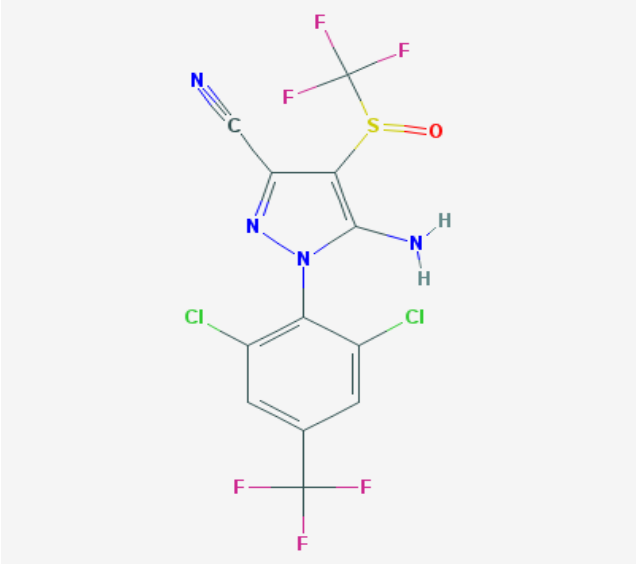
FIPRONIL

RISK CHARACTERIZATION DOCUMENT



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LIST OF ABBREVIATIONS

AADD	Annual average daily dose
ADI	Accepted daily intake
AFSSA	French Food Health Safety Agency
AFSSE	French Environmental Health Safety Agency
AI	Pesticidal active ingredient
APVMA	Australian Pesticide and Veterinary Medicine Authority
aRfD	Acute reference dose
BMD	Benchmark dose
BMDL	Lower 95% confidence limit of the benchmark dose
BMDS	Benchmark Dose Software
BMR	Benchmark response level
BW	Body weight
C_{max}	Maximum concentration
CNS	Central nervous system
CTA	Comparative Thyroid Assay
CYP	Cytochrome P450 enzyme
DEEM™	Dietary Exposure Evaluation Model
DNA	Deoxynucleic Acid
DNT	Developmental Neurotoxicity
DPR	Department of Pesticide Regulation
EAD	Exposure Assessment Document
FCID	Food Commodity Intake Database
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FOB	Functional Observational Battery
FQPA	Food Quality Protection Act
GABA	Gamma-aminobutyric acid
GD	Gestation day
HPT	Hypothalamic-pituitary-thyroid
IP	Intraperitoneal
LD_{50}	Median lethal dose
LC_{50}	Median lethal concentration
LC	Liquid concentrate
LC-MS	Liquid chromatography mass spectrophotometry
LD	Lactation day
LOD	Limit of detection
LOEL	Lowest-observed-effect-level
MMAD	Median mass aerodynamic diameter
MOE	Margin of exposure

NOEL	No-observed-effect-level
PDP	Pesticide Data Program
PISP	Pesticide Illness Surveillance Program
PND	Postnatal day
POD	Point of departure
ppm	Part per million
ppb	Part per billion
PSN	Progressive senile nephropathy
RCD	Risk Characterization Document
RDL	Resistant to dieldrin GABA receptor
RfC	Reference concentration
RfD	Reference dose
SENSOR	Sentinel Event Notification System for Occupational Risk
SADD	Seasonal average daily dose
STADD	Short-term absorbed daily dose
SULT	Sulfotransferase enzyme
$T_{1/2}$	Half-life
T3	Triiodothyronine
T4	Thyroxine
T_{max}	Time to maximum concentration
TOPE	Time to peak effect
ToxCast™	Toxicity Forecaster™
TSH	Thyroid stimulating hormone
UF	Uncertainty factor
UGT	Uridine diphospho-glucuronosyltransferase
USDA	US Department of Agriculture
US EPA	US Environmental Protection Agency

I. EXECUTIVE SUMMARY

The purpose of this Risk Characterization Document (RCD) is to evaluate potential risks to human health that result from occupational and residential uses and exposures to fipronil.

