pesticide registration purposes were reviewed. These consisted of a uterotrophic assay, a multi-generation reproductive toxicity study; two perinatal studies; two developmental toxicity studies; three chronic toxicity and/or oncogenicity studies; and five subchronic toxicity studies. The majority of these studies were acceptable to EPA. These studies are discussed below in terms of quality and functional equivalence for assessing possible endocrine modulation by pyriproxyfen.

1. Uterotrophic, Reproductive Toxicity and Peri-natal or Developmental with Post-natal component Studies

These studies examined a wide range of pyriproxyfen doses, and although the studies evaluated an extensive array of parameters, only a very few potentially endocrine-related endpoints in each study exhibited statistically significant differences from control in the exposed groups. Specific details regarding the study designs, test species, number of animals per group, doses, and route and duration of exposure can be found in Table 2, along with information regarding the various endpoints of relevance to the issue of endocrine modulation in these studies. The 2-generation study was conducted by the dietary route of administration; the uterotrophic study, and the perinatal or developmental exposure studies were dosed by gavage.

The 2-generation study with rats (Robinson et al. 1991) and uterotrophic assay with rats (Ose 2005) are cited as OSRI. Also cited are two non-guideline developmental or perinatal exposure studies in which non-exposed offspring from dams exposed either during gestation or perinatally were mated and F1 pup development, reproductive performance, and F2 fetal development were evaluated (Saegusa 1988a,b), and one non-guideline study in which rats were exposed from prior to mating to GD 7, with fetuses evaluated on GD 21 (Saegusa 1988c). Note the developmental components of the Saegusa 1988a and 1988c studies are discussed in Section 4 below.

Table 2. Experimental Details and Selected Reproductive Toxicity Endpoints of Potential Relevance to Endocrine Modulation Evaluated in Pyriproxyfen Toxicity Studies

	Robinson et al. (1991)	Ose (2005)	Saegusa et al. (1988a)	Saegusa et al. (1988b)	Saegusa et al. (1988c)
EPA MRID #	42178313	48066201	41321719 42178312	44985001	44985002
Study design	2-generation reproductive (83-4)	Uterotrophic assay in juvenile rats	GD 7-17 exposure with F1 evaluated PND21, PND56; and F1 reproductive component	GD17-PND 20 Perinatal and lactation exposure with postnatal component; F1 evaluated PND21; F1 reproductive component	Premating (♂ 9wk, ♀ 2wk), and mating (3 wk) to GD7 for ♀; dams c-sectioned at GD 21
Species (strain)	Crl:CD(SD) rat	Crl:CD(SD) rat	Slc:SD rat	Slc:SD rat	Slc:SD rat
# animals	26/sex/dose	6/dose	F0: 10–13 dams/dose; F1: 10-13/sex/dose mated (no exposure)	F0: 23–24 dams/dose; F1: 20-23/sex/dose, 14/sex high dose mated (no exposure)	24/sex/dose
Doses (ppm)	0, 200, 1000, 5000 ppm				
Targeted Doses (mg/kg/day)	0, 11–23 (43), 58–115 (220), 289–557 (1086) mg/kg/day premating and gestation (lactation)	0, 250, 500, 1000 mg/kg/day	0, 100, 300, 1000 mg/kg/day	0, 30, 100, 300, 500 mg/kg/day	0, 100, 300, 500, 1000 mg/kg/day
Route of exposure	Diet	Gavage	Gavage	Gavage	Gavage

	Robinson et al. (1991)	Ose (2005)	Saegusa et al. (1988a)	Saegusa et al. (1988b)	Saegusa et al. (1988c)
Exposure duration	10 wks (F0) or 11–13 wks (F1) plus mating, gestation and lactation for both generations	3 days	GD7-GD17 (to F0 dams)	GD17-PND20 (to F0 dams, F1 potentially exposed during lactation)	Premating (♂ 9wk, ♀ 2wk), and mating (3 wk) to GD7 for ♀
Selected Endpoints					
Body weights – adults (F0, F1)	X (F0, F1)	X	X (F0 dams, F1)	X (F0 dams, F1)	X (F0)
Body weights – weanlings	X (F1, F2)		X (F1)	X (F1)	
Clinical observations – dams	X (F0, F1)		X	X	X (F0)
Clinical observations – offspring	X (F1, F2)		X (F1)	X (F1)	
Male mating index			X (F1)	X (F1)	X (F0)
Female mating index	X (F0, F1)		X (F1)	X (F1)	X (F0)
Estrous Cyclicity	X (F0, F1)				
Time to mating	X (F0, F1)				
Male fertility			X (F1)	X (F1)	X (F0)
Female fertility	X (F0, F1)		X (F1)	X (F1)	X (F0)
Gestation duration	X (F0, F1)		X (F0)	X (F0)	
Gestation index	X (F0, F1)		X (F0)	X (F0, F1)	X (F0)
Dystocia					
Mean # live pups/litter	X (F0, F1)		X (F0)	X (F0)	
Mean # live fetuses/liter			X (F1, F2)	X (F2)	X (F1)
Mean # dead pups/litter	X (F0, F1)		X (F0)	X (F0)	
Live birth index	X (F0, F1)		X (F0)	X (F0)	
Litter weights	X (F1, F2)		X (F0)	X (F0)	
Pup sex ratio	X (F1, F2)		X (F1)	X (F1)	X (F1)

	Robinson et al. (1991)	Ose (2005)	Saegusa et al. (1988a)	Saegusa et al. (1988b)	Saegusa et al. (1988c)
Testicular descent			X (F1)	X (F1)	
Vaginal opening			X (F1)	X (F1)	
Pup viability index	X (F1, F2)		X (F1)	X (F1)	
Lactation index	X (F1, F2)				
Gross pathology (F0, F1 adults)	X (F0, F1)	X	X (F0 dams, F1)	X (F0 dams, F1)	X (F0)
Gross pathology (F1, F2) weanlings)	X (F1, F2)		X (F1)	X (F1)	
Organ weights (F0, F1 adult) – testes (T), epididymides (E), seminal vesicles (S), prostate (P)	T, E, P, S		T (F1 PND21, PND56)	T (F1 PND21, PND56)	T (F0)
Organ weights (F0, F1) – ovaries (O), uterus (U)		U	O (F0 dams, F1 PND 21, 56)	O (F0 dams, F1 PND21, 56)	
Organ weights (F0, F1) – adrenal (A), pituitary (Pit)	Pit	A	A (F0 only)	A (F0 only)	A
Organ weights (F0, F1) – liver (L) kidney(K), brain (B), spleen (Sp), thymus (Thy), Heart (H), Lung (Lu)	L, K, B	L, K	L, K, H, Lu, Sp (B at PND56 F1 only)	L, K, H, Lu, Sp (B at PND56 F1 only)	L, K H, Lu, Sp, Thy
Histopathology (F0, F1 adult) – testes (T), epididymides (E), seminal vesicles (S), prostate (P)	T, E, S, P				
Histopathology (F0, F1 adult) – ovaries (O), uterus (U), vagina (V), mammary (M)	O, U, V, M				

	Robinson et al. (1991)	Ose (2005)	Saegusa et al. (1988a)	Saegusa et al. (1988b)	Saegusa et al. (1988c)
Histopathology (F0, F1 adult) –pituitary (Pit)	Pit				
Histopathology (F0, F1 adult) – liver (L), kidney (K), colon (C), eye (Ey), fat (F), lung (Lu), spleen (Sp), thymus (Thy), stomach (St), lymph node (LN), heart (H)	L, K (C, Ey, F, Sp, St, H Thy, LN, Lu if gross lesions)				

Rat Dietary Two-Generation Reproductive Toxicity (Robinson et al. 1991) **[MRID 42178313]**

Sprague Dawley (Crl:CD(SD)) F0 rats (26/sex) 6 weeks old were treated with pyriproxyfen by diet at 0, 200, 1000, and 5000 ppm for 10 weeks prior to and throughout mating, gestation, and lactation. F1 offspring (26/sex) were treated identically to F0 rats from weaning for 11–13 weeks and throughout mating, gestation, and lactation. Adult F0 and F1 males were sacrificed approximately 3 weeks after mating and adult F0 and F1 females were sacrificed after weaning. F2 pups were sacrificed at weaning (PND21).

- There were no treatment-related deaths.
- Statistically significant decreases in body weight for F0 and F1 males (~10%) and females (~9%) were observed in the 5000 ppm-treated groups at various times during treatment. Feed consumption was also decreased at various times during treatment (7-9% decrease).
- No exposure-related gross pathological findings were observed. No treatment-related histopathological changes were found in the adult F0 generation. F1 males had increased relative kidney weights at 1000 and 5000 ppm. F1 adult males treated with 5000 ppm had a higher incidence and severity of chronic interstitial nephritis; this finding was considered exposure-related but not endocrine-related.
- F1 adult liver weights (absolute and relative) were increased in 5000 ppm-treated rats; males had increased relative liver weight at 1000 ppm. These findings were considered exposure-related but no endocrine-related.
- Parental performance (mating and fertility indices and conception rate) and maternal performance (gestation length, gestation index, number of live and dead pups at birth, pup sex

ratios, implantation sites and post-implantation loss) were unaffected by treatment. Estrous cyclicity evaluation showed no evidence of prolonged or persistent estrus.

- F1 and F2 male, female, and total pup weights were significantly reduced at PND 14 and 21 with 5000 ppm treatment. Litter weights were statistically significantly reduced at these days as well.
- The viability, survival, and lactation indices of F1 and F2 generation pups were not different from controls.
- There were no effects on the clinical or pathological findings for pups in the treated groups. There was no evidence of genital malformations in the F1 or F2 pups.

The maternal reproductive NOAEL was 5000 ppm (289–557 mg/kg/day), the highest dose tested, and the pup NOAEL for F1 and F2 pups was 1000 ppm (58–115 mg/kg/day) based on reduced pup body weight at 5000 ppm. These were well above the parental NOAEL of 200 ppm (11-23 mg/kg/day), based on liver effects at 1000 ppm. The decreased pup weight at weaning at the high dose is considered likely to be exposure-related; however, this is attributed to systemic toxicity from the high consumption late in lactation on a mg/kg/day basis, and is unlikely to have been caused by an endocrine-disrupting mechanism.

The Robinson et al. (1991) study satisfies EPA guideline (83-4) requirement for a 2-generation reproductive study. The 2-generation reproductive study examined endpoints from pyriproxyfen doses ranging from 11–556 mg/kg/day during premating and gestation. For females during lactation doses ranged from 18 to 1086 mg/kg/day. No potentially endocrine-related endpoints exhibited significant differences from control in the exposed groups, other than overall decrease in body weights of parents and pups at the high exposure level.

This study was considered acceptable by EPA and it is cited as OSRI.

Rat Uterotrophic Assay (Ose 2005) **[MRID 48066201]**

Pyriproxyfen was tested in a uterotrophic assay in immature female rats at doses of 250, 500 and 1000 mg/kg/day administered by gavage in corn oil for 3 days to 6 females/group starting on PND 20. Animals were sacrificed at the day following the end of exposure and uterus (blotted and wet), liver, adrenal and kidney weights collected. The high dose had been shown to be toxic but not lethal in a previous study; it was also the Limit Dose for this assay under the draft OECD Guideline. 17α-ethynyl estradiol was used as a positive control.

Pyriproxyfen showed no evidence of uterotrophic activity in this assay. There were some indications of toxicity: at ≥500 mg/kg/day feed consumption was decreased and absolute and relative liver weights increased; at 1000 mg/kg/day body weight was decreased (approximately 10% compared to corn oil control) and relative and absolute kidney weights were increased.

There were some deviations in this study from the current Tier 1 endocrine-screening Guidelines:

- The corn oil vehicle was not tested versus untreated control. This deviation is not considered significant.

- Some uterine control weights were outside the range specified in the Uterotrophic Assay Guideline for immature rat blotted uterine weights. The EPA guideline indicates that if control uterine weights are above 45 mg it "may lead to re-run the test." But the guideline also indicates these studies should be reviewed on a case-by-case basis. In the case of pyriproxyfen there were only small excursions in the control blotted uterine weights greater than the specified weight range. Further, there was no indication of a dose related increase in absolute (wet or blotted) or relative uterine weights in pyriproxyfen-exposed rats. In contrast, the positive control uterine weight was increased 1.6 fold from the vehicle control. Thus there is confidence that the model would have detected any estrogenic activity of pyriproxyfen.

- The report did not describe the phytoestrogen levels in the diet. Information obtained subsequently from the published literature indicates that the rats in this study were fed CRF-1 feed (Oriental Yeast Co., Ltd.) which has been characterized by Owens et al., 2003 and shown to contain ≤ 350 µg of genistein equivalents per gram, which falls within the Guideline requirement.

We consider that this study fulfills the requirement for a uterotrophic assay in the rat for purposes of the Tier 1 screening battery. The NOAEL for uterotrophic activity is 1000 mg/kg/day (highest dose tested or HDT). EPA has not yet reviewed this study. It is cited as OSRI.

Developmental Exposure; Post-natal Component (Saegusa et al. 1988a) [MRID 41321719; 42178312]

In a teratogenicity study with a post-natal toxicity and reproductive evaluation component, pregnant rats (F0) were gavaged with pyriproxyfen at 0 (corn oil), 100, 300 or 1000 mg/kg/day during gestational days (GD) 7–17. Selected dams (20-23 dams/group) underwent C-section on GD 21 (see Table 3 and discussion below for developmental effects). The remaining 10-13 dams/group delivered naturally to produce F1 offspring; dams and pups were not further dosed with pyriproxyfen. At PND4 the litters were culled to 8 animals (4/sex) and culled pups were examined for anomalies. Dams were sacrificed at weaning (PND21) and 4 offspring (F1, 2/sex) per litter were also sacrificed at PND 21, and organ weights (including liver, kidneys, testes with epididymides, and ovaries) and external, visceral and skeletal anomalies recorded. Two offspring (F1, 1/sex) per litter were retained for learning ability, 'emotional' and motor coordination tests until 8 weeks of age (PND56) at which time animals were necropsied, gross lesions and organ weights recorded. F1 animals also had reproductive function evaluated; 2 offspring (F1, 1/sex) per litter were retained until 11 weeks of age then paired for mating within the same group. After confirmed copulation, males were sacrificed; pregnant F1 dams were retained until cesarean section at GD21. Number of corpora lutea, implantation sites, live and dead fetuses, resorbed fetuses, fetal weights and sex ratio were recorded. EPA indicated this study was acceptable.

- Of the high dose F0 dams (1000 mg/kg/day), 12 of the 42 animals died.

- Decreased body weight and body weight gain of F0 dams at ≥300 mg/kg/day was observed during gestation, extending post partum (and post treatment) until sacrifice at PND 21. Body weight gain was decreased in F0 dams at 100 mg/kg/day during gestation.
- No differences in delivery parameters were noted for F0 dams (number of pregnant dams, number of dams with live newborns, delivery rate, and length of gestation, number of implantations, birth rate, and number of still births or live newborns).
- There were no treatment related differences in delivery parameters of pup body weights at birth.
- No change in viability of F1 pups was observed on days 4, 7, 14, or 21, at weaning, or beyond (up to PND 77 and mating).
- There was no difference in time of vaginal opening for pups from treated dams compared to those from control dams. The mean number of days for vaginal opening for F1 pups was 37.1, 34.8, 35.2, and 36.7 for pups whose dams (P) received 0, 100, 300, and 1000 mg/kg/day, respectively.
- Pups from dams at 300 mg/kg/day showed a statistically significant accelerated descent of testes compared to pups from control dams. The mean number of days for descent of testis for F1 pups was 27.8, 25.8, 24.5* ($p < 0.05$), and 25.7 for pups whose dams (P) received 0, -100, 300, and 1000 mg/kg/day, respectively. The control in this study appears delayed (compared to controls in Saegusa et al. 1988b, conducted in the same laboratory and discussed below).
- A statistically significant increase in absolute and relative mean testis weights was observed in treated F1 males at 300 mg/kg/day at PND 21 and PND 56. Body weights were significantly higher at 300 mg/kg/day at PND 21, but not statistically significant different from PND 28 on. There was no dose-response, and histopathological evaluation of the testes was not conducted, so the biological significance of the difference in organ weights at 300 mg/kg/day is unclear, and it is considered unlikely that this finding is exposure related.
- There were no exposure related changes in F1 liver, kidney, ovary or weights at PND21 or PND56.
- No differences in the ability to reproduce were noted among F1 offspring from control or treated dams based on mating and pregnancy rates.
- Body weights in female offspring (F1) during pregnancy did not differ between the control and dosed groups.
- There were no differences in the ability of F1 offspring to reproduce nor were there fetal effects in the F2 generation.

The maternal NOAEL is ≤100 mg/kg/day; the F1 NOAEL 1000 mg/kg/day (exposed during gestation). This study is cited as OSRI.

Perinatal; Post-natal Reproduction Component (Saegusa et al. 1988b) [MRID 44985001]

In a perinatal study (see Table 2) with a post-natal reproductive evaluation component, pregnant rats (F0) were gavaged with pyriproxyfen at 0 (corn oil), 30, 100, 300, or 500 mg/kg/day during GD17–PND20. Each dose group had 23–24 pregnant dams, which were allowed to deliver naturally. At PND4 litters were culled to 8 offspring; sacrificed pups were examined for anomalies. After sacrifice of F0 dams and F1 pups at weaning (PND21), one male and one female F1 offspring from each litter was maintained without exposure for 11 weeks and then used in reproductive performance tests. Two of each sex were also maintained until 8 weeks of age and used to assess learning ability. F1 animals also had reproductive function evaluated; 2 offspring (F1, 1/sex) per litter were retained until 11 weeks of age then paired for mating within the same group. After confirmed copulation, males were sacrificed; pregnant F1 dams were retained until cesarean section at GD21. Number of corpora lutea, implantation sites, live and dead fetuses, resorbed fetuses, fetal weights and sex ratio were recorded. EPA indicated this study was acceptable, non-guideline.

- Weight gain was inhibited at ≥ 300 mg/kg/day F0 dams.
- There were 26 stillbirths in the 500 mg/kg/day group compared to 6 in the control group, the majority of which were delivered by 2 dams that showed severe toxicity. The mean number of liveborn pups, however, at 500 mg/kg/day was not statistically significantly different from control.
- The survival rate of F1 female offspring was lower in the 500 mg/kg/day group from PND 0-PND 4, and the weanling survival rate post-cull to PND21 was lower in males (74.4%) and females (78.1%) in this group when compared to controls (97.4% and 98.9%, respectively).
- The mean body weight of F1 offspring was decreased at ≥ 300 mg/kg/day.
- The mean day of vaginal opening was slightly but statistically significantly delayed at 500 mg/kg/day; this finding was probably related to developmental delay including decreased body weight in this group. The mean day of vaginal opening was 35.6, 36.8, 36.9, 36.9, 37.3*for the 0, 30, 100, 300 and 500 mg/kg/day groups, respectively (*$p \leq 0.05$). It should be noted, however, that the control mean day for vaginal opening in another study in the same laboratory (Saegusa et al. 1988a) was 37.1, so this finding is of questionable biological significance, particularly considering the pre-weaning and continuing body weight deficit in the 500 mg/kg/day offspring.
- The mean day of testicular descent was statistically significantly delayed at 300 mg/kg/day but not 500 mg/kg/day; mean day of testicular descent was 23.0, 24.1, 23.8, 24.9*, and 24.6 for the 0, 30, 100, 300 and 500 mg/kg/day groups, respectively (*$p \leq 0.05$). This slight non-dose-related difference is considered unlikely to represent a treatment-related effect.

- There were no statistically significant decreases in mating or fertility rates in the F1 mating.
- The number of implantations was slightly, but statistically significantly, lower in pregnant F1 dams at 500 mg/kg/day group. The number of live fetuses/dam in this group was also slightly, but significantly low. The percent of resorbed or dead fetuses, however, were not increased in treated animals compared to control and fetal body weights were not affected.
- No exposure-related differences were observed in sex ratios in any dose group or external abnormalities

The maternal and F1 NOAEL are 100 mg/kg/day. This study is cited as OSRI.

Perinatal Exposure to GD7, Reproductive Component (Saegusa et al. 1988c) [MRID 44985002]

Sprague Dawley rats (24/sex) were administered pyriproxyfen via gavage at 0 (corn oil), 100, 300, 500 or 1000 mg/kg/day. Males received doses for 9 weeks prior to mating and an additional 3 weeks during mating through necropsy (total of 12 weeks). Females received doses for two weeks prior to mating, and through mating until GD7; dams were caesarian sectioned at GD 21. No histopathology was performed. The developmental toxicity component of this study is discussed in section (b) below. EPA indicated this study was acceptable non-guideline.

- There was no treatment related F0 mortality. Clinical observations at 300 (females), 500 and 1000 mg/kg/day reported anal area irritation/inflammation.
- Body weight and body weight gain of F0 males were decreased in the 300 (starting at day 45), 500 (starting at day 28) and 1000 mg/kg/day (starting at day 2) groups and remained decreased until sacrifice.
- Body weights of F0 females were decreased at ≥ 500 mg/kg/day starting on day 3 of the premating period and the decreases continued until GD21. Decreases in body weight occurred at 100 and 300 mg/kg/day inconsistently during gestation. Body weight gains were decreased during premating at ≥ 100 mg/kg/day.
- Kidney weights were increased in F0 females at ≥ 100 mg/kg/day.
- Adrenal gland weights (absolute and relative) in F0 male rats were statistically significantly increased at doses of ≥ 100 mg/kg/day. During necropsy the adrenal glands were noted as enlarged at ≥300 mg/kg/day (0/24, 0/24, 12/24, 18/24, 24/24 for the 0, 100, 300, 500 or 1000 mg/kg/day dose groups, respectively). F0 female adrenal gland weights (absolute and relative) incidence only were increased at 1000 mg/kg/day. Adrenal enlargement was noted in only 2/24 of the high dose females. The adrenal weight increases in the higher dosed males are likely attributable to stress (males were dosed through necropsy).

- Absolute testes weights in F0 males were decreased at ≥500 mg/kg/day; relative weights were increased at ≥300 mg/kg/day. These findings were attributed to the decreased body weights in males.

- No differences between dosed and control groups was noted for the mating index or gestation index from the F0 mating.

The maternal NOAEL during gestation was ≤100 mg/kg/day; the reproductive effect NOAEL was 1000 mg/kg/day (HDT). This study is cited as OSRI.

Discussion

Reduced offspring body weights were seen in the two-generation reproductive study, which may be an indication of general toxicity rather than an effect of endocrine disruption. Reduced pup body weights and reduced survival was also seen in the perinatal exposure study where dams were directly dosed during lactation with high gavage doses of test material. There were no indications of endocrine-specific effects. Adrenal weights were increased at toxic doses, likely due to stress. Changes in rate of testicular descent were considered due to a markedly delayed control, in one study, in which non-dose related acceleration occurred, and to chance in another study, in which a non-dose related delay was observed. The latter study also showed a slight delay in vaginal opening, likely attributable to decreased body weight. We are not aware of an alternative mechanism at which both testicular descent and vaginal opening would be delayed.

Other than systemic toxicity effects, there was notable lack of effect on parental and maternal performance and reproductive endpoints. Other more specific indications of potential endocrine modulation – such as altered pup sex ratios; altered reproductive organ weights, external genital malformations were not observed. It should be noted that the reproductive toxicity study design exposes animals peri-natally, which is a lifestage that is considered potentially the most susceptible to endocrine-mediated effects. This lifestage is not evaluated in the Tier 1 screening battery.

2. Developmental Toxicity Studies

Two developmental toxicity studies have been conducted with pyriproxyfen. These studies include definitive studies in the rat (Saegusa et al. 1988a) and rabbit (Hirohashi 1988, 1994). Both of the studies satisfy EPA guideline requirements and are being cited as OSRI. Additionally, Saegusa et al. 1988c included evaluations of fetuses in a study in which the F0 rats were exposed prior to mating, with female exposure continuing to GD7; developmental findings from that study are also summarized in this section. Specific details regarding each study's design, test species, number of animals per group, doses, route and duration of exposure can be found in Table 3, along with information regarding the various endpoints of relevance to the issue of endocrine modulation examined in these studies.

Table 3. Experimental Details and Selected Developmental Toxicity Endpoints of Potential Relevance to Endocrine Modulation Evaluated in Pyriproxyfen Toxicity Studies

	Hirohashi (1988) Hirohashi (1994) addendum	Saegusa et al. (1988a)	Saegusa et al. (1988c)
EPA MRID #	41321720, 42178311 43215402, 43215401	41321719 42178312	44985002
Study design	Teratogenicity (83-3)	Perinatal exposure with postnatal exposure; F1(GD21) and F2(GD21) no exposure	Premating (9 week ♂and 2 week ♀ exposure) exposure through mating and for females to GD 7; dams c-sectioned at GD 21
Species (strain)	JW-NIBS Rabbit	Slc:SD rats	Slc:SD rats
# animals/group	12-14 dams	F0 20-23 dams (cesarean); F1 1 pair/litter (10-13/sex) mated at PND77 (cesarean, no exposure)	23-24/sex
Doses (mg/kg/d)	0, 100, 300, 1000	0, 100, 300, 1000	0, 100, 300, 500, 1000
Route of exposure	Gavage	Gavage	Gavage
Exposure duration	GD6–18	GD7–17	Premating (♂9wks, ♀2wks) and mating to GD7
Selected Endpoints			
Body weights – adult (F0, F1)	X	X	X
Clinical observations – dams	X	X	X
Post-implantation loss	X	X	X
Mean # resorptions	X		
# abortions	X		
Mean # live fetuses/litter	X	X	X
Mean # dead fetuses/litter	X	X	X
Litter weights	X	X	X
Fetal weights	X	X	X
Fetal sex ratio	X	X	X
Fetal abnormalities (external, skeletal, visceral)	X	X	X

Rabbit Oral Teratogenicity (Hirohashi 1989) [MRID 41321720, 42178311, 43215402, 43215401]

In a teratogenicity study, pyriproxyfen was administered by oral gavage to female rabbits once a day from GD6–18 at 0, 100, 300, or 1000 mg/kg/day. Dams were caesarean-sectioned on day 28 of gestation, and fetuses evaluated. EPA indicated the study was core minimum (83-3).

- There were an increased number of dams which aborted or prematurely delivered or which died or were sacrificed early and a decreased number of dams with live fetuses at 1000 mg/kg/day.
- Decreased body weight and body weight gain were seen in dams receiving 1000 mg/kg/day during treatment up to day GD25; however, body weight was not statistically significantly different from controls at sacrifice (GD28).
- There were no treatment-related changes in the number of corpora lutea, number of implantations, and total number of live fetuses, sex ratio or fetal litter body weight.
- There were no treatment-related changes in fetal external, visceral or skeletal malformations, anomalies or variations. There was no evidence of teratogenicity in this study.

The maternal NOAEL was 100 mg/kg/day based on decreased weight gain at 300 and 1000 mg/kg/day. Increased abortion and premature delivery at 1000 mg/kg/day were considered secondary effects of decreased feed consumption induced by administration of the test substance and are considered indications of maternal toxicity. The developmental NOAEL for embryos and fetuses was 300 mg/kg/day. This study is cited as OSRI.

Rat Oral Teratogenicity plus F1 Mating (Saegusa et al. 1988a) [MRID 41321719, 42178312]

In a teratogenicity study with a postnatal evaluation component, pregnant rats (F0) were gavaged with pyriproxyfen at 0 (corn oil), 100, 300 or 1000 mg/kg/day during gestational days (GD) 7–17. Each dose group had 36 females except for the high dose group which had 42 females. At caesarean section (GD21) 20–23 dams (F0) per dose were sacrificed and number of corpora lutea, implantation sites, live and dead fetuses, resorbed fetuses, fetal weights and sex ratio were recorded, and fetuses evaluated for external, visceral and skeletal anomalies. Further details on the reproductive components of this study are described above EPA indicated that this study was acceptable for an 83-3 guideline teratogenicity study.

- Of the high dose F0 dams (1000 mg/kg/day), 12 of the 42 animals died.
- Decreased body weight and body weight gain of F0 dams was seen at ≥100 mg/kg/day.
- In F0 dams, increased liver and kidney weights were found at 300 and 1000 mg/kg/day; In F0 dams treated with 1000 mg/kg/day, gross necropsy showed enlarged adrenal glands, confirmed by increased adrenal weights. The increased adrenal weights at the high dose level were considered stress-related.

- No differences between dosed and control F0 dams were seen for number of pregnant dams, number of dams with live newborns, delivery rate, and length of gestation, number of corpora lutea, implantations or resorptions, or number of live or dead fetuses.

- There were no differences between exposed and control groups for fetal (F1) findings including number of live fetuses, sex ratio, or body weight of live fetuses.

- There were no treatment-related increases in the number of litters or fetuses with external malformations, visceral malformations, or skeletal abnormalities. The number of skeletal variations increased at 300 and 1000 mg/kg/day (opening of foramen transversarium of 7th cervical vertebrae). No teratogenicity was evident in this study and there was no increase in genital malformations.

The maternal NOAEL is ≤ 100 mg/kg/day and the developmental NOAEL is 100 mg/kg/day. This study is cited as OSRI.

Developmental toxicity study with premating, mating, and perinatal exposure to GD7 (Saegusa et al. 1988c) [MRID 44985002]

Sprague Dawley rats (24/sex) were administered pyriproxyfen via gavage at 0 (corn oil), 100, 300, 500 or 1000 mg/kg/day. Males received doses for 9 weeks prior to mating and an additional 3 weeks during mating (total of 12 weeks). Females received doses for two weeks prior to mating, and through mating until GD7; dams were caesarian sectioned at GD 21. No histopathology was performed. F0 findings are discussed above; in brief there was no treatment-related mortality, but there were effects on body weight at ≥300 mg/kg/day in males and females. Decreased body weight gains were also observed at 100 mg/kg/day but were considered incidental. EPA considered this study Acceptable, non-guideline.

- There were no differences between dosed groups and control for: number of implantations, implantation rate or rate of resorbed or dead fetuses (%).

