

Pesticide Exposure and Risk Assessment Evaluation

Document Review

Department of Pesticide
Regulation's Draft Risk
Characterization of Fipronil

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Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

LIST OF CONTRIBUTORS

Peer Reviewers

Toxicology and Risk Assessment

Jocelyn Claude, Ph.D.

Rima Woods, Ph.D.

Jennifer Hsieh, Ph.D., DABT

Kate Li, Ph.D.

Worker and Bystander Exposure Assessment

James Nakashima, Ph.D.

Jing Tao, Ph.D.

Report Reviewers

Katherine Sutherland-Ashley, Ph.D.

Ouahiba Laribi, Ph.D.

Meng Sun, Ph.D.

Martha Sandy, Ph.D.

David Ting, Ph.D.

Vincent Cogliano, Ph.D.

Director

Lauren Zeise, Ph.D.

PREFACE

Under the authority of California Food and Agricultural Code Section 11454.1, the Office of Environmental Health Hazard Assessment (OEHHA) conducts scientific peer review of human health risk assessments prepared by the Department of Pesticide Regulation (DPR). DPR reports the risk assessment in two documents:

- The Risk Characterization Document (RCD), which summarizes the toxicology database of the chemical; discusses hazard identification and dose-response analyses; assesses dietary exposure, when appropriate; and characterizes the risk associated with the various exposure scenarios (dietary, occupational, residential, and aggregate exposures).
- The Human Exposure Assessment Document (EAD), which describes non-dietary exposure scenarios and estimates exposure levels of workers and residents.

This report is a review of the draft RCD for the pesticide fipronil provided by DPR (dated and received January 20, 2021). The draft EAD was included as Appendix 1 in the draft RCD.

This peer review report has four parts:

- I. Summary of Review
- II. Detailed Comments
- III. Response to Charge Statements
- IV. Other Comments

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I. SUMMARY OF REVIEW

This report presents the review by the Office of Environmental Health Hazard Assessment (OEHHA) on the Department of Pesticide Regulation's (DPR) draft Risk Characterization Document (RCD) for fipronil, a broad-spectrum pesticide registered for multiple non-food uses in California. The draft RCD characterizes human health risks associated with exposures to fipronil from its uses as flea and tick control treatments for dogs and cats, and for structural pest control and lawn treatments. Risks were assessed for acute, subchronic, and chronic exposures to workers, and acute and subchronic exposures to adult and child residents following home uses, including flea and tick treatments, in and around treated structures, and on turf. The main document includes toxicity evaluation, risk assessment, and risk appraisal; Appendix 1 includes the human exposure assessment document (EAD).

OEHHA's principal comments are summarized here in Section I; they focus on issues that are likely to impact the key findings and conclusions of the assessment. Detailed comments are provided in Section II. Responses to DPR's charge statements (descriptions of scientific assumptions, findings and conclusions to be addressed by peer reviewers) are provided in Section III, and minor comments are provided in Section IV.

A. Toxicity Evaluation

1. The draft RCD adequately described the oral toxicity database and presented acceptable rationale for applying oral point of departures (PODs) to dermal and inhalation exposure routes. OEHHA agrees that PODs for the parent compound would be health protective for its major metabolites.
2. OEHHA disagrees with the identification of the acute oral POD of 0.87 milligrams per kilogram per day (mg/kg-day), which was based on decreased hindlimb splay in rats following a single oral gavage dose (Hughes 1997). There are several short-term studies available that showed adverse health effects near or below 0.87 mg/kg-day; thus, OEHHA suggests DPR re-evaluate these studies and identify a more health-protective acute oral POD.
3. OEHHA agrees with DPR's approach in deriving the subchronic oral POD from the chronic oral POD. Based on the available data from subchronic toxicity studies, the chronic oral POD should be protective of subchronic exposures. DPR's subchronic POD is more health protective and more appropriate than the one established by the US Environmental Protection Agency (US EPA) in their most recent draft human health risk assessment (US EPA, 2020).
4. The draft RCD determined the thyroid follicular cell tumors reported in rat cancer bioassays (Aughton, 1993) can be explained by a thyroid hormone

disruption mechanism and used a threshold approach in addressing cancer risk. OEHHA's analysis found that in addition to the thyroid follicular cell tumors in the rat, fipronil induced hepatocellular carcinomas in the mouse. OEHHA also concluded that the weight of evidence indicates that fipronil is genotoxic and recommends using a non-threshold approach for estimating cancer risk.

B. Risk Characterization

1. OEHHA recommends an additional uncertainty factor (UF) of 3 be applied to increase the intraspecies pharmacokinetic UF from $\sqrt{10}$ to 10 to account for sensitive subpopulations such as the elderly, pregnant woman, and children.
2. OEHHA recommends an additional UF of 3 be applied to acute exposure scenarios for infants, children, and women of childbearing age if the proposed acute POD of 0.87 mg/kg-day based on hindlimb splay in adult rats is retained. Developmental toxicity studies showed effects in fetuses and offspring at dose levels lower than the dam and lower than the acute POD.

C. Exposure Assessment

1. OEHHA suggests that the draft EAD aggregate exposures from all relevant pathways and sources, regardless of whether exposure levels exceed level of concern.
2. OEHHA suggests that the draft EAD explain why exposure to fipronil degradants were included in some exposure pathways but not in others.
3. OEHHA suggests that the draft EAD consider using high-end exposure duration rather than using arithmetic means for estimating acute post-application exposure for pet owners.

II. DETAILED COMMENTS

Our comments on the draft RCD for fipronil are grouped into A) Toxicity Evaluation and Risk Assessment and B) Exposure Assessment.

A. Toxicity Evaluation and Risk Assessment

1. Non-cancer Toxicity Evaluation and Point of Departure Determination

a. Pharmacokinetics

The absorption, distribution, metabolism, and excretion of fipronil are adequately addressed in the draft RCD. In addition to the parent compound, fipronil, the draft RCD includes discussion of two metabolites, fipronil sulfone and fipronil sulfide, and the degradation product fipronil-desulfinyl. Studies in rats showed that oral absorption of fipronil is over 80%. Data in rats, goats and hens also showed a high oral absorption rate and elimination primarily through the feces. OEHHA agrees with DPR's decision to assume 100% oral absorption based on this information. Once absorbed, fipronil is rapidly metabolized to fipronil sulfone and many other metabolites, such as fipronil sulfide and fipronil amide. These chemicals are distributed throughout the body. Fipronil sulfone is the major metabolite in mammals and has a median lethal dose (LD₅₀) value similar to that of the parent compound. Limited toxicity data are available for fipronil sulfide. Dermal studies in rats were used by DPR to estimate a dermal absorption rate of 4.3% for fipronil. There are no studies available on inhalation absorption, so a default absorption rate of 100% was used. OEHHA agrees with these two determinations.