Fipronil (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfinyl]-1H-pyrazole-3-carbonitrile) is a broad spectrum pesticide that is effective against various insect and arachnid pests. The pesticidal action is attributed to inhibition of the gamma-aminobutyric acid (GABA)-gated chloride channels in pests and in mammals.

Fipronil was first registered in the US in 1996 for use on golf courses and commercial turf. It was registered in California in 1997 to control termites, ants, and cockroaches in residential, industrial, and institutional sites. It is also used as the active ingredient in topical spot-on and spray anti-flea and tick formulations for companion animals (dogs, cats). There are no food uses of fipronil permitted in California.

Technical grade fipronil causes moderate acute mammalian toxicity by the oral, dermal, and inhalation routes. Fipronil is designated as a Category II toxicant based on median lethal dose and concentration (LD_{50} and LC_{50})[1]. It is a Category III eye irritant and a Category IV dermal irritant. Fipronil is classified as a Group C carcinogen, i.e., a "Possible Human Carcinogen" by the US Environmental Protection Agency (US EPA). Fipronil was prioritized for risk assessment by the California Department of Pesticide Regulation (DPR) in 2009 for several reasons:

1. It is acutely neurotoxic in rats
2. It has low acute, subchronic, and chronic lowest-observed-effect levels and no-observed-effect levels (LOELs, NOELs) compared to other pesticides
3. It causes convulsions and other neurological disturbances in chronic toxicity studies in rats and dogs, and is oncogenic in rats (thyroid tumors), and
4. It is the source of a large number of adverse human health effects reports.

Sixty-eight cases of possible, probable, or definite illness in humans from fipronil exposure were submitted to the DPR Pesticide Illness Surveillance Program (PISP) between 1999 and 2017 (most currently data available[2]). More than 6000 possible adverse human health effects reports have been submitted to DPR by the pesticide registrants since 2008.

[1] Acute Toxicity Categories. US EPA Label Review Manual Chapter 7: Precautionary Statements. US Environmental Protection Agency, Office of Pesticide Programs, Registration Division. Revised March 2018. https://www.epa.gov/sites/production/files/2018-04/documents/chap-07-mar-2018.pdf

[2] California law requires physicians to report any known or suspected illness caused by a pesticide exposure. The DPR Pesticide Illness Surveillance Program (PISP) is tasked with collecting and evaluating these reports. Database queries and the latest reports are available at https://www.cdpr.ca.gov/docs/whs/pisp.htm

Scope of Risk Assessment

The health effects and the risks of exposure to fipronil by both workers and the general public are detailed in this assessment. Risks were calculated for occupational handlers, home users, and for adult and child residents following the use of flea and tick treatments, bait gels, dusts, and liquid concentrates that are approved for use in and around structures, and for turf applications. Fipronil does not have any approved food uses in California. However, because US EPA has established tolerances for several crops and because fipronil is approved for food use in other states, this RCD includes a dietary and drinking water assessment to characterize both acute and chronic risks for the general population and sensitive subpopulations. Toxicity data are available for fipronil as well as for its two main metabolites (fipronil-sulfone and fipronil-sulfide) and an environmental photodegradate (fipronil-desulfinyl). Data used in this assessment came from registrant-submitted studies, published scientific studies, and documents from other regulatory and scientific bodies. A systematic review of published literature was conducted through June 2022, as detailed in Appendix I. Qualitative data not useful for determining the points of departure were analyzed using a weight-of-evidence approach to understand modes of action and potential effects in humans.

Findings

Critical points of departure (PODs) were established for fipronil from acute, subchronic and chronic studies in laboratory rats for non-oncogenic effects by all routes of exposure. For purposes of this risk assessment, PODs are doses or air concentrations that do not produce toxicologically significant effects following a specific duration of exposure.