- The number of corpora lutea (14.2) was slightly but statistically significantly decreased at 1000 mg/kg/day when compared to control (15.8), but was within historical control range for the laboratory. The mean number of live fetuses was statistically significantly decreased at 100 (12.6) and 1000 mg/kg/day (12.6), when compared to control, but this finding was not dose-related. The mean number of live fetuses was also within the historical control range for the laboratory. Therefore these findings are considered unlikely to be treatment-related.

- Body weight of F1 female live fetuses were statistically significantly increased at ≥ 100 mg/kg/day. Body weights of male live fetuses were increased at 100 and 1000 mg/kg/day, but no dose response was observed in the males. Differences in litter sizes could have contributed to the body weight differences. Therefore this finding is considered unlikely to be treatment-related.

This study is cited as OSRI.

Discussion

Reduced maternal weight gains were observed in both rats and rabbits at the high dose (1000 mg/kg/day) and at 300 and 100 mg/kg/day in the rats during exposure and immediately after (dose-related decrease, statistically significant at 100 mg/kg/day in the rat (Saegusa et al. 1988 a). This is likely to be a non-specific finding, particularly at a dose level at which both reduced maternal weight gains and decreased feed consumption were evident. The decreased number of rabbit dams with live fetuses at 1000 mg/kg/day appears to be related to systemic toxicity evidenced by an increased number of aborted or prematurely delivered fetuses. The rat developmental study showed a slight increase in fetal skeletal anomalies at the maternally toxic doses of 300 and 1000 mg/kg/day. There were no exposure-related reproductive tract anomalies, and no other parameters including number of corpora lutea, number of implantations, and number of live fetuses, sex ratio or fetal body weight were affected by treatment during gestation, showing a lack of endocrine-mediated effects of pyriproxyfen on development.

3. Subchronic and Short-term Toxicity Studies

Five subchronic toxicity studies of pyriproxyfen were reviewed for evidence of potential endocrine modulation. These studies included three 90-day oral toxicity studies in the rat, mouse and dog (Cox 1989, 1990 and Yamada 1988); one 28-day inhalation toxicity study in the rat (Kawaguchi et al. 1988); and one 21-day dermal study in the rat (Moore 1993). Details regarding each study's design, test species, number of animals per group, doses, route and duration of exposure can be found in Table 4, along with endpoints of relevance to potential endocrine modulation. The three oral 90-day subchronic studies (rat, mouse and dog) were considered acceptable by EPA, and are cited as OSRI. The 28-day rat inhalation study was considered supplemental by EPA as various exposure parameters were not well characterized, and the study is not cited as OSRI. The 21-day dermal study (Moore 1993) is considered acceptable and is cited as OSRI.

Table 4. Experimental Details and Selected Endpoints of Potential Relevance to Endocrine Modulation from Pyriproxyfen Subchronic Toxicity Studies

	Cox 1989	Cox 1990	Yamada 1988	Kawaguchi et al. 1988	Moore 1993
EPA MRID #	41321716	43210504	41321717 42178307	41321718 42178308	43004101
Study design	Subchronic toxicity	Subchronic toxicity	Subchronic toxicity	Subacute inhalation	Dermal toxicity
Species (strain)	Crl:CD BR rat	Crl:CD-1(ICR)BR	Beagle dog	Sprague-Dawley rat	Sprague-Dawley CD

	Cox 1989	Cox 1990	Yamada 1988	Kawaguchi et al. 1988	Moore 1993
		mouse			rat
# animals/ group	10/sex	10/sex	4/sex	10/sex	5/sex
Doses (mg/kg/day unless noted)	0, 400, 2000, 5000, 10000 ppm	0, 200, 1000, 5000, 10000 ppm	0, 100, 300, 1000 mg/kg-day	0, 269, 482, 1000 mg/m^3	0, 100, 300, 1000 mg/kg/day
Route of exposure	diet	diet	capsule	inhalation	dermal
Exposure duration	13 weeks	13 weeks	13 weeks	28 days 4 hr/day	21 days 6 hr/day
Selected Endpoints					
Body weights	X	X	X	X	X
Mortality	X	X	X	X	X
Organ weights – ovaries (O), uterus (U)			O, U	O	
Organ weights – testes (T), epididymides (E), prostate (P)	T	T	T, P	T,P	T(E)
Organ weights – Adrenal (A), pituitary (Pit) Thyroid, (Th), parathyroid (pTh)	A	A	A, Pit, Th(pTh)	A, Pit, Th	
Gross pathology – ovaries (O), uterus (U), vagina (V), mammary glands (M)	O, U, M	O, U, M	O, U,V,M	O, U, V,M	O, U, V, M
Gross pathology – testes (T), epididymides (E), prostate (P), seminal vesicles (S)	T, E	T, E, S	T, E, P, S	T, E, P, S	T, E, P, S
Gross pathology – adrenal (A), pituitary (Pit) thyroid (Th), parathyroid (pTh)	A, Pit, Th, pTh	A, Pit, Th, pTh	A, Pit, Th, pTh	A, Pit, Th, pTh	A, Pit, Th, pTh
Histopathology – ovaries (O), uterus (U), vagina (V), mammary glands (M)	O, U, M	O, U, M	O, U, V, M	O, U, V, M	
Histopathology – testes (T), epididymides (E), seminal vesicles (S), prostate (P)	T, E	T, E, S	T, E, S,P	T, E, S,P	
Histopathology – adrenal (A), pituitary (Pit), thyroid (Th), parathyroid (pTh)	A, Pit, Th, pTh	A, Pit, Th, pTh	A, Pit, Th, pTh	A, Pit, Th	

Rat 90-Day Dietary Subchronic Toxicity (Cox 1989) **[MRID 41321716]**

Rats (10/sex) were administered pyriproxyfen in the diet at levels of 0, 400, 2000, 5000, or 10000 ppm in the diet for 90 days.

- No treatment-related deaths were observed.
- Body weight decrease was observed in males and females at 5000 and 10000 ppm.
- Liver weight (absolute and relative) increased at 2000 ppm (relative weight in males only), 5000, and 10000 ppm; relative kidney weight increased at 5000 ppm (males only) and 10000 ppm (males and females).
- There were no organ weight changes in testes. Increased relative adrenal weight was observed in males at 10000 ppm; this finding was considered related to the decreased body weight at that dose.
- No gross or histopathological changes were observed in the following tissues: adrenal glands, pituitary, thyroid, parathyroid, testes, epididymides, ovaries, uterus, or mammary glands.

The NOAEL of 400 ppm (23 mg/kg/day for males) was based on increased blood biochemical changes (cholesterol and phospholipids), hematology changes, and increased liver weight with accompanied histopathological changes in hepatocyte cytoplasm at 2000 ppm (118 gm/kg-day in males); females did not exhibit these statistically significant effects until 5000 ppm.

EPA considered this study acceptable; it is cited as OSRI.

Mouse 90-Day Dietary Subchronic Toxicity (Cox 1990) **[MRID 43210504]**

Mice (10/sex) were administered pyriproxyfen in the diet at levels of 0, 200 (28–38 mg/kg/day), 1000 (149–197 mg/kg/day), 5000 (838–964 mg/kg/day), or 10000 ppm (2035–2345 mg/kg/day) in the diet for 90 days.

- Mortality was observed in the high dose males and females.
- Body weight decreased in males fed 5000 and 10000 ppm (12 and 31% decreased body weight, respectively) Body weight was also decreased (16%) in females at 10,000 ppm at week 4, but was not statistically significantly decreased at week 13.
- Kidney lesions were present at 5000 and 10000 ppm.
- Decreased trend in absolute mean testes weight in treated males (pairwise statistically significant at 10000 ppm); relative mean testes weight did not change with treatment. No histopathological changes noted. This decrease is attributable to the severe body weight deficit at 10000 ppm.

- There was an increased trend in absolute adrenal gland weight in males; relative adrenal weights were statistically significantly increased at 5000 and 10000 ppm in males when compared to controls. This adrenal enlargement is likely attributable to stress including decreased body weight. No changes in adrenal weights were observed in females.

- Decreased size of seminal vesicles in 4/10 males was noted in gross observations of males fed 10000 ppm; (seminal vesicle weights not recorded); histopathology indicated reduced secretion in 4/10 males fed 10000 ppm. This change may be related to the severe body weight deficit in this dose group.

- No histopathological changes in epididymides, thyroid/parathyroid glands, ovary, uterus or mammary glands.

The NOEL for systemic toxicity was 200 ppm (28-38 mg/kg/day) based on hematology and blood chemistry findings at 1000 ppm (149–197 mg/kg/day). EPA considered the NOEL for systemic toxicity to be 1000 ppm (149–197 mg/kg/day).

EPA considered this study acceptable; it is cited as OSRI.

Dog 90-Day Oral Subchronic 90-day Toxicity (Yamada 1998) **[MRID 41321717, 42178307]**

In a 90-day subchronic study, beagle dogs (4/sex) were orally administered pyriproxyfen by capsule at 0, 100, 300 or 1000 mg/kg/day.

- No deaths occurred.

- In dogs which received the 1000 mg/kg/day dose, a yellow viscous substance which was believed to be the test substance was noted in the stool; it was judged that the change in fecal property (soft stool and diarrhea) were not a result of toxicity but the physical properties of the test substance. The test material may not have been absorbed completely thus reducing the effective dose.

- Blood biochemistry changes (cholesterol and phospholipids) in females at 300 and 1000 mg/kg/day

- Absolute liver weights were increased in the male dogs receiving 300 and 1000 mg/kg/day; Relative liver weight was increased only at 300 mg/kg/day. No changes were observed in females. In males, histopathological examination indicated enlarged hepatocytes at 1000 mg/kg/day; females showed similar results at 300 and 1000 mg/kg/day. Along with electron microscopy of the 1000 mg/kg/day livers, it was judged that changes in the liver were an adaptation of the smooth endoplasmic reticulum to administration of the compound rather than an indication of hepatotoxicity.

The NOEL was 100 mg/kg/day based on adaptation of liver-related detoxification of the test material in animals receiving 300 mg/kg/day or more. EPA considered this study acceptable. It is cited as OSRI.

28-Day Inhalation Toxicity (Kawaguchi et al. 1988) **[MRID 41321718, 42178308]**

In a subacute inhalation study, pyriproxyfen was administered to rats (10/sex) in a mist with corn oil as the vehicle at 0, 269, 482, or 1000 mg/m^3 for 4 hours a day for 28 consecutive days. EPA classified the study as supplementary and not upgradeable because of lack of characterization of the test material, inadequate air flow dynamics, and less exposure frequency and duration than required by Guideline (6 hours a day, 5 days a week for 90 days).

- There were no treatment-related deaths.
- Body weight decreased overall for males and females exposed to 1000 mg/m^3; however, statistical significance was not consistent with time.
- Relative liver weight was increased in male rats exposed to 1000 mg/m^3.
- There were no endocrine-modulated effects on reproductive organs.

The NOEL was 482 mg/m^3 based on salivation, decreased body weight in males, and elevated serum lactate dehydrogenase in males at 1000 mg/m^3. This study is not cited as OSRI due to EPA-identified study deficiencies.

21-Day Rat Dermal Toxicity Study (Moore 1993) **[MRID 43004101]**

Daily application of pyriproxyfen to the dorsal skin of Sprague-Dawley rats (5/sex/group) produced no evidence of dermal or systemic toxicity at dose levels of 100, 300, or 1000 mg/kg/day. The NOEL for dermal as well as systemic toxicity in both males and females was 1000 mg/kg/day, which was the highest dose tested.

- There were no effects on mortality or body weight.
- No treatment related effects observed with gross pathology (all organs) or histopathology (liver or kidney).

EPA considered this study acceptable. It is cited as OSRI.

Discussion

Of the oral 90-day rat, mouse, and dog studies, the systemic toxicity NOAEL levels ranged from 23–197 mg/kg/day and were consistently based on liver weight and histopathological changes and related blood biochemistry effects; the NOEL in the mouse 90-study was also based on renal effects. The mouse study was the only study that showed possibly endocrine-related findings – such as decreased testes weight (absolute but not relative) at the highest dose, 10000 ppm (2035–2345 mg/kg/day) and decreased

seminal vesicle size and reduced secretion in 4/10 high dose males. These changes, however, can be associated with overall systemic toxicity and marked decreases in body weight, and are not considered to represent specific endocrine toxicity. Increased adrenal organ weights (absolute and relative) at 5000 and 10000 ppm were also observed, likely stress-related including decreased body weight. There was no correlative adrenal histopathology. It should be noted that the 10000 ppm high dose in mice approached a lethal dose with high mortality.

Although the dermal studies evaluated few of the endpoints relevant to assessing endocrine toxicity, it provides a NOEL for dermal testicular toxicity and is cited as OSRI. The inhalation study was considered supplementary by EPA; although it provides data on several endocrine-related endpoints (showing no endocrine-related effects) it is not cited as OSRI.

4. Chronic Toxicity/Oncogenicity Studies

Three studies examining the chronic toxicity and/or oncogenicity of pyriproxyfen were reviewed. These include two bioassays in the rat and mouse (Osheroff 1991a,b) and one one-year chronic study in the dog (Chapman 1991). All three of these studies were considered guideline-compliant by EPA. Specific details regarding each study's design: species, number of animals per group, doses, route and duration of exposure can be found in Table 5 along with information regarding endpoints relevant to assessing potential endocrine modulation. These studies are cited as OSRI.

Table 5. Experimental Details and Selected Endpoints of Potential Relevance to Endocrine Modulation from Pyriproxyfen Chronic Toxicity/Oncogenicity Studies.

	Osheroff (1991a)	Osheroff (1991b)	Chapman (1991)
EPA MRID #	42178314 43210501 43210502 43210503	42178310 43413202 43210501 43413201	42178309
Study design	Chronic Toxicity/ Carcinogenicity	Carcinogenicity	Chronic Toxicity
Species (strain)	Crl:CD BR rat	Crl:CD-1(ICR)BR mouse	Beagle dog
Number animals/group	50/sex (104 wks) 30/sex (52 wks)	50/sex (78 wks) 10/sex (52 wks)	4/sex
Doses	0, 120, 600, 3000 ppm (Approx 5–7, 27–35, 138–182 mg/kg/day)	0, 120, 600, 3000 ppm (Approx 17-22, 84-110, 420-547 mg/kg/day)	0, 30, 100, 300, 1000 mg/kg/day
Route of exposure	Diet	Diet	Oral capsule
Exposure duration	104 weeks	78 weeks	52 weeks

Selected Endpoints	Osheroff (1991a)	Osheroff (1991b)	Chapman (1991)
Body weights	X	X	X
Mortality	X	X	X
Organ weights – ovaries (O), uterus (U), cervix (C)	O	O	O, U (+C)
Organ weights – testes (T) , prostate (P)	T	T	P, T
Organ weights – adrenal (A), pituitary (Pit), thyroid(Th), parathyroid(pTh)	A, Th(pTh)	A	Th(pTh), Pit, A
Gross pathology – ovaries (O), uterus (U), cervix (C), vagina (V), mammary glands (M)	O, U(V+C), M	O, U(+C), V, M	M
Gross pathology – testes (T) , epididymides (E), seminal vesicles (SV), prostate (P), penis (Pe)	T, E, SV, P, Pe	T, E, SV, P, Pe	T, E, P
Gross pathology – adrenal (A), pituitary (Pit), thyroid (Th), parathyroid (pTh)	Pit, A, Th, pTh	Pit, A, Th, pTh	Pit, A, Th
Histopathology – ovaries (O), uterus (U), cervix (C), vagina (V), mammary glands (M)	O, U(V+C), M	O, U(V+C), M,	O, U, V, M
Histopathology – testes (T) , epididymides (E), prostate (P), seminal vesicles (SV)	T, E, SV, P	T, E, SV, P	T, E, P
Histopathology – adrenal (A), pituitary (Pit), thyroid (Th), parathyroid (pTh)	Pit, A, Th(pTh)	Pit, A, Th(pTh)	Pit, A, Th(pTh)

Rat Dietary Chronic Toxicity/Oncogenicity Study (Osheroff 1991a) [MRID 42178314, 43210501, 43210502, 43210503]

Pyriproxyfen was administered to rats (50/sex/dose) in the diet at 0, 120, 600, or 3000 ppm for at least 104 weeks (approximately equivalent to 0, 5.4, 27, 140 mg/kg/day in males and 0, 7.0, 35, and 180 mg/kg/day in females). In a satellite study rats (30/sex/dose) were administered pyriproxyfen in similar concentration for 52 weeks. There was no treatment-related effect on survival.

- Overall growth rates were significantly depressed in males fed 3000 ppm (8%) and females fed 600 and 3000 ppm (10 and 23% respectively). The finding at 600 ppm in females was transient and was not seen in the satellite group at the same dose level; therefore this finding was not considered an adverse effect.
- At 3000 ppm, changes in several hematology and serum chemistry parameters and increased liver weight appeared to be transient, inconsistent, and not manifested at the end of the study.
- There were no treatment-related organ weight changes in adrenals, thyroid, ovary, testes or kidney.
- There were no gross or histopathological tissue alterations which could be attributed to administration of test material.
- There was no evidence of increased or decreased oncogenicity of endocrine-related tissues, including testes, prostate, seminal vesicles, epididymides, uterus, ovaries, pituitary or mammary glands.

The report defined the NOEL as 600 ppm (27 mg/kg/day) for males and 120 ppm (7.0 mg/kg/day) for females based on decreased body and growth rates. EPA concluded the NOEL was 600 ppm in both sexes; we believe this can be considered the NOAEL. There is no indication of endocrine-related toxicity in this study. EPA indicated that study was guideline (83-5) acceptable. It is cited as OSRI.

Mouse Dietary Oncogenicity Study (Osheroff 1991b) [MRID 42178310, 43413202, 43210501, 43413201]

Pyriproxyfen was administered to Crl:CD-1(ICR)BR mice (50/sex) in the diet at 0, 120, 600, or 3000 ppm for at least 78 weeks (equivalent to 0, 17, 84 or 420 mg/kg/day and 0, 22, 110 or 547 mg/kg/day in males and females, respectively). In a satellite study mice (10/sex) per dose were administered pyriproxyfen at similar concentrations for 52 weeks.

- Decreased survival was seen in male mice fed 600 or 3000 ppm and female mice fed 3000 ppm.
- Decreased body weight was noted in males and females receiving 3000 ppm
- Exposure to pyriproxyfen was associated with an accelerated development of systemic amyloidosis at ≥600 ppm in males and 3000 ppm in females, chronic progressive nephropathy at 3000 ppm in both males and females, and increased incidence of renal tubular mineralization at 3000 ppm in females; all of these diseases are known to occur spontaneously in these mice.
- There were no exposure-related changes in adrenal, ovaries or testes organ weights.

- There was no evidence of increased or decreased tumor incidence in endocrine-related tissues, including thyroids, testes, prostate, seminal vesicles, epididymides, uterus, ovaries, pituitary or mammary glands.

The report defined the NOEL as 120 ppm (17 mg/kg/day) based on decreased survival at 600 ppm (for male mice, and decreased body weight and growth as a result of increased incidence/severity of systemic amyloidosis at 3000 ppm in both sexes. EPA concluded that the NOEL was 600 ppm; this dose can be regarded as a NOAEL in this study. This study showed no evidence of EAT endocrine effects. EPA indicated that study was guideline (83-2) acceptable. It is cited as OSRI.

Dog Oral Chronic Toxicity Study (Chapman 1991) **[MRID 42178309]**

Beagle dogs (4/sex) were administered pyriproxyfen via oral capsule at doses of 0 (empty gelatin capsule), 30, 100, 300, or 1000 mg/kg/day for 52 weeks. EPA considered the study acceptable per 83-1 guideline.

- At 1000 mg/kg/day, males had decreased survival (2/4); hematology, serum chemistry, urine and liver histopathology changes were noted.
- Overall body weight gain of surviving males receiving 300 or 1000 mg/kg/day were low during the first 13 weeks compared to controls.
- Increased liver weights were noted in animals receiving ≥30 mg/kg/day; histopathology revealed significant liver damage in all animals receiving 1000 mg/kg/day; serum biochemistry (alkaline phosphatase, alanine amino-transferase, aspartate amino-transferase, and cholesterol, triglyceride, and chloride concentrations) also confirmed liver effects at doses of 100 mg/kg/day and higher.
- Slightly elevated relative kidney weights were noted in animals at ≥ 300 mg/kg/day.
- Slight but statistically significant increased thyroid weight (absolute and relative) was seen in females at ≥ 100 mg/kg/day (not dose-dependent); this finding was considered related to low control thyroid weights. There were no correlating histopathological findings. Increased parafollicular cell hyperplasia was seen in 300 mg/kg/day treated males only (not dose dependent). This finding is not typically associated with anti-thyroid hormone activity.
- No changes in organ weights or histopathology of adrenals, pituitary, ovary, uterus, testes, or prostrate were observed.

The NOAEL was 30 mg/kg/day based on hepatic toxicity at 100 and 300 mg/kg/day. This study is cited as OSRI.

Discussion

The body weight reductions observed in these three studies are considered indicators of systemic toxicity. The only finding related to potential endocrine modulation in these studies was a relatively slight increase in thyroid weight in female dogs at ≥100 mg/kg/day (though not dose dependent); there were no histopathological correlates to this finding and it is considered unlikely to be exposure-related.

B. Ecotoxicological Studies

1. Fish

There are three fish guidelines studies with pyriproxyfen, a lifecycle study with Japanese medaka (*Oryzias latipes*), an early life stage (ELS) study with 0-61 days post hatch (dph) rainbow trout (*Oncorhynchus mykiss*), and a 21-day survival study with rainbow trout. The test with Japanese medaka provides all of the endpoints required for the fish short-term reproduction assay (FSRA), and is the study reviewed in depth and cited as providing functional equivalence for OSRI. EPA has not yet reviewed this study. The fish ELS study and 21-day studies are also cited as OSRI, primarily because findings in these studies support the high dose selection for the Medaka study.

Life-cycle Study with Japanese Medaka (Gries, 2007) **[MRID 48066202]**

Springborn Smithers Laboratory (Europe) conducted a full lifecycle study with Japanese medaka under flow-through conditions to determine lethal and sublethal effects of exposure to pyriproxyfen, with an add-on portion to determine fecundity and fertility via an additional 30-day exposure period. The test was compliant with "The Medaka (*Oryzias latipes*) Full Life Cycle Test Guideline" (Ministry of the environment, Japan, Annex 6-2, November 2002) which states that "The method is applicable to a variety of chemicals, including endocrine disrupters and general toxicants." Endpoints measured at 60-day (larval maturation) and 114-day (end of study for parental generation) post hatch included: length, weight, condition factor, deformities, genetic sex (coloration of the body), secondary sexual characteristics (fin morphology), liver weight (and hepato-somatic index), gonad weight (and gonadal-somatic index), hepatic vitellogenin, and gonad histopathology. This encompasses all the endpoints required in the Tier 1 FSRA study. Four replicates of 15 fish were used for each test concentration for both parental and F1 generation fish. For the reproduction portion of the study, an additional 8 pairs (1 male and 1 female) were used per test concentration. The F1 generation fish were subjected to the same monitoring and measurement endpoints as the parental generation until they were 60-days post hatch (dph). Average measured test concentrations were 0.84, 2.7, and 8.6 µg/L based on weekly measurements.

Statistically significant, dose-responsive findings were limited.

- Hepatic vitellogenin in F0 males was increased in the high-dose 8.6 µg/L group at 60 dph; however, no statistically significant differences between dosed and control fish were seen at

114 dph. Further, no statistically significant differences were seen in F1 males at 60 dph. Therefore, the difference in vitellogenin in high dose F0 males at 60 dph was not considered exposure related.

- The hatchability of the F1 generation 8.6 µg/L group was decreased at 99 dph in comparison to the solvent control; however, no statistically significant differences were found for the hatchability of eggs from 100 and 101 dph. Therefore the findings at 99 dph are considered an artifact and not related to pyriproxyfen exposure.
- Other findings were non-dose dependent; most were seen in the F0 generation at 60 dph but were not replicable at 114 dph or in the F1 generation.

Therefore, the authors concluded that 8.6 µg/L represents an unbounded NOEC for fish exposed to pyriproxyfen.

Fish Early Life Stage Study in Rainbow Trout (Rhodes and Cramer, 1991) [MRID 42178319]

ABC Laboratories conducted an early life stage study under 40 CFR Part 158.145 (Guideline 72-4) with rainbow trout (*Oncorhynchus mykiss*) embryos and fry exposed to 1.8, 4.3, 6.7, 14, and 26 µg/L pyriproxyfen in a 96-day flow-through study design (from 4 hrs post fertilization to 61 dph).

- Egg hatchability and fry swim-up was not affected at any dose.
- Fry survival (at 35 and 61 dph) was significantly reduced in the 14 µg/L treatment group but not in the 26 µg/L group.
- Reduced growth (length and weight) was documented at ≥6.7 µg/L at 35 and 61 dph.
- Behavior and coloration changes were observed occasionally at 6.7 µg/L, but all reversed by the termination of the study.

The authors concluded that growth was the most sensitive endpoint in this ELS rainbow trout study, with a NOEC of 4.3 µg/L and a LOEC of 6.7 µg/L, with the point estimate MATC being 5.4 µg/L.

Although endpoints assessed included some of those required in the Tier 1 FSRA, this study did not include observations of adults (e.g., number of spawns and number of eggs per female reproductive day, fertilization success, etc.) and therefore does not provide information for all required FSRA test endpoints. This study was termed "scientifically sound, non-guideline" by EPA. It is cited as OSRI because it supports dose selection for the Medaka study, showing a sub-lethal effect concentration under long-term exposure conditions.

21-Day LC50 with Rainbow Trout (Sword and Northrup, 1992) [MRID 48066203]

ABC Laboratories conducted a 21-day LC50 test following OECD Guideline 204, exposing rainbow trout fry to 11, 21, 46, 88, and 180 µg/L (measured) pyriproxyfen in a flow-through study design with 20 fish

per concentration. The highest concentration (nominal and mean measured concentrations of 200 and 180 µg/L, respectively) appeared to slightly exceed the solubility limit, as a slight surface film (precipitate) was observed on the diluter face of the mixing cell during the final week of the test. The 21-day LC50 was calculated to be 90 µg/L; the NOEC was 21 µg/L and the LOEC was 46 µg/L. Mortality and abnormal behavior was observed in 0, 0, 1, 8, and 20 fish per concentration, respectively. Because survival, body length, and weight were the only measurement endpoints, this study provides no information to support fulfilling the requirements for the FSRA. It is cited as OSRI, however, because it supports dose selection for the Medaka study, defining a lethal effect concentration under long-term exposure conditions.

2. Avian Studies

Mallard Avian Reproductive Toxicity Study (Beaver et al. 1994a) **[MRID 44036908]**

A guideline (71-4) avian 1-generation reproduction study with Mallard ducks (*Anas platyrhynchos*) was conducted by Wildlife International. Birds (16/sex/group) were exposed to 0, 120, 360, 600 ppm pyriproxyfen in the diet for 21 weeks. F0 endpoints included: mortality, feed consumption, final body weight, reproductive parameters (egg production, fertilization success, embryo viability, 3-week embryo viability, eggshell thickness, number set/hen, number hatching/hen), and F1 chick weight, 14-day weight, and chick survival. There was no mortality or exposure-related change in body weight. There were no differences in hatching success or fecundity. No biologically significant gross findings were made; some ovaries and testes were labeled "small" including in control birds. There were no statistically significant, dose-response relationships for any measured endpoint in either the parental or F1 generations. The authors concluded 600 ppm represents an unbounded NOAEC for Mallard ducks exposed to pyriproxyfen. EPA considers this study Core Guideline, and it is cited as OSRI.

Bobwhite Quail Avian Reproduction Study (Beaver et al. 1994b) **[MRID 44036906]**

Wildlife International Ltd. conducted a guideline (71-4) avian 1-generation reproduction studies with Bobwhite quail (*Colinus virginianus*).

Birds (16/sex/group) were exposed to 0, 120, 360, 600 ppm pyriproxyfen in the diet for 22 weeks. F0 endpoints included: mortality, feed consumption, final body weight, reproductive parameters (egg production, fertilization success, embryo viability, 3-week embryo viability, eggshell thickness, number set/hen, number hatching/hen), and F1 chick weight, 14-day weight, and chick survival. There was no mortality or exposure related change in body weight. There were no differences in hatching success, fecundity. No biologically significant gross findings were observed; some ovaries and testes were labeled "small" including in control. There were no statistically significant, dose-response relationships for any measured endpoint in either the parental or F1 generations. The authors concluded 600 ppm represents an unbounded NOAEC for Bobwhite quail exposed to pyriproxyfen. EPA considers this study Core Guideline, and it is cited as OSRI.

Summary

The available ecotoxicology studies are considered adequate to address the possibility of endocrine modulation from pyriproxyfen exposures to fish. In particular the full-life cycle study with Japanese medaka followed an accepted, standard protocol and measured all the endpoints required in a FSRA. Therefore, The Pyriproxyfen Task Force requests a waiver from the fish short-term reproduction study. However, there are no studies on pyriproxyfen effects on frogs; although birds and mammals showed no evidence of thyroid disruption, the species are not similar enough to draw conclusions regarding potential effects on the amphibian. Therefore, The Pyriproxyfen Task Force will conduct an amphibian metamorphosis assay of pyriproxyfen as specified in the Tier 1 Guideline (OPPTS 890.1100).