The pharmacokinetics of fipronil-desulfinyl, the photodegradation product of fipronil, are also described in Appendix III of the draft report, with experimental data indicating a similar half-life for elimination in rats. Fipronil-desulfinyl is potentially more toxic than fipronil based on several studies in animals showing acute No-Observed-Adverse-Effect Level (NOAEL) values lower than that of the parent compound, as presented in the draft RCD; subchronic and chronic NOAELs are similar. However, fipronil-desulfinyl is a photodegradation product, not a metabolite found in humans or animals.

b. General Approaches

The draft RCD derives critical toxicity endpoints for only the parent compound fipronil; OEHHA agrees that PODs based on the parent compound will be protective of the major metabolites as well. There is also limited information regarding the toxicity of fipronil through the dermal and inhalation routes, but the available data suggest these routes are not more toxic than the oral route. For this reason, OEHHA agrees with the use of the oral PODs to assess inhalation and dermal exposure pathways.

c. Acute Toxicity

The draft RCD selected the acute neurotoxicity study reported by Hughes (1997) as the critical study and the decreased hindlimb splay reported in male rats 7 hours post-dosing via oral gavage as the critical endpoint. The study NOAEL was 2.5 mg/kg-day with a Lowest-Observed-Adverse-Effect Level (LOAEL) of 7.5 mg/kg-day. The NOAEL was also based on decreased weight gain and food consumption in females during week 1 following treatment. DPR used Benchmark Dose (BMD) modeling with a 10% benchmark response (BMDL₁₀) and derived a critical acute POD of 0.87 mg/kg-day. The critical acute POD is higher than other potential acute PODs discussed in the draft, but DPR's rationale for choosing the study over others included less uncertainty in the dose range between the NOAEL and LOAEL, the relevance of the critical effect to human health, and uncertainties in the other studies that limited their utility for acute POD derivation.

OEHHA concurs with DPR's use of BMD modeling for this dataset, as BMD modeling can overcome some of the limitations of the NOAEL/LOAEL approach, and male rats exhibited a dose-dependent but non-statistically significant decrease in hindlimb splay at the study NOAEL. The draft RCD cites more confidence in this study over other potential PODs in large part due to it being amenable to BMD modeling. However, as discussed in the following paragraphs, OEHHA does not agree with the acute POD selected

OEHHA notes there are other studies and considerations that may lead to a more health-protective acute oral POD. An acute neurotoxicity study in rats (Gill, 1993) with a similar study design showed a lower NOAEL of 0.5 mg/kg-day for the same endpoint. The draft RCD cited the 10-fold difference between the NOAEL and LOAEL as a source of uncertainty and reasoning for not using this lower value to derive an acute POD. However, it should be noted that there are uncertainties in the study design and NOAELs in both the Hughes (1997) and Gill (1993) studies because they might not have captured the peak effects of the treatment. Based on the Time Of Peak Effects (TOPE) probe study conducted by Gill (1993), at 50 and 80 mg/kg, neurotoxic effects were observed as early as 2 hours post dosing, with convulsions and tremors readily apparent at 4-5 hours post dosing. In a similar TOPE study conducted by Hughes (1997), at 25 mg/kg, neurotoxic effects were seen at 4 hours post dosing. It is possible that for both studies, neurotoxicity testing using a functional observation battery (FOB) at 7 hours post-dosing might have missed the most severe effects.

With uncertainty in the ability of the two acute neurotoxicity studies to capture peak effects resulting from acute fipronil exposure, and a number of acute and short-term toxicity studies showing effects lower than 0.87 mg/kg-day (shown in Table 1 below), OEHHA suggests DPR to re-evaluate these studies and consider a more health-protective acute oral POD.

Table 1. Effects reported in toxicity studies that support a more health protective acute oral POD

| Study | Effect | NOAEL/LOAEL (mg/kg-day) |
|-----------------|--|-------------------------|
| Gill (1993) | Decreased hindlimb splay in male rats | 0.5 / 5.0 |
| Aughton (1993) | Decreased T4 in male rats after 1 week of exposure | 0.02 / 0.06 |
| Aughton (1993) | Decreased T4 in female rats after 1 week of exposure | 0.08 / 1.6 |
| Coder (2019) | Decreased T4 in fetal rats (gestational day 20) | 0.3 / 1.0 (dams) |
| King (1990) | Reduced body weight gain in female rabbits | 0.1 / 0.2 |
| Mandella (1995) | Delays in preputial separation, altered startle response, decrements in body weight (rat pups) | 0.05 / 0.9 |

T4=thyroid hormone thyroxine

In a chronic oral study, Aughton (1993) observed significantly decreased thyroid hormone thyroxine (T4) levels after one week of fipronil exposure at 0.06 mg/kg-day in male rats and 1.6 mg/kg-day in female rats, with NOAELs of 0.02 mg/kg-day and 0.08 mg/kg-day for males and females, respectively. In addition to lower T4 levels, convulsions were observed in three males in the 0.06 mg/kg-day dose group during the first few weeks of treatment. There was discussion in the draft RCD surrounding the decision not to use this dataset to derive an acute POD, which includes the NOAEL being higher in females, and the reasoning that short-term changes in thyroid hormone levels are not likely deleterious to adults. OEHHA disagrees with these statements. Placental transfer of maternal thyroid hormones are critical in early embryonic development and up until maturation of the fetal thyroid gland. A decrease in maternal serum T4 even for a short period can have detrimental effect on the neurodevelopment of the fetus (OEHHA, 2015; Miranda and Sousa, 2018). If decreased serum T4 were selected as the critical endpoint, an acute oral POD of 0.02 mg/kg-day or 0.08 mg/kg-day could be determined.

The importance of protecting the fetus and developing neonatal brain is highlighted by a comparative thyroid assay (CTA) in pregnant rats and their offspring reported by Coder (2019). The study showed dosing at the LOAEL of 1 mg/kg-day fipronil in pregnant female rats had a non-significant effect on T4 in the dams at gestational day 20, yet caused a statistically significant 19% reduction in T4 in their fetuses at the same time point. The NOAEL for T4 effects in the fetus equated to a maternal dose of 0.3 mg/kg-day. This shows the rat fetus is more susceptible to thyroid hormone disruption caused by fipronil than the dam, and there is a potential hazard to the fetus at the LOAEL of 1 mg/kg-day which is close to the acute POD of 0.87 mg/kg-day.

The draft RCD cites uncertainty in the thyroid hormone measurements from this study as a basis for not considering it for POD selection, based on an ion ratio analysis requested by US EPA. However, the toxicological significance of the findings of this study are supported by statements in the draft RCD, stating that the changes in measured thyroid hormone levels following treatment with fipronil in the CTA study were consistent with effects measured in similar dose groups in other animal toxicity studies, that many of the failed samples were just outside of the tolerable range for the ion ratio analysis, and the accompanying effects on thyroid weight and histopathology at higher doses in the study suggested that changes in thyroid hormone levels were representative of potential physiological or pathological change. While the draft RCD used the ion ratio analysis to “preclude the use of the acute results from the CTA to derive a quantitative acute POD,” this approach is inconsistent with US EPA who selected NOAELs for thyroid hormone disruption from Coder (2019) for both maternal (0.3 mg/kg-day) and offspring (1 mg/kg-day) as critical PODs for short and intermediate term assessments, depending on the population being assessed.

A teratology study in rabbits showed a decrease in maternal body weight gain within two days of treatment, with a NOAEL of 0.1 mg/kg-day (King, 1990). While the effect at 0.1 mg/kg-day was not statistically significant, it still represented a 33% reduction in body weight gain at that dose. The higher doses, at 0.2 mg/kg-day, 0.5 mg/kg-day, and 1.0 mg/kg-day, all caused statistically significant reductions in body weight gain over the first 2 days of fipronil treatment. Maternal T4 was not measured in this study. While no teratogenicity from fipronil exposure was observed in the fetuses in this study, decrements in body weight gain in the pregnant dam suggest pregnancy may be an especially susceptible lifestage to fipronil toxicity. Furthermore, severe effects on maternal body weight during pregnancy could lead to adverse developmental or neurodevelopmental effects of offspring.