Acute Toxicity:

The critical acute oral POD of 0.77 mg/kg/day was based on neuromuscular effects seen in an acute oral neurotoxicity study. Healthy rats splay their back legs and feet when they land from a height. Fipronil caused a significant dose-dependent reduction in the ability of rats to properly orient their back legs and feet (hindlimb splay) 7 hours after dosing (Hughes, 1997). The acute oral POD was the threshold modeled dose BMDL10 (lower 95% confidence limit of the benchmark dose) which reduced hindlimb splay by 10%. The acute oral POD was also used to evaluate the acute dermal and inhalation risk to humans.

Subchronic Toxicity:

The critical subchronic oral POD of 0.02 mg/kg/day was based on decreases in serum thyroxine (T4) levels, convulsions, and death in treated rats. The critical subchronic oral POD was the no observed effect level (NOEL) in a chronic toxicity study in which effects were seen at the lowest observed effect level (LOEL) of 0.06 mg/kg/day within one year of exposure (Aughton, 1993). The subchronic oral POD was also used to evaluate the subchronic dermal and inhalation risks to humans.

Chronic Toxicity:

The critical chronic oral POD of 0.02 mg/kg/day was based on convulsions and mortality, sustained decreases in T4, and increased progressive senile nephropathy in a chronic oral study in rats (Aughton, 1993). The POD was based on the study NOEL. The chronic oral POD was also used to evaluate dermal and inhalation risk to humans, as chronic inhalation and dermal toxicity studies were not available.

Oncogenicity:

This RCD does not include a cancer risk estimate for fipronil. Fipronil exposure resulted in significant increases in thyroid follicular cell adenomas and carcinomas in male and female CD rats when compared to controls (Aughton, 1993). Based on the current fipronil database, there is a high degree of confidence that fipronil-induced thyroid tumors arise in rats from hypothalamic-pituitary-thyroid (HPT) axis disruption. The weight of evidence suggests that fipronil-induced hepatic clearance of serum T4 is an upstream effect required in the development of thyroid tumors in rats. Humans are less sensitive to thyroid tumor development from thyroid-pituitary disruption because of the body's ability to efficiently buffer thyroid hormone changes in the blood. Establishing the critical chronic POD at 0.02 mg/kg/day, a level which does not affect T4 levels and well below a level at which tumors formed, will be protective of tumor formation in humans.

Thyroid and Liver Toxicity:

Repeated exposures to fipronil caused increased thyroid weight and histopathology in adult and developing rats as well as thyroid tumors at the high dose in one study. However, fipronil did not act directly on the thyroid. Instead, it induced hepatic (liver) metabolism, which led to increased elimination of T4. Sustained T4 elimination is the required first step for all subsequent thyroid pathologies observed in rats. In humans, decreased T4 levels are generally not considered deleterious to adults in the short-term. During human development, however, thyroid hormone deficiencies of even a short duration during development can lead to irreversible brain damage. Analysis by DPR suggested that fipronil-induced thyroid effects occur at doses and exposure durations above the critical PODs.

Reference doses (RfDs) are target levels that are likely to be without appreciable risk of deleterious effects. These values are calculated by dividing the critical endpoint values by uncertainty factors (UFs). Commonly used default uncertainty factors are 10x to account for interspecies sensitivity (UF_A) and 10x to account for intraspecies (human) variability (UF_H). Both uncertainty factors are themselves products of two separate components, a pharmacokinetic uncertainty factor of 3x and a pharmacodynamic uncertainty factor of 3x. Default uncertainty factors are used in this assessment as the current database does not warrant alterations to these values.

Acute, subchronic, and chronic oral and dermal RfDs were generated using a total uncertainty factor of 100. RfDs were normalized using fipronil absorption rates established by DPR. The oral

absorption rate of 100% and the dermal absorption rate of 4.3% were determined experimentally from registrant-submitted data. While inhalation exposure and risk were calculated for fipronil products, DPR found that the inhalation risk was minimal compared to the dermal risk for adults and compared to the dermal and incidental oral risk for children. In addition, when dermal and inhalation exposures occurred in tandem, dermal exposure drove the risk. Therefore, a separate reference concentration (RfC) was not calculated for inhalation. Values found in Summary Table 1 represent internal doses.