C. Published Literature on Pyriproxyfen Relevant to Evaluation of Potential Endocrine Effects

1. Literature search methodology

An extensive literature search was conducted on pyriproxyfen (CAS# 95737-68-1) using the following databases and keywords:

Databases:

- CAS, "CAplus," STN online (2009)
- CAS, "TOXCENTER," STN online (2009)
- CABI Publishing, "CABA," STN online (2009)
- JST, "JSTplus," JdreamII online (2009)
- JST, "JMEDplus," JdreamII online (2009)

Abbreviations:

- CAS: Chemical Abstracts Service
- STN: The Scientific and Technical Information Network
- JST: Japan Science and Technology Agency

Keywords:

endocrine disruptor, hormone disruptors, environmental hormones, endocrine disrupting substances, hormone disrupting compounds, xenoestrogens

estrogen, estrogen antagonists, antiestrogen, estrogen inhibitors, estrogen receptor antagonists

androgen, antiandrogens, androgen antagonists, androgen inhibitors, testosterone receptor

thyroid

steroidogenesis

aromatase

uterotrophic

Hershberger

pubertal

amphibian metamorphosis

reproduction, fertility, insemination, oogenesis, ovarian cycle, estrus, luteinization, menstruation, oviposition, spermatogenesis

reprotox, reproduction toxicity

Results of the public literature search are briefly summarized below for *in vitro* assays, *in vivo* mammalian assays and *in vivo* ecotoxicological studies.

2. In Vitro Assays

Three *in vitro* assays were identified (Kojima et al. 2005a and 2005b, and Manabe et al., 2006) assessing estrogen receptor transactivation. These studies are summarized in Appendix IV. All three showed a relatively weak positive response which may not reflect specific activity of the compound to the receptor. Two of the assays, the Kojima et al. 2005a and 2005b studies are generally similar in design to the Tier 1 estrogen receptor transcriptional activation assay and are cited as OSRI. The Manabe et al. 2006 study is cited as OSRI, but is not considered similar in design to the Tier 1 ER transcriptional activation assay. Positive findings in these assays, however, only resulted from concentrations not reasonably anticipated to be reachable *in vivo*; thus they are not considered to support potential estrogenicity of pyriproxyfen under normal use or environmental conditions.

3. *In Vivo* Mammalian Studies

No published mammalian *in vivo* studies of pyriproxyfen were identified.

4. *In Vivo* Ecotoxicological Studies

a. Frogs

No published studies were identified on the effects of pyriproxyfen on thyroid function or metamorphosis in frogs or other amphibians.

b. Fish

A single study was identified in the public literature about pyriproxyfen effects to fish. Brown et al. (2002) conducted laboratory toxicity studies to determine the acute lethal effects of a 1-hour pulse exposure of selected insecticides, including pyriproxyfen (as formulated product), on crimson-spotted rainbowfish (*Melanotaenia duboulayi*), a species native to the Australia-Papua New Guinea region. Laboratory bred adult and larval (< 72-hour post hatch) rainbowfish were exposed to Sumilarv (2% a.i. pyriproxyfen) for 1 hour. After the 1-hour pulse exposure, the fish were moved into tanks with clean water and the number that survived to 24-hours was measured. Ten adult fish (5 males and 5 females) in each of three replicates were exposed to the expected environmental concentration (EEC) of 8 µg/L. Larvae were exposed to 10 and 100 µg/L, with four replicates of 10 fish at each concentration. None of the adult fish died, and neither of the test concentrations was toxic to the larval fish. This study has significant short-comings that make it unsuitable for use as a screen for potential endocrine effects of pyriproxyfen. Specifically, the test article was a formulated product, not the active ingredient, only a single pulsed exposure dose was used, and survival was the only endpoint measured. Because of the identified deficiencies, this study is not cited as OSRI.

c. Birds

No published studies were identified relating to effects of pyriproxyfen on birds.

d. Invertebrates

Pyriproxyfen is an insect growth regulator and is known to modulate the endocrine system of insects. The effects of Juvenile Hormone (JH) in insects and related arthropods are well known and have been extensively studied. (See Takimoto et al. 1998 (MRID # 44647801) for a general review of Juvenile Hormone's effects in arthropods.)

JH regulates development and differentiation in immature insects, and plays a critical role in adult reproduction, feeding, diapause, and cast determination in social insects. JH is a sesquiterpenoid hormone that is specific to insects and other arthropods and is not found in vertebrate systems. JH's specificity to arthropods and the central role that it plays in insect growth and development are key reasons why compounds mimicking its effects were developed as insecticides. The resulting "JH mimic" insecticides, including pyriproxyfen, have enjoyed commercial success because of their low acute mammalian toxicity, their unique mode of action, low use rates, and specificity to insects and some related arthropods.

Because Juvenile Hormone is unique to arthropods and does not exist in vertebrates, there is no *a priori* reason to believe that pyriproxyfen would disrupt vertebrate endocrine systems.

It should also be noted that, because of the mode of action of pyriproxyfen, extensive regulatory toxicology testing has been conducted on Mysid and *Daphnia*. These studies are not cited as OSRI because of the lack of relevance to vertebrate endocrine modulation.

IV. Analysis of Regulatory Toxicology Data and Published Studies for Assessment of Endocrine Function

A. Indicators of Endocrine Modulation in Guideline Mammalian Toxicological Studies

As previously mentioned, a large body of guideline-compliant mammalian and ecological toxicity studies must be conducted for each food-use pesticide registered for use in the US. The endpoints examined in these assays generally are not identical to those measured in the Tier I screening assays; however, the information derived from these studies in many cases is similar enough to provide confidence that the study results adequately characterize whether the compound is likely to be an endocrine disruptor at environmentally relevant exposure levels. For example, the male pubertal assay, which is part of the Tier 1 screening battery, measures exposure-related changes in the organ weights and histopathological changes in various estrogen, androgen or thyroid hormone-producing or impacted tissues following exposure to an immature intact animal through the time of sexual maturation. Many of these endpoints receive redundant assessments across multiple life stages and in several species in testing of a food use pesticide, as many of the same organs are also evaluated following subchronic and chronic exposures. Further, clinical, gross and histopathological evaluations conducted as part of these guideline studies measure many related endpoints, which provide additional confidence that potential endocrine-related effects have not been missed.

Even the EPA Guideline 83-4 reproductive toxicity study, which lacks some of the critical endocrine-related endpoints in the newer OPPTS 870.3800 Guideline study, has been demonstrated as competent and sensitive for detecting aromatase inhibitors, anti-androgens and estrogenic compounds. It may not provide sufficient information to assess the mechanism of toxicity, but it provides significant sensitivity to detect the potential hazard. For example, the adverse effects of linuron, which EPA characterizes as a weak anti-androgen (and uses as a weak positive control), were detected in an 83-4 guideline reproductive toxicity study (EPA, 1995).

Furthermore, the data derived from studies done for guideline purposes are more informative than much of the data from the Tier I screen in that they address whether exposure is associated with an actual functional endocrine—rather than mechanistic—change in the intact animal system, at what doses, and how these doses compare to those shown to cause other toxic effects that may drive risk assessment. Thus they provide information on the potential for endocrine disruption as defined in the Weybridge conference:

> *"An endocrine disrupter is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function."*
> - European Workshop on the Impact of Endocrine Disrupters on Human Health and Wildlife (Weybridge, UK; 1996). European Union Report EUR17459

Table 6 provides a bulleted list of some (but not all) of the indicators from guideline-compliant mammalian studies that assess pesticide exposure-related disruption of the endocrine system. These indicators may be summarized as follows.

Estrogenic activity is indicated based on estrous cyclicity evaluations (or time to mating plus in some cases stage of estrous at necropsy), with prolonged or persistent estrous the most characteristic finding. Timing of vaginal opening may be accelerated and balano-preputial separation or testicular descent delayed in offspring in reproductive or perinatal toxicity DNT studies. Other signs of estrogenicity would include reduced gestational length, uterine weight changes and histopathological alterations in tissues of the reproductive system (e.g., vaginal cornification or uterine hypertrophy/hyperplasia), increased incidence of mammary tumors (adenocarcinomas or carcinomas), or reproductive tract tumors in female offspring. In males, reduced fertility, decreased reproductive organ weights and increased histopathological changes in the testes (e.g., Leydig cell proliferation or tumors) or prostate might be observed.

Alternatively, if a chemical acted as an estrogen antagonist, then female animals would likely exhibit reduced fertility, decreased corpora lutea and implantations in reproductive and developmental toxicity studies, delayed vaginal opening in female offspring, decreased female reproductive organ weights, decreases in ovarian follicle counts or accelerated reproductive senescence. Strains of rats prone to mammary tumors (such as Sprague Dawley rats) would be predicted to show a decreased incidence of mammary tumors compared to control in oncogenicity studies. The incidence of pituitary pheochromocytomas might also be decreased. In males, anti-estrogenic effects would be more limited. The epithelium of the testicular tubules may be reduced in height. Also, testicular weights may be increased following short-term exposures, or alternatively, testicular atrophy and infertility may be observed after long-term exposures.

If a chemical exhibited androgenic activity, then altered reproductive organ weights would likely be observed in male animals, the direction of the change depending on the period of exposure and when the animals were examined. Preputial separation or testicular descent would be predicted to be accelerated. Other changes would be expected in males as well, including testicular atrophy, decreased fertility and sperm counts. Female animals might exhibit male sex accessory tissues, reduced fertility, altered differential ovarian follicle count, and histopathological changes to the reproductive tissues.

In contrast, exposure to an anti-androgenic compound is likely to cause numerous changes in male offspring, including markedly delayed preputial separation, reduced anogenital distance, an increase in pups missexed at birth (a useful parameter if anogenital distance was not evaluated), ectopic testes, hypospadias or epispadias (apparent on examination of external genitalia), retained nipples (apparent on detailed necropsy of F1 weanlings), decreased reproductive organ weights and histopathological alterations of the reproductive tissues (e.g., epididymal agenesis, rat Leydig cell or interstitial testicular tumors), and decreased fertility.

If aromatase is inhibited, females might exhibit increased body weights, altered uterine weights, increased weights and histopathological changes in the ovary (e.g., polycystic changes, stromal hyperplasia), and possibly an increased incidence of ureter and bladder infections. Strains of rats prone to mammary tumors (such as SD) would be predicted to show a decreased incidence compared to control in oncogenicity studies. In males, body weight might be reduced, testicular weights increased, time to mating increased and male mounting behavior decreased.

Modulations of steroidogenesis could affect one or more of the above parameters, depending where in the process of steroidogenesis hormone production was inhibited or enhanced. It should also be noted that some compounds, e.g., tamoxifen, have been identified that have a mixed profile of responses.

If the chemical modulates thyroid hormone activity, resulting alterations would be predicted to be similar in both males and females, unless the metabolism of the compound differs between sexes. Indicators of decreased thyroid function include a pattern of decreased T_4 and/or T_3 levels, increased TSH levels, increased thyroid weights, decreased colloid content of follicular cells, thyroid follicular cell hyperplasia, and possibly thyroid follicular cell tumors on prolonged exposure. 28-day studies are generally adequate to detect thyroid hormone alterations.

Table 6 is not a complete list and other endocrine-related changes may be observed in mammalian studies conducted for pesticide registration purposes; however, the table captures the effects most commonly observed in response to endocrine modulation. Consequently, this table serves as a useful starting point for the evaluation of EAT from the mammalian toxicology studies available for pyriproxyfen.

Table 6. Indicators of Potential EAT Endocrine Disruption from Mammalian Studies

Mechanism	Potential Effects in Males	Potential Effects in Females
Estrogenicity	• Delayed preputial separation (marked, or in absence of significant body weight decreases) • Reduced fertility • Reduced sperm counts • Decreased male reproductive organ weights • Histopathological findings of the testes (*e.g.,* Leydig cell proliferation and/or tumors) • Increased incidence, growth of pituitary tumors • Increased prostate weight	• Precocious vaginal opening • Persistent estrus • Increased time to mating • Reduced gestation duration • Increased uterine weights • Histopathological findings of the female reproductive organs (*e.g.,* vaginal cornification, uterine hypertrophy and hyperplasia) • Increased mammary tumors (adenomas and/or adenocarcinomas) • Increased incidence, growth of pituitary tumors • Ovarian, uterine and vaginal tumors in female offspring
Anti-estrogenicity	• Decreased height of epithelium in testicular tubules • Testicular weight increases (short term) • Testicular atrophy (long term) • Infertility and testicular atrophy (moderate term)	• Delayed vaginal opening • Delayed start of estrous cycling and irregular or absent estrous cyclicity • Reduced fertility • Decreased corpora lutea, implantations • Decreased female reproductive organ weights • Decreased mammary tumor incidence • Decreased incidence of estrogen-responsive pituitary tumors
Androgenicity	• Increased or decreased male reproductive organ weights • Reduced sperm counts • Testicular atrophy	• Increased anogenital distance • Accelerated vaginal opening • Reduced fertility • Altered differential follicle count • Histopathological findings of the female reproductive organs (*e.g.,* vaginal agenesis) • Induced male sex accessory tissues

Table 6 (continued). Indicators of Potential EAT Endocrine Disruption from Mammalian Studies

Anti-androgenicity	• Delayed preputial separation • Reduced anogenital distance • Ectopic testes • Hypospadias/epispadias • Reduced fertility • Reduced reproductive organ weight (particularly prostate, seminal vesicles) • Retained nipples/areolas • Histopathological findings of the *reproductive organs (e.g.,* epididymal agenesis, testicular tumors)	• Altered pup sex ratios between external and internal sexing
Reduced steroid biosynthesis	• Similar to anti-androgenicity • Possible increased serum cholesterol levels • Increased Leydig cell tumors	• Similar to anti-estrogenicity/ aromatase inhibition
Aromatase inhibition	• Increased time to mating • Decreased male mounting behavior • Decreased body weight (chronic) • Increased testis weight (chronic)	• Increased body weight • Decreased uterine weight • Increased ovary size • Polycystic ovaries • Stromal hyperplasia in ovary (chronic) • Hyalinization in ovary (chronic) • Increased ureter and bladder infection • Decreased mammary tumor incidence (SD)
Thyroid hormone modulation	• Decreased T_3 and/or T_4 levels, increased TSH levels • Increased thyroid weights • Decreased colloid content and thyroid follicle size • Follicular cell hypertrophy • Thyroid follicular cell hyperplasia • Thyroid follicular cell tumors	

Further information may be obtained from ecotoxicological assessments of fish (particularly if a full life-cycle study is available). Endocrine modulation in fish will vary depending on the species tested, but for Medaka sensitive endpoints could include changes in vitellogenin concentrations, with biologically significant increases in males signifying estrogenicity, or decreased fertility or fecundity, or alterations in secondary sex characteristics. Avian reproductive studies provide somewhat less useful information, although major changes in egg-laying or hatching success in the absence of marked systemic toxicity may predict endocrine modulating effects. Egg-shell thinning has been used in the past as a marker for

endocrine disruption; however this parameter may also be affected by non-specific influences such as poor nutrition.

B. Analysis of Pyriproxyfen Data for Potential Estrogen or Androgen Modulation

As seen in Table 6, modulation of either estrogen or androgen function will often affect many of the same endpoints or parameters; however, the direction of the change will often depend on which hormone system is affected, whether the chemical mimics or blocks the hormone's function, and whether male or female animals are being evaluated. Endpoints that provide information regarding potential estrogen or androgen modulation of pyriproxyfen include reproductive organ weights, reproductive tissue histopathology, gross findings in reproductive organs, mammary gland, adrenal and pituitary (including increases or decreases in hormonally sensitive tumors), parameters related to reproductive function (*e.g.,* estrous cycle length, fertility), parameters related to reproductive development (*e.g.,* day and body weight at time of vaginal opening and testicular descent), and endpoints related to pregnancy and *in utero* development (*e.g.,* gestational length, number of corpora lutea and implantations, and fetal and pup sex ratio). Reproductive tract malformations in F1 fetuses or pups may also provide indications of endocrine disruption.

Of the extensive parameters measured in the body of pyriproxyfen studies conducted for pesticide registration purposes, only limited findings related to possible modulation of estrogen and/or androgen function were noted.

- Reproductive organ weights: testes weight changes following high dose exposure ≥5000 ppm in diet or 300 mg/kg/day by gavage; no correlating histopathological findings; attributable to marked body weight deficits
- Reproductive tissue histopathology: no exposure related (neoplasms or non-neoplasms)
- Endpoints related to reproductive function:
    - decreased pup weight and increased mortality at high maternally toxic doses (pup mortality only when dam was directly administered pyriproxyfen at high doses via gavage)
    - no evidence of prolonged or persistent estrus in estrous cyclicity data
    - no effect on time to mating, mating, fertility indices
- Endpoints related to reproductive development:
    - Non-dose-related accelerated testicular descent in one study following gestational exposure (attributable to control being markedly "delayed" compared to what would normally be expected)
    - Slight delayed testicular descent in another perinatal study, not dose-related
    - Slight non-dose related delayed vaginal opening in the latter perinatal study, associated with body weight deficit
- Endpoints related to pregnancy and *in utero* development:
    - decreased corpora lutea at high maternally toxic dose 1000 mg/kg/day

- decreased dams with live fetuses at high maternally toxic dose 1000 mg/kg/day (rabbits)

There is no pattern of effects in these studies indicating either estrogenicity, anti-estrogenicity, androgenicity or anti-androgenicity as a potential mechanism of action. Findings in pups were at maternally toxic doses. It is questionable whether these changes may be endocrine mediated, but are unlikely to involve the estrogen, androgen or thyroid mechanisms screened for in the Tier 1 assays.

All other parameters related to estrogen and androgen hormone function—including estrous cyclicity, reproductive organ histopathology, estrogen-responsive mammary gland or pituitary tumor incidence, anti-androgen or estrogen responsive testicular Leydig cell tumors, time to mating, and gestational length showed no effects. In conclusion, review of the reproductive and developmental toxicity and subchronic and chronic toxicity and/or oncogenicity data for pyriproxyfen cited as OSRI do not show any consistent patterns of effects suggesting a possible interaction with the estrogen or androgen receptor, either agonistic or antagonistic. The potential anti-androgenicity or androgenicity of pyriproxyfen will also be further evaluated in a Hershberger assay, which The Pyriproxyfen Task Force intends to conduct.

A Medaka life cycle study showed no dose-related pattern of effects indicating endocrine modulation. A slight dose related increase in male vitellogenin was seen in F0 males at 60 dph, but was not replicated at 114 dph, or in the F1 males at 60 dph. It is considered related to a low control value. A decrease in fertility was seen in one of three consecutive days at which this parameter was evaluated; this is considered an artifact. Other statistically significant findings were not dose related, and also were generally not replicable within the assay.

Table 7 shows the historical hepatic vitellogenin data in the laboratory. These data confirm that the F0 male control value in the pyriproxyfen study is at the lower end of the range.

Table 7. Historical Control Data for Hepatic Vitellogenin[1]

Study	Sex	F0		F1
		ca. 60 dph	Ca. 110-120 dph	ca. 60 dph
Study 1 (May-Nov., 2005)	M	4.5-13.1	1.6-1.9	2.0-2.6
MRID 46793501	F	1676-2145	757-1241	637-1270
Study 2 (Apr.-Oct., 2006)	M	0.17-0.27	0.21-0.41	0.28-0.57
MRID 48066202	F	907-1202	767-1310	482-595

[1] **ng/mg; control range (dilution water and solvent)**

Avian reproductive toxicity studies showed no effects of pyriproxyfen on parameters that could be related to endocrine modulation. No amphibian studies providing a reliable evaluation of the potential for endocrine effects were identified in the regulatory data base or in the published literature.

Review of *in vitro* data also fails to show potential binding to estrogen receptors, and ER transactivation assay results were mixed. The US EPA ToxCast™ assays cited as OSRI reviewed in Appendix V showed no evidence of binding to ERα or ERβ in a cell free system or of interacting with ERα in a cell based system, with the exception of a weak estrogenic response in the Multiplex assay. It was concluded that the concentration required *in vitro* to elicit this response could not reasonably be achieved *in vivo*, so the response was considered not relevant. Three ER transactivation assays in the published literature showed similar weak evidence of estrogen receptor transactivation at high concentrations not considered relevant to *in vivo* exposure. The lack of relevance of these findings was confirmed by the negative uterotrophic assay of pyriproxyfen, which showed no indication of estrogenic activity at the highest concentration tested (limit dose of 1000 mg/kg/day), which produced other indications of toxicity, as well as by the lack of other indications of estrogenic potential in mammalian and aquatic *in vivo* studies. No published studies were identified that assessed androgen receptor binding or transactivation; both, however, were assessed in the ToxCast™ assays and were negative. The ToxCast™ battery also included an assay of aromatase, which was negative; The Pyriproxyfen Task Force intends to repeat this assay and also to conduct a steroidogenesis assay.

C. Analysis of Pyriproxyfen Data for Potential Thyroid Hormone Modulation

A number of parameters related to thyroid function were measured in the pyriproxyfen studies conducted for pesticide registration purposes, including thyroid weight and thyroid histopathology in multiple studies. No findings suggest that pyriproxyfen adversely affects thyroid hormones based on the lack of evidence of follicular cell hypertrophy, hyperplasia or neoplasia in any of the studies. Increased thyroid weight was noted in the chronic dog study in females; this was attributed to a low control thyroid weight and there was no histopathological correlate.

The ToxCast™ *in vitro* assay testing binding to the human thyroid hormone receptor was negative. As a whole, the body of studies indicates that pyriproxyfen exposure is not associated with an alteration in thyroid hormone function. Potential for thyroid effects will be further evaluated in the amphibian metamorphosis assay, which The Pyriproxyfen Task Force is planning to conduct, also providing the redundancy appropriate to a screening battery.

D. Modulation of Other Endocrine Systems

The following parameters related to adrenal function were measured in the pyriproxyfen studies conducted for pesticide registration purposes. These include adrenal weights and histopathology in multiple studies.

The only findings to suggest a possible effect of pyriproxyfen treatment on adrenal function were adrenal weight increases attributed to stress, including decreased body weight, at doses ≥ 5000 ppm or ≥ 100 mg/kg/day (gavage), without histopathological correlates. All other adrenal evaluations were negative. As a whole, the body of studies indicates that pyriproxyfen exposure is not associated with an alteration in adrenal function relevant to EAT evaluation. Furthermore, these studies provide sufficient information for hazard and risk assessment and indicate that additional screening to address potential adrenal modulation should not be required for pyriproxyfen.

V. Conclusions

Pyriproxyfen has a high quality current toxicological data base including a uterotrophic assay, a two-generation reproductive toxicity study and three other mammalian reproductive developmental or peri-natal studies, and in a full set of sub-chronic and chronic toxicity or chronic toxicity/oncogenicity studies in three species, which provide functionally equivalent data to the Tier 1 assays evaluating androgenicity, anti-androgenicity, estrogenicity and anti-estrogenicity. The Pyriproxyfen Task Force plans to evaluate aromatase inhibition and steroidogenesis and to conduct a Hershberger assay to supplement this data base and to provide the redundancy desired in a testing screen.

Data developed by ToxCast™ under the auspices of EPA show no effects on endocrine parameters in assays similar to those required under Tier 1 screening for estrogen receptor binding or transactivation or for androgen receptor binding. An additional Multiplex assay showed weak evidence of estrogen receptor transactivation; there were similar findings in three published studies; the concentration range at which effects were seen, however, were not considered relevant to *in vivo* exposure. As a whole, there is very little evidence in the mammalian toxicology studies to suggest that pyriproxyfen is a potential endocrine modulator. Additionally, a fish life cycle study is available for pyriproxyfen and shows no evidence of exposure-related endocrine modulation from this compound up to the highest concentration tested. Further, data from avian reproduction studies do not provide any evidence of pyriproxyfen toxicity to the endocrine system.

Thyroid evaluations have been conducted in several studies, and the lack of thyroid follicular cell changes is considered evidence that biologically significant changes in thyroid hormone levels have not occurred.

In contrast, the available ecotoxicology studies are not considered adequate to address the possibility of endocrine modulation from pyriproxyfen exposures to frogs. Therefore, The Pyriproxyfen Task Force is planning to conduct an Amphibian Metamorphosis assay as specified in the Tier 1 Guideline (OPPTS 890.1100). This will also supplement the evaluation of potential thyroid modulation by pyriproxyfen.

Pyriproxyfen is an insect growth regulator and modifies juvenile hormone in insects. Predictably it is also toxic to aquatic invertebrates; potential toxicity to Mysid shrimp and *Daphnia* is well characterized and suitable for risk assessment. However, this information is not considered relevant to assessment of endocrine toxicity in vertebrate species.

The Pyriproxyfen Task Force believes that sufficient data for identification of potential endocrine effects in mammals and fish are available from the studies cited as OSRI, particularly from the extensive regulatory mammalian toxicology data base developed for supporting the registration of pyriproxyfen and from the Medaka life-cycle study, in conjunction with the Tier 1 studies that the Task Force has committed to conduct. We believe these data provide information which may be used in lieu of a full Tier 1 screening battery, to conserve animal usage, use existing information appropriately, and still adequately identify any potential relevant endocrine modulating effects of pyriproxyfen.

The following table (Table 8) summarizes the assays that The Pyriproxyfen Task Force will conduct and those assays for which we are requesting waivers.

Table 8. Tier 1 Test Battery and Requested EPA Action Based on OSRI Weight of the Evidence

Endocrine Disruptor Screening Program Tier 1 Test Guideline	Requested Action
OPPTS 890.1250: Estrogen Receptor Binding Assay Using Rat Uterine Cytosol (ER-RUC). EPA 2009a	WAIVER requested based on Functional Equivalence of OSRI
OPPTS 890.1300: Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa-9903)). EPA 2009b	WAIVER requested based on Functional Equivalence of OSRI
OPPTS 890.1150: Androgen Receptor Binding (Rat Prostate Cytosol). EPA 2009c	WAIVER requested based on Functional Equivalence of OSRI
OPPTS 890.1200: Aromatase (Human Recombinant). EPA 2009d	The Pyriproxyfen Task Force will conduct this study.
OPPTS 890.1550: Steroidogenesis (Human Cell Line –H295R). EPA 2009e	The Pyriproxyfen Task Force will conduct this study.
OPPTS 890.1600: Uterotrophic Assay. EPA 2009f	WAIVER requested based on Uterotrophic assay in existing data set and Functional Equivalence of OSRI.
OPPTS 890.1450: Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Female Rats. EPA 2009g	WAIVER requested based on Functional Equivalence of OSRI
OPPTS 890.1400: Hershberger Bioassay.EPA 2009h	The Pyriproxyfen Task Force will conduct this study.
OPPTS 890.1500: Pubertal Development and Thyroid Function in Intact Juvenile/ Peripubertal Male Rats. EPA 2009i	WAIVER requested based on Functional Equivalence of OSRI
OPPTS 890.1100: Amphibian Metamorphosis (Frog) EPA 2009j	The Pyriproxyfen Task Force will conduct this study.
OPPTS 890.1350: Fish Short-Term Reproduction Assay EPA 2009k	WAIVER requested based on Functional Equivalence of OSRI.

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Appendix I. Regulatory Studies and Published Studies Cited as OSRI

Table I-1: Previously Submitted Regulatory Toxicological or Ecotoxicological Studies of Pyriproxyfen Cited as OSRI

Study Type	Reference	MRID	EPA Classification
Reproductive/Developmental Toxicity			
Two-generation reproductive toxicity study	Robinson, 1991	42178313	Acceptable, Core Minimum
Uterotrophic assay rat	Ose, 2005	48066201	Not yet reviewed
Rat developmental study with post-natal component	Saegusa et al., 1988a	41321719; 42178312	Acceptable
Rabbit developmental toxicity study and addendum	Hirohashi, 1988; Hirohashi, 1994	41321720; 42178311; 43215401; 43215402	Acceptable
Perinatal and postnatal study	Saegusa et al., 1988b	44985001	Acceptable, non-guideline
Exposure to rats prior to and in the early stage of pregnancy	Saegusa et al., 1988c	44985002	Acceptable, non-guideline
Sub-chronic Toxicity			
Subchronic toxicity study rats	Cox, 1989	41321716	Acceptable
Subchronic toxicity study mice	Cox, 1990	43210504	Acceptable
Subchronic toxicity study dog	Yamada, 1988	41321717; 42178307	Acceptable
21-day dermal toxicity study in rats	Moore, 1993	43004101	Acceptable
Chronic Toxicity			
Chronic toxicity and oncogenicity study in rats	Osheroff, 1991a	42178314; 43210501; 43210502; 43210503	Acceptable, Core minimum

Oncogenicity study in mice	Osheroff, 1991b	42178310; 43413202; 43210501; 43413201	Acceptable, Core minimum
52 week toxicity study beagle dogs	Chapman, 1991	42178309	Acceptable
Ecotoxicology Studies			
Pyriproxyfen: Full life cycle toxicity test with medaka	Gries, 2007	48066202	Not yet reviewed
Early life-stage toxicity study rainbow trout	Rhodes and Cramer, 1991	42178319	Scientifically sound, non-guideline
21-day Flow through toxicity of pyriproxyfen to rainbow trout	Sword and Northrup, 1992	48066203	Not yet reviewed
Reproduction study with Mallard duck	Beavers et al. 1994a	44036908	Acceptable
Reproduction study with Bobwhite quail	Beavers et al. 1994b	44036906	Acceptable

Table I-2. Literature Studies Cited as OSRI.