Lastly, OEHHA suggests including the results from the developmental neurotoxicity (DNT) study in rats (Mandella, 1995) when considering the health protectiveness of the acute oral POD. The study derived a NOAEL for developmental neurotoxicity of 0.05 mg/kg-day and a LOAEL of 0.9 mg/kg-day. Even though a repeated exposure protocol was used in the study, we cannot be certain that the developmental neurotoxicity observed in the offspring was not caused by a single or short-term exposure on a sensitive day (whichever day that may have been during the prenatal or lactation periods) for the observed outcomes. Because the acute oral POD of 0.87 mg/kg-day is so close to the LOAEL of 0.9 mg/kg-day, there is a concern that the POD is not sufficiently health protective.

In summary, several adverse effects were seen following short-term exposures to fipronil, on the order of hours to days. Many of the effects were seen at levels lower than the acute oral POD of 0.87 mg/kg-day determined in the draft RCD (Table 1). While OEHHA finds that there are strengths and weaknesses in the studies discussed, collectively they indicate the acute POD may not be health protective, particularly of the

most susceptible lifestages, notably pregnancy, in utero fetal development, and early infancy. OEHHA recommends DPR re-evaluate the available data, apply BMD modeling when appropriate, and choose a more health-protective POD. Alternatively, the draft RCD could apply an additional UF to the currently proposed acute POD to address the uncertainties and concern for developmental and neurodevelopmental toxicities described above.

d. Subchronic and Chronic Toxicity

Generally, effects of subchronic and chronic fipronil exposure included toxicity to the liver, thyroid and kidneys, as well as effects on body weight and evidence of neurotoxicity. The most sensitive subchronic and chronic LOAELs/NOAELs available ranged from 0.01 to 0.06 mg/kg-day, with the lowest NOAEL (0.01 mg/kg-day) being from a subchronic neurotoxicity toxicity study (Driscoll and Hurley, 1993). The draft RCD did not select this value as the critical subchronic POD since the value was based on a single endpoint derived from an indirect observation. Rather, the draft RCD identified a subchronic POD derived from an oral chronic toxicity study in rats (Aughton, 1993). A slightly higher oral POD of 0.02 mg/kg/day was chosen based on mortality, convulsions, and decreases in thyroid hormones seen starting 1 week after the initiation of fipronil administration and persisting throughout the chronic study in rats (Aughton, 1993). OEHHA agrees that this critical POD is appropriate and health protective for assessing both subchronic and chronic exposures to fipronil.

e. Reproductive and Developmental Toxicity

The developmental study database for fipronil includes teratology studies in rats and rabbits, a developmental neurotoxicity (DNT) study in rats, and the CTA assay in pregnant rats. In general, OEHHA agrees with the interpretation of the major effects of these studies. However, OEHHA suggests that all developmental toxicity studies (including DNT) and the CTA assay (Coder, 2019) be considered quantitatively for acute and subchronic PODs, as we have outlined in the acute toxicity section (II.A.1.c) of this report. This is supported by section VII.A.5. of the draft fipronil RCD, where it is clearly stated that even short duration deficits in thyroid hormone during specific times in development can cause irreversible brain damage, and that damagingly low levels of thyroid hormone in the neonate can be associated with maternal levels appearing in the normal range (Bernal, 2015 and OEHHA, 2015, as cited in DPR, 2020).

1. Genotoxicity

OEHHA disagrees with the conclusion in the draft RCD that fipronil is not genotoxic. There are five in vivo studies that showed fipronil was genotoxic in mammals (four included in the draft RCD and one additional study identified by OEHHA below) causing DNA strand breaks, and some of the studies showed positive results in chromosomal

aberration or micronuclei tests (Appendix I). In other *in vivo* studies, the chemical was also shown to be genotoxic in other species, such as bird, fish, and fruit fly.

In many *in vitro* test systems, fipronil caused DNA damage, DNA alterations, chromosomal aberration, micronuclei, and other chromosomal effects (Appendix 1). In particular, fipronil induced DNA strand breaks and chromosomal damage in human peripheral blood lymphocytes and laryngeal mucosal cells. These positive studies in primary human cells are important per IARC's Preamble, which states that in evaluating mechanistic data for carcinogenicity, "[s]tudies in exposed humans and in human primary cells or tissues that incorporate end-points relevant to key characteristics of carcinogens are emphasized when available". OEHHA found that no cytotoxicity or presence of oxidative stress markers were reported in most of these studies at the lowest doses that indicated positive results for genotoxicity. A summary table of the *in vivo* and *in vitro* genotoxicity tests as listed in the draft RCD and OEHHA's interpretation of them is included in Appendix 1 of this report. Using the weight of evidence approach, OEHHA determined there is evidence to show fipronil is genotoxic.

OEHHA identified several additional genotoxicity studies that are not in the draft RCD through a quick review of the literature. They are listed below. We suggest a thorough search to identify any additional genotoxicity studies be performed.

- Girgis and Yassa (2013) reported that fipronil induced significant increases in CA and micronuclei in bone marrow cells of albino rats treated with 25 and 50 mg/kg of fipronil for 24, 48 or 96 hours *in vivo*.
- Mohammed et al. (2016) reported that fipronil induced dose-dependent increases of DNA strand breaks (measured by comet assays) in the liver cells of Japanese quails, 96 hours after administration of a single oral dose of fipronil at 1.13, 2.26, 5.65, or 11.3 mg/kg *in vivo*.
- Ardeshir et al. (2019) reported that at 1, 5, and 10 µg/L, fipronil induced DNA strand breaks (measured by comet assays) in the liver of Caspian white fish *in vivo*.
- de Castilhos Ghisi et al. (2011) found that at 0.10 and 0.23 mg/ml, fipronil was able to cause *in vivo* clastogenic and/or aneugenic effects (measured by micronucleus test and nuclear morphological alterations) in the fish *Rhamdia quelen*.
- Karaismailoglu (2017) reported that fipronil induced significant dose-dependent increases in CA and micronuclei in the somatic cells of the plant *Allium cepa*, at non-cytotoxic doses of 1, 2.5, 5, and 10 ppm for 6, 12, and 24 hours.
- Ucar et al (2020) reported that fipronil induced statistically significant increases of micronucleus formation at all doses tested in the erythrocytes of treated rainbow trout.
- Zilio et al. (2017) reported that a single dose (6.7 mg/kg) of a formulation of fipronil (Frontline plus®) applied on the dorsal neck region of dogs did not induce statistically significant increases in DNA strand breaks (measured by comet assay) in peripheral blood samples at 3, 8, or 24 hours after application. A small increase was observed at 3 and 8 hours each.

2. Mechanistic Data

OEHHA suggests that the analysis of data for fipronil in ToxCast/Tox21 be updated. The draft RCD states that fipronil is active in 134 of 667 high-throughput screening assays in ToxCast mostly associated with metabolism, elimination, inflammation, cell cycle regulation, and fatty liver disease. However, as of March 25, 2021, fipronil is active in 292/957 ToxCast/Tox21 assays. The present data include active assays for DNA binding and other important biological endpoints. These mechanistic data should be reviewed and added to the weight of evidence in determining the carcinogenicity of fipronil.

3. Carcinogenicity

OEHHA reviewed the cancer bioassay in rats (Aughton, 1993) and found fipronil caused thyroid follicular cell adenomas and carcinomas in male and female rats. OEHHA disagrees with the draft RCD that these tumors are not likely relevant to humans and can be evaluated using a threshold approach. The guidance from IARC (1999) discusses the induction of follicular cell tumors in rodents through various mechanisms (e.g., genotoxicity, thyroid hormone imbalance). Specifically, IARC noted all the following criteria have to be met for identifying a chemical as causing thyroid follicular-cell neoplasia in rats “solely through hormonal imbalance.”