Summary Table 1. Points of Departure (PODs), Uncertainty Factors (UFs), and Reference Doses (RfDs) for Exposure to Fipronil

Duration	Route	POD (mg/kg/day)	% Abs.[a]	UF_{TOTAL}	RfD[a] (mg/kg/day)
Acute	Oral[b]	0.77	100	100	0.008
Acute	Dermal	0.77	4.3	100	0.18
Subchronic and chronic	Oral	0.02	100	100	0.0002
Subchronic and chronic	Dermal	0.02	4.3	100	0.005

POD: Point of Departure; % Abs: percent absorption; UF: Uncertainty factors: UF_{TOTAL}: total UF, includes 10x for intrahuman variability and 10x for interspecies extrapolation from animal to human; RfD: reference dose = (POD x % Abs)/UF_{TOTAL}.

[a]Oral percent absorption from (Powles, 1992); dermal percent absorption from (Cheng, 1995; DPR, 1999a). No data were available for inhalation percent absorption, so DPR used 100% by default.

[b]Oral route is for incidental or non-dietary oral exposure.

The margin of exposure (MOE) is a quantitative tool used by DPR to determine the potential risk arising from exposure to a pesticide. An MOE is defined as the ratio of the critical POD value derived from the definitive acute, subchronic, or chronic studies to the estimated human exposure. The resulting value is compared to the acceptable or target MOE which, for purposes of this risk assessment, is equivalent to the total uncertainty factor (UF_{TOTAL}) of 100. Values at or above the target MOE are generally considered protective against the toxicity of fipronil.

Margin of Exposure (MOE) = POD / Exposure concentration

Risks from single and multiple routes (that is, combined or aggregate exposure) were calculated for occupational handlers, home users, and adult and child residents. Exposure scenarios included use of pet spray and spot-on treatments, applications of structural dust, structural liquid concentrate and turf granules, and the use of structural bait gel. Exposure durations included short-term/acute (up to one week), seasonal/subchronic (7 days to one year), and annual/chronic (sum of monthly exposure values amortized over one year) (DPR, 2017a). Exposure calculations are described in the Exposure Assessment Document (Jiang, 2022). Exposure scenarios resulting in MOEs lower than the target of 100 indicate a potential health risk to humans.

Risks from single-route exposure scenarios

Summary Table 2 details scenarios that resulted in MOEs lower than the target of 100. The table is organized by target population (the group of people expected to be exposed to fipronil), fipronil use scenario (the specific person using a specific product), the duration (length) of exposure, the route of exposure, and the calculated MOE. Of these, the most common risk for occupational handlers results from dermal exposure to pet products regardless of exposure duration, and liquid concentrate over subchronic and chronic exposure durations. Inhalation of fipronil from pet spray products also pose both subchronic and chronic risks to occupational handlers. Pet spray poses an acute risk to adults applying products at home on their own pet from dermal exposure. Dermal exposure resulting from applications of pet spray products also pose subchronic risks to both children and adults. Finally, this analysis shows risks to children from non-dietary oral exposure (incidental or hand-to-mouth) from two different products (pet spray and turf granules). Overall, pet products accounted for 11 of 17 (9 pet spray scenarios and 2 spot-on scenarios) and dermal exposure accounted for 13 of 17 scenarios which indicated potential risk to human health. These exposure scenarios track directly with those noted in over 95% of adverse effects reports submitted to DPR between 2009 and 2015 for fipronil-containing flea and tick products.