Authors	Title	Journal	Year
Manabe, M., Kanda, S., Fukunaga, K., Tsubura, A., and Nishiyama, T.	Evaluation of the estrogenic activities of some pesticides and their combinations using MtT/Se cell proliferation assay	*Int. J. Hyg. Environ. Health.* 209:413-421	2006
Kojima, M., K. Fukunaga, M. Sasaki, M. Nakamura, M. Tsuji, and T. Nishiyama.	Evaluation of estrogenic activities of pesticides using an *in vitro* reporter gene assay	*Int. J. Environ. Health Res.* 15:271-280.	2005a
Kojima, M., M. Manabe, S. Kanda, K. Fukunaga, M. Nakamura, M. Tuji, and T. Nishiyama	Additive effects of estrogenic activity by the combination of E_2 and pesticides.	*Kansai Ika Daigaku Zasshi* (The Journal of Kansai Medical University) 57:165-170.	2005b

Appendix II. (Mammalian Matrices-for Hershberger's, Male Pubertal, Uterotrophic and Female Pubertal Assays)

Abbreviation/ Notation	Definition
—	No statistically significant findings
↑	Statistically significant increase
↓	Statistically significant decrease
♂	Male
♀	Female
abs	Absolute
adj	Adjusted for terminal body weight
F0	Parental generation
F1	1st generation offspring
GD	Gestation day
HDT	Highest dose tested
LD	Lactation Day
mkd	mg/kg/day
ND	Not determined
PND	postnatal day
rel	Relative
TWA	Time-weighted average
unk	Unknown
VC	Vehicle control

Color Key

HEADER: Key studies cited as OSRI

HEADER: Not cited as OSRI

Evaluated, **NO EFFECT:** no statistically significant difference

Evaluated, **NO EFFECT:** statistically significant difference but no dose response and/or no biological significance

Evaluated, Effect: **NOT Endocrine-related**

Evaluated, Effect: **Not likely Endocrine-related; related to systemic toxicity**

Evaluated, Effect: **Possible Endocrine-related**

Evaluated, Effect: **Clearly Endocrine-related**

Background
Background

NOT Evaluated

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Uterotrophic

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect	Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats	Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats	Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy	Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)
MRID Number	42178313	48066201	41321719 42178312	44985001	44985002	41321720 42178311 43215402 43215401
Study acceptability	Acceptable/ Core minimum	DER not available	Acceptable	Acceptable/ non-guideline	Acceptable/ non-guideline	Core minimum (upgraded with additional data)
Study report date	23-Sep-91	2-Aug-05	28-Mar-88	28-Mar-88	21-Apr-88	30-Aug-89
Study design	2-generation reproductive (83-4)	Uterotrophic assay in juvenile (20-day old) rats	GD7-17 exposure with developmental component (cesarean GD21); F1 evaluation PND21, PND56; F1 reproductive component (cesarean GD21)	GD17-PND20 perinatal and lactation exposure with postnatal component; F1 evaluated PND21, PND56; F1 reproductive component (cesarean GD21)	Premating and mating (♂♀ exposure) to GD7 (♀) (cesarean GD21)	Teratogenicity 83-3
Test material purity	95.3%	98.7%	97.2%	97.2%	97.2%	97.2%
Route of exposure	Dietary	Gavage (corn oil vehicle)	oral gavage	oral gavage	oral gavage	oral gavage
Exposure duration	70 day pre-breed♂♀, through mating gestation, lactation of F0 dams through prebreed exposure, mating, gestation, lactation of F1 dams; F2 pups terminated LD21	3 day	GD7-17	GD17-PND20	♂ F0 9 weeks premating and 3 weeks mating; ♀ 2 weeks premating, mating and gestation until GD7	GD6-18
Animal species/strain	Crl:CD(SD) Rat	Crl:CD(SD) Rat	Slc:SD rats (SPF)	Slc:SD rats (SPF)	Slc:SD rats (SPF)	JW-NIBS Rabbit
No. animals per sex per group	26/sex	6 ♀	F0 20-23 dams (cesarean); F0 10-13 dams (delivered); F1 PND21: 9-12/sex; F1 PND56: 10-13/sex; F1 10-13/sex mated (cesarean)	F0 23-24 dams (delivered); F1 PND21: 13-22/sex; F1 PND56: 13-23/sex; F1 14-23/sex mated (cesarean)	24/sex	15-18 dams copulated; 12-14 dams used for anlaysis
Dose levels (ppm, unless otherwise noted)	0, 200, 1000, 5000	NA				

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Uterotrophic

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	**Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect**	**Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats**	**Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats**	**Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy**	**Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)**
Dose (mg/kg/day)	F0 ♂prebreed 11.6-23.1, 59.8-112.7, 288.5-549.5; F0♀ prebreed-gestation 11.4-23.3, 59.8-115.1, 307.2-556.7; F1♂ prebreed 11.7-36.6, 58.0-178.2, 306-956.9, F1♀ prebreed-gestation 10.5-37.3, 52.9-184.1, 281.2-992.2 for 200, 1000, 5000 ppm, respectively	0, 250, 500, and 1000 mkd pyriproxyfen; 0.001 mkd 17α-ethynyl estradiol (EE) as positive control)	0 (corn oil), 100, 300, 1000	0 (corn oil), 30, 100, 300, 500	0 (corn oil), 100, 300, 500, 1000	0 (corn oil), 100, 300, 1000
NOAEL / NOEL / Effect	NOAEL 5000 ppm (reproductive) NOAEL 1000 ppm (pups) NOAEL 200 ppm (parental)	NOAEL ↑ 1000 mkd (uterine wt) NOAEL 500 mkd (bw) NOEL 100 mkd (liver wt)	maternal NOEL 100mkd, LOEL 300 mkd; fetus NOEL 100mkd, LOEL 300 mkd; pup NOEL 1000 mkd (EPA pup NOEL 300 mkd)	Maternal NOAEL 100 mkd (based on clincial, ↓bwg, organ weights at 300 mkd); Devopmental NOAEL 100 mkd based on ↓bw and ↑fetal dilation of renal pelvis	Parental NOAEL 100 mkd based on clincial, ↓bw, organ weights at 300 mkd); Devopmental NOAEL 1000 mkd based on ↓BW & renal toxicity	maternal NOEL 100 mkd, LOEL 300 mkd; fetal NOEL 300, LOEL 1000 mkd

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Uterotrophic

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect	Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats	Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats	Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy	Hirohashi (1988) Sumilarv - study of S-31183 by oral admnsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)
Endpoint Correlates to Tier 1 Screening Assay						
Uterus (wet) wt.		abs rel —; abs rel ↑ for positive control				
Uterus (blotted) wt.		abs rel —; abs rel ↑ for positive control				
Body weight (female)	F0↓ F1↓ (both at 5000 ppm)	bw bwg ↓ at 1000 mkd	ND/—	F1↓PND 28-42 at ≥ 300 mkd then recovery	bw bwg↓at ≥ 300 mkd (through premating and mating)	
Other Relevant Endpoints						
Mammary gland histopath						
Uterus histopath	F0 ♀ —; F1 ♀ —					
Vagina histopath	F0 ♀ —; F1 ♀ —					
Cervix histopath						
Estrous cyclicity (age, length, % animals)	F0 ♀ —; F1 ♀ —					
Normal external genitalia (pups)	No exposure-related abnormalities noted (10/sex/dose weanlings necropsied)					
Fetal/pup reproductive tract anomolies	None noted		—/—	"no differencences... In sexual development at PND21, 56 or post reproduction"		—
Ovaries (paired) wt.			F0(PND21) — F1(PND21) — F1(PND56) —	F0 —; F1(PND21) ↓abs at 30, 300, 500 (not 100) mkd, rel at 30 and 100 mkd only; F1(PND56) —		
Uterus (gravid, w/ or w/out placenta) wt.						
Vagina wt.						
Pituitary histopath						
Ovary histopath	F0 ♀—; F1 ♀—					

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Uterotrophic

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect	Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats	Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats	Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy	Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)
Oviducts histopath						
Time to mate (pre-coital interval)	F0 —; F1 —					
Gestation duration	F0 ♀ —; F1 ♀ —		—/ND	F0 ↓ at 300 mkd, no dose response		
Female reproductive indices: (mating, conception, fertility, gestation)	F0 ♀ —; F1 ♀ —		—/—	F0 birth and delivery rate —, ↑ # stillbirths F1 mating, copulation, conception, fertility rate —	F0 mating, copulation, conception, fertility rate —	↓ number dams with live fetuses at 1000 mkd (due to excessive maternal toxicity)
# Corpora lutea			—/—	F0 —; F1 ↓ at 30mkd , no dose response	↓ # corpora lutea/ dam at 1000 mkd	—
# Implantation sites	F0 —; F1 —		—/—	F0 —; F1 ↓ at 30 and 500 mkd, no dose response	—	—
# Fetuses			—/—	F1 — ↓ fetuses/dam at 30 and 500 mkd, no dose resonse)	↓ # live fetuses/ dam at 100 and 1000 mkd (no dose response)	—
Dystocia	None noted					
Fetal/Pup sex ratio	F0 —; F1 —		—/—	F1 —	—	—
Ovarian eval for follicles (qual or quant)						
Lactation/nursing (behavior or indices)						
No. pups at birth	F0 —; F1—		—/ND	F1 at 500 mkd: ↑ stillborn index, ↓mean litter size, ↓ # live pups, ↑ # deaths (data examined by DER); F2 ↓ # live pups at 500 mkd		
Pup survival (early vs. late)	F0 —; F1 —		—/ND	F1 ↓♀ survival rate at 500 mkd		
No. pups "mis-sexed" @ birth vs. @ necropsy	None noted					
Anogenital distance						
Age and weight at vaginal opening			—/ND	delayed vaginal separation at 500 mkd but minor (growth retardation)		

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Uterotrophic

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect	Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats	Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats	Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy	Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)
Nipple retention (necropsy or quant)	None noted (10/sex/dose weanlings necropsied)					
Fetal weight			—/—	F1 ♂♀ pups ↓bw at ≥ 300 mkd; F2 —	♂ ↑ at 100, 500, and 1000 mkd (no dose dependence); ♀ ↑ ≥ 100 mkd	—
Luteinizing hormone, serum						
Follicular stimulating hormone, serum						
Pituitary wt.	F1 —					
Liver wt.	F1 ♀ abs rel ↑at 5000 ppm, F1 ♂abs ↑ at 5000 ppm, rel ↑ at 1000 and 5000 ppm	abs ↑at 1000 mkd rel ↑at 500, 1000 mkd	F0(GD21) abs— rel↑ 300 and 1000 mkd' F0(PND21) — / F1(PND21) — / F1(PND56) —	F0 abs rel ↑ at ≥300 mkd F1(PND21) ♂↓ abs rel at ≥300 mkd, rel at 300 mkd only, ♀↓abs at ≥ 300 mkd rel at 300 mkd only; F1(PND56) ♂↓ abs at 300 mkd, ♀↓rel at 500 mkd only	♂↑ abs rel at ≥ 300 mkd, rel at ≥100 mkd also; ♀ abs rel —	
Hypothalamus / brain histopath						
Liver histopath	F0 ND ; F1 ♂↑ clear cells at 5000 ppm (not considered treatment related), F1 ♀—					
Gross lesions	F0 ♂ —; F0 ♀ — F1 ♂ —; F1 ♀ —	Enlarged kidney at 1000 mkd	F0(GD21) enlarged adrenal at 1000 mkd	F0 liver congestion, liver enlargement, spleen atrophy, adrenal enlargement, thymus atrophy, stomach hemorrhage at 500 mkd	♂enlarged and dark red liver at ≥300 mkd; enlargement kidney at ≥300 mkd; pitted surface of kidney at ≥300 mkd; enlarged adrenal glands at ≥300 mkd	—

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Uterotrophic

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	**Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect**	**Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats**	**Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats**	**Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy**	**Hirohashi (1988) Sumilarv - study of S-31183 by oral admnsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)**
Gestation bw, bw gain	bw F0↓ F1↓ (both at 5000 ppm)		bw↓ 300 and 1000; bwg↓ 100, 300 and 1000 mkd /—	F0 bw ↓ at 500 mkd GD20-22; bwg↓ at ≥300 mkd on GD19, 20, 21 and 22 (500 mkd only)	♀ bw ↓at ≥ 500 mkd, bw ↓ at 100 and 300 with recovery by GD21; ♀ bwg ↑at ≥500 mkd with recovery by GD5, ↓ at 100 mkd only at GD7-8 (no dose dependence)	bw bwg ↓ at ≥300 mkd (statistically significant at 1000 mkd)
Lactation bw, bw gain	bw F0↓ F1↓ (both at 5000 ppm) during early lactation		bw↓300 and 1000 mkd ;bwg ↓ 1000 mkd	F0 bw —, bwg ↑at 500 mkd on PND4-21		
Kidney (paired) wt.	F1 ♂ abs —, rel ↑ 1000 and 5000 ppm; F1 ♀abs rel —	abs — rel ↑ at 1000 mkd	F0(GD21) abs↑ 1000 mkd, rel ↑ 300 and 1000 mkd; F0(PND21) —/F1(PND21) —, F1(PND56) ♂ — ♀ ↑ rel 1000 mkd	F0 —; F1(PND21) ♂↓ abs at ≥ 300 mkd, rel —, ♀↓abs at ≥ 300 mkd, rel at 500 mkd; F1(PND56) ↓abs at ≥ 300,↑ rel at 100 mkd	♂↑ abs at 300 and 1000 mkd only, rel at ≥ 100 mkd; ♀ ↑ abs at 1000 mkd, rel at ≥ 100 mkd	
Kidney histopath	F0 ND ; F1 ♂ ↑interstitial nephritis at 5000 ppm; F1 ♀—				♂ ↑ regenerative changes and dilation of tubules with accumulation of neutrophils at ≥300 mkd (in text not tables, DER could not confirm)	

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Uterotrophic

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	Cox (1990) Sumilarv - subchronic study in mice	Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs	Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats	Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]
MRID Number	41321716	43210504	41321717 42178307	41321718 42178308	43004101
Study acceptability	Acceptable	Acceptable	Acceptable	Supplemental	Acceptable
Study report date	8-Mar-89	23-Jan-90	6-May-88	14-Apr-88	11-Jan-93
Study design	Subchronic diet 82-1(a)	Subchronic diet 82-1(a)	Subchronic oral (capusle)	28-day inhalation 82-4	21-day dermal toxicity (82-2)
Test material purity	95.3%	95.3%	97.2%	97.0%	97.2%
Route of exposure	diet	diet	capsule	inhalation	dermal
Exposure duration	13 weeks	13 weeks	13 weeks	28 days 4 hours/day	21 day 6 hours/day
Animal species/strain	Crl:CD BR Rat	Crl:CD-1(ICR)BR Mouse	Beagle dogs	Sprague-Dawley Rat	Sprague Dawley CD Rat
No. animals per sex per group	10/sex	10/sex	4/sex	10/sex	5/sex
Dose levels (ppm, unless otherwise noted)	0, 400, 2000, 5000, 10000	0, 200, 1000, 5000, 10000	NA	0, 269, 482, 1000 mg/m3	NA

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Uterotrophic

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	Cox (1990) Sumilarv - subchronic study in mice	Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs	Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats	Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]
Dose (mg/kg/day)	Mean intake: ♂ 23.49, 117.79, 309.05, 641.81 mg/kg/day ♀ 27.68, 141.28, 356.30, 783.96 mg/kg/day	Mean intake: ♂ 28.2, 149.4, 838.1, or 2034.5 mg/kg/day ♀ 37.9, 196.5, 963.9, 2345.3 mg/kg/day	0, 100, 300, 1000		0, 100, 300, 1000
NOAEL / NOEL / Effect	NOAEL 400 ppm	NOAEL 200 ppm	NOEL 100 mkd based on changes in liver for detoxification	NOEL 482 mg/m3	1000 mkd (100 mkd for skin irritation)

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Uterotrophic

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	Cox (1990) Sumilarv - subchronic study in mice	Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs	Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats	Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]
Endpoint Correlates to Tier 1 Screening Assay					
Uterus (wet) wt.			–		
Uterus (blotted) wt.					
Body weight (female)	↓ at 5000 and 10000 ppm (significant trend)	–	–	–	–
Other Relevant Endpoints					
Mammary gland histopath	–	–	–	–	
Uterus histopath	–	–	–	–	
Vagina histopath			–	–	
Cervix histopath					
Estrous cyclicity (age, length, % animals)					
Normal external genitalia (pups)					
Fetal/pup reproductive tract anomolies					
Ovaries (paired) wt.			–	–	
Uterus (gravid, w/ or w/out placenta) wt.					
Vagina wt.					
Pituitary histopath	–	♂♀+ congestion at 10000 ppm	–	–	
Ovary histopath	–	–	–	–	

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Uterotrophic

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	Cox (1990) Sumilarv - subchronic study in mice	Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs	Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats	Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]
Oviducts histopath					
Time to mate (pre-coital interval)					
Gestation duration					
Female reproductive indices: (mating, conception, fertility, gestation)					
# Corpora lutea					
# Implantation sites					
# Fetuses					
Dystocia					
Fetal/Pup sex ratio					
Ovarian eval for follicles (qual or quant)					
Lactation/nursing (behavior or indices)					
No. pups at birth					
Pup survival (early vs. late)					
No. pups "mis-sexed" @ birth vs. @ necropsy					
Anogenital distance					
Age and weight at vaginal opening					

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Uterotrophic

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	Cox (1990) Sumilarv - subchronic study in mice	Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs	Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats	Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]
Nipple retention (necropsy or quant)					
Fetal weight					
Luteinizing hormone, serum					
Follicular stimulating hormone, serum					
Pituitary wt.			—	—	
Liver wt.	abs rel ♂♀ ↑at 5000 and 10000 ppm; rel ♂↑ at 2000 ppm	abs ♂— ♀↑ at 5000 ppm (not enough animals at 10000 ppm for statistical analysis), significant increase trend; rel ♂♀ ↑at 5000 and 10000(♂only) ♂♀significant increase trend	abs ♂↑at 300 and 1000 mkd, rel —; ♀—	abs ♂♀—; rel ♂↑ at 1000 mg/m3, ♀—	—
Hypothalamus / brain histopath	ND/— (brain	ND /— (brain)	ND /— (brain)	ND / — (brain)	
Liver histopath	♂♀↑ of cytoplasmic change at 2000, 5000, 10000 ppm	♂♀↑congestion at 5000 and 10000 ppm (♂ only) for unscheduled deaths	♂♀↑hepatocellular enlargement at 300 (♀ only) and 1000 mkd; ♂♀↑ of smooth endoplasmic reticulum at 1000 mkd	—	—
Gross lesions	—	♀↓ovarian cysts (not dose dependent); kidney ♂♀↑dilated pelvis and ↑cysts and pale tissue	—	♂♀ liver slightly large	—

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Uterotrophic

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	Cox (1990) Sumilarv - subchronic study in mice	Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs	Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats	Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]
Gestation bw, bw gain					
Lactation bw, bw gain					
Kidney (paired) wt.	abs ♂♀— rel ♂♀↑ at 5000 (♂only) and 10000 ppm	—	—	—	—
Kidney histopath	—	♂♀↑microcysts/dilated tubules, pelvis dilation, tubular nephrosis, tubule mineralization at 5000 and 10000 ppm	—	—	—

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Uterotrophic

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)	Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks
MRID Number	42178314 43210501 43210503	42178310 43413202 43210501 43413201	42178309
Study acceptability	Acceptable/Core minimum (upgraded)	Acceptable/Core minimum	Acceptable
Study report date	6-Sep-91	23-Jul-91	1-Aug-91
Study design	Chronic/ carcinogenicty	Carcinogenicity	Chronic non-rodent
Test material purity	95.3%	95.3%	94.9%
Route of exposure	Diet	Diet	Capsule undiluted
Exposure duration	104 weeks	78 weeks	52 weeks
Animal species/strain	Crl:CD BR Rat	Crl: CD-1 (ICR) BR mouse	Beagle Dogs
No. animals per sex per group	50/sex (104 weeks) 30/sex (52 weeks)	50/sex (78 wks) 10/sex (52 wks)	4/sex
Dose levels (ppm, unless otherwise noted)	0, 120, 600, 3000	0, 120, 600, 3000	

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Uterotrophic

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	**Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)**	**Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks**
Dose (mg/kg/day)	♂: 0, 5, 27, 138 mkd ♀: 0, 7, 35, 182 mkd	♂: 0, 17, 84, 420 mkd ♀: 0, 22, 110, 547 mkd	0, 30, 100, 300, 1000 mkd
NOAEL / NOEL / Effect	NOEL ♀ 7 mkd (bw ↓, bwg ↓, blood chemistry); NOEL ♂ 27 mkd (bw ↓, bwg ↓, blood chemistry) (EPA concluded NOEL 600ppm)	NOEL 3000 ppm HDT for cancer effects; NOEL 120 ppm for non-cancer effects (EPA concluded non-cancer NOEL 600ppm)	NOAEL 30 mkd LOEL 100 mkd ↑ liver wt (slight ↑ kidney wt)

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Uterotrophic

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)	Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks
Endpoint Correlates to Tier 1 Screening Assay			
Uterus (wet) wt.			— (with cervix)
Uterus (blotted) wt.			
Body weight (female)	bw ↓, bwg ↓at 3000 ppm bwg ↓at 600 ppm	bw ↓ at 3000 ppm	bwg ↓ ≥ 300 mkd
Other Relevant Endpoints			
Mammary gland histopath	—	—	—
Uterus histopath	—	—	— (with cervix)
Vagina histopath	—	—	—
Cervix histopath	—	—	
Estrous cyclicity (age, length, % animals)			
Normal external genitalia (pups)			
Fetal/pup reproductive tract anomolies			
Ovaries (paired) wt.	—	—	—
Uterus (gravid, w/ or w/out placenta) wt.			
Vagina wt.			
Pituitary histopath	—	—	—
Ovary histopath	—	amyloidosis related finding not endocrine related	—

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Uterotrophic

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	**Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)**	**Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks**
Oviducts histopath			
Time to mate (pre-coital interval)			
Gestation duration			
Female reproductive indices: (mating, conception, fertility, gestation)			
# Corpora lutea			
# Implantation sites			
# Fetuses			
Dystocia			
Fetal/Pup sex ratio			
Ovarian eval for follicles (qual or quant)			
Lactation/nursing (behavior or indices)			
No. pups at birth			
Pup survival (early vs. late)			
No. pups "mis-sexed" @ birth vs. @ necropsy			
Anogenital distance			
Age and weight at vaginal opening			

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Uterotrophic

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	**Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)**	**Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks**
Nipple retention (necropsy or quant)			
Fetal weight			
Luteinizing hormone, serum			
Follicular stimulating hormone, serum			
Pituitary wt.			—
Liver wt.	abs ♂♀ — (52 wk), rel ♂— ♀rel ↑ at 3000 ppm (52 wk); abs rel ♂♀ — (104 wk)	abs rel ♂ — , abs rel ♀ ↑at 3000 ppm (52 wk); abs rel ♂♀ — (78 wk)	↑abs rel ♂♀ at ≥ 100 mkd
Hypothalamus / brain histopath	ND/— (brain)	ND/— (brain)	ND/— (brain)
Liver histopath	—	amyloidosis related finding not endocrine related	↑♂♀ centriacinar fibrosis and bile duct hyperplasia at 1000 mkd
Gross lesions	—	amyloidosis related finding not endocrine related	♂ livers appear large, ♂♀ liver irregular surface

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Uterotrophic

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	**Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)**	**Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks**
Gestation bw, bw gain			
Lactation bw, bw gain			
Kidney (paired) wt.	—	abs rel ♂♀— (52 wk), abs ♂↓ at 3000 ppm, rel—; abs rel ♀— (78 wk)	abs — ↑rel ♂300 mkd ♀ ≥ 300 mkd
Kidney histopath	—	♂♀↑ chronic progressive nephropathy at 3000 ppm; ♀↑renal tubular mineralization at 3000 ppm	—

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Female Purbertal

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect	Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats	Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats	Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy	Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)
MRID Number	42178313	48066201	41321719 42178312	44985001	44985002	41321720 42178311 43215402 43215401
Study acceptability	Acceptable/ Core minimum	DER not available	Acceptable	Acceptable/ non-guideline	Acceptable/ non-guideline	Core minimum (upgraded with additional data)
Study report date	23-Sep-91	2-Aug-05	28-Mar-88	28-Mar-88	21-Apr-88	30-Aug-89
Study design	2-generation reproductive (83-4)	Uterotrophic assay in juvenile (20-day old) rats	GD7-17 exposure with developmental component (cesarean GD21); F1 evaluation PND21, PND56; F1 reproductive component (cesarean GD21)	GD17-PND20 perinatal and lactation exposure with postnatal component; F1 evaluated PND21, PND56; F1 reproductive component (cesarean GD21)	Premating and mating (♂♀ exposure) to GD7 (♀) (cesarean GD21)	Teratogenicity 83-3
Test material purity	95.3%	98.7%	97.2%	97.2%	97.2%	97.2%
Route of exposure	Dietary	Gavage (corn oil vehicle)	oral gavage	oral gavage	oral gavage	oral gavage
Exposure duration	70 day pre-breed♂♀, through mating gestation, lactation of F0 dams through prebreed exposure, mating, gestation, lactation of F1 dams; F2 pups terminated LD21	3 day	GD7-17	GD17-PND20	♂ F0 9 weeks premating and 3 weeks mating; ♀ 2 weeks premating, mating and gestation until GD7	GD6-18
Animal species/strain	Crl:CD(SD) Rat	Crl:CD(SD) Rat	Slc:SD rats (SPF)	Slc:SD rats (SPF)	Slc:SD rats (SPF)	JW-NIBS Rabbit
No. animals per sex per group	26/sex	6 ♀	F0 20-23 dams (cesarean); F0 10-13 dams (delivered); F1 PND21: 9-12/sex; F1 PND56: 10-13/sex; F1 10-13/sex mated (cesarean)	F0 23-24 dams (delivered); F1 PND21: 13-22/sex; F1 PND56: 13-23/sex; F1 14-23/sex mated (cesarean)	24/sex	15-18 dams copulated; 12-14 dams used for anlaysis
Dose levels (ppm, unless otherwise noted)	0, 200, 1000, 5000	NA				

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Female Purbertal

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	**Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect**	**Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats**	**Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats**	**Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy**	**Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)**
Dose (mg/kg/day)	F0 ♂prebreed 11.6-23.1, 59.8-112.7, 288.5-549.5; F0♀ prebreed-gestation 11.4-23.3, 59.8-115.1, 307.2-556.7; F1♂ prebreed 11.7-36.6, 58.0-178.2, 306-956.9, F1♀ prebreed-gestation 10.5-37.3, 52.9-184.1, 281.2-992.2 for 200, 1000, 5000 ppm, respectively	0, 250, 500, and 1000 mkd pyriproxyfen; 0.001 mkd 17α-ethynyl estradiol (EE) as positive control)	0 (corn oil), 100, 300, 1000	0 (corn oil), 30, 100, 300, 500	0 (corn oil), 100, 300, 500, 1000	0 (corn oil), 100, 300, 1000
NOAEL / NOEL / Effect	NOAEL 5000 ppm (reproductive) NOAEL 1000 ppm (pups) NOAEL 200 ppm (parental)	NOAEL ↑ 1000 mkd (uterine wt) NOAEL 500 mkd (bw) NOEL 100 mkd (liver wt)	maternal NOEL 100mkd, LOEL 300 mkd; fetus NOEL 100mkd, LOEL 300 mkd; pup NOEL 1000 mkd (EPA pup NOEL 300 mkd)	Maternal NOAEL 100 mkd (based on clincial, ↓bwg, organ weights at 300 mkd); Devopmental NOAEL 100 mkd based on ↓bw and ↑fetal dilation of renal pelvis	Parental NOAEL 100 mkd based on clincial, ↓bw, organ weights at 300 mkd); Devopmental NOAEL 1000 mkd based on ↓BW & renal toxicity	maternal NOEL 100 mkd, LOEL 300 mkd; fetal NOEL 300, LOEL 1000 mkd

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Female Purbertal

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect	Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats	Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats	Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy	Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)
Endpoint Correlates to Tier 1 Screening Assay						
Thyroid wt. (after fixation)						
Uterus (blotted) wt.		abs rel —; abs rel ↑ for positive control				
Thyroid histopath (colloid area, FC ht.)						
Ovary histopath	F0 ♀—; F1 ♀—					
Uterus histopath	F0 ♀ —; F1 ♀ —					
Estrous cyclicity (age, length, % animals)	F0 ♀ —; F1 ♀ —					
Age and weight at vaginal opening			—/ND	delayed vaginal separation at 500 mkd but minor (growth retardation)		
Thyroid hormone levels (T_4, TSH)						
Adrenals (paired) wt.		abs rel —; abs — rel ↑ for positive control	F0(GD21) abs rel ↑ 1000 mkd/ F0(PND21) —/ND	F0 —; F1 ND	♂↑abs rel at ≥ 100 mkd; ♀↑ abs rel at 1000 mkd	
Ovaries (paired) wt.			F0(PND21) — F1(PND21) — F1(PND56) —	F0 —; F1(PND21) ↓abs at 30, 300, 500 (not 100) mkd, rel at 30 and 100 mkd only; F1(PND56) —		
Pituitary wt.	F1 —					
Liver wt.	F1 ♀ abs rel ↑at 5000 ppm, F1 ♂abs ↑ at 5000 ppm, rel ↑ at 1000 and 5000 ppm	abs ↑at 1000 mkd rel ↑at 500, 1000 mkd	F0(GD21) abs— rel↑ 300 and 1000 mkd' F0(PND21) — / F1(PND21) — / F1(PND56) —	F0 abs rel ↑ at ≥300 mkd F1(PND21) ♂↓ abs rel at ≥300 mkd, rel at 300 mkd only, ♀↓abs at ≥ 300 mkd rel at 300 mkd only; F1(PND56) ♂↓ abs at 300 mkd, ♀↓rel at 500 mkd only	♂↑ abs rel at ≥ 300 mkd, rel at ≥100 mkd also; ♀ abs rel —	
Body weight (female)	F0↓ F1↓ (both at 5000 ppm)	bw bwg ↓ at 1000 mkd	ND/—	F1↓PND 28-42 at ≥ 300 mkd then recovery	bw bwg↓at ≥ 300 mkd (through premating and mating)	

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Female Purbertal

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect	Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats	Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats	Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy	Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)
Standard blood chemistry (creatinine, BUN, and urea)						
Kidney (paired) wt.	F1 ♂ abs —, rel ↑ 1000 and 5000 ppm; F1 ♀abs rel —	abs — rel ↑ at 1000 mkd	F0(GD21) abs↑ 1000 mkd, rel ↑ 300 and 1000 mkd; F0(PND21) —/F1(PND21) —, F1(PND56) ♂ — ♀ ↑ rel 1000 mkd	F0 —; F1(PND21) ♂↓ abs at ≥ 300 mkd, rel —, ♀↓abs at ≥ 300 mkd, rel at 500 mkd; F1(PND56) ↓abs at ≥ 300,↑ rel at 100 mkd	♂↑ abs at 300 and 1000 mkd only, rel at ≥ 100 mkd; ♀ ↑ abs at 1000 mkd, rel at ≥ 100 mkd	
Kidney histopath	F0 ND ; F1 ♂ ↑interstitial nephritis at 5000 ppm; F1 ♀—				♂ ↑ regenerative changes and dilation of tubules with accumulation of neutrophils at ≥300 mkd (in text not tables, DER could not confirm)	
Other Relevant Endpoints						
Uterus (wet) wt.		abs rel —; abs rel ↑ for positive control				
Vagina wt.						
Adrenal histopath						
Mammary gland histopath						
Vagina histopath	F0 ♀ —; F1 ♀ —					
Cervix histopath						
Lactation/nursing (behavior or indices)						
Pup growth (to PND 21)	F0↓ at 5000 ppm (LD 14-21 ♀, LD 21 ♂); F1 ↓at 5000 ppm (PND 14-21)		—/ND	F1 ↓ in bw bwg at ≥ 300 mkd		
Anogenital distance						
Normal external genitalia (pups)	No exposure-related abnormalities noted (10/sex/dose weanlings necropsied)					