- “•There is a lack of genotoxic activity (agent and/or metabolite) based on an overall evaluation of in-vitro and in-vivo data.
- The presence of hormone imbalance has been demonstrated under the conditions of the carcinogenicity assay.
- The mechanism whereby the agent leads to hormone imbalance has been defined.”

As discussed in the genotoxicity section, OEHHA has determined that fipronil is genotoxic and this finding makes the first criterion not fulfilled. It is possible that multiple mechanisms are operative in the induction of thyroid tumors by fipronil, including genotoxic mechanisms, such as chromosomal changes, as well as mechanisms resulting in disruption of the hypothalamus-pituitary-thyroid axis. This possibility is strengthened by the observation of liver hepatocellular carcinomas in male mice in other cancer bioassays (Broadmeadow, 1993) and the data from ToxCast (see II.A.3) indicating direct DNA interacting mechanisms could be operative.

The draft RCD does not conduct a linear dose response analysis of the rat thyroid tumor data, and instead states that “the critical chronic POD of 0.02 mg/kg/day based partly on the precursor event for tumors at 0.06 mg/kg/day will be protective of any possible tumor formation in humans.” For the reasons provided, OEHHA recommends the thyroid follicular cell adenoma and carcinoma data should be evaluated by a linearized multistage model.

OEHHA has additional concerns about the adequacy of the rat cancer bioassays to fully assess the carcinogenic potential of fipronil, as the study duration was only 89-91 weeks, which is shorter than the recommended 104 weeks for a rat cancer bioassay and considered a less-than-lifetime study. It is possible that more thyroid tumors might have been observed if the study duration had been extended to 104 weeks. This is particularly concerning as the LOAEL for thyroid hormone disruption, which was considered the precursor event in the draft RCD, is the same as the LOAEL for tumor formation (0.06 mg/kg-day).

OEHHA also reviewed the cancer bioassays in mice (Broadmeadow, 1993) and found fipronil caused hepatocellular carcinomas in male mice. OEHHA agrees with the determination in the draft RCD that these tumors were treatment related, but has several issues with the analysis and interpretation of this dataset.

In its Table 17, the draft RCD only included mice that were killed after 78 weeks, and excluded animals that died before 78 weeks, resulting in a significant number of liver tumors observed before week 78 not being included in the analysis. OEHHA believes all the liver tumors need to be considered, whether they were discovered at the 78-week sacrifice or earlier in the study. OEHHA re-analyzed the male CD-1 mouse bioassay data and determined that there were altogether 10, 3, 2, 6, and 5 hepatocellular adenomas and 1, 1, 2, 1, 5 hepatocellular carcinomas in the control, 0.1, 0.5, 10, and 30 ppm groups, respectively.

In addition to the early mortality in some control animals (as noted in the draft RCD), there were significant differences of early mortality (greater than 15%) between the control group and some treatment groups (0.5 and 10 ppm groups) of male mice from 40 to 68 weeks. As mentioned earlier, when reporting tumor incidence, all hepatocellular adenomas and carcinomas observed throughout the study should be included. Because detailed data on individual animals are available, OEHHA calculated the number of tumor-bearing animals and compared them to the effective animal number, which is the number of animals alive at the first occurrence of the tumor. In male mice, the first occurrence of hepatocellular carcinoma was on day 409 (at 58 weeks). OEHHA's analysis identified a statistically significant dose-dependent trend in hepatocellular carcinomas in male mice ($p = 0.025$). The information is summarized in Table 2.

Table 2: Liver tumor incidence^a in male CD-1 mice administrated fipronil via diet for 78 weeks

| Tumor Type (day of first tumor) | Administered Dose (ppm) | Achieved Dose (mg/kg-day) | Number of Tumor-Bearing Animals | Effective Animal Number | Trend and Pair-wise p-value ^{a,b} |
|------------------------------------|-------------------------|---------------------------|---------------------------------|-------------------------|--|
| Hepatocellular Adenoma (day 317) | 0 | 0 | 10 | 47 | np |
| | 0.1 | 0.01 | 3 | 50 | np |
| | 0.5 | 0.06 | 2 | 45 | np |
| | 10 | 1.18 | 6 | 40 | np |
| | 30 | 3.42 | 5 | 46 | np |
| Hepatocellular Carcinoma (day 409) | 0 | 0 | 1 | 41 | 0.025* |
| | 0.1 | 0.01 | 1 | 39 | >0.05 |
| | 0.5 | 0.06 | 2 | 34 | >0.05 |
| | 10 | 1.18 | 1 | 32 | >0.05 |
| | 30 | 3.42 | 5 | 42 | >0.05 |

^a The effective animal number represents the number of animals alive at the time of first occurrence of tumor;

^b p-values for the exact trend test are presented on the control row (conducted by OEHHA); p-values of treatment group tumor incidences were from Fisher Exact pairwise comparison with controls (performed by OEHHA); np=not performed. * p < 0.05

OEHHA also notes that the incidence rate of hepatocellular adenoma in the control group was unusually high; it is outside the range of historical controls of the laboratory, and over four times higher than the average historical control rate. It is unclear why there was such high incidence of hepatocellular adenoma in the control group, yet a very similar dose in the treated animals (0.01 mg/kg-day) had a much lower rate. This could pose a problem for linearized cancer risk model should DPR chose to model the combined incidence of hepatocellular adenoma and carcinoma. To overcome this problem, OEHHA suggests DPR evaluate the hepatocellular carcinoma data using a linearized multistage model. However, due to early mortality between controls and some treatment groups, it is possible that the regular linearized multistage model may not be suitable for cancer dose-response assessment. An alternative approach such as the multistage Weibull time-to-tumor model may be more appropriate for the cancer dose-response analysis. Similar to the cancer bioassay in the rat, another issue with the cancer bioassay in the mouse is that the study duration was only 78 weeks, also a less-than-lifetime study. This is particularly important because hepatocellular adenomas can progress to carcinomas over time. It is possible that if the study duration were 104 weeks, more hepatocellular carcinomas might have been observed.

Collectively, OEHHA presents evidence that fipronil-induced thyroid follicular cell tumors in rats could be due to a genotoxic mechanism of action in addition to thyroid hormone disruption, and that there is concern that the threshold approach taken in the draft RCD may be inadequate to protect human health. When constructing a carcinogenicity mode

of action network for fipronil, OEHHA suggests that the thyroid follicular cell tumors in rats and the liver carcinomas in mice be considered relevant for human cancer risk assessment. OEHHA also suggests that the genotoxicity (see II.A.4) and recent data from ToxCast (see II.A.3) be included in the mechanistic considerations.

4. Extrapolation, Variability, and Uncertainty

a. Duration Extrapolation

No duration extrapolations were used in the draft RCD. The chronic POD is protective of subchronic health effects, and is used as the subchronic POD in the draft RCD.

b. Intraspecies Extrapolation

In the draft RCD, a default UF of 10-fold was applied to account for intraspecies variability within the human population (UF_H). This is generally considered to be a factor of $\sqrt{10}$ for pharmacokinetics and $\sqrt{10}$ for pharmacodynamics. It is OEHHA's opinion that an intraspecies UF of 10 is insufficient as there are many factors affecting human variability in response to a chemical exposure (OEHHA, 2008; Zeise et al. 2013). The scientific basis for this recommendation is detailed in OEHHA's peer reviewed Air Toxics Hot Spots Risk Assessment Guidelines, Technical Support Document for the Derivation of Reference Exposure Levels (OEHHA, 2008). Based on analyses of human pharmacokinetic variability, OEHHA's practice is to increase the traditional intraspecies pharmacokinetic UF of $\sqrt{10}$ to 10. This increase would account for the wide variability in pharmacokinetics in the population, especially for individuals who are more sensitive to toxic exposures due to life-stage, health or immune status, genetic and epigenetic variability, or individuals and communities disproportionately burdened by multiple sources of pollution. Thus, OEHHA recommends addressing these concerns by increasing the intraspecies pharmacokinetic UF to 10, resulting in a total UF_H of 30.

c. Sensitive Populations and Life-Stages

As discussed in the acute toxicity and reproductive and developmental toxicity sections above, infants and fetuses appear to be especially vulnerable to fipronil toxicity, and thyroid hormone disruption during critical development periods can cause neurological and developmental effects in offspring. The acute POD selected in the draft RCD is higher than multiple potential PODs derived from developmental studies, as described above. OEHHA suggests that the toxicity database be re-evaluated and a more health protective acute POD be selected.