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Female Purbertal

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect	Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats	Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats	Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy	Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)
Fetal/pup reproductive tract anomolies	None noted		—/—	"no differencences... In sexual development at PND21, 56 or post reproduction"		—
Uterus (gravid, w/ or w/out placenta) wt.						
Oviducts histopath						
Gestation duration	F0 ♀ —; F1 ♀ —		—/ND	F0 ↓ at 300 mkd, no dose response		
Female reproductive indices: (mating, conception, fertility, gestation)	F0 ♀ —; F1 ♀ —		—/—	F0 birth and delivery rate —, ↑ # stillbirths F1 mating, copulation, conception, fertility rate —	F0 mating, copulation, conception, fertility rate —	↓ number dams with live fetuses at 1000 mkd (due to excessive maternal toxicity)
# Corpora lutea			—/—	F0 —; F1 ↓ at 30mkd , no dose response	↓ # corpora lutea/ dam at 1000 mkd	—
Dystocia	None noted					
Ovarian eval for follicles (qual or quant)						
Pup survival (early vs. late)	F0 —; F1 —		—/ND	F1 ↓♀ survival rate at 500 mkd		
No. pups "mis-sexed" @ birth vs. @ necropsy	None noted					
Nipple retention (necropsy or quant)	None noted (10/sex/dose weanlings necropsied)					
Fetal weight			—/—	F1 ♂♀ pups ↓bw at ≥ 300 mkd; F2 —	♂ ↑ at 100, 500, and 1000 mkd (no dose dependence); ♀ ↑ ≥ 100 mkd	—
Luteinizing hormone, serum						
Follicular stimulating hormone, serum						
Thyroid hormone levels (T_3)						
Auditory or acoustic startle						
Brain myelination (special stain)						
Pituitary histopath						
Hypothalamus / brain histopath						

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Female Purbertal

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect	Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats	Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats	Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy	Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)
Liver histopath	F0 ND ; F1 ♂↑ clear cells at 5000 ppm (not considered treatment related), F1 ♀—					
Gross lesions	F0 ♂ —; F0 ♀ — F1 ♂ —; F1 ♀ —	Enlarged kidney at 1000 mkd	F0(GD21) enlarged adrenal at 1000 mkd	F0 liver congestion, liver enlargement, spleen atrophy, adrenal enlargement, thymus atrophy, stomach hemorrhage at 500 mkd	♂enlarged and dark red liver at ≥300 mkd; enlargement kidney at ≥300 mkd; pitted surface of kidney at ≥300 mkd; enlarged adrenal glands at ≥300 mkd	—
Time to mate (pre-coital interval)	F0 —; F1 —					
# Implantation sites	F0 —; F1 —		—/—	F0 —; F1 ↓ at 30 and 500 mkd, no dose response	—	—
# Fetuses			—/—	F1 — ↓ fetuses/dam at 30 and 500 mkd, no dose resonse)	↓ # live fetuses/ dam at 100 and 1000 mkd (no dose response)	—
Fetal/Pup sex ratio	F0 —; F1 —		—/—	F1 —	—	—
Gestation bw, bw gain	bw F0↓ F1↓ (both at 5000 ppm)		bw↓ 300 and 1000; bwg↓ 100, 300 and 1000 mkd /—	F0 bw ↓ at 500 mkd GD20-22; bwg↓ at ≥300 mkd on GD19, 20, 21 and 22 (500 mkd only)	♀ bw ↓at ≥ 500 mkd, bw ↓ at 100 and 300 with recovery by GD21; ♀ bwg ↑at ≥500 mkd with recovery by GD5, ↓ at 100 mkd only at GD7-8 (no dose dependence)	bw bwg ↓ at ≥300 mkd (statistically significant at 1000 mkd)
Lactation bw, bw gain	bw F0↓ F1↓ (both at 5000 ppm) during early lactation		bw↓300 and 1000 mkd ;bwg ↓ 1000 mkd	F0 bw —, bwg ↑at 500 mkd on PND4-21		
No. pups at birth	F0 —; F1—		—/ND	F1 at 500 mkd: ↑ stillborn index, ↓mean litter size, ↓ # live pups, ↑ # deaths (data examined by DER); F2 ↓ # live pups at 500 mkd		

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Female Purbertal

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	Cox (1990) Sumilarv - subchronic study in mice	Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs	Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats	Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]
MRID Number	41321716	43210504	41321717 42178307	41321718 42178308	43004101
Study acceptability	Acceptable	Acceptable	Acceptable	Supplemental	Acceptable
Study report date	8-Mar-89	23-Jan-90	6-May-88	14-Apr-88	11-Jan-93
Study design	Subchronic diet 82-1(a)	Subchronic diet 82-1(a)	Subchronic oral (capusle)	28-day inhalation 82-4	21-day dermal toxicity (82-2)
Test material purity	95.3%	95.3%	97.2%	97.0%	97.2%
Route of exposure	diet	diet	capsule	inhalation	dermal
Exposure duration	13 weeks	13 weeks	13 weeks	28 days 4 hours/day	21 day 6 hours/day
Animal species/strain	Crl:CD BR Rat	Crl:CD-1(ICR)BR Mouse	Beagle dogs	Sprague-Dawley Rat	Sprague Dawley CD Rat
No. animals per sex per group	10/sex	10/sex	4/sex	10/sex	5/sex
Dose levels (ppm, unless otherwise noted)	0, 400, 2000, 5000, 10000	0, 200, 1000, 5000, 10000	NA	0, 269, 482, 1000 mg/m3	NA

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Female Purbertal

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	**Cox (1990) Sumilarv - subchronic study in mice**	**Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs**	**Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats**	**Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]**
Dose (mg/kg/day)	Mean intake: ♂ 23.49, 117.79, 309.05, 641.81 mg/kg/day ♀ 27.68, 141.28, 356.30, 783.96 mg/kg/day	Mean intake: ♂ 28.2, 149.4, 838.1, or 2034.5 mg/kg/day ♀ 37.9, 196.5, 963.9, 2345.3 mg/kg/day	0, 100, 300, 1000		0, 100, 300, 1000
NOAEL / NOEL / Effect	NOAEL 400 ppm	NOAEL 200 ppm	NOEL 100 mkd based on changes in liver for detoxification	NOEL 482 mg/m3	1000 mkd (100 mkd for skin irritation)

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Female Purbertal

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	Cox (1990) Sumilarv - subchronic study in mice	Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs	Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats	Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]
Endpoint Correlates to Tier 1 Screening Assay					
Thyroid wt. (after fixation)			— (and parathyroids)	—	
Uterus (blotted) wt.					
Thyroid histopath (colloid area, FC ht.)	— (and parathyroid)	— (and parathyroid)	— (and parathyroids)	—	
Ovary histopath	—	—	—	—	
Uterus histopath	—	—	—	—	
Estrous cyclicity (age, length, % animals)					
Age and weight at vaginal opening					
Thyroid hormone levels (T_4, TSH)					
Adrenals (paired) wt.	abs ♂♀—; rel ♀— rel ♂↑ at 10000 ppm	abs ♂♀— rel ♀— rel ♂↑ at ≥ 5000 ppm	—	—	
Ovaries (paired) wt.			—	—	
Pituitary wt.			—	—	
Liver wt.	abs rel ♂♀ ↑at 5000 and 10000 ppm; rel ♂↑ at 2000 ppm	abs ♂— ♀↑ at 5000 ppm (not enough animals at 10000 ppm for statistical analysis), significant increase trend; rel ♂♀ ↑at 5000 and 10000(♂only) ♂♀significant increase trend	abs ♂↑at 300 and 1000 mkd, rel —; ♀—	abs ♂♀—; rel ♂↑ at 1000 mg/m3, ♀—	—
Body weight (female)	↓ at 5000 and 10000 ppm (significant trend)	—	—	—	—

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Female Purbertal

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	**Cox (1990) Sumilarv - subchronic study in mice**	**Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs**	**Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats**	**Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]**
Standard blood chemistry (creatinine, BUN, and urea)	BUN ♂ — ♀ ↑at 10000 ppm creatinine ♂— ♀ ↑ at 10000 ppm	BUN ♂♀ ↑ (significant trend) creatinine not measured	BUN — creatinine —	BUN — creatinine —	BUN — creatinine —
Kidney (paired) wt.	abs ♂♀— rel ♂♀↑ at 5000 (♂only) and 10000 ppm	—	—	—	—
Kidney histopath	—	♂♀↑microcysts/dilated tubules, pelvis dilation, tubular nephrosis, tubule mineralization at 5000 and 10000 ppm	—	—	—
Other Relevant Endpoints					
Uterus (wet) wt.			—		
Vagina wt.					
Adrenal histopath	—	♂♀+ cortex congestion at 10000 ppm	—	—	
Mammary gland histopath	—	—	—	—	
Vagina histopath			—	—	
Cervix histopath					
Lactation/nursing (behavior or indices)					
Pup growth (to PND 21)					
Anogenital distance					
Normal external genitalia (pups)					

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Female Purbertal

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	Cox (1990) Sumilarv - subchronic study in mice	Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs	Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats	Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]
Fetal/pup reproductive tract anomolies					
Uterus (gravid, w/ or w/out placenta) wt.					
Oviducts histopath					
Gestation duration					
Female reproductive indices: (mating, conception, fertility, gestation)					
# Corpora lutea					
Dystocia					
Ovarian eval for follicles (qual or quant)					
Pup survival (early vs. late)					
No. pups "mis-sexed" @ birth vs. @ necropsy					
Nipple retention (necropsy or quant)					
Fetal weight					
Luteinizing hormone, serum					
Follicular stimulating hormone, serum					
Thyroid hormone levels (T_3)					
Auditory or acoustic startle					
Brain myelination (special stain)					
Pituitary histopath	—	♂♀+ congestion at 10000 ppm	—	—	
Hypothalamus / brain histopath	ND/— (brain	ND /— (brain)	ND /— (brain)	ND / — (brain)	

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Female Purbertal

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	Cox (1990) Sumilarv - subchronic study in mice	Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs	Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats	Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]
Liver histopath	♂♀↑ of cytoplasmic change at 2000, 5000, 10000 ppm	♂♀↑congestion at 5000 and 10000 ppm (♂ only) for unscheduled deaths	♂♀↑hepatocellular enlargement at 300 (♀ only) and 1000 mkd; ♂♀↑ of smooth endoplasmic reticulum at 1000 mkd	—	—
Gross lesions	—	♀↓ovarian cysts (not dose dependent); kidney ♂♀↑dilated pelvis and ↑cysts and pale tissue	—	♂♀ liver slightly large	—
Time to mate (pre-coital interval)					
# Implantation sites					
# Fetuses					
Fetal/Pup sex ratio					
Gestation bw, bw gain					
Lactation bw, bw gain					
No. pups at birth					

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Female Purbertal

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)	Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks
MRID Number	42178314 43210501 43210503	42178310 43413202 43210501 43413201	42178309
Study acceptability	Acceptable/Core minimum (upgraded)	Acceptable/Core minimum	Acceptable
Study report date	6-Sep-91	23-Jul-91	1-Aug-91
Study design	Chronic/ carcinogenicty	Carcinogenicity	Chronic non-rodent
Test material purity	95.3%	95.3%	94.9%
Route of exposure	Diet	Diet	Capsule undiluted
Exposure duration	104 weeks	78 weeks	52 weeks
Animal species/strain	Crl:CD BR Rat	Crl: CD-1 (ICR) BR mouse	Beagle Dogs
No. animals per sex per group	50/sex (104 weeks) 30/sex (52 weeks)	50/sex (78 wks) 10/sex (52 wks)	4/sex
Dose levels (ppm, unless otherwise noted)	0, 120, 600, 3000	0, 120, 600, 3000	

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Female Purbertal

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	**Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)**	**Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks**
Dose (mg/kg/day)	♂: 0, 5, 27, 138 mkd ♀: 0, 7, 35, 182 mkd	♂: 0, 17, 84, 420 mkd ♀: 0, 22, 110, 547 mkd	0, 30, 100, 300, 1000 mkd
NOAEL / NOEL / Effect	NOEL ♀ 7 mkd (bw ↓, bwg ↓, blood chemistry); NOEL ♂ 27 mkd (bw ↓, bwg ↓, blood chemistry) (EPA concluded NOEL 600ppm)	NOEL 3000 ppm HDT for cancer effects; NOEL 120 ppm for non-cancer effects (EPA concluded non-cancer NOEL 600ppm)	NOAEL 30 mkd LOEL 100 mkd ↑ liver wt (slight ↑ kidney wt)

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Female Purbertal

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	**Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)**	**Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks**
Endpoint Correlates to Tier 1 Screening Assay			
Thyroid wt. (after fixation)	—		♂— ; ♀↑ abs rel thyr wt (prob due to low control value)
Uterus (blotted) wt.			
Thyroid histopath (colloid area, FC ht.)	—	amyloidosis related finding not endocrine related	—
Ovary histopath	—	amyloidosis related finding not endocrine related	—
Uterus histopath	—	—	— (with cervix)
Estrous cyclicity (age, length, % animals)			
Age and weight at vaginal opening			
Thyroid hormone levels (T_4, TSH)			
Adrenals (paired) wt.	—	—	—
Ovaries (paired) wt.	—	—	—
Pituitary wt.			—
Liver wt.	abs ♂♀ — (52 wk), rel ♂— ♀rel ↑ at 3000 ppm (52 wk); abs rel ♂♀ — (104 wk)	abs rel ♂ — , abs rel ♀ ↑at 3000 ppm (52 wk); abs rel ♂♀ — (78 wk)	↑abs rel ♂♀ at ≥ 100 mkd
Body weight (female)	bw ↓, bwg ↓at 3000 ppm bwg ↓at 600 ppm	bw ↓ at 3000 ppm	bwg ↓ ≥ 300 mkd

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Female Purbertal

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	**Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)**	**Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks**
Standard blood chemistry (creatinine, BUN, and urea)	BUN — creatinine —		urea — creatinine —
Kidney (paired) wt.	—	abs rel ♂♀— (52 wk), abs ♂↓ at 3000 ppm, rel—; abs rel ♀— (78 wk)	abs — ↑rel ♂300 mkd ♀ ≥ 300 mkd
Kidney histopath	—	♂♀↑ chronic progressive nephropathy at 3000 ppm; ♀↑renal tubular mineralization at 3000 ppm	—
Other Relevant Endpoints			
Uterus (wet) wt.			— (with cervix)
Vagina wt.			
Adrenal histopath	—	amyloidosis related finding not endocrine related	—
Mammary gland histopath	—	—	—
Vagina histopath	—	—	—
Cervix histopath	—	—	
Lactation/nursing (behavior or indices)			
Pup growth (to PND 21)			
Anogenital distance			
Normal external genitalia (pups)			

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Female Purbertal

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	**Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)**	**Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks**
Fetal/pup reproductive tract anomolies			
Uterus (gravid, w/ or w/out placenta) wt.			
Oviducts histopath			
Gestation duration			
Female reproductive indices: (mating, conception, fertility, gestation)			
# Corpora lutea			
Dystocia			
Ovarian eval for follicles (qual or quant)			
Pup survival (early vs. late)			
No. pups "mis-sexed" @ birth vs. @ necropsy			
Nipple retention (necropsy or quant)			
Fetal weight			
Luteinizing hormone, serum			
Follicular stimulating hormone, serum			
Thyroid hormone levels (T_3)			
Auditory or acoustic startle			
Brain myelination (special stain)			
Pituitary histopath	—	—	—
Hypothalamus / brain histopath	ND/— (brain)	ND/— (brain)	ND/— (brain)

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Female Purbertal

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	**Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)**	**Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks**
Liver histopath	—	amyloidosis related finding not endocrine related	↑♂♀ centriacinar fibrosis and bile duct hyperplasia at 1000 mkd
Gross lesions	—	amyloidosis related finding not endocrine related	♂ livers appear large, ♂♀ liver irregular surface
Time to mate (pre-coital interval)			
# Implantation sites			
# Fetuses			
Fetal/Pup sex ratio			
Gestation bw, bw gain			
Lactation bw, bw gain			
No. pups at birth			

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Hershberger

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect	Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats	Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats	Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy	Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)
MRID Number	42178313	48066201	41321719 42178312	44985001	44985002	41321720 42178311 43215402 43215401
Study acceptability	Acceptable/ Core minimum	DER not available	Acceptable	Acceptable/ non-guideline	Acceptable/ non-guideline	Core minimum (upgraded with additional data)
Study report date	23-Sep-91	2-Aug-05	28-Mar-88	28-Mar-88	21-Apr-88	30-Aug-89
Study design	2-generation reproductive (83-4)	Uterotrophic assay in juvenile (20-day old) rats	GD7-17 exposure with developmental component (cesarean GD21); F1 evaluation PND21, PND56; F1 reproductive component (cesarean GD21)	GD17-PND20 perinatal and lactation exposure with postnatal component; F1 evaluated PND21, PND56; F1 reproductive component (cesarean GD21)	Premating and mating (♂♀ exposure) to GD7 (♀) (cesarean GD21)	Teratogenicity 83-3
Test material purity	95.3%	98.7%	97.2%	97.2%	97.2%	97.2%
Route of exposure	Dietary	Gavage (corn oil vehicle)	oral gavage	oral gavage	oral gavage	oral gavage
Exposure duration	70 day pre-breed♂♀, through mating gestation, lactation of F0 dams through prebreed exposure, mating, gestation, lactation of F1 dams; F2 pups terminated LD21	3 day	GD7-17	GD17-PND20	♂ F0 9 weeks premating and 3 weeks mating; ♀ 2 weeks premating, mating and gestation until GD7	GD6-18
Animal species/strain	Crl:CD(SD) Rat	Crl:CD(SD) Rat	Slc:SD rats (SPF)	Slc:SD rats (SPF)	Slc:SD rats (SPF)	JW-NIBS Rabbit
No. animals per sex per group	26/sex	6 ♀	F0 20-23 dams (cesarean); F0 10-13 dams (delivered); F1 PND21: 9-12/sex; F1 PND56: 10-13/sex; F1 10-13/sex mated (cesarean)	F0 23-24 dams (delivered); F1 PND21: 13-22/sex; F1 PND56: 13-23/sex; F1 14-23/sex mated (cesarean)	24/sex	15-18 dams copulated; 12-14 dams used for anlaysis
Dose levels (ppm, unless otherwise noted)	0, 200, 1000, 5000	NA				

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Hershberger

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	**Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect**	**Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats**	**Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats**	**Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy**	**Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)**
Dose (mg/kg/day)	F0 ♂prebreed 11.6-23.1, 59.8-112.7, 288.5-549.5; F0♀ prebreed-gestation 11.4-23.3, 59.8-115.1, 307.2-556.7; F1♂ prebreed 11.7-36.6, 58.0-178.2, 306-956.9, F1♀ prebreed-gestation 10.5-37.3, 52.9-184.1, 281.2-992.2 for 200, 1000, 5000 ppm, respectively	0, 250, 500, and 1000 mkd pyriproxyfen; 0.001 mkd 17α-ethynyl estradiol (EE) as positive control)	0 (corn oil), 100, 300, 1000	0 (corn oil), 30, 100, 300, 500	0 (corn oil), 100, 300, 500, 1000	0 (corn oil), 100, 300, 1000
NOAEL / NOEL / Effect	NOAEL 5000 ppm (reproductive) NOAEL 1000 ppm (pups) NOAEL 200 ppm (parental)	NOAEL ↑ 1000 mkd (uterine wt) NOAEL 500 mkd (bw) NOEL 100 mkd (liver wt)	maternal NOEL 100mkd, LOEL 300 mkd; fetus NOEL 100mkd, LOEL 300 mkd; pup NOEL 1000 mkd (EPA pup NOEL 300 mkd)	Maternal NOAEL 100 mkd (based on clincial, ↓bwg, organ weights at 300 mkd); Devopmental NOAEL 100 mkd based on ↓bw and ↑fetal dilation of renal pelvis	Parental NOAEL 100 mkd based on clincial, ↓bw, organ weights at 300 mkd); Devopmental NOAEL 1000 mkd based on ↓BW & renal toxicity	maternal NOEL 100 mkd, LOEL 300 mkd; fetal NOEL 300, LOEL 1000 mkd

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Hershberger

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect	Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats	Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats	Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy	Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)
Endpoint Correlates to Tier 1 Screening Assay						
Ventral prostate wt.						
Seminal vesicles and coagulating gland (w/ and w/out fluid) wt.						
Cowper gland wt.						
Levator Ani-bulbocavernosus muscle wt.						
Glans penis wt. (castrated male)						
Testes wt. (left and right)	F1 abs rel — (right); abs — rel ↑ at 5000 ppm (left)		F1(PND21) abs rel ↑300 mkd; F1(PND56) abs rel ↑300 mkd	F1 (PND21) abs ↓ at ≥ 300 mkd, rel at 300 mkd only; F1(PND56)—		
Epididymides wt. (left and right)	F1 abs rel — (right); abs — rel ↑ at 5000 ppm (left)					
Body weight (male)	F0↓ F1↓ (both at 5000 ppm)		ND/—	F1 ↓PND28-56 at ≥ 300 mkd then recovery,	bw bwg↓ at ≥ 300 mkd	
Testosterone, total serum						
Luteinizing hormone, serum						
Follicular stimulating hormone, serum						
Adrenals (paired) wt.		abs rel —; abs — rel ↑ for positive control	F0(GD21) abs rel ↑ 1000 mkd/ F0(PND21) —/ND	F0 —; F1 ND	♂↑abs rel at ≥ 100 mkd; ♀↑ abs rel at 1000 mkd	
Kidney (paired) wt.	F1 ♂ abs —, rel ↑ 1000 and 5000 ppm; F1 ♀abs rel —	abs — rel ↑ at 1000 mkd	F0(GD21) abs↑ 1000 mkd, rel ↑ 300 and 1000 mkd; F0(PND21) —/F1(PND21) —, F1(PND56) ♂ — ♀ ↑ rel 1000 mkd	F0 —; F1(PND21) ♂↓ abs at ≥ 300 mkd, rel —, ♀↓abs at ≥ 300 mkd, rel at 500 mkd; F1(PND56) ↓abs at ≥ 300,↑ rel at 100 mkd	♂↑ abs at 300 and 1000 mkd only, rel at ≥ 100 mkd; ♀ ↑ abs at 1000 mkd, rel at ≥ 100 mkd	
Liver wt.	F1 ♀ abs rel ↑at 5000 ppm, F1 ♂abs ↑ at 5000 ppm, rel ↑ at 1000 and 5000 ppm	abs ↑at 1000 mkd rel ↑at 500, 1000 mkd	F0(GD21) abs— rel↑ 300 and 1000 mkd' F0(PND21) — / F1(PND21) — / F1(PND56) —	F0 abs rel ↑ at ≥300 mkd F1(PND21) ♂↓ abs rel at ≥300 mkd, rel at 300 mkd only, ♀↓abs at ≥ 300 mkd rel at 300 mkd only; F1(PND56) ♂↓ abs at 300 mkd, ♀↓rel at 500 mkd only	♂↑ abs rel at ≥ 300 mkd, rel at ≥100 mkd also; ♀ abs rel —	

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Hershberger

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect	Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats	Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats	Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy	Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)
Other Relevant Endpoints						
Prostate wt.	F1 abs ↓ at 5000 ppm, rel —					
Dorsolateral prostate wt.						
Seminal vesicles wt.	F1 —					
Prostate histopath	F0 ♂ —; F1 ♂ —					
Seminal vesicles histopath	F0 ♂ —; F1 ♂ —					
Coagulating gland histopath						
Age and weight at preputial separation			accelerated descent of testis at 300 mkd, no dose response	delayed descent of testis at 300 mkd, no dose response		
Testis + epididymides wt.					↓ abs ≥ 500 mkd, ↑ rel ≥ 300 mkd	
Testes histopath	F0 ♂ —; F1 ♂ —					
Epididymides histopath	F0 ♂ —; F1 ♂ —					
Sperm count, motility, and anomolies						
Anogenital distance						
Nipple retention (necropsy or quant)	None noted (10/sex/dose weanlings necropsied)					
Normal external genitalia (pups)	No exposure-related abnormalities noted (10/sex/dose weanlings necropsied)					
Normal male external genitalia (F1 adults)	No exposure-related abnormalities noted		—			
Pituitary wt.	F1 —					
Pituitary histopath						
Hypothalamus / brain histopath						

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Hershberger

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect	Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats	Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats	Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy	Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)
Gross lesions	F0 ♂ —; F0 ♀ — F1 ♂ —; F1 ♀ —	Enlarged kidney at 1000 mkd	F0(GD21) enlarged adrenal at 1000 mkd	F0 liver congestion, liver enlargement, spleen atrophy, adrenal enlargement, thymus atrophy, stomach hemorrhage at 500 mkd	♂enlarged and dark red liver at ≥300 mkd; enlargement kidney at ≥300 mkd; pitted surface of kidney at ≥300 mkd; enlarged adrenal glands at ≥300 mkd	—
Time to mate (pre-coital interval)	F0 —; F1 —					
Male reproductive indices: (mating, conception, fertility)	F0 ♂ —; F1 ♂ —		ND/—		F0 —	
# Implantation sites (♂ exposed)						
# Fetuses (♂ exposed)						
Fetal/Pup sex ratio	F0 —; F1 —		—/—	F1 —	—	—
No. pups at birth	F0 —; F1—		—/ND	F1 at 500 mkd: ↑ stillborn index, ↓mean litter size, ↓ # live pups, ↑ # deaths (data examined by DER); F2 ↓ # live pups at 500 mkd		
Pup growth (to PND 21)	F0↓ at 5000 ppm (LD 14-21 ♀, LD 21 ♂); F1 ↓at 5000 ppm (PND 14-21)		—/ND	F1 ↓ in bw bwg at ≥ 300 mkd		
Ectopic testes at necropsy (F1 adults)	None noted					
Fetal/pup reproductive tract anomolies	None noted		—/—	"no differencences... In sexual development at PND21, 56 or post reproduction"		—

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Hershberger

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	Cox (1990) Sumilarv - subchronic study in mice	Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs	Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats	Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]
MRID Number	41321716	43210504	41321717 42178307	41321718 42178308	43004101
Study acceptability	Acceptable	Acceptable	Acceptable	Supplemental	Acceptable
Study report date	8-Mar-89	23-Jan-90	6-May-88	14-Apr-88	11-Jan-93
Study design	Subchronic diet 82-1(a)	Subchronic diet 82-1(a)	Subchronic oral (capusle)	28-day inhalation 82-4	21-day dermal toxicity (82-2)
Test material purity	95.3%	95.3%	97.2%	97.0%	97.2%
Route of exposure	diet	diet	capsule	inhalation	dermal
Exposure duration	13 weeks	13 weeks	13 weeks	28 days 4 hours/day	21 day 6 hours/day
Animal species/strain	Crl:CD BR Rat	Crl:CD-1(ICR)BR Mouse	Beagle dogs	Sprague-Dawley Rat	Sprague Dawley CD Rat
No. animals per sex per group	10/sex	10/sex	4/sex	10/sex	5/sex
Dose levels (ppm, unless otherwise noted)	0, 400, 2000, 5000, 10000	0, 200, 1000, 5000, 10000	NA	0, 269, 482, 1000 mg/m3	NA

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Hershberger

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	Cox (1990) Sumilarv - subchronic study in mice	Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs	Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats	Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]
Dose (mg/kg/day)	Mean intake: ♂ 23.49, 117.79, 309.05, 641.81 mg/kg/day ♀ 27.68, 141.28, 356.30, 783.96 mg/kg/day	Mean intake: ♂ 28.2, 149.4, 838.1, or 2034.5 mg/kg/day ♀ 37.9, 196.5, 963.9, 2345.3 mg/kg/day	0, 100, 300, 1000		0, 100, 300, 1000
NOAEL / NOEL / Effect	NOAEL 400 ppm	NOAEL 200 ppm	NOEL 100 mkd based on changes in liver for detoxification	NOEL 482 mg/m3	1000 mkd (100 mkd for skin irritation)

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Hershberger

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	Cox (1990) Sumilarv - subchronic study in mice	Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs	Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats	Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]
Endpoint Correlates to Tier 1 Screening Assay					
Ventral prostate wt.					
Seminal vesicles and coagulating gland (w/ and w/out fluid) wt.					
Cowper gland wt.					
Levator Ani-bulbocavernosus muscle wt.					
Glans penis wt. (castrated male)					
Testes wt. (left and right)	—	abs ↓ at 10000 ppm; rel —	—	—	
Epididymides wt. (left and right)					
Body weight (male)	↓ at 5000 and 10000 ppm (significant trend)	↓ at 5000 and 10000 ppm significant trend	—	—	—
Testosterone, total serum					
Luteinizing hormone, serum					
Follicular stimulating hormone, serum					
Adrenals (paired) wt.	abs ♂♀—; rel ♀— rel ♂↑ at 10000 ppm	abs ♂♀— rel ♀— rel ♂↑ at ≥ 5000 ppm	—	—	
Kidney (paired) wt.	abs ♂♀— rel ♂♀↑ at 5000 (♂only) and 10000 ppm	—	—	—	—
Liver wt.	abs rel ♂♀ ↑at 5000 and 10000 ppm; rel ♂↑ at 2000 ppm	abs ♂— ♀↑ at 5000 ppm (not enough animals at 10000 ppm for statistical analysis), significant increase trend; rel ♂♀ ↑at 5000 and 10000(♂only) ♂♀significant increase trend	abs ♂↑at 300 and 1000 mkd, rel —; ♀—	abs ♂♀—; rel ♂↑ at 1000 mg/m3, ♀—	—

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Hershberger

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	Cox (1990) Sumilarv - subchronic study in mice	Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs	Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats	Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]
Other Relevant Endpoints					
Prostate wt.			—	—	
Dorsolateral prostate wt.					
Seminal vesicles wt.					
Prostate histopath			—	—	
Seminal vesicles histopath		♂↑ reduced secretion 4/10 at 10000 ppm		—	
Coagulating gland histopath					
Age and weight at preputial separation					
Testis + epididymides wt.					—
Testes histopath	—	—	—	—	
Epididymides histopath	—	—	—	—	
Sperm count, motility, and anomolies					
Anogenital distance					
Nipple retention (necropsy or quant)					
Normal external genitalia (pups)					
Normal male external genitalia (F1 adults)					
Pituitary wt.			—	—	
Pituitary histopath	—	♂♀+ congestion at 10000 ppm	—	—	
Hypothalamus / brain histopath	ND/— (brain	ND /— (brain)	ND /— (brain)	ND / — (brain)	

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Hershberger

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	Cox (1990) Sumilarv - subchronic study in mice	Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs	Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats	Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]
Gross lesions	–	♀↓ovarian cysts (not dose dependent); kidney ♂♀↑dilated pelvis and ↑cysts and pale tissue	–	♂♀ liver slightly large	–
Time to mate (pre-coital interval)					
Male reproductive indices: (mating, conception, fertility)					
# Implantation sites (♂ exposed)					
# Fetuses (♂ exposed)					
Fetal/Pup sex ratio					
No. pups at birth					
Pup growth (to PND 21)					
Ectopic testes at necropsy (F1 adults)					
Fetal/pup reproductive tract anomolies					

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Hershberger

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)	Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks
MRID Number	42178314 43210501 43210503	42178310 43413202 43210501 43413201	42178309
Study acceptability	Acceptable/Core minimum (upgraded)	Acceptable/Core minimum	Acceptable
Study report date	6-Sep-91	23-Jul-91	1-Aug-91
Study design	Chronic/ carcinogenicty	Carcinogenicity	Chronic non-rodent
Test material purity	95.3%	95.3%	94.9%
Route of exposure	Diet	Diet	Capsule undiluted
Exposure duration	104 weeks	78 weeks	52 weeks
Animal species/strain	Crl:CD BR Rat	Crl: CD-1 (ICR) BR mouse	Beagle Dogs
No. animals per sex per group	50/sex (104 weeks) 30/sex (52 weeks)	50/sex (78 wks) 10/sex (52 wks)	4/sex
Dose levels (ppm, unless otherwise noted)	0, 120, 600, 3000	0, 120, 600, 3000	

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Hershberger

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	**Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)**	**Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks**
Dose (mg/kg/day)	♂: 0, 5, 27, 138 mkd ♀: 0, 7, 35, 182 mkd	♂: 0, 17, 84, 420 mkd ♀: 0, 22, 110, 547 mkd	0, 30, 100, 300, 1000 mkd
NOAEL / NOEL / Effect	NOEL ♀ 7 mkd (bw ↓, bwg ↓, blood chemistry); NOEL ♂ 27 mkd (bw ↓, bwg ↓, blood chemistry) (EPA concluded NOEL 600ppm)	NOEL 3000 ppm HDT for cancer effects; NOEL 120 ppm for non-cancer effects (EPA concluded non-cancer NOEL 600ppm)	NOAEL 30 mkd LOEL 100 mkd ↑ liver wt (slight ↑ kidney wt)

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Hershberger

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	**Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)**	**Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks**
Endpoint Correlates to Tier 1 Screening Assay			
Ventral prostate wt.			
Seminal vesicles and coagulating gland (w/ and w/out fluid) wt.			
Cowper gland wt.			
Levator Ani-bulbocavernosus muscle wt.			
Glans penis wt. (castrated male)			
Testes wt. (left and right)	—	—	—
Epididymides wt. (left and right)			
Body weight (male)	bw ↓, bwg ↓at 3000 ppm	bw ↓ bwg ↓at 3000 ppm	—
Testosterone, total serum			
Luteinizing hormone, serum			
Follicular stimulating hormone, serum			
Adrenals (paired) wt.	—	—	—
Kidney (paired) wt.	—	abs rel ♂♀— (52 wk), abs ♂↓ at 3000 ppm, rel—; abs rel ♀— (78 wk)	abs — ↑rel ♂300 mkd ♀ ≥ 300 mkd
Liver wt.	abs ♂♀ — (52 wk), rel ♂— ♀rel ↑ at 3000 ppm (52 wk); abs rel ♂♀ — (104 wk)	abs rel ♂ — , abs rel ♀ ↑at 3000 ppm (52 wk); abs rel ♂♀ — (78 wk)	↑abs rel ♂♀ at ≥ 100 mkd

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Hershberger

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)	Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks
Other Relevant Endpoints			
Prostate wt.			—
Dorsolateral prostate wt.			
Seminal vesicles wt.			
Prostate histopath	— inflammatory change at 3000 ppm not treatment related	—	—
Seminal vesicles histopath	—	—	
Coagulating gland histopath			
Age and weight at preputial separation			
Testis + epididymides wt.			
Testes histopath	—	amyloidosis related finding not endocrine related	—
Epididymides histopath	—	—	—
Sperm count, motility, and anomolies			
Anogenital distance			
Nipple retention (necropsy or quant)			
Normal external genitalia (pups)			
Normal male external genitalia (F1 adults)			
Pituitary wt.			—
Pituitary histopath	—	—	—
Hypothalamus / brain histopath	ND/— (brain)	ND/— (brain)	ND/— (brain)

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Hershberger

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	**Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)**	**Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks**
Gross lesions	—	amyloidosis related finding not endocrine related	♂ livers appear large, ♂♀ liver irregular surface
Time to mate (pre-coital interval)			
Male reproductive indices: (mating, conception, fertility)			
# Implantation sites (♂ exposed)			
# Fetuses (♂ exposed)			
Fetal/Pup sex ratio			
No. pups at birth			
Pup growth (to PND 21)			
Ectopic testes at necropsy (F1 adults)			
Fetal/pup reproductive tract anomolies			

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Male Pubertal

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect	Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats	Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats	Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy	Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)
MRID Number	42178313	48066201	41321719 42178312	44985001	44985002	41321720 42178311 43215402 43215401
Study acceptability	Acceptable/ Core minimum	DER not available	Acceptable	Acceptable/ non-guideline	Acceptable/ non-guideline	Core minimum (upgraded with additional data)
Study report date	23-Sep-91	2-Aug-05	28-Mar-88	28-Mar-88	21-Apr-88	30-Aug-89
Study design	2-generation reproductive (83-4)	Uterotrophic assay in juvenile (20-day old) rats	GD7-17 exposure with developmental component (cesarean GD21); F1 evaluation PND21, PND56; F1 reproductive component (cesarean GD21)	GD17-PND20 perinatal and lactation exposure with postnatal component; F1 evaluated PND21, PND56; F1 reproductive component (cesarean GD21)	Premating and mating (♂♀ exposure) to GD7 (♀) (cesarean GD21)	Teratogenicity 83-3
Test material purity	95.3%	98.7%	97.2%	97.2%	97.2%	97.2%
Route of exposure	Dietary	Gavage (corn oil vehicle)	oral gavage	oral gavage	oral gavage	oral gavage
Exposure duration	70 day pre-breed♂♀, through mating gestation, lactation of F0 dams through prebreed exposure, mating, gestation, lactation of F1 dams; F2 pups terminated LD21	3 day	GD7-17	GD17-PND20	♂ F0 9 weeks premating and 3 weeks mating; ♀ 2 weeks premating, mating and gestation until GD7	GD6-18
Animal species/strain	Crl:CD(SD) Rat	Crl:CD(SD) Rat	Slc:SD rats (SPF)	Slc:SD rats (SPF)	Slc:SD rats (SPF)	JW-NIBS Rabbit
No. animals per sex per group	26/sex	6 ♀	F0 20-23 dams (cesarean); F0 10-13 dams (delivered); F1 PND21: 9-12/sex; F1 PND56: 10-13/sex; F1 10-13/sex mated (cesarean)	F0 23-24 dams (delivered); F1 PND21: 13-22/sex; F1 PND56: 13-23/sex; F1 14-23/sex mated (cesarean)	24/sex	15-18 dams copulated; 12-14 dams used for anlaysis
Dose levels (ppm, unless otherwise noted)	0, 200, 1000, 5000	NA				

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Male Pubertal

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	**Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect**	**Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats**	**Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats**	**Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy**	**Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)**
Dose (mg/kg/day)	F0 ♂prebreed 11.6-23.1, 59.8-112.7, 288.5-549.5; F0♀ prebreed-gestation 11.4-23.3, 59.8-115.1, 307.2-556.7; F1♂ prebreed 11.7-36.6, 58.0-178.2, 306-956.9, F1♀ prebreed-gestation 10.5-37.3, 52.9-184.1, 281.2-992.2 for 200, 1000, 5000 ppm, respectively	0, 250, 500, and 1000 mkd pyriproxyfen; 0.001 mkd 17α-ethynyl estradiol (EE) as positive control)	0 (corn oil), 100, 300, 1000	0 (corn oil), 30, 100, 300, 500	0 (corn oil), 100, 300, 500, 1000	0 (corn oil), 100, 300, 1000
NOAEL / NOEL / Effect	NOAEL 5000 ppm (reproductive) NOAEL 1000 ppm (pups) NOAEL 200 ppm (parental)	NOAEL ↑ 1000 mkd (uterine wt) NOAEL 500 mkd (bw) NOEL 100 mkd (liver wt)	maternal NOEL 100mkd, LOEL 300 mkd; fetus NOEL 100mkd, LOEL 300 mkd; pup NOEL 1000 mkd (EPA pup NOEL 300 mkd)	Maternal NOAEL 100 mkd (based on clincial, ↓bwg, organ weights at 300 mkd); Devopmental NOAEL 100 mkd based on ↓bw and ↑fetal dilation of renal pelvis	Parental NOAEL 100 mkd based on clincial, ↓bw, organ weights at 300 mkd); Devopmental NOAEL 1000 mkd based on ↓BW & renal toxicity	maternal NOEL 100 mkd, LOEL 300 mkd; fetal NOEL 300, LOEL 1000 mkd

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Male Pubertal

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	**Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect**	**Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats**	**Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats**	**Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy**	**Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)**
Endpoint Correlates to Tier 1 Screening Assay						
Thyroid wt. (after fixation)						
Testes wt. (left and right)	F1 abs rel — (right); abs — rel ↑ at 5000 ppm (left)		F1(PND21) abs rel ↑300 mkd; F1(PND56) abs rel ↑300 mkd	F1 (PND21) abs ↓ at ≥ 300 mkd, rel at 300 mkd only; F1(PND56)—		
Epididymides wt. (left and right)	F1 abs rel — (right); abs — rel ↑ at 5000 ppm (left)					
Ventral prostate wt.						
Dorsolateral prostate wt.						
Seminal vesicles and coagulating gland (*w/ and w/out fluid*) wt.						
Levator Ani-bulbocavernosus muscle wt.						
Thyroid histopath (colloid area, FC ht.)						
Testes histopath	F0 ♂ —; F1 ♂ —					
Epididymides histopath	F0 ♂ —; F1 ♂ —					
Age and weight at preputial separation			accelerated descent of testis at 300 mkd, no dose response	delayed descent of testis at 300 mkd, no dose response		
Testosterone, total serum						
Thyroid hormone levels (T_4, TSH)						
Adrenals (paired) wt.		abs rel —; abs — rel ↑ for positive control	F0(GD21) abs rel ↑ 1000 mkd/ F0(PND21) —/ND	F0 —; F1 ND	♂↑abs rel at ≥ 100 mkd; ♀↑ abs rel at 1000 mkd	
Pituitary wt.	F1 —					
Liver wt.	F1 ♀ abs rel ↑at 5000 ppm, F1 ♂abs ↑ at 5000 ppm, rel ↑ at 1000 and 5000 ppm	abs ↑at 1000 mkd rel ↑at 500, 1000 mkd	F0(GD21) abs— rel↑ 300 and 1000 mkd' F0(PND21) — / F1(PND21) — / F1(PND56) —	F0 abs rel ↑ at ≥300 mkd F1(PND21) ♂↓ abs rel at ≥300 mkd, rel at 300 mkd only, ♀↓abs at ≥ 300 mkd rel at 300 mkd only; F1(PND56) ♂↓ abs at 300 mkd, ♀↓rel at 500 mkd only	♂↑ abs rel at ≥ 300 mkd, rel at ≥100 mkd also; ♀ abs rel —	

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Male Pubertal

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect	Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats	Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats	Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy	Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)
Body weight (male)	F0↓ F1↓ (both at 5000 ppm)		ND/—	F1 ↓PND28-56 at ≥ 300 mkd then recovery,	bw bwg↓ at ≥ 300 mkd	
Standard blood chemistry (creatinine, BUN, and urea)						
Kidney (paired) wt.	F1 ♂ abs —, rel ↑ 1000 and 5000 ppm; F1 ♀abs rel —	abs — rel ↑ at 1000 mkd	F0(GD21) abs↑ 1000 mkd, rel ↑ 300 and 1000 mkd; F0(PND21) —/F1(PND21) —, F1(PND56) ♂ — ♀ ↑ rel 1000 mkd	F0 —; F1(PND21) ♂↓ abs at ≥ 300 mkd, rel —, ♀↓abs at ≥ 300 mkd, rel at 500 mkd; F1(PND56) ↓abs at ≥ 300,↑ rel at 100 mkd	♂↑ abs at 300 and 1000 mkd only, rel at ≥ 100 mkd; ♀ ↑ abs at 1000 mkd, rel at ≥ 100 mkd	
Kidney histopath	F0 ND ; F1 ♂ ↑interstitial nephritis at 5000 ppm; F1 ♀—				♂ ↑ regenerative changes and dilation of tubules with accumulation of neutrophils at ≥300 mkd (in text not tables, DER could not confirm)	
Other Relevant Endpoints						
Testis + epididymides wt.					↓ abs ≥ 500 mkd, ↑ rel ≥ 300 mkd	
Prostate wt.	F1 abs ↓ at 5000 ppm, rel —					
Seminal vesicles wt.	F1 —					
Cowper gland wt.						
Glans penis wt. (castrated male)						
Adrenal histopath						
Pituitary histopath						
Prostate histopath	F0 ♂ —; F1 ♂ —					
Seminal vesicles histopath	F0 ♂ —; F1 ♂ —					
Coagulating gland histopath						
Sperm count, motility, and anomolies						

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Male Pubertal

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect	Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats	Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats	Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy	Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)
Pup growth (to PND 21)	F0↓ at 5000 ppm (LD 14-21 ♀, LD 21 ♂); F1 ↓at 5000 ppm (PND 14-21)		—/ND	F1 ↓ in bw bwg at ≥ 300 mkd		
Anogenital distance						
Nipple retention (necropsy or quant)	None noted (10/sex/dose weanlings necropsied)					
Normal external genitalia (pups)	No exposure-related abnormalities noted (10/sex/dose weanlings necropsied)					
Normal male external genitalia (F1 adults)	No exposure-related abnormalities noted		—			
Fetal/pup reproductive tract anomolies	None noted		—/—	"no differencences... In sexual development at PND21, 56 or post reproduction"		—
Male reproductive indices: (mating, conception, fertility)	F0 ♂ —; F1 ♂ —		ND/—		F0 —	
No. pups "mis-sexed" @ birth vs. @ necropsy	None noted					
Ectopic testes at necropsy (F1 adults)	None noted					
Luteinizing hormone, serum						
Follicular stimulating hormone, serum						
Thyroid hormone levels (T_3)						
Auditory or acoustic startle						
Brain myelination (special stain)						
Hypothalamus / brain histopath						
Liver histopath	F0 ND ; F1 ♂↑ clear cells at 5000 ppm (not considered treatment related), F1 ♀—					

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Male Pubertal

	Reproductive Studies		Reproductive/Developmental Studies			
	Robinson et al. (1991) Dietary 2-generation (1 litter) reproduction study of S-31183 in the rat	**Ose (2005) Uterotrophic assay by oral route using juvenile rat: investigation on estrogenic effect**	**Saegusa et al. (1988a) Sumilarv - study of S-31183 by oral administration during the period of fetal organogenesis in rats**	**Saegusa et al. (1988b) Perinatal and postnatal study of S-31183 orally administered to rats**	**Saegusa et al. (1998c) Study by oral administration of S-31183 to rats prior to and in the early stage of pregnancy**	**Hirohashi (1988) Sumilarv - study of S-31183 by oral adminsitration during the period of organogenesis in rabbits (plus addendum and response to EPA)**
Gross lesions	F0 ♂ —; F0 ♀ — F1 ♂ —; F1 ♀ —	Enlarged kidney at 1000 mkd	F0(GD21) enlarged adrenal at 1000 mkd	F0 liver congestion, liver enlargement, spleen atrophy, adrenal enlargement, thymus atrophy, stomach hemorrhage at 500 mkd	♂enlarged and dark red liver at ≥300 mkd; enlargement kidney at ≥300 mkd; pitted surface of kidney at ≥300 mkd; enlarged adrenal glands at ≥300 mkd	—
Time to mate (pre-coital interval)	F0 —; F1 —					
# Implantation sites (♂ exposed)						
# Fetuses (♂ exposed)						
Fetal/Pup sex ratio	F0 —; F1 —		—/—	F1 —	—	—
No. pups at birth	F0 —; F1—		—/ND	F1 at 500 mkd: ↑ stillborn index, ↓mean litter size, ↓ # live pups, ↑ # deaths (data examined by DER); F2 ↓ # live pups at 500 mkd		

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Male Pubertal

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	Cox (1990) Sumilarv - subchronic study in mice	Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs	Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats	Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]
MRID Number	41321716	43210504	41321717 42178307	41321718 42178308	43004101
Study acceptability	Acceptable	Acceptable	Acceptable	Supplemental	Acceptable
Study report date	8-Mar-89	23-Jan-90	6-May-88	14-Apr-88	11-Jan-93
Study design	Subchronic diet 82-1(a)	Subchronic diet 82-1(a)	Subchronic oral (capusle)	28-day inhalation 82-4	21-day dermal toxicity (82-2)
Test material purity	95.3%	95.3%	97.2%	97.0%	97.2%
Route of exposure	diet	diet	capsule	inhalation	dermal
Exposure duration	13 weeks	13 weeks	13 weeks	28 days 4 hours/day	21 day 6 hours/day
Animal species/strain	Crl:CD BR Rat	Crl:CD-1(ICR)BR Mouse	Beagle dogs	Sprague-Dawley Rat	Sprague Dawley CD Rat
No. animals per sex per group	10/sex	10/sex	4/sex	10/sex	5/sex
Dose levels (ppm, unless otherwise noted)	0, 400, 2000, 5000, 10000	0, 200, 1000, 5000, 10000	NA	0, 269, 482, 1000 mg/m3	NA

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Male Pubertal

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	**Cox (1990) Sumilarv - subchronic study in mice**	**Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs**	**Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats**	**Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]**
Dose (mg/kg/day)	Mean intake: ♂ 23.49, 117.79, 309.05, 641.81 mg/kg/day ♀ 27.68, 141.28, 356.30, 783.96 mg/kg/day	Mean intake: ♂ 28.2, 149.4, 838.1, or 2034.5 mg/kg/day ♀ 37.9, 196.5, 963.9, 2345.3 mg/kg/day	0, 100, 300, 1000		0, 100, 300, 1000
NOAEL / NOEL / Effect	NOAEL 400 ppm	NOAEL 200 ppm	NOEL 100 mkd based on changes in liver for detoxification	NOEL 482 mg/m3	1000 mkd (100 mkd for skin irritation)

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Male Pubertal

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	Cox (1990) Sumilarv - subchronic study in mice	Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs	Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats	Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]
Endpoint Correlates to Tier 1 Screening Assay					
Thyroid wt. (after fixation)			— (and parathyroids)	—	
Testes wt. (left and right)	—	abs ↓ at 10000 ppm; rel —	—	—	
Epididymides wt. (left and right)					
Ventral prostate wt.					
Dorsolateral prostate wt.					
Seminal vesicles and coagulating gland (w/ and w/out fluid) wt.					
Levator Ani-bulbocavernosus muscle wt.					
Thyroid histopath (colloid area, FC ht.)	— (and parathyroid)	— (and parathyroid)	— (and parathyroids)	—	
Testes histopath	—	—	—	—	
Epididymides histopath	—	—	—	—	
Age and weight at preputial separation					
Testosterone, total serum					
Thyroid hormone levels (T_4, TSH)					
Adrenals (paired) wt.	abs ♂♀—; rel ♀— rel ♂↑ at 10000 ppm	abs ♂♀— rel ♀— rel ♂↑ at ≥ 5000 ppm	—	—	
Pituitary wt.			—	—	
Liver wt.	abs rel ♂♀ ↑at 5000 and 10000 ppm; rel ♂↑ at 2000 ppm	abs ♂— ♀↑ at 5000 ppm (not enough animals at 10000 ppm for statistical analysis), significant increase trend; rel ♂♀ ↑at 5000 and 10000(♂only) ♂♀significant increase trend	abs ♂↑at 300 and 1000 mkd, rel —; ♀—	abs ♂♀—; rel ♂↑ at 1000 mg/m3, ♀—	—

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Male Pubertal

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	Cox (1990) Sumilarv - subchronic study in mice	Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs	Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats	Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]
Body weight (male)	↓ at 5000 and 10000 ppm (significant trend)	↓ at 5000 and 10000 ppm significant trend	—	—	—
Standard blood chemistry (creatinine, BUN, and urea)	BUN ♂ — ♀ ↑at 10000 ppm creatinine ♂— ♀ ↑ at 10000 ppm	BUN ♂♀ ↑ (significant trend) creatinine not measured	BUN — creatinine —	BUN — creatinine —	BUN — creatinine —
Kidney (paired) wt.	abs ♂♀— rel ♂♀↑ at 5000 (♂only) and 10000 ppm	—	—	—	—
Kidney histopath	—	♂♀↑microcysts/dilated tubules, pelvis dilation, tubular nephrosis, tubule mineralization at 5000 and 10000 ppm	—	—	—
Other Relevant Endpoints					
Testis + epididymides wt.					—
Prostate wt.			—	—	
Seminal vesicles wt.					
Cowper gland wt.					
Glans penis wt. (castrated male)					
Adrenal histopath	—	♂♀+ cortex congestion at 10000 ppm	—	—	
Pituitary histopath	—	♂♀+ congestion at 10000 ppm	—	—	
Prostate histopath			—	—	
Seminal vesicles histopath		♂↑ reduced secretion 4/10 at 10000 ppm		—	
Coagulating gland histopath					
Sperm count, motility, and anomolies					

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Male Pubertal

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	Cox (1990) Sumilarv - subchronic study in mice	Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs	Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats	Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]
Pup growth (to PND 21)					
Anogenital distance					
Nipple retention (necropsy or quant)					
Normal external genitalia (pups)					
Normal male external genitalia (F1 adults)					
Fetal/pup reproductive tract anomolies					
Male reproductive indices: (mating, conception, fertility)					
No. pups "mis-sexed" @ birth vs. @ necropsy					
Ectopic testes at necropsy (F1 adults)					
Luteinizing hormone, serum					
Follicular stimulating hormone, serum					
Thyroid hormone levels (T_3)					
Auditory or acoustic startle					
Brain myelination (special stain)					
Hypothalamus / brain histopath	ND/— (brain	ND /— (brain)	ND /— (brain)	ND / — (brain)	
Liver histopath	♂♀↑ of cytoplasmic change at 2000, 5000, 10000 ppm	♂♀↑congestion at 5000 and 10000 ppm (♂ only) for unscheduled deaths	♂♀↑hepatocellular enlargement at 300 (♀ only) and 1000 mkd; ♂♀↑ of smooth endoplasmic reticulum at 1000 mkd	—	—

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Male Pubertal

	Subchronic Toxicity Studies				
	Cox (1989) Subchronic study with S-31183 in rats	Cox (1990) Sumilarv - subchronic study in mice	Yamada et al. (1988) Three-month oral toxicity study of S-31183 in dogs	Kawaguchi et al. (1988) Sumilarv - subacute inhalation toxicity stuyd of S-31183 in rats	Moore (1993) Sumilarv - 21-day dermal toxicity study with rats with S-31183 [technical grade]
Gross lesions	—	♀↓ovarian cysts (not dose dependent); kidney ♂♀↑dilated pelvis and ↑cysts and pale tissue	—	♂♀ liver slightly large	—
Time to mate (pre-coital interval)					
# Implantation sites (♂ exposed)					
# Fetuses (♂ exposed)					
Fetal/Pup sex ratio					
No. pups at birth					

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Male Pubertal

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	**Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)**	**Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks**
MRID Number	42178314 43210501 43210503	42178310 43413202 43210501 43413201	42178309
Study acceptability	Acceptable/Core minimum (upgraded)	Acceptable/Core minimum	Acceptable
Study report date	6-Sep-91	23-Jul-91	1-Aug-91
Study design	Chronic/ carcinogenicty	Carcinogenicity	Chronic non-rodent
Test material purity	95.3%	95.3%	94.9%
Route of exposure	Diet	Diet	Capsule undiluted
Exposure duration	104 weeks	78 weeks	52 weeks
Animal species/strain	Crl:CD BR Rat	Crl: CD-1 (ICR) BR mouse	Beagle Dogs
No. animals per sex per group	50/sex (104 weeks) 30/sex (52 weeks)	50/sex (78 wks) 10/sex (52 wks)	4/sex
Dose levels (ppm, unless otherwise noted)	0, 120, 600, 3000	0, 120, 600, 3000	

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Male Pubertal

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	**Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)**	**Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks**
Dose (mg/kg/day)	♂: 0, 5, 27, 138 mkd ♀: 0, 7, 35, 182 mkd	♂: 0, 17, 84, 420 mkd ♀: 0, 22, 110, 547 mkd	0, 30, 100, 300, 1000 mkd
NOAEL / NOEL / Effect	NOEL ♀ 7 mkd (bw ↓, bwg ↓, blood chemistry); NOEL ♂ 27 mkd (bw ↓, bwg ↓, blood chemistry) (EPA concluded NOEL 600ppm)	NOEL 3000 ppm HDT for cancer effects; NOEL 120 ppm for non-cancer effects (EPA concluded non-cancer NOEL 600ppm)	NOAEL 30 mkd LOEL 100 mkd ↑ liver wt (slight ↑ kidney wt)

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Male Pubertal

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)	Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks
Endpoint Correlates to Tier 1 Screening Assay			
Thyroid wt. (after fixation)	—		♂— ; ♀↑ abs rel thyr wt (prob due to low control value)
Testes wt. (left and right)	—	—	—
Epididymides wt. (left and right)			
Ventral prostate wt.			
Dorsolateral prostate wt.			
Seminal vesicles and coagulating gland (w/ and w/out fluid) wt.			
Levator Ani-bulbocavernosus muscle wt.			
Thyroid histopath (colloid area, FC ht.)	—	amyloidosis related finding not endocrine related	—
Testes histopath	—	amyloidosis related finding not endocrine related	—
Epididymides histopath	—	—	—
Age and weight at preputial separation			
Testosterone, total serum			
Thyroid hormone levels (T_4, TSH)			
Adrenals (paired) wt.	—	—	—
Pituitary wt.			—
Liver wt.	abs ♂♀ — (52 wk), rel ♂— ♀rel ↑ at 3000 ppm (52 wk); abs rel ♂♀ — (104 wk)	abs rel ♂ — , abs rel ♀ ↑at 3000 ppm (52 wk); abs rel ♂♀ — (78 wk)	↑abs rel ♂♀ at ≥ 100 mkd

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Male Pubertal

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	**Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)**	**Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks**
Body weight (male)	bw ↓, bwg ↓at 3000 ppm	bw ↓ bwg ↓at 3000 ppm	—
Standard blood chemistry (creatinine, BUN, and urea)	BUN — creatinine —		urea — creatinine —
Kidney (paired) wt.	—	abs rel ♂♀— (52 wk), abs ♂↓ at 3000 ppm, rel—; abs rel ♀— (78 wk)	abs — ↑rel ♂300 mkd ♀ ≥ 300 mkd
Kidney histopath	—	♂♀↑ chronic progressive nephropathy at 3000 ppm; ♀↑renal tubular mineralization at 3000 ppm	—
Other Relevant Endpoints			
Testis + epididymides wt.			
Prostate wt.			—
Seminal vesicles wt.			
Cowper gland wt.			
Glans penis wt. (castrated male)			
Adrenal histopath	—	amyloidosis related finding not endocrine related	—
Pituitary histopath	—	—	—
Prostate histopath	— inflammatory change at 3000 ppm not treatment related	—	—
Seminal vesicles histopath	—	—	
Coagulating gland histopath			
Sperm count, motility, and anomolies			

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Male Pubertal

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	**Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)**	**Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks**
Pup growth (to PND 21)			
Anogenital distance			
Nipple retention (necropsy or quant)			
Normal external genitalia (pups)			
Normal male external genitalia (F1 adults)			
Fetal/pup reproductive tract anomolies			
Male reproductive indices: (mating, conception, fertility)			
No. pups "mis-sexed" @ birth vs. @ necropsy			
Ectopic testes at necropsy (F1 adults)			
Luteinizing hormone, serum			
Follicular stimulating hormone, serum			
Thyroid hormone levels (T_3)			
Auditory or acoustic startle			
Brain myelination (special stain)			
Hypothalamus / brain histopath	ND/— (brain)	ND/— (brain)	ND/— (brain)
Liver histopath	—	amyloidosis related finding not endocrine related	↑♂♀ centriacinar fibrosis and bile duct hyperplasia at 1000 mkd