However, if the acute POD based on hindlimb splay in adults is retained, OEHHA suggests an additional UF be applied for exposure scenarios that include infants,

children, and women of childbearing age to protect them from potential developmental or neurodevelopmental effects resulting from in utero or early-life fipronil exposure.

d. Risk Characterization

The Margin of Exposure (MOE) approach was used to evaluate non-cancer hazards. The draft RCD characterized whether an exposure is likely to cause adverse health effects using a target MOE of 100 for all age groups. OEHHA recommends a target MOE of 300 for all age groups, occupational and non-occupational, to take into account the recommended increase in the intraspecies pharmacokinetic UF from $\sqrt{10}$ to 10. An additional UF may also be warranted for exposure scenarios that include women of child-bearing age and children due to developmental and neurodevelopmental concerns following in utero and early life exposures if the acute POD based on hindlimb splay is retained, as described above.

B. Exposure Assessment

1. Aggregate Exposure

OEHHA has concerns about the potential for underestimation of aggregate exposure from multiple sources that are not addressed by the draft EAD. Residents could receive cumulative exposure from multiple sources. Therefore, OEHHA suggests the draft EAD aggregate exposures from all relevant pathways and sources regardless of whether exposure levels exceed the level of concern.

For example, residents who treat their own pets (applicators) or bathe their pets (table 35, using surrogate scenario as recommended by US EPA) would likely also be exposed to additional post-application sources such as indoor dust and contaminated surfaces or residues that remain on the treated pet. This scenario is supported by a study cited in the draft EAD (Bigelow-Dyk et al., 2012), which found fipronil residues on the indoor areas frequently visited by treated pets and that these residues are transferable to humans. In another study, Mahler (2009) showed fipronil residues on indoor surfaces came from multiple sources that included transport from pet treatment. Among the five residences with the highest fipronil indoor dust concentrations, which ranged between 1100 to 9800 $\mu\text{g}/\text{kg}$, three residences reported regular use of fipronil on dogs. OEHHA suggests the draft EAD consider using this study to estimate residents' post-application exposure through contact with indoor surfaces or ingestion of house dust and include the result in the aggregate exposure assessment. .

Lastly, since no post-application study data were available, the environmental monitoring data in the Mahler study (2009) was used as surrogate data to estimate post-application exposure. Because fipronil levels on outdoor surfaces were lower than on indoor surfaces the draft EAD conducted the exposure assessment for indoor surfaces but did not estimate any additional exposure from outdoor surfaces. The

exposure assessment followed the US EPA SOP guidance (2012) and considered 4-hr exposure time for children exposed to indoor surfaces. However, besides the 4-hr indoor exposure, children may also be active outdoors and be further exposed to fipronil and its degradants through contact with outdoor surfaces.

2. Fipronil Degradants

OEHHA suggests that the draft EAD explain why exposure to fipronil degradants were included in some exposure pathways but not in others. DPR provided the following information on degradants in the draft RCD:

- For indoor surfaces, two degradants from the Mahler study were included in the exposure assessment. For residential outdoor surfaces, degradant levels were reported in Jiang et al. 2016a (EAD, page 11 of 66, Table 3) but these degradants were not considered in the assessment.
- For the drinking water exposure, fipronil degradants were reported in the DPR surface water database (SURF), (EAD Table 4 Page 12 of 66), but were not included in the drinking water exposure assessment (EAD Page 48).
- The draft RCD considered degradants in food. However, degradant residues in the USDA Pesticide Data Program (PDP) data were all non-detects and therefore the degradants were not quantitatively evaluated in the dietary exposure assessment.
- For spray pet products (post-application), two degradants were measured on gloves in the de Fontenay et al. study (1997a), but it is not clear if they were included in the exposure assessment (draft EAD page 34-35 of 66). The appraisal (page 55 of 66) mentioned other data available on degradants in spray pet products in the Bigelow-Dyk et al. study (2012) that were not considered in the draft EAD.

3. Exposure duration assumption for pet owners

OEHHA is concerned that the assumption to use arithmetic means to set time spent with animals may lead to an underestimation of post-application exposure from pet products. The draft EAD sets the time spent with animals at 1 hr for children and 0.77 hr for adults using the arithmetic means found on Tables 8-2, 8-5 and 8-6 of US EPA SOP (2012). The 95th percentile are respectively 2.3 and 2.5. Since this is for estimating acute exposure, OEHHA suggests it is more appropriate to use high-end exposure duration rather than using arithmetic means.

III. RESPONSE TO CHARGE STATEMENTS

DPR asked OEHHA to address charge statements in our peer review of the draft RCD and EAD. The answers provided in this section are purposely brief with more in-depth discussion of these answers and OEHHA's other comments in Section II, Detailed Comments.

A. Toxicity

- 1) **All critical points of departure (PODs) used in this assessment were established using the parent compound fipronil.**

OEHHA concurs with the use of the parent compound, fipronil, for the purposes of deriving critical PODs for human health risk assessment (see section II.A.1a).

B. Hazard Identification

- 1) **The acute oral POD of 0.87 mg/kg-day was based on neurotoxic effects observed in the adult rat.**

The draft RCD chose a dose-dependent reduction in hindlimb splay in rats observed seven hours post-administration as the critical effect and estimated an acute oral POD of 0.87 mg/kg-day using BMD modeling. There is evidence that certain effects occurred earlier than seven hours, and there is uncertainty about whether a lower POD would be estimated if the optimal time were chosen. Furthermore, endpoints observed in several acute or short-term studies suggest a lower POD. OEHHA recommends the acute oral POD be re-evaluated. There is further discussion on this in the following charge statement response and under the detailed comments sections of this report (II.A.1.c and II.A.1.e).

- 2) **Three repeated dose studies in rats identified PODs lower than the critical acute POD of 0.87 mg/kg-day for effects that could potentially result from acute to short-term exposures. However, DPR did not consider these PODs as appropriate critical values to characterize the risk from acute exposures to humans.**

The three repeat dose studies cited by DPR with PODs lower than the critical acute oral POD are Mandella (1995), Coder (2019), and Aughton (1993). The developmental neurotoxicity study (Mandella, 1995) identified several endpoints, the most sensitive being delayed preputial separation, decreased maximum startle response, and decreased body weight in male pups at a LOAEL of 0.9 mg/kg-day. The study NOAEL was 0.05 mg/kg-day. As discussed under the detailed comments sections, there is concern that acute POD of 0.87 mg/kg-day, similar to the LOAEL from Mandella (1995), is not sufficiently protective of the fetus.

The CTA study (Coder, 2019) reported decreased T4 hormone levels and decreased thyroid gland weight in fetuses at gestational day 20 from dams exposed to 1 mg/kg-day fipronil resulting in a NOAEL of 0.3 mg/kg-day. OEHHA disagrees that this study is inadequate for critical POD determination and recommends that this study be reconsidered. This would be consistent with the most recent US EPA (2020) draft risk assessment on fipronil which found the study acceptable for quantitative POD determination.