Tier 1 Mammalian Screening Evaluation: PYRIPROXYFEN - Male Pubertal

	Chronic Toxicity Studies		
	Osheroff (1991a) Combined chronic and oncogenicity study in rats with S-31183 (plus addendum and ammendments)	**Osheroff (1991b) Oncogenicity study in mice with S-31183 (plus supplemental data, addendum and ammendment)**	**Chapman (1991) S-31183: Toxicity study by oral (capsule) administration to beagle dogs for 52 weeks**
Gross lesions	—	amyloidosis related finding not endocrine related	♂ livers appear large, ♂♀ liver irregular surface
Time to mate (pre-coital interval)			
# Implantation sites (♂ exposed)			
# Fetuses (♂ exposed)			
Fetal/Pup sex ratio			
No. pups at birth			

Appendix III. Ecotoxicological Study Matrix

Spreadsheet Key - Tier 1 Ecological Screening Evaluation: PYRIPROXYFEN

Abbreviation/ Notation	Definition
—	No statistically significant findings
↑	Statistically significant increase
↓	Statistically significant decrease
X	Reported in methods, no details provided in report
XX	Data reported on endpoint, but interpretation or calculations needed
♂	Male
♀	Female
abs	Absolute
adj	Adjusted for terminal body weight
dph	day post-hatch
F0	Parental generation
F1	1st generation offspring
HD	High dose
HDT	Highest dose tested
LD	Low dose
MD	Intermediate dose
mkd	mg/kg/day
ND	Not determined
rel	Relative
TWA	Time-weighted average
unk	Unknown
VC	Vehicle control

Color Key

HEADER: Key studies cited as OSRI

HEADER: Not cited as OSRI

Evaluated, **NO EFFECT:** no statistically significant difference

Evaluated, **NO EFFECT:** statistically significant difference but no dose response and/or no biological significance

Evaluated, Effect: **NOT Endocrine-related**

Evaluated, Effect: **NOT likely Endocrine-related; related to systemic toxicity**

Evaluated, Effect: **Possible Endocrine-related**

Evaluated, Effect: **Clearly Endocrine-related**

Background
Background

NOT Evaluated

Tier 1 Ecological Screening Evaluation: PYRIPROXYFEN

	Fish Studies				Avian Reproduction	
	Gries (2007) Full life cycle toxicity test with Medaka under flow-through conditions	**Rhodes and Cramer (1991) Early life-stage toxicity of Sumilarv technical to the Rainbow Trout under flow-through conditions**	**Sword and Northrup (1992) 21-Day flow-through toxicity of pyriproxyfen to Rainbow Trout**	**Brown et al. (2002) Pulse-exposure effects of selected insecticides to juvenile Crimson-Spottted Rainbowfish**	**Beavers et al. (1994a) Reproduction study with the Mallard**	**Beavers et al. (1994b) Reproduction study with the Bobwhite Quail**
MRID	48066202	42178319	48066203	NA	44036908	44036906
Study acceptability	Study not yet reviewed by EPA	Scientifically sound non-guideline; data requested	DER not available	NA (publication)	Acceptable/ Core guideline	Acceptable/ Core guideine
Study design	Full life cycle; Ministry of the Environment, Japan, Annex 6-2	Early Life-stage; 40 CFR 158.145 (Guideline 72-4)	21-day prolonged toxicity; OECD (Guideline 204)	1-hr pulse exposure followed by 24 hr recovery in clean water	Reproduction (Guideline 71-4)	Reproduction (Guideline 71-4)
Route of exposure	Flow-through	Flow-through	Flow-through	Static	diet	diet
Exposure duration	F0 114 dph F1 60 dph	95 days (61 dph)	21 days	1 hr	21 weeks	22 weeks
Animal species/strain	Japanese medaka (*Oryzias latipes)*	Rainbow trout (*Oncorhynchus mykiss)*	Rainbow trout (*Oncorhynchus mykiss)*	Australian crimson-spotted rainbowfish (*Melanotaenia duboulayi)*	Mallard duck *(Anas platyrhynchos)*	Northern Bobwhite Quail *(Colinus virginianus)*
No. animals per sex per group	F0: 4 replicates of 20 eggs and 9-15 fry and 8 adult breeding pairs; F1: 4 replicates of 20 eggs and 15 fry	4 replicates of 35 eggs & 15 fry	20 fry	adult: 3 replicates of 5/sex juvenile: 10	16/sex	16/sex
Test material purity	98.7%	97.2%	97.2%	2%	95.3%	95.3%
Dose levels	0.84, 2.7, and 8.6 µg/L (measured)	1.8, 4.3, 6.7, 14, and 26 µg/L (measured)	11, 21, 46, 88, and 180 µg/L (measured)	8 µg/L (adult); 10 and 100 µg/L (juvenile)	0, 120, 360, 600 ppm	0, 120, 360, 600 ppm
Doses (mg/kg/day)	NA	NA	NA	NA	NA	NA
NOAEL/NOEC/Effect	NOEC 8.6 µg/L	NOEC 4.3 µg/L LOEC 6.7 µg/L MATC 5.4 µg/L	LC50 90 µg/L NOEC 21 µg /L LOEC 46 µg/L	NOAEC 8 µg/L (adult) NOAEC 100 µg/L (juvenile)	600 ppm	600 ppm

Tier 1 Ecological Screening Evaluation: PYRIPROXYFEN

	Fish Studies				Avian Reproduction	
	Gries (2007) Full life cycle toxicity test with Medaka under flow-through conditions	**Rhodes and Cramer (1991) Early life-stage toxicity of Sumilarv technical to the Rainbow Trout under flow-through conditions**	**Sword and Northrup (1992) 21-Day flow-through toxicity of pyriproxyfen to Rainbow Trout**	**Brown et al. (2002) Pulse-exposure effects of selected insecticides to juvenile Crimson-Spottted Rainbowfish**	**Beavers et al. (1994a) Reproduction study with the Mallard**	**Beavers et al. (1994b) Reproduction study with the Bobwhite Quail**
Mortality, daily (F0)	♂♀–	–	↑ at 46, 88 and 180 µg/L	– (adult and juvenile)	–	–
Mortality (F1)	♂♀–				–	–
Daily observations:						
Feed consumption			↓ at 88 and 180 µg/L (subjective)		F0 –	F0 –
Abnormal behavior	F0 ♂♀–; F1 ♂♀–	↑ at 6.7, 14, and 26 µg/L	↑ at 46, 88 and 180 µg/L		F0 –	F0 –
Gross malformations/lesions	F0 ♂♀–; F1 ♂♀–					
External abnormalities	F0 ♂♀–; F1 ♂♀–				F0 –	F0 –
Egg production (fecundity)	F0 –				–	–
Body weight	F0 ♂♀–; F1 ♂♀–	↓ at 6.7, 14, and 26 µg/L on 61 dph	↓ at 180 µg/L		F0 –	F0 –
Gonadosomatic index (GSI)	F0 ♂↑at 2.7 µg/L at 60 dph, ♀–;♂♀ – 114 dph; F1 ♂♀–					
Reproductive parameters (fertility, hatching)						
Hatching success	F0 – F1 ↓ (difference was artifact on 1 of 3 days measured)	–			F1 —	F1 —
Fertilitization success	F0 ↓ at 2.7 µg/L				F0 –	F0 –
Body length (F0)	♂↑at 0.84 µg/L at 60 dph (length) ♀–; ♂♀ – 114 dph	↓ at 6.7, 14, and 26 µg/L 35 dph (length)	↓ at 180 µg/L			
Body length (F1)	♂ ↓ at 2.7 µg/L at 60 dph; ♀—					

Tier 1 Ecological Screening Evaluation: PYRIPROXYFEN

	Fish Studies				Avian Reproduction	
	Gries (2007) Full life cycle toxicity test with Medaka under flow-through conditions	**Rhodes and Cramer (1991) Early life-stage toxicity of Sumilarv technical to the Rainbow Trout under flow-through conditions**	**Sword and Northrup (1992) 21-Day flow-through toxicity of pyriproxyfen to Rainbow Trout**	**Brown et al. (2002) Pulse-exposure effects of selected insecticides to juvenile Crimson-Spottted Rainbowfish**	**Beavers et al. (1994a) Reproduction study with the Mallard**	**Beavers et al. (1994b) Reproduction study with the Bobwhite Quail**
Secondary sexual characteristics (terminal)	F0 change at 60 dph, — 114 dph; F1 — (fin morphology)					
Vitellogenin (VTG)	*F0 ↑♂ at 0.84 and* 8.6 µg/L 60 dph within control range, — 114 dph; F1 — (hepatic)					
17B-Estradiol concentrations						
Testosterone concentrations						
Ovary size (R&L)					F0 —	F0 —
Ovary wt. (R&L)	F0 F1 —					
Ovarian histopathology	F0 F1 —					
Testes size (R&L)					F0 —	F0 —
Testes wt. (R&L)	F0 ↓ at 0.84 µg/L 60 dph, — at 114 dph; F1 —					
Testes histopathology	F0 F1 —					
Gross pathology (avian)					F0 —	F0 —
Chick wt. gain					—	—
Chick survival					—	—
Hepato-somatic index (HSI%)	F0 ♀↓ at 0.84 µg/L at 60 dph, ♂—, — at 114 dph; F1 ♀ ↑at 0.84 µg/L at 60 dph, ♂—					

Appendix IV. Review of Published Literature on Pyriproxyfen Relevant to Evaluation of Potential Endocrine Effects

Pyriproxyfen has been examined for activity at the estrogen receptor in three published *in vitro* studies and at a variety of endocrine-related receptors as part of the EPA ToxCast™ Program. Descriptions of these studies, their results as they relate to pyriproxyfen and how closely the described assays correspond to those required in EDSP Tier 1 screening can be found below.

I. Published *in vitro* studies

a. Kojima et al., 2005a

This study specifically addresses one of the data requirements of Tier 1 screening (ER transactivation). Thirty-two pesticides, including pyriproxyfen (purity of 99%), were tested for the ability to transactivate or antagonize the human estrogen receptor (ER) using the E-CALUX assay. This assay system uses a human ovarian carcinoma cell line, BG1, which expresses the human ER and has been stably transfected with a luciferase reporter plasmid containing estrogen response elements upstream of the luciferase gene to assess the estrogenic/antiestrogenic activity of test compounds. The cells were plated in 96-well plates at a density of 2×10^5 cells/well in RPMI 1640 medium containing 5% carbon-stripped fetal calf serum (FCS) with penicillin/streptomycin and L-glutamine. Twenty-four hours later, the cells were treated with 0.7-367 pM estradiol (E_2) or 19.5-10,000 nM of test chemical for an additional 24 hours. To test for antagonist activity, the test chemicals were run in the presence of 10^{-11} M E_2. After incubation, the cells were lysed and luciferase activity was measured using a luminometer.

The EC_{10} for E_2 was reported to be 3.2×10^{-12} M; however, an EC_{50} could not be calculated because, "a plateau in the reporter gene activity was not reached." Consequently, it is possible that the true EC_{10} is higher than that reported in the study. The concentration of pyriproxyfen reported as equivalent to the E_2 EC_{10} was 2.9×10^{-5} M, which is considerably higher than tissue concentrations that could be achieved from *in vivo* exposures. These data are based on five replicate runs of the assay. Pyriproxyfen was not reported to demonstrate antagonist activity.

This study was conducted using a different model system and a slightly narrow concentration range of test chemicals than that specified in the ER transcriptional activation guidelines (EPA, 2009a). Further, the number of replicates of assays was not reported. It is also not known if the solubility and/or cytotoxicity of the test chemicals were evaluated. Nevertheless, this study went further than required in the EDSP Tier 1 guidelines to show a lack of antagonist activity at the human ER. In summary, pyriproxyfen was weakly estrogenic at concentrations not anticipated to be reached *in vivo* and negative for anti-estrogenic activity.

b. Kojima et al., 2005b

The main purpose of this study was to evaluate the mRNA and protein expression patterns for ERα and ERß in the human ovarian carcinoma (BG1) cells of the E-CALUX assay system. This assay system is used to assess the estrogenic activity of test compounds, as described above in the summary for Kojima et al. (2005a). Using real-time PCR and immunohistochemistry, BG1 cells were demonstrated to express both ER receptor types, but the expression of ERα appeared to be many times that of ERß, suggesting that the estrogenic activity detected using the E-CALUX system was most likely dominated by ERα. In further experiments, the estrogenic activity of three pesticides, including pyriproxyfen (purity not reported), was evaluated. BG1 cells were plated in 96-well plates at a density of 2×10^5 M in RPMI 1640 medium (supplements to the medium were not reported). Twenty-four hours later, the cells were treated with 10^{-11} M E_2 and/or 5×10^{-5} M of test chemical. After 24 hours, the cells were lysed and luciferase activity measured using a luminometer. E_2 induced luciferase activity equal to 4,653 relative light units (RLU), pyriproxyfen alone induced activity equal to approximately 2,700 RLU, and E_2 and pyriproxyfen combined induced activity equal to approximately 13,000 RLU. It should be noted that the concentration of pyriproxyfen tested is substantially greater than that which could be achieved from *in vivo* exposure.

This study was conducted using a different model system than specified in the ER transcriptional activation test guidelines (EPA, 2009a) and both E_2 and pyriproxyfen were tested at one concentration only. Further, many experimental details are missing from the study report. Thus, it is not known if the RPMI medium was supplemented with carbon-stripped fetal bovine serum, if the solubility and/or cytotoxicity of the test chemical were evaluated, or what were the number of sample replicates and assay runs used. Consequently, this study provides supplementary information only regarding the possible estrogenic activity of pyriproxyfen.

c. Manabe et al., 2006

The estrogenic activity of six pesticides, including pyriproxyfen (purity 99%), was assessed based on the proliferative response of the rat pituitary tumor cell line, MtT/Se. These cells were first evaluated for ERα and ERß expression using real-time PCR and immunohistochemistry. These investigations showed that MtT/Se cells express both receptor types, but that ERα is expressed more abundantly, suggesting that the proliferative response to estrogen treatment is primarily mediated by ERα. In further studies, MtT/Se cells were plated in 96-well plates at a density of 2×10^3 cells/well in phenol red-free DMEM medium containing 5% charcoal dextran-treated fetal bovine serum and 10% horse serum. From the methods description, incubation times are not clear; however, it appears that, 24 hours after plating, the cells were incubated with E_2 or test chemicals (concentrations of $10^{-13} - 10^{-3}$ M) for an additional 3 days (72 hours), after which cell viability and proliferation were assessed. Compounds that induced cell proliferation at a level that was equivalent or greater than 10% of that induced by E_2 were considered to be estrogenic and the 10% relative effective concentration, or REC_{10}, was reported. Reported results

were the mean of three triplicate assays. Pesticide mixtures, including the combination of prothiofos and pyriproxyfen, were also tested. The threshold E_2 concentration for induction of cell proliferation was 10^{-13} M, and proliferation showed a plateau at 10^{-10} M E_2. The REC_{10} concentrations of pyriproxyfen and prothiofos + pyriproxyfen were reported to be 5.5×10^{-5} M and 8.6×10^{-5} M, respectively. It should be noted that these concentrations are substantially greater than those which could be achieved from *in vivo* exposures. Results of cell viability evaluations were not reported.

This study assesses estrogenic activity based on the proliferative response of MtT/Se cells. This endpoint is substantially different and less specific than those assessed in the EDSP Tier 1 *in vitro* screens. Nevertheless, this study provides some information regarding the potential estrogenicity of pyriproxyfen.

The following table provides a comparison of the two Kojima et al. studies discussed above with the EDSP Tier 1 screening guidelines for the ER transactivation assay (Table IV-1).

The Manabe et al. study differs markedly from the Tier 1 screening assay and no comparison of methods was attempted.

Table IV-1: Published *In Vitro* ER Transactivation Studies of Pyriproxyfen Referenced as OSRI: Comparison to EDSP Screening Guidelines[1]

Assay Parameters	Guideline Requirements	Kojima et al., 2005a[2]	Kojima et al., 2005b
Model System			
Cell line	hERα-HeLa-9903 (stably transfected)	BG1 cells (stably transfected)	
Estrogen receptor	Human ERα	Human ER	
Reporter construct	Luciferase gene w/ vitellogenin ERE	Luciferase gene w/ upstream ERE	
Characterization	Mycoplasma-free; passaged <40 X	Unknown	
Culturing Conditions			
Media	EMEM w/o phenol red	RPMI (phenol red status unknown)	
Antibiotic	60 mg/L Kanamycin	1% penicillin-streptomycin	Penicillin-streptomycin
FBS	10% dextran-coated-charcoal-treated FBS	5% carbon-stripped FCS	8% FBS
Plating vessel	Plastic micropl. free of estrogenicity	96-well plate	
Plating density	1 x 10^4 / 100 µL/ well	200,000 cells/well	
CO_2 concentration	5%	5%	
Temperature	37°C	37°C	
Pre-incubation time	3 hrs	24 hrs	
Assay Conditions			
Maximum test conc.	1 µl or mg/ml or 1 mM	10 µM	50 µM
Dilutions	Log serial dilutions (7 conc.)	19.5 – 10,000 nM (exact conc. unknown)	None
No. wells per test conc.	3 (run in triplicate)	5	Unknown
Test limits	Insolubility, cytotoxicity	Unknown	
Cytotoxicity cut-off	80%	Unknown	
Final test volume	150 µl	Unknown	
Incubation period	20-24 hrs	24 hrs	
No. of replicates	2-3	Unknown	Unknown

Table IV-1 (continued): Published *In Vitro* ER Transactivation Studies of Pyriproxyfen Referenced as OSRI: *Comparison to EDSP Screening Guidelines*[1]

Assay Parameters	Guideline Requirements	Kojima et al., 2005a[2]	Kojima et al., 2005b
Data Analysis			
For all chemicals	Report RPC_{Max}, PC_{Max}	No	No
For all positive chemicals	Report PC_{10}, PC_{50} (if appropriate)	Yes	No
Data Interpretation			
Positive finding	$RPC_{Max} \geq PC_{10}$ in 2/2 or 2/3 replicate runs.	Yes	Data not reported in this format.
Negative finding	$RPC_{Max} < PC_{10}$ in 2/2 or 2/3 replicate runs.	No	
Reference Chemicals			
Positive control	17ß-estradiol (10^{-14} - 10^{-8} M)	Yes	Yes
Weak estrogen	17α-estradiol (10^{-12} - 10^{-6} M)	No	No
Very weak agonist	17α-methyltestosterone (10^{-10} - 10^{-4} M)	No	No
Negative control	Corticosterone (10^{-11} - 10^{-5} M)	No	No
Controls			
Positive control	1 nM 17ß-estradiol (run in triplicate)	0.7-367 pM 17ß-estradiol	0.01 nM 17ß-estradiol
Vehicle control	DMSO (preferred; H_2O & EtOH acceptable)	DMSO	DMSO

1 Boxes highlighted in yellow indicate where the study does not match the Tier 1 Guideline or the data is not available from the study report.

2 Also tested for antagonist activity at ER in the presence of 10^{-11} M estradiol.

II. *In Vivo* Mammalian Studies

No published mammalian *in vivo* studies of pyriproxyfen were identified.

III. *In Vivo* Ecotoxicological Studies

Frogs

There are no studies of the effects of pyriproxyfen on thyroid function or metamorphosis in frogs or other amphibians.

Fish

There is a single study in the published literature about pyriproxyfen effects to fish. Brown et al. (2002) conducted laboratory toxicity studies to determine the acute lethal effects of a 1-hour pulse exposure of selected insecticides, including pyriproxyfen (as formulated product), on crimson-spotted rainbowfish (*Melanotaenia duboulayi*), a species native to the Australia-Papua New Guinea region. Laboratory bred adult and larval (< 72-hour post hatch) rainbowfish were exposed to Sumilarv (2% a.i. pyriproxyfen) for 1 hour. After the 1-hour pulse exposure, the fish were moved into tanks with clean water and the number that survived to 24-hours was measured. Ten adult fish (5 males and 5 females) in each of three replicates were exposed to the expected environmental concentration (EEC) of 8 µg /L. Larvae were exposed to 10 and 100 µg/L, with four replicates of 10 fish at each concentration. None of the adult fish died, and neither of the test concentrations was toxic to the larval fish. This study has significant short-comings that make it unsuitable for use as a screen for potential endocrine effects of pyriproxyfen. Specifically, the test article was used as a formulated product, not the active ingredient, only a single pulsed exposure dose was used, and survival was the only endpoint measured. Because of the identified deficiencies, this study is not cited as OSRI.

Appendix V. ToxCast™ Studies of Pyriproxyfen

ToxCast™ is a US EPA program that includes a battery of *in vitro*, high-throughput screening (HTS) assays to screen chemicals for potential human toxicity. Over 300 chemicals have undergone HTS under the ToxCast™ program, the majority of these being pesticides. A number of these chemicals, including pyriproxyfen, have current requirements for Tier 1 screening.

Potential redundancies exist between some of the ToxCast™ assays and the Tier 1 screening objectives; thus, ToxCast™ data provide useful OSRI (Other Scientifically Relevant Information) in lieu of or to supplement Tier 1 required tests. Information regarding the ToxCast™ Program results became generally available to the public through *Environmental Health Perspectives* (EHP) and the EPA ToxCast™ Program website (www.epa.gov/ncct/toxcast/ index.html) in mid-December 2009, with the raw data for many of the assays being posted on the ToxCast™ website in mid-February 2010. The data presentation in the EHP article (Judson et al., 2009) and supplementary materials and on the ToxCast™ Program website is fairly opaque, with individual chemical data tabulated and much of the detail on assay methodologies provided only through website links.

This review was developed to evaluate the data most relevant to potential endocrine modulation and the results specific to pyriproxyfen. This review describes briefly the HTS assay technologies incorporated in ToxCast™ relevant to the EDSP Tier 1 screening requirements, provides an overview of the HTS assays most relevant to potential estrogen/androgen/thyroid hormone (EAT) endocrine modulation, discusses the EDSP Tier 1 screening assays that could be potentially replaced, and provides a specific review of pyriproxyfen results based on the data available from EPA.

a. ToxCast™ HTS Test Battery and Relevance to Potential Endocrine Modulation

The ToxCast™ HTS test battery includes nine separate *in vitro* assay technologies:

- Cell-free HTS assays to measure binding constants and enzyme inhibition values;
- Cell-based HTS assays to measure binding constants and enzyme inhibition values;
- High content cell-imaging assays to test for effects on a range of phenotypes in either the human hepatoma cell line HepG2 or rat primary hepatocytes;
- Gene expression in human primary hepatocytes, focused on Phase I and II metabolic enzymes and transporters;
- Multiplex transcription reporter assays to evaluate 48 transcription factor binding sites;
- Biologically multiplexed activity profiling (BioMAP) to characterize effects potentially relevant to human tissue and inflammatory disease biology;
- Phase I & II xenobiotic metabolizing enzyme (XME) cytotoxicity assays based on microarray technology;

- High-throughput genotoxicity screening based on the GreenScreen high content *GADD45A-GFP* HTS genotoxicity assay; and
- Real-time cell electronic sensing (RT-CES) to measure cellular changes in response to chemicals.

Of these assay technologies, those that provide information most directly relevant to the issue of potential EAT endocrine modulation include the cell-free HTS assays, the cell-based HTS assays, and the multiplex transcription reporter assays. Each of these assays and their data relevant to potential endocrine modulation is described below.

1. Cell-Free HTS Assays

These assays were run under contract to EPA by Caliper Life Sciences (formally NovaScreen) and methods information was confirmed through personal communications with Arthur Weissman and David Manyak, both of Caliper Life Sciences. Of the 239 endpoints examined, binding to the following receptor proteins relevant to the endocrine system was evaluated:

- Bovine estrogen receptor from uterine membrane (bER);
- Human estrogen receptor from breast cancer cells (hER);
- Bovine progesterone receptor from uterine membrane (bPR);
- Human progesterone receptor from T47-D[1] cells (hPR);
- Rat androgen receptor (rAR; recombinant);
- Human androgen receptor from LnCAP cells (hAR);
- Rat thyrotropin-releasing hormone receptor from forebrain membranes (rTRH); and
- Human thyroid hormone receptor-alpha (hTRa; recombinant).

All of these proteins are nuclear receptors, with the exception of the rat thyrotropin-releasing hormone receptor (rTRH), which is a G-protein coupled receptor. Additionally, all of these, with the exception of the hTRa, were evaluated in competitive binding assays that measured the displacement of radiolabeled ligand from the receptor proteins. The hTRa assay, in contrast, examined receptor activation by measuring the luminescent output from an hTRa-responsive reporter gene. Inhibition of the aromatase enzyme (CYP19A1 or CYPC19) was also evaluated. Assay parameters specific to each of these endpoints are detailed in Table 2.

[1] EPA indicated that the human progesterone receptor was derived from MCF-7 cells; however, according to Caliper Life Sciences, the receptor protein was derived from T-47D cells.

Table 2: Parameter Specifics Regarding Endocrine-Related Endpoints of the Cell-Free HTS Assays

Receptor/enzyme		Assay type	Reference compound	Positive control compound	Ligand (conc., M)	Kd (M)	Bmax (fmol/mg protein)
Estrogen receptor	bER	Competitive binding	N/A[a]	17ß-estradiol	^{3}H-estradiol (7×10^{-10})	7×10^{-10}	406
	hER	Competitive binding	17ß-estradiol	17ß-estradiol	^{3}H-estradiol (1×10^{-10})	5×10^{-11}	3,844
Progest-erone receptor	bPR	Competitive binding	promegestone	promegestone	^{3}H-promegestone (7×10^{-10})	6×10^{-9}	152
	hPR	Competitive binding	promegestone	promegestone	^{3}H-promegestone (7×10^{-10})[b]	3×10^{-10}	152
Androgen receptor	rAR	Competitive binding	methyl-trienolone	methyl-trienolone	^{3}H-methyl-trienolone (1×10^{-9})	4.6×10^{-9}	103,928
	hAR	Competitive binding	methyl-trienolone	methyl-trienolone	^{3}H-methyl-trienolone (5×10^{-10})	3×10^{-10}	270
Thyroid related	rTRH	Competitive binding	thyroid releasing hormone (TRH)	TRH	^{3}H-3-meHis$_2$-TRH (2×10^{-9})	2.3×10^{-9}	34
	hTRa[c]	Receptor activation	bisphenol-A	N/A	triiodothyronine (T_3) (1.5×10^{-8})	3.5×10^{-9}	N/A
Aromatase	hCYP C19	Enzyme inhibition	ketoconazole	N/A	di(benzyloxy-methoxy) fluorescein[d] (2.5×10^{-6})	N/A	N/A

[a] N/A = not applicable.

[b] EPA lists the ligand concentration as 3×10^{-10} M; however, according to Caliper Life Sciences, the ligand concentration was 7×10^{-10} M.

[c] Assay of antagonism to ligand-dependent binding of co-factor to the receptor.

[d] Aromatase substrate binding affinity = 4.4×10^{-6} M and vmax = 0.03 pmol product/pmol CYPC19/min.

In all cases, the data were normalized to those of the solvent controls from the same plate as the tested chemicals and results expressed as the percent of neutral control. For the parameters above, chemicals were initially screened at a single concentration of 25 µM (10 µM for aromatase) in duplicate wells. Those chemicals that were positive in the initial screen were then run using eight concentrations in the range of 0.0229-50 µM (0.00914-20 µM for aromatase) to determine the AC_{50} (i.e., the half maximal activity concentration).

2. Cell-Based HTS Assays

These assays were conducted by the NIH Chemical Genomics Center (NCGC). Both agonist and antagonist activity at various nuclear receptors, including the human androgen receptor (hAR), human ERα (hERα), and human thyroid hormone receptor-β (hTRβ), were examined using a reporter gene assay system (misidentified in EPA documents as the " Invitrogen™ GeneGLAzer;" actually the" Invitrogen™ GeneBLAzer® " reporter assay system).

According to the Invitrogen™ website (http://www.invitrogen.com/), the GeneBLAzer® technology uses a beta-lactamase reporter system, but specifics about how the system works are not provided. The nuclear receptor sources and the assay parameters related to each of the examined endpoints, as provided by the EPA, are detailed in Table 3.

Table 3: Parameter Specifics Regarding Endocrine-Related Endpoints of the Cell-Based HTS Assays

	Activity	Target source	Reference compound	Positive control compound	Ligand (conc., M)
Androgen related	hAR agonist	HEK293H cells	R1881	N/A[a]	N/A
	hAR antagonist	HEK293H cells	N/A	N/A	R1881 (1×10^{-8})
Estrogen related	hERα agonist	HEK293H cells	17β-estradiol	N/A	N/A
	hERα antagonist	HEK293H cells	4-hydroxy-tamoxifen	4-hydroxy-tamoxifen	17β-estradiol (5×10^{-10})
Thyroid related	hTRβ agonist	HEK293T cells	triiodothyronine (T_3)	N/A	N/A
	hTRβ antagonist	HEK293T cells	N/A	N/A	T_3 (4×10^{-10})

[a] N/A = not applicable.

Exact concentrations of chemicals tested in this assay system are not clear. A recent publication indicates that chemicals were tested at fifteen concentrations in the range of 0.0012-92 μM (Judson et al., 2009). However, the summary data file for these assays provided on the ToxCast™ website (http://www.epa.gov/ncct/toxcast/index.html) indicates that chemicals were tested to a maximum concentration of 200 μM. In the case of pyriproxyfen, concentrations in the range of 0.0010 – 76.6 μM were tested. A minimum of 25% of the control efficacy was used as the cut-off for considering a chemical to be active. The supplemental information to Judson et al. (2009) indicates that, "values where only the highest concentration exceeded 50% activity were excluded to eliminate weak or false positives." Cell viability was also evaluated in this assay system; however, the criteria for excluding concentrations to be tested in the assay system based on cytotoxicity are not clear.

3. Multiplex Transcription Reporter Assays

These assays were performed by Attagene, Inc. using a multiple reporter transcription unit (MRTU) library consisting of 48 transcription factor binding sites transfected into HepG2 cells (Martin et al., 2010). Cis-acting reporter genes examined the activities of transcription factors endogenous to the HepG2 cells. Trans genes evaluated the activities of exogenous, chimeric proteins. Each chimera was comprised of the ligand-binding domain of a nuclear receptor and a yeast Gal4 DNA-binding domain. When activated, these chimeric proteins activated transcription of a reporter gene expressed downstream of a 5X-UAS-TATA promoter. In all cases, the reporter gene was identical in sequence and included a restriction enzyme cleavage site; the position of the restriction site, however, was unique to each reporter gene. The system allowed for reporter gene products to be identified through sequencing and separation via high resolution electrophoresis. Using this system, the following endpoints relevant to endocrine modulation were evaluated (listed in Table 4, along with their respective reference compounds, as provided in EPA materials).

Table 4: Endocrine-Related Endpoints and Reference Compounds Tested in the Multiplex Transcription Reporter Assays

	Endpoint	Reference Compounds
Androgen related	Human androgen receptor	dioxin, FIC
Estrogen related	Human ER-α	17β-estradiol, diethylstilbestrol
	Human estrogen response element[a]	estrogens
	Human estrogen-related receptor-α	N/A[b]
	Human estrogen-related receptor-γ	4-hydroxytamoxifen
Thyroid related	Human thyroid hormone receptor-α	triiodothyronine (T_3)

[a] The human estrogen response element was tested as a cis element; all other endpoints relevant to endocrine modulation were tested as trans factors.
[b] Not applicable.

Cytotoxicity was first determined using the MTT tetrazolium assay and 24-hour chemical exposure to five test concentrations, with an upper concentration of 50 µM and 10-fold serial dilutions. The maximum tolerated concentration (MTC) was derived as one-third the cytotoxic IC_{50}, or if no IC_{50} value was determined, then the MTC was set at 100 µM. Test compounds were then assayed at seven different concentrations in the range of 0.0014-100 µM, starting at the MTC, followed by three-fold serial dilutions. Pyriproxyfen, specifically, was tested at concentrations of 0.129-100 µM. After a 24-hour exposure, cells were harvested, reporter gene products isolated and reverse-transcribed using fluorescent-tagged primers, and resolved by high resolution gel electrophoresis. From the resulting data, the lowest effective concentration that activated a cis-acting response element or transcription factor was determined (LEL; lowest concentration at which there was a statistically significant change from the concurrent negative control).

b. Comparison to Tier 1 *In Vitro* Screening Requirements

The ToxCast™ data supplement, and in some cases, may fulfill the *in vitro* data requirements of the EDSP Tier 1 ER binding assay, AR binding assay, ER transactivation assay and aromatase inhibition assay. In addition, these data provide important OSRI that should be considered in a weight-of–the-evidence evaluation of functionally equivalent information supporting the absence of need for many of the Tier 1 mammalian *in vivo* screening tests.

ER binding assay: The EDSP Tier 1 ER binding assay requires that binding be evaluated using rat ER derived from uterine tissue taken from ovariectomized Sprague Dawley rats (EPA, 2009b). In the ToxCast™ cell-free HTS assays, binding to bovine and human, but not rat, ER was evaluated. In the Tier 1 screening assay, the concentration of ^{3}H-17β-estradiol ligand used in the assay is 1 nM and the test chemicals are evaluated at concentrations of 1×10^{-10} to 1×10^{-3} M (EPA, 2009c). In the ToxCast™ assay, the concentration of radiolabeled estradiol varied depending on the ER source (bER: 7×10^{-10} M; hER: 1×10^{-10} M), but was generally similar to that recommended in the EDSP Tier 1 ER binding assay; however, compounds were tested at an initial concentration of 2.5×10^{-5} M, and if positive for binding activity, were further evaluated at concentrations of $2.3 \times 10^{-8} - 5 \times 10^{-5}$ M. This concentration range is narrower than the range recommended in the Tier 1 screening assay, but still should be adequate to detect activity in a moderate concentration range.

Androgen receptor binding assay: The EDSP Tier 1 screen AR binding assay requires that binding be evaluated using rat AR derived from prostate tissue taken from castrated Sprague Dawley rats (EPA, 2009c). The concentration of ^{3}H-R1881 ligand used in the assay is 1 nM and the test chemicals are evaluated at concentrations of 1×10^{-9} to 1×10^{-4} M. In the ToxCast™ cell-free HTS assay, binding to both rat and human androgen receptors was evaluated, so it provides a more comprehensive screen than does the Tier 1 battery. Radiolabeled R1881 was used as the radioligand in this assay and concentrations varied depending on the AR source (rAR: 4.59×10^{-9} M; hAR: 3×10^{-10} M), but were similar to that recommended in the EDSP Tier 1 AR binding assay. Finally, compounds were tested in the ToxCast™ cell-free HTS assay at an initial concentration of 2.5×10^{-5} M, and if positive for binding activity, further evaluated at concentrations of $2.3 \times 10^{-8} - 5 \times 10^{-5}$ M. This concentration range is slightly narrower that that recommended in the EDSP Tier 1 screen AR binding assay guideline ($1 \times 10^{-9} - 1 \times 10^{-4}$ M; EPA, 2009b), but still should be adequate to detect activity in a moderate concentration range.

ER transactivation assay: The EDSP Tier 1 screen ER transactivation assay requires that the assay be conducted in hER-HELA-9903 cells, which express the human ER-α and have been stably transfected with a luciferase reporter gene linked to an upstream vitellogenin estrogen-responsive element. Test compounds are evaluated at concentrations of 1×10^{-9} to 1×10^{-3} M. Finally, a test chemical is considered positive for ER transactivation in the Tier 1 assay if the maximum response induced is at least 10% of that induced by 1 nM 17β-estradiol (EPA, 2009a).

In the case of the ToxCast™ cell-based HTS assays, details regarding the gene reporter system were not available; however, activity at the human ER-α was assessed and test chemicals were evaluated at a similar range of concentrations ($1.2 \times 10^{-9} - 0.9 \times 10^{-4}$ M) as recommended in the EDSP Tier 1 screen. The data outputs from the ToxCast™ cell-based HTS assays (AC_{50} values) are different than those required in Tier 1 screening, and in most cases, positive control compounds were not tested. However, if a chemical elicited a low level of activity at the highest concentration tested in the cell-based assay, it can be assumed that it would be negative according to the EDSP Tier 1 screening criteria. It should be noted that the ToxCast™ program's cell-based HTS assay provides more robust data than that required in the EDSP Tier 1 ER transactivation assay because it also examined the test chemical's ability to act as an antagonist at the ER-α.

The multiplex transcription reporter assay also examined transactivation of the human ER-α. In this case, the assay is conducted in HepG2 cells (instead of HELA-9903 cells), and the output from the reporter gene and how it is measured is different from that indicated in the EDSP Tier 1 screening assay. HepG2 is a perpetual cell line, which was derived from the liver tissue of a 15-year old Caucasian American male with a well differentiated hepatocellular carcinoma. The HepG2 cells and its derivatives are often used as a model system for studies of liver metabolism and xenobiotics toxicity because of their metabolic capabilities. The range of test chemical concentrations tested ($1.4 \times 10^{-9} - 1 \times 10^{-4}$ M) is similar to that recommended in the EDSP Tier 1 screen. Finally, as with the ToxCast™ cell-based HTS assay, the data outputs (LEL values) are different than those required in EDSP Tier 1 screening, and positive control compounds were not tested. However, if a chemical elicited a low level of activity at the highest concentration tested in the multiplex transcription reporter assay, it can be assumed that it would be negative according to the EDSP Tier 1 screening criteria.

Aromatase assay: The EDSP Tier 1 screen aromatase assay requires that enzyme activity be evaluated using human recombinant microsomes containing CYP19 plus reductase. Radio-labeled androst-4-ene-3,17-dione at a concentration of 1×10^{-8} M is used as the enzyme substrate and test compounds are evaluated at concentrations of 1×10^{-10} M - 1×10^{-3} M (EPA. 2009d). In the ToxCast™ cell-free HTS assay, human aromatase activity was evaluated using di(benzyloxymethoxy)fluorescein, which produces a fluorescent product as the enzyme substrate. Compounds were initially screened at a single concentration of 1×10^{-5} M, and those chemicals that were positive in the initial screen were then run using eight concentrations in the range of 9.14×10^{-9} M – 2×10^{-5} M. This concentration range is narrower than the range recommended in the Tier 1 screening assay, but still adequate to detect activity in a moderate concentration range.

It should be noted that the ToxCast™ HTS data set includes information that should be useful to submit as OSRI, and in fact, goes beyond the *in vitro* testing required in the Tier 1 screening program to provide additional information on the potential to interact with the EAT systems, including thyroid-related receptors, as well as progesterone receptors. It does not, however, directly address Tier 1 steroidogenesis *in vitro* test requirements.

c. Data Specific to Pyriproxyfen

Pyriproxyfen of 90% or greater purity was tested in all ToxCast™ assays.

In the cell-free HTS assays, pyriproxyfen was tested at eight concentrations in the range of 0.02-50 µM at all receptor proteins of relevance to potential endocrine modulation, except the rat thyrotropin-releasing hormone receptor (rTRH). In the raw data files provided by EPA, no rTRH data for pyriproxyfen are listed, suggesting that either pyriproxyfen was not tested for binding at this receptor protein, or more likely, that pyriproxyfen did not provide a positive binding signal at the initial test concentration of 25 µM and was not further tested; however, this cannot be confirmed from the available data. At the other seven receptor proteins, inhibition of radio-ligand binding was less than 50% at all pyriproxyfen concentrations tested. This includes the bovine and human estrogen receptors; bovine and human progesterone receptors; rat and human androgen receptors; and human thyroid hormone receptor-α. Additionally, pyriproxyfen tested at eight concentrations in the range of 0.00914-20 µM exhibited less than 50% inhibition of human aromatase enzyme activity, and is considered negative for inhibition of aromatase.

In the cell-based HTS assays, pyriproxyfen was tested at 15 concentrations in the range of 0.0010-76.6 µM (two replicates at each concentration). Under these conditions, pyriproxyfen was considered inactive for agonist activity at the human AR, human ER-α, and the human thyroid hormone receptor-β. Furthermore, it did not block (or antagonize) the activity of established ligands at these receptor sites.

In the multiplex transcription reporter assay, pyriproxyfen did not activate chimeric transcriptional proteins containing ligand-binding domains for the human AR, human estrogen-related receptor-α, human estrogen-related receptor-γ, or human thyroid hormone receptor-α. Furthermore, pyriproxyfen did not activate transcription at a human estrogen response element. However, pyriproxyfen activated a chimeric transcriptional protein containing the ligand-binding domain of the human ERα, with an LEL of 51 µM. This value is similar to the EC_{10} values for pyriproxyfen reported in Kojima et al. (2005a) and Manabe et al. (2006).

Overall, the ToxCast™ data for pyriproxyfen related to endocrine-associated endpoints is primarily negative. Pyriproxyfen did not bind to human or bovine progesterone receptors; did not bind to, activate or antagonize human or rat thyroid-related receptors; and did not inhibit aromatase activity. Pyriproxyfen also did not bind to, activate or antagonize the human or rat androgen receptor or a chimeric transcription factor containing the ligand-binding site for the human androgen receptor.

Results regarding activity at the estrogen receptor, however, are conflicting, which makes their interpretation difficult. While pyriproxyfen did not bind to bovine or human estrogen receptors in the cell-free HTS assay or act as an agonist or antagonist at the human estrogen receptor-α in the cell-based HTS assay, it activated a chimeric transcription factor containing the ligand binding domain of the human estrogen receptor-α in the multiplex assay. The nature of the data reported in the cell-free and

cell-based assays (AC_{50} values) is different from that reported for the multiplex assay (LEL values), suggesting that the multiplex assay may be more sensitive. Alternatively, it is possible that the positive results from the multiplex assay are an anomaly. However, the LEL value reported in the multiplex assay for activation of the chimeric ERα protein is similar to the EC_{10} values reported for pyriproxyfen in two recent studies of estrogenic activity (Kojima et al., 2005a; Manabe et al., 2006), suggesting that pyriproxyfen may possess weak estrogenic activity, albeit only at extremely high concentrations that would not be achievable following *in vivo* exposures.

These data are considered to fulfill the data requirements of the EDSP Tier I estrogen receptor and androgen receptor binding assays. It is suggested estrogen receptor transcriptional activity is negative with the exception of weak activity at unanticipated concentration *in vivo*. It appears that pyriproxyfen is negative for aromatase inhibition; however, the aromatase assay will be repeated using the Tier 1 guideline because the available ToxCast™ documentation of the assay methodology is considered limited.

Appendix VI. Cover Pages for Regulatory Studies submitted as OSRI

MRID 41321716

NNT-91-0045
9400023-6

HAZLETON
LABORATORIES AMERICA, INC.
9200 LEESBURG TURNPIKE, VIENNA, VIRGINIA 22180, U.S.A.

Sponsor:

Sumitomo Chemical Co., Ltd.
Kitahama, 4-Chome 5-33
Chuo-Ku, Osaka 541, Japan

FINAL REPORT

Study Title:

Subchronic Toxicity Study with S-31183 in Rats

Data Requirement:

EPA Guideline 82-1

Author:

Raymond H. Cox, Ph.D.

Study Completion Date:

March 8, 1989

Performing Laboratory:

Hazleton Laboratories America, Inc.
9200 Leesburg Turnpike
Vienna, Virginia 22180

Laboratory Project Identification:

HLA Study No. 343-208

Page 1 of 334

9400023-7

NNT-80-0037

Three-month Oral Toxicity Study of S-31183 in Dogs

Study No.: 220

Date: May 6, 1988

Facility Management: Hirohiko Yamada, M. Sc.
Research Director
Biochemistry and Toxicology Laboratory
Takarazuka Research Center
Sumitomo Chemical Co., Ltd.

SOP/REC/013 RS-1

9500960

APPLICATION FOR PESTICIDE REGISTRATION
SUMILARV TECHNICAL GRADE

Volume 14

SUMILARV -- STUDY OF S-31183 BY ORAL ADMINISTRATION DURING THE PERIOD OF FETAL ORGANOGENESIS IN RATS

AMENDED MRID # 413217-19

Data Requirements

Teratogenicity
Guideline 83-3

Author

Tadashi Saegusa

Date Completed

March 28, 1988

Performing Laboratory

Sumitomo Chemical Co., Ltd.
5-33, Kitahama 4-chome, Chuo-Ku
Osaka 541, Japan

Laboratory Project I.D. No.

NNT-80-0029

Submitted by

Sumitomo Chemical Company, Limited
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Osaka 541, Japan

Prepared by

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1101 17th Street, N.W., Suite 500
Washington, D.C. 20036

9400029-13

NNT-80-0033

Study of S-31183 by Oral Administration During the Period of Fetal Organogenesis in Rabbits

Study No.: 376

Date: March 7, 1988

Facility Management: Hirohiko Yamada, M. Sc.
Research Director,
Biochemistry and Toxicology Laboratory
Takarazuka Research Center
Sumitomo Chemical Co., Ltd.

SOP/REC/013 RS-01

MRID 42178307

APPLICATION FOR PESTICIDE REGISTRATION
SUMILARV TECHNICAL GRADE

9500475

Volume 9

SUMILARV -- THREE MONTH ORAL TOXICITY STUDY OF S-31183 IN DOGS

AMENDED MRID # 413217-17

Data Requirements

90-Day Feeding Studies - Non-rodent
Guideline 82-1

Author

Minoru Nakano

Date Completed

May 6, 1988

Performing Laboratory

Sumitomo Chemical Company, Limited
5-33, Kitahama 4-Chome, Chuo-Ku
Osaka 541, Japan

Laboratory Project I.D. No.

NNT-80-0037

Submitted by

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42178307

APPLICATION FOR PESTICIDE REGISTRATION
SUMILARV TECHNICAL GRADE

Volume 11

S31183: TOXICITY STUDY BY ORAL (CAPSULE) ADMINISTRATION TO BEAGLE DOGS FOR 52 WEEKS

Data Requirements

Chronic Toxicity -- Dogs
Guideline 83-1

Author

E. A. Chapman

Date Completed

August 1, 1991

Performing Laboratory

Life Science Research Limited
Eye
Suffolk IP23 7PX England

Laboratory Project I.D. No.

LSR Report 91/0776

Submitted by

Sumitomo Chemical Company, Limited
5-33, Kitahama 4-Chome, Chuo-Ku
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Prepared by

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MRID 42178310

NNT-11-0084
(1/4)
9400025 11

HAZLETON
LABORATORIES AMERICA, INC.

Sponsor:

Sumitomo Chemical Company, Ltd.
5-33 Kitahama 4-Chome, Chuo-Ku
Osaka, Japan (541)

FINAL REPORT

Study Title:

Oncogenicity Study in Mice with S-31183

Data Requirement:

EPA Guideline 83-2

Author:

Merrill R. Osheroff, Ph.D., D.A.B.T.

Study Completion Date:
July 23, 1991

Performing Laboratory:

Hazleton Laboratories America, Inc.
9200 Leesburg Turnpike
Vienna, Virginia 22182

Laboratory Project Identification:

HLA Study No. 343-215

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APPLICATION FOR PESTICIDE REGISTRATION
SUMILARV TECHNICAL GRADE

Volume 13

SUMILARV -- STUDY OF S-31183 BY ORAL ADMINISTRATION DURING THE PERIOD OF FETAL ORGANOGENESIS IN RABBITS

AMENDED MRID # 413217-20

Data Requirements

Teratogenicity
Guideline 83-3

Author

Atsuko Hirohashi

Date Completed

August 30, 1989

Performing Laboratory

Sumitomo Chemical Co., Ltd.
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Laboratory Project I.D. No.

NNT-80-0003

Submitted by

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MRID 42178312

9500960

APPLICATION FOR PESTICIDE REGISTRATION
SUMILARV TECHNICAL GRADE

Volume 14

SUMILARV -- STUDY OF S-31183 BY ORAL ADMINISTRATION DURING THE PERIOD OF FETAL ORGANOGENESIS IN RATS

AMENDED MRID # 413217-19

Data Requirements

Teratogenicity
Guideline 83-3

Author

Tadashi Saegusa

Date Completed

March 28, 1988

Performing Laboratory

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Laboratory Project I.D. No.

NNT-80-0029

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APPLICATION FOR PESTICIDE REGISTRATION
SUMILARV TECHNICAL GRADE

Volume 15

A DIETARY 2-GENERATION (1 LITTER) REPRODUCTION STUDY OF S-31183 IN THE RAT

BOOK 1 of 5

Data Requirements

Reproduction, 2-generation
Guideline 83-4

Authors

K. Robinson, B.Sc.
G. Washer, B.Sc.
J. Noveroske, Ph.D.

Date Completed

September 23, 1991

Performing Laboratory

Bio-Research Laboratories Ltd.
87 Senneville Rd.
Senneville, Quebec H9X 3R3, Canada

Performing Laboratory Project I.D. No.

83963

Submitted by

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MRID 42178314

9400032-15

NNT-11-0085 ①

HAZLETON WASHINGTON

Sponsor:

Sumitomo Chemical Co., Ltd.
5-33 Kitahama 4-Chome, Chuo-Ku
Osaka, Japan (541)

FINAL REPORT

Study Title:

Combined Chronic Toxicity and Oncogenicity Study in Rats with S-31183

Data Requirement:

EPA Guideline 83-5

Author:

Merrill R. Osheroff, Ph.D., D.A.B.T.

Study Completion Date:

September 6, 1991

Performing Laboratory:

Hazleton Washington, Inc.
9200 Leesburg Turnpike
Vienna, Virginia 22182

Laboratory Project Identification:

HWA Study No. 343-214

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APPLICATION FOR PESTICIDE REGISTRATION
SUMILARV TECHNICAL GRADE

Volume 23

Early Life-State Toxicity of Sumilarv Technical
To the Rainbow Trout (*Oncorhynchus mykiss*) Under
Flow-Through Conditions

Data Requirements

Early Life Stage and Aquatic Invertebrate Life Cycle
Guideline 72-4

Authors

Jon E. Rhodes
Diana Cramer

Date Completed

November 27, 1991

Performing Laboratory

ABC Laboratories
7200 E. ABC Lane
P.O. Box 1097
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Laboratory Project I.D. No.

39377

Submitted by

Sumitomo Chemical Company, Limited
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APPLICATION FOR PESTICIDE REGISTRATION
SUMILARV TECHNICAL GRADE
EPA Reg. No. 10308-RR

Volume 34

SUMILARV – 21-DAY DERMAL TOXICITY STUDY IN RATS WITH S-31183

Data Requirements

21-Day Dermal Toxicity
Guideline 82-2

Author

Michael R. Moore, Ph.D., D.A.B.T.

Date Completed

January 11, 1993

Performing Laboratory

Hazleton Washington, Inc
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Laboratory Project I.D. No.

HWA 343-244

Reference No.

NNT-31-0094

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MRID 43210501

APPLICATION FOR PESTICIDE REGISTRATION
SUMILARV TECHNICAL GRADE, EPA Reg. No. 10308-RR **9500481**

Volume 38

SUMILARV -- RESPONSE TO EPA REVIEW OF CHRONIC TOXICITY AND/OR ONCOGENICITY STUDIES OF SUMILARV (S-31183) IN RATS AND MICE

Data Requirements

Chronic Feeding/Oncogenicity
Guideline Series 83

Authors

Christopher F. Wilkinson, Ph.D.
Jeffrey Driver, Dr.P.H., D.A.B.T., M.T., C.L.S.
Gary Whitmyre, M.A., D.A.B.T.
Colleen E. Dragula, M.S.

Date Completed

April 7, 1994

Performing Laboratory

N/A

Reference No.

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APPLICATION FOR PESTICIDE REGISTRATION
SUMILARV TECHNICAL GRADE, EPA Reg. No. 10308-RR

9500477 V

Volume 39

ADDENDUM TO THE FINAL REPORT: SUMILARV -- COMBINED CHRONIC TOXICITY AND ONCOGENICITY STUDY IN RATS WITH S-31183, MRID NO. 42178314

Data Requirements

Chronic Feeding/Oncogenicity
Guideline 83-2(a)

Author

Merrill R. Osheroff, Ph.D., D.A.B.T.

Date Addendum Completed

March 31, 1994

Performing Laboratory

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Laboratory I.D. No.

HWA 343-214

Reference No.

NNT-11-0085

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APPLICATION FOR PESTICIDE REGISTRATION
SUMILARV TECHNICAL GRADE, EPA Reg. No. 10308-RR

9500479

Volume 40

AMENDMENTS 1 & 2 TO THE FINAL REPORT: SUMILARV -- COMBINED CHRONIC TOXICITY AND ONCOGENICITY STUDY IN RATS WITH S-31183, MRID NO. 42178314

Data Requirements

Chronic Feeding/Oncogenicity
Guideline 83-2(a)

Author

Merrill R. Osheroff, Ph.D., D.A.B.T.

Date Amendment Completed

March 31, 1994

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NNT-11-0085

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MRID 43210504

APPLICATION FOR PESTICIDE REGISTRATION
SUMILARV TECHNICAL GRADE, EPA Reg. No. 10308-RR

Volume 41

9400259

Final Report:
Sumilarv -- Subchronic Toxicity Study in Mice

Data Requirements

Subchronic Feeding, Rodent
Guideline 82-1

Author

Raymond H. Cox, Ph.D.

Date Completed

January 23, 1990

Performing Laboratory

Hazleton Laboratories America, Inc.
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Laboratory I.D. No.

HLA 343-209

Reference No.

NNT-01-0066

Sponsored/Submitted by

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APPLICATION FOR PESTICIDE REGISTRATION
SUMILARV TECHNICAL GRADE, EPA Reg. No. 10308-RR

9500505

Volume 42

Response to EPA Review of Teratogenicity/Developmental Toxicity Study of Sumilarv (S-31183) in Rabbits

Data Requirements

Teratogenicity, Rabbits
Guideline 83-3(b)

Authors

Christopher F. Wilkinson, Ph.D.
Jeffery Driver, Dr.P.H., D.A.B.T., M.T., C.L.S.
Gary Burin, Ph.D.
Gary Whitmyre, M.A. D.A.B.T.
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Date Completed

April 20, 1994

Original Refrence No.

NNT-80-0033

Sponsored/Submitted by

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APPLICATION FOR PESTICIDE REGISTRATION
SUMILARV TECHNICAL GRADE, EPA Reg. No. 10308-RR

Volume 43

Addendum to the Final Report:
Study of S-31183 by Oral Administration During the Period of Fetal Organogensis in Rabbits

Data Requirements

Teratogenicity, Rabbits
Guideline 83-3(b)

Author

Atsuko Hirohashi

Date Completed

April 27, 1994

Performing Laboratory

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Reference No.

NNT-80-0033

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MRID 43413201

9500513

Application for Pesticide Registration
Sumilarv Technical Grade
EPA Registration No 10308-RR

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VOLUME 45

SUPPLEMENTAL DATA AND REVIEW OF ONCOGENICITY STUDY WITH S-31183 (SUMLIARV) IN MICE (MRID No. 421783-10)

Response to EPA Review of Chronic and/or Oncogenicity Studies of Sumilarv in Rats and Mice

Data Requirement:
83-2(b) Oncogenicity (Mouse)

Authors:
R. Cardy, D.V.M, D.A.B.T., M. Moore, Ph.D., D.A.B.T., C. Murphy, B.S. and A. Thakur, Ph.D.
(Hazleton Washington);
C. Tellone, Ph.D., D.A.B.T.
(Valent U.S.A.);
S. Ito, D.V.M., M.Sc.
(Sumitomo Chemical Company, Ltd.);
P. Lang, Ph.D.
(Consultant in Toxicology);
M. Ginevan, Ph.D.
(Step 5 Corporation); and
J. Driver, Dr.P.H., D.A.B.T., M.T., C.L.S., R. Stewart, Ph.D. and C. Wilkinson, Ph.D.
(Technology Sciences Group Inc.)

Date Completed:
October 11, 1994

Submitted By:
Sumitomo Chemical Company, Ltd.
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Osaka 541, Japan

Prepared By:
Technology Sciences Group Inc.
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Page 1 of 79

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Application for Pesticide Registration
Sumilarv Technical Grade
EPA Registration No. 10308-RR

VOLUME 46

AMENDMENT TO FINAL REPORT:
ONCOGENICITY STUDY IN MICE WITH S-31183 (SUMILARV)
(MRID No. 421783-10)

Data Requirement:
83-2(b) Oncogenicity (Mouse)

Authors:
Michael R. Moore, Ph.D., D.A.B.T.
Merril R. Osherhoff, Ph.D., D.A.B.T

Date Completed:
October 3, 1994

Submitted By:
Sumitomo Chemical Company, Ltd.
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Page 1 of 24

NNW-41-0114

SUMILARV T.G.:

A REPRODUCTION STUDY WITH THE BOBWHITE

9400293-2

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 166-146

FIFRA GUIDELINE 71-4

AUTHORS: Joann B. Beavers
Oscar Sipler
Gloria A. Marselas
Mark Jaber

STUDY INITIATION: December 18, 1991

STUDY COMPLETION: April 5, 1994

SUBMITTED TO

Sumitomo Chemical Company, Ltd.
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CHR15

MRID 44036908

NNW-41-0115

9400293-4

SUMILARV T.G.:

A REPRODUCTION STUDY WITH THE MALLARD

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 166-147

FIFRA GUIDELINE 71-4

AUTHORS: Joann B. Beavers
Oscar Sipler
Gloria A. Marselas
Mark Jaber

STUDY INITIATION: December 18, 1991

STUDY COMPLETION: April 5, 1994

SUBMITTED TO

Sumitomo Chemical Company, Ltd.
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Chuo-ku, Osaka 541, Japan

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CHR15

Perinatal and Postnatal Study of S-31183 Orally Administered to Rats

March 28, 1988

Hamamatsu Seigiken Research Co., Ltd.

MRID 44985002

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Study by Orally Administration of S-31183 to Rats Prior to and in the Early Stage of Pregnancy

April 21, 1988

Hamamatsu Seigiken Research Co., Ltd.

1 of 209

MRID 48066201

DATA SUBMISSION VOLUME 1

201000114

DATA REQUIREMENT:

None

STUDY TITLE:

Uterotrophic Assay of Pyriproxyfen by Oral Route Using Juvenile Rat: Investigation on Estrogenic Effect

AUTHOR:

Keiko Ose

STUDY COMPLETED:

August 2, 2005

PERFORMING LABORATORY:

Environmental Health Science Laboratory
Sumitomo Chemical Co., Ltd.
1-98, Kasugadenaka 3-chome,
Konohana-ku, Osaka
Japan

LABORATORY PROJECT IDENTIFICATION:

Study No. S1198

STUDY VOLUME:

1 of 1

TOTAL PAGES:

32

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DATA SUBMISSION VOLUME 2

201000086

DATA REQUIREMENT:

Ministry of the Environment,
Japan Annex 6-2
(November 2002)

STUDY TITLE:

Pyriproxyfen:
Full Life Cycle Toxicity Test with Medaka (*Oryzias latipes*)
Under Flow-Through Conditions

AUTHOR:

Dr. Thomas Gries

STUDY COMPLETED:

February 16, 2007

PERFORMING LABORATORY:

Springborn Smithers Laboratories (Europe)
Seestrasse 21, Postfach
CH-9326 Horn
Switzerland

LABORATORY PROJECT IDENTIFICATION:

Springborn Smithers Labs. Study # 1043.035.123

STUDY VOLUME:

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ABC LABORATORIES

SPONSOR

Sumitomo Chemical Company, Limited
5-33, Kitahama, Japan
Chuo-Ku, Osaka 541, Japan,

STUDY TITLE

21-Day Flow-Through Toxicity of Pyriproxyfen
to Rainbow Trout (*Oncorhynchus mykiss*)

DATA REQUIREMENT

OECD Guideline 204

AUTHORS

Marc Sword
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and

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Laboratory Technician II

REPORT SUBMITTED ON

January 8, 1992

PERFORMING LABORATORY

ABC Laboratories, Inc.
Aquatic Toxicology Programs Division
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Columbia, Missouri 65205

ABC LABORATORY PROJECT ID

Final Report No. 39544

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