The Aughton (1993) study showed effects in the range of 0.06 – 1.6 mg/kg-day, during the first week of treatment, significantly decreased T4 levels. Convulsions were also observed in 3 male animals during the first few weeks of treatment. OEHHA disagrees with the rationale presented in the draft RCD for not selecting this endpoint to characterize acute risk. Thyroid hormone disruption seems to be one of the most sensitive effects at any exposure duration, and an acute POD based on this endpoint would be more health protective for sensitive populations. DPR should reconsider these, as well as other studies outlined above, in the determination of an acute oral POD. Additional discussion of these points can be found in our detailed comments (section II.A.1).

3) PODs from dermal and inhalation studies were not used to establish critical PODs.

While route-specific studies are available for acute and subchronic inhalation and dermal exposures, OEHHA agrees that the oral studies are more suitable for POD derivation, and that the approach of route-to-route extrapolation is appropriate.

4) This RCD did not include a cancer risk estimate for fipronil.

OEHHA disagrees with the draft RCD finding that thyroid follicular cell tumors are not relevant to humans and can be evaluated using a threshold approach. OEHHA suggests that the thyroid follicular cell tumors in male and female rats and liver tumors in male mice should be considered relevant for human cancer risk assessment, and the risk should be evaluated by the linearized cancer risk model. This approach is supported by the positive genotoxicity data (see II.A.4) and recent mechanistic data from ToxCast (see II.A.3).

C. Exposure

1) Due to a lack of fipronil monitoring data, handler exposures for structural liquid concentrate (LC), structural dust and turf granule products were assessed using surrogate data.

OEHHA agrees with DPR's use of approaches from the Pesticide Exposure Handlers Database and surrogate chemical for estimating handler exposure to structural LC, structural dust, and turf granular products.

- 2) **Due to lack of post-application monitoring data, environmental sampling data at residential homes were used to assess post-application dermal and oral exposures for structural LC products.**

Since the USGS residential indoor surface monitoring dataset does not have information about the source or the timing of fipronil LC product applications, the acute dermal and oral exposure estimates based on this dataset would have large uncertainties. However, this issue is mitigated to some extent by using the highest estimates.

- 3) **The drinking water assessment only relied on a subset of measured water samples.**

OEHHA agrees that using data from the SURF to estimate fipronil concentration in drinking water is a conservative approach. However, OEHHA is concerned that the analysis method as described in Appendix I (Assessment of human exposure to fipronil) did not include several key details, as described in the Minor comments in this report (section IV.B).

D. Risk Characterization

- 1) **The target margin of exposure (MOE) was set at 100, reflecting the default assumption that humans are 10-fold more sensitive than animals, and that a 10-fold range of sensitivity exists within the human population.**

OEHHA agrees with the use of 10-fold UF for interspecies extrapolation.

However, as described in section (II.A.5.b), OEHHA generally uses a combined intraspecies UF of 30 to account for wide variability in pharmacokinetics in the human population, especially due to susceptible life-stages, health, immune, and genetic factors, and disproportionate pollution burden.

Additionally, for acute exposure scenarios that include infants, children, and women of childbearing age, OEHHA recommends an additional UF if the acute oral POD of 0.87 mg/kg-day is retained due to concern for developmental and neurodevelopmental toxicities (see section II.A.5.c).

- 2) **Risks to workers were estimated for short-term, seasonal and annual exposures.**

For the worker exposure estimates, OEHHA concurs with the approach used in the draft RCD and EAD, but has noted several issues that affect the structural bait gel, structural LC and structural dust estimates that may lead to an underestimation of worker exposure (see section IV.B).

3) Risks to home users were estimated for short-term exposures.

OEHHA is concerned the draft EAD assessed only short-term exposure for home users. Home users of pet products likely receive additional exposure due to post-application contact with residues on treated pets and indoor surfaces. OEHHA suggests risk be assessed for aggregate exposures for home user from all relevant pathways and sources, regardless of whether exposure levels exceed level of concern. OEHHA also recommends reconsidering the assumptions for assessing only acute exposure.

4) Post-exposure risks to child and adult residents were estimated for short-term and seasonal exposures.

For the pet products, estimates for post-application exposure to pest products were solely based on the amount of fipronil transferred to receptors due to direct dermal contact with treated pets and incidental oral contact (for children). As noted in the detailed comments (section II.B.1), indoor dust from homes with treated pets contains high levels of fipronil and its degradants (Mahler, 2009) and this may provide an additional exposure source, especially for children. For structural LC products, the draft EAD did not consider child residents. OEHHA suggests estimating risk for aggregate exposures from all relevant pathways and sources, regardless of whether exposure levels exceed level of concern.

IV. OTHER COMMENTS

A. Toxicity Evaluation and Risk Assessment

- The critical acute POD lists a BMDL₁₀ of 0.87 mg/kg-day. This value appears to be from modeling the hindlimb splay dataset using the Exponential4 model assuming non-constant variance, not assuming constant variance as listed in Table 2 (Appendix IV) of the draft RCD. OEHHA modeled the same dataset using a constant variance model and returned lower BMD and BMDL₁₀ values of 2.09 mg/kg-day and 0.77 mg/kg-day, respectively, from the best-fit model—Exponential4. OEHHA recommends the BMD modeling be verified in the final RCD for accuracy and recommends using constant variance, which appears to be the most appropriate for this dataset.

B. Exposure Assessment

- OEHHA is concerned that some of the assumptions made in the exposure assessment may lead to an underestimation of exposure.
 - For pet product applicators (home users), the draft EAD assumed 2 dogs per house referring to an average found in American Veterinary Medical Association sourcebook (AVMA, 2012).

The most recent AVMA publication reports an average of 2.2 pets per household (AVMA, 2018). In addition, over 35% of pet-owning households have more than 2 pets. OEHHA suggests DPR consider the possibility of more than 2 pets/household. The draft EAD did not calculate seasonal and lifetime exposure because it assumed one application per month. A pet owner can have many pets sequentially and treat them with fipronil products. This could lead to chronic or even lifetime exposure. Moreover, if fipronil persists on pets, even once a month application may lead to seasonal exposure for pet owners. OEHHA suggests DPR reconsider this assumption.

- For the structural bait gel scenario, the draft EAD states that fipronil content ranges from 0.001--0.01% (page 43). However, the DPR Product database shows that two products - Maxforce FC Magnum Roach Killer Bait Gel (432-1460-AA) and Nouvel Sales Fipronil Roach Bait Gel (92028-4-AA) contain 0.05% fipronil. OEHHA recommends that DPR update the dose estimates for the bait gel scenario.
- For the structural LC handler scenario, the draft EAD assumed handling amount 40 gallon/day as recommended by US EPA. Assuming 2 or 4 gallons per 10 linear feet as suggested by the draft EAD, 40 gallons/day means 200 linear feet/day or less, which is much less than the linear feet treated for most of houses listed in Table 11. Therefore, the draft EAD may have assumed

multiple applicators are needed to treat a house within a day. OEHHA recommends the draft EAD include additional information about the application practice to support the assumption of 40 gallons/day per handler.

- For the structural dust scenario, the draft EAD cited 2012-2014 Pesticide Use Report (PUR) data and used a median value to estimate the amount of product handled. The draft EAD noted this amount, 0.04g/day AI (~8g/day of product), was roughly consistent with US EPA's default value of 2 "cans" of product used per application (US EPA, 2012). PUR data from 2014 and 2018 shows that many applications were substantially larger than this median amount and 20-25% of all applications exceeded 0.227g AI (equal to 0.1 lb product). Given the wide range shown by the PUR data, OEHHA is concerned that using median values to estimate the usage amount (product g/day) may underestimate exposure. OEHHA recommends that DPR use the average for acute and chronic exposure assessments.
- OEHHA suggests the draft EAD include more details to support the approach and datasets used in the exposure assessment. For example:
 - Fipronil residue in drinking water: the fipronil concentration in drinking water was estimated using the SURF database, which contains monitoring results from a wide variety of surface water sources. OEHHA suggests the draft EAD include more details on data analysis such as the period or years of extracted data, the approach used to determine the monitoring site source categories (canal, ditch, storm drain, slough, and other), and the rationale for including waterways such as canals and ditches as representative drinking water sources.
 - All the 95th percentiles estimates summarized in the draft EAD cited the method introduced in Frank (2009) and were not the commonly known 95th percentiles in statistical analysis. OEHHA suggests the draft EAD clearly state the difference between these two estimations as recommended by OEHHA in the review for AITC (OEHHA, 2020).
 - In some instances, DPR refers to an external document (US EPA 2012) which makes it hard for the reader to verify assumptions and calculations. That is the case for Tables 15, 21, 28, 30, and 32 in Appendix 1. OEHHA suggests the draft EAD provide all the equations used to calculate exposure including the ones for estimating oral exposures.
 - Similarly, some estimates could not be reproduced because product-specific data were not provided or cited. For example, the specific gravity values for individual fipronil pet products were necessary to replicate dose estimates, but they were not provided. OEHHA recommends that these values be provided in the draft EAD.

- The draft RCD did not include an assessment of the swimmer scenario. The draft EAD stated that a preliminary swimmer assessment was conducted, however the model inputs and results were not reported. OEHHA recommends the model inputs and results be included.
- For the structural dust scenario, OEHHA is concerned about the assumed size of the product container as it relates to the calculation of the amount of fipronil handled. The US EPA SOP (2012) used to evaluate this scenario assumes each handler applies 2 “cans” per application. However, it is unclear if this product is only available in 5g “cans” as the product label does not indicate container size or amount. Also, in response to a recent OEHHA query, the registrant, BASF, indicated that production of the Termidor Dry California product ceased in 2018. OEHHA suggests that DPR include additional information about the product container size.

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VI. APPENDIX I. OEHHA's evaluation of the in vivo and in vitro genotoxicity assays for Fipronil as included in Table 19 of the draft RCD

| Test Type, System | Species or Culture | Exposure Regime | S9 ^a | Results | Cytotoxicity | Reference | OEHHA Comments |
|---|--|---|-----------------|---|---|-----------------------------|---|
| In vivo Bone Marrow Micronucleus Test | CD-1 mouse bone marrow erythrocytes | 0 (0.5% methylcellulose), 1, 5 or 25 mg/kg, gavage, single dose | n/a | Negative | No | Edwards (1993) ^b | FIFRA guideline genotoxicity study. |
| In vivo Bone Marrow Micronucleus Test | CD-1 mouse bone marrow erythrocytes | 0 (0.5% methyl cellulose), 1, 12.5, 25 or 50 mg/kg, gavage, single dose | n/a | Negative | No | Edwards (1995) ^b | FIFRA guideline genotoxicity study. |
| In vivo Comet Assay and Micronucleus Test | Peripheral blood samples of Swiss mice | 0 (water), 15, 25 or 50 mg/kg, IP, one dose; blood taken after 24 (Comet) or 24, 48 or 72 hr (MN) | n/a | Positive at 50 mg/kg after 24 hr | Not tested | de Oliveira (2012) | Only 50 mg/kg fipronil caused significant increase in the frequency of MN (after 24 hr treatment) and DNA strand breaks in treated mice. |
| In vivo Comet Assay and Chromatin structure Assay | Wistar rat (spermatozoa) | 0 (corn oil), 2.5, 5 or 10 mg/kg/day, gavage, 28 days | n/a | Comet positive at 2.5 mg/kg/day and higher; damage to sperm chromatin at 5 mg/kg/day and higher | Significant increase in cytotoxic effects in terms of loss of mitochondria membrane potential (MMP) and apoptosis at 5 and 10 mg/kg/day | Khan (2015) | Higher doses (5 and 10 mg/kg) markedly reduced the DNA integrity of spermatozoa. All 3 doses cause significant increase of sperm DNA strand breaks (starting at low dose 2.5 mg/kg). However, there were no significant ROS and apoptotic cells increases in the 2.5 mg/kg treatment group. This indicates the DNA damage in this group was not caused by either ROS or loss of MMP-induced apoptosis (cytotoxicity). |

| Test Type, System | Species or Culture | Exposure Regime | S9 ^a | Results | Cytotoxicity | Reference | OEHHA Comments |
|---|--|---|-----------------|--|--------------|-------------------|---|
| In vivo Comet Assay and Micronucleus Test and CA | Blood cells of Holtzman rat (Comet), bone marrow of Holtzman rat (CA), and bone marrow of Swiss albino mice (MN) | 0 (corn oil), 2.5, 12.5 or 25 mg/kg, gavage, a single dose | n/a | Positive at 2.5, 12.5 and 25 mg/kg | No. | Badgujar (2016) | Fipronil (3 doses) caused significant increase in the frequency of MN in mouse polychromatic erythrocytes. Structural CAs in bone marrow cells and DNA strand breaks in the lymphocytes was significantly higher in the fipronil (3 doses) treated rats as compared to their respective controls. No cytotoxicity was observed, as noted by the authors' statement "In the present study, fipronil did not significantly alter PCE/NCE ratio indicating lack of cytotoxicity at the dose levels tested." No oxidative stress markers were tested in this study. |
| In vivo Mammalian Bone Marrow and germ cell Chromosome Aberration Test and Comet Assay in liver, lung, and spleen | BALB/cYwal mice | 0, 4.75, 9.5, 19, 31.7mg/kg, IP, one single dose (Comet); 9.5 mg/kg, IP, one single dose or daily for 10 days (bone marrow CA); 19, 31.7mg/kg, IP, one single dose (germ cell CA) | n/a | Comet positive in liver: 9.5-31.7 mg/kg (6h), 4.75- 31.7 mg/kg (24h); lung: 4.75- 31.7 mg/kg (6h), 9.5- 31.7 mg/kg (24h); spleen: 9.5- 31.7 mg/kg (6h), 9.5- 31.7 mg/kg (24h); CA test positive at 9.5 mg/kg (IP, one dose), 9.5 mg/kg (repeated); germ cell CA test positive at 19 and 31.7 mg/kg IP (one dose) | Not tested. | Lovinskaya (2016) | This paper showed the pronounced genotoxic effect of fipronil at all tested doses (4.75, 9.50, 19.00, and 31.70 mg/kg) with a single exposure or at dose of 9.5 mg/kg with repeated exposure (low dose range as compared to other mice in vivo studies listed in the table). Fipronil induced genotoxicity in both somatic and germ cells in mice in vivo. There were no cytotoxicity or oxidative stress markers tested in this study. |

| Test Type, System | Species or Culture | Exposure Regime | S9 ^a | Results | Cytotoxicity | Reference | OEHHA Comments |
|---|--|---|---|--|---|---------------------------|---|
| In vivo Somatic Mutation and Recombination Test (SMART) | 4 different strains of <i>Drosophila melanogaster</i> third instar larvae [mwh/flr ³ (MH) standard (ST) cross, mwh/TM3 (BH) (ST) cross, MH bioactivation (HB) cross, BH (HB) cross] | 0.3, 0.7, 1.5 or 3.0× 10 ⁻⁵ mM, 48 hours | Yes (HB strain has a high level of CYP 6A2) | Increased somatic mutation and recombination at all doses tested in MH ST cross and MH HB cross strains; Increased mutations at 1.5 x 10 ⁻⁵ mM in BH HB cross strain; | Fipronil did not cause the cytotoxicity in ST and HB cross strain pupae formation process. But, concentrations equal or higher than 6 x 10 ⁻⁵ mM reduced the survival rate of ST adults and only the lowest concentration (0.3 x 10 ⁻⁵ mM) evaluated in this study was not toxic in HB cross strains. | de Morais (2016) | Fipronil in the lowest concentration tested (0.3 x 10 ⁻⁵ mM) induced the highest frequency of DNA damage in MH ST cross and MH HB cross strains. Overall, the results showed mutagenic effects at all concentrations tested in the HB cross; and all concentrations tested in the ST cross, except at concentration of 0.7 x 10 ⁻⁵ mM. The mutagenicity data and carcinogenicity data of fipronil are in agreement in this <i>D. melanogaster</i> models. Fipronil has been proved to be mutagenic, recombinogenic and carcinogenic in somatic cells of <i>D. melanogaster</i> . No oxidative stress markers were measured in this study. |
| Ames Test | <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537 | 0 (DMSO), 0.8, 2, 20, 100, 400 or 500 µg/plate, 48 hr | ± | Negative | No | Clare (1988) ^b | This is a FIFRA guideline genotoxicity study. There are big gaps in dose between the 20 and 100 µg/plate doses and between the 100 and 400 µg/plate doses used in the study. These missing ranges of test doses may be the effective doses. Cytotoxicity in high dose groups was not report by authors, but cytotoxicity is possible. |
| HGPRT Forward Mutation | Chinese hamster lung cell line V79 | 0 (DMSO), 0.8, 4, 20, 100 or 500 µg/mL, 3 hr | ± | Negative | No | Lloyd (1993) ^b | This is a FIFRA guideline genotoxicity study. The dose range in this study is a concern as compared to the treated doses 30, 45, 60 µg/ml in Wright <i>et al.</i> , 1995 using the same cell line. Wright <i>et al.</i> , 1995 showed positive effects on chromatid |

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| | | | | | | | breaks and exchanges in 45-60 µg/ml groups; however, in this study there is a big dose gap between 20 and 100 µg/ml fipronil. High dose treatments (above 100 µg/mL) are also a concern. Cytotoxicity in high dose groups was not reported by authors, but cytotoxicity is possible. |
| In vitro Chromosome Aberration | Human lymphocytes | 0 (DMSO), 75, 150 or 300 µg/ml, 3 hr | ± | Negative | Decreased cell viability | Marshall (1988) ^b | This is a FIFRA guideline genotoxicity study. The quality of primary culture from various donors may be an issue and the cellular mitotic index presents a large variability between male and female donors. This variability made the CA and cytotoxicity data hard to be interpreted. There are big gaps between 0 and 75 µg/ml dose ranges in the study, which may be the effective doses. High dose treatments (above 75 µg/ml) are a concern. Authors reported significant cytotoxicity occurred at 300 µg/ml (around 70% mitotic inhibition). |
| In vitro Chromosome Aberration | Chinese hamster lung cell line CHL | 0, (DMSO), 30, 45, or 60 µg/ml for 6, 24 or 48 hr | ± | Positive at 45 and 60 µg/ml after 6 hrs only, no S9 | Yes (≥ 60 µg/ml) | Wright, (1995) ^b | This is a FIFRA guideline genotoxicity study. The assay without S9 showed a sharp dose-response for chromatid breaks and exchanges in the 45-60 µg/ml groups. |
| In vitro Comet Assay | Human laryngeal mucosal cells | 0, 0.05, 0.1, 0.5, 0.75 or 1 mM, 1 hr | n/a | Positive at all doses | n/a | Tisch (2007) | Written in German. The draft RCD states cytotoxicity is "likely." Please specify what are the cytotoxicity data in different treatment doses as reported by study authors to support this statement. |

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| In vitro Sister Chromatid Exchange and Micronucleus Test and DNA break (comet assay) | Human lymphocytes | 0, 0.1, 0.3 or 0.7 µg/ml, 72 hr | n/a | SCE and Comet positive at all doses; MN positive at two higher doses. | Decreased mitotic index, indicating high cellular toxicity at concentrations ≥ 0.7µg/ml | Celik (2014) | Fipronil induced a statistically significant increase in the MN and SCE frequency and DNA strand breaks in a dose-dependent manner. At 0.1 µg/ml, Survival around 99.3%, 74%, 51% at 0.1, 0.3, and 0.7 µg/ml, respectively. No oxidative stress markers were tested in this study. Authors reported that fipronil was obtained from FIBREX 75 (5.5mL contains 412.5mg of FP). Controls were appropriate. |
| In vitro Micronucleus Test and γH2AX marker (DNA strand breaks) | Human HepaRG cells | 0, 5, 10, 15 or 25 µM for 1 day or 0, 5, 10, 20 µM for 7 days or 14 days | n/a | MN test positive at 15 and 20 µM after 7 days; γH2AX marker positive at 20 µM after 7 and 14 days | Increased cytotoxicity (MTT assay) observed at doses higher than 20 µM in all time points | Quesnot (2016) | No cytotoxicity was observed at doses lower than 20 µM. No oxidative stress markers were measured in this study. Induction of γH2AX was observed with fipronil treatments only after 7 days, highlighting the importance of studying long-term effects in low doses. γH2AX is an indicator of DNA strand breaks. The results from γH2AX and MN with low dose long-term treatment of fipronil are consistent. |
| In vivo Random Amplified Polymorphic DNA analysis (RAPD) | <i>Vicia faba</i> seedlings | 0, 0.5, 1, 2, 3 or 4 ppm for 7 days. Fipronil was obtained from Sigma Chemical Company (St Louis, Missouri, USA). It is around 90 to 100% purity. The negative control is the sterile distilled water solution | n/a | Genomic template stability (GTS) was decreased in a dose-dependent manner for all doses tested. | Not tested. | Yildirim (2016) | The decrease in GTS values is likely due to addition of all DNA alterations induced by fipronil (e.g. mutations, rearrangements, and structural modifications). These events can affect polymerization of DNA in the PCR reaction; therefore can be measured by changes of RAPD band numbers and intensity. Increased fipronil concentration caused decreasing GTS values and increasing polymorphism values. The degree of |

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| | | contained zero amount of fipronil. | | | | | polymorphism values and GTS were clearly dose dependent. No cytotoxicity and oxidative stress markers were tested in this study. |
| In vivo Micronucleus Test | <i>Tradescantia pallida</i> flowering plantstems | 0.025, 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 g/L solution for 8 hr, with 24-hr recovery phase | n/a | Increased formation of MN at ≥ 0.2 g/L compared to the negative control | Not tested. | de Morais (2019) | T. pallida has been shown to be a good model organism in the screening of insecticide-induced genotoxicity. The results reveal that, under the experimental conditions, fipronil was genotoxic at concentrations of 0.2, 0.4, 0.8 , and 1.6 g/L. No cytotoxicity and oxidative stress markers were tested in this study. |

^aS9 is liver homogenate used for biological activation of xenobiotics in DNA damage testing. ± indicates the test was done in the absence (-) or presence (+) of S9. ROS: reactive oxygen species.

^bRegistrant-submitted studies.