Summary Table 2. Single-Route Exposure Scenarios with Potential Risk to Humans as Identified in the RCD

Target Population	Fipronil Use Scenario	Duration of Exposure	Route of Exposure	MOE
occupational	pet spray, groomer	acute	dermal	15
occupational	pet spot-on, groomer	subchronic	dermal	28
occupational	pet spray, groomer	subchronic	dermal	2
occupational	structural LC – no overhead	subchronic	dermal	41
occupational	structural LC – overhead	subchronic	dermal	15
occupational	turf granules	subchronic	dermal	83
occupational	pet spray, groomer	subchronic	inhalation	51
occupational	pet spot-on, groomer	chronic	dermal	28
occupational	pet spray, groomer	chronic	dermal	2
occupational	structural LC – no overhead	chronic	dermal	71
occupational	structural LC – overhead	chronic	dermal	27
occupational	pet spray, groomer	chronic	inhalation	51
home user, adult	pet spray, pet owner	acute	dermal	41
post-app, adult	pet spray	subchronic	dermal	39
post-app, child	turf granules	acute	oral	33
post-app, child	pet spray	subchronic	oral	91
post-app, child	pet spray	subchronic	dermal	21

Margin of Exposure (MOE) < 100 represents a potential health risk. MOE = point of departure/estimated exposure; LC: liquid concentrate; post-app: post-application, i.e., exposures following application

Acute and chronic risk from combined exposure to fipronil residues in food and drinking water exposures was calculated for the general population and sensitive subgroups including infants, children 1–2 years old, and women of childbearing age (13–49). The US Department of Agriculture (USDA) Pesticide Data Program database was used as a source for fipronil residues on food commodities. Food consumption data came from the National Health and Nutrition Examination Survey (NHANES, 2005–2010). All acute dietary MOEs at the 95th exposure percentile and all chronic dietary MOEs were above the target of 100, and therefore are not considered a health concern.

Risks from aggregate exposure (via a multiple exposure routes)

This assessment also calculated aggregate MOEs which represent risk from combined exposure scenarios. For workers, adult home users, and adult residents, MOEs were calculated using simultaneous dermal and inhalation exposures. For children, MOEs were calculated based on dermal, inhalation and oral (non-dietary incidental) exposures. Overall, dermal exposure was the main contributor to total fipronil risk for adults. For children, non-dietary oral (incidental and hand-to-mouth) and dermal exposure contributed the most to the total risk. Exposure from diet and drinking water was not included in the aggregate MOE calculations because the dietary exposure estimates had little impact on either total exposure or on the overall aggregate MOE calculations.

Summary Table 3. Aggregate Margins of Exposure for Occupational Handlers, Home Users, and Adult and Child Residents as Identified in the RCD

Target Population	Fipronil Use Scenario	Duration of Exposure	Routes of Exposure	MOE
occupational	pet spray, groomer	acute	dermal+inhalation	14
occupational	pet spot-on, groomer	subchronic	dermal+inhalation	28
occupational	pet spray, groomer	subchronic	dermal+inhalation	1
occupational	structural LC – no overhead	subchronic	dermal+inhalation	29
occupational	structural LC – overhead	subchronic	dermal+inhalation	15
occupational	turf granules	subchronic	dermal+inhalation	78
occupational	pet spot-on, groomer	chronic	dermal + inhalation	28
occupational	pet spray, groomer	chronic	dermal+inhalation	1
occupational	structural LC – no overhead	chronic	dermal+inhalation	51
occupational	structural LC – overhead	chronic	dermal+inhalation	26
home user, adult	pet spray, pet owner	acute	dermal+inhalation	40
post-app, adult	pet spray	subchronic	dermal+inhalation	39
post-app, child	turf granules	acute	oral+dermal+inhalation	33
post-app, child	pet spot-on	subchronic	oral+dermal+inhalation	95
post-app, child	pet spray	subchronic	oral+dermal+inhalation	17

Margin of Exposure (MOE) < 100 represents a potential health risk. MOE = point of departure/estimated exposure; LC: liquid concentrate; post-app: post-application, i.e., exposures following application

II. TECHNICAL SUMMARY

II.A. Toxicological Profile

II.A.1. Human Illness Reports and Epidemiology

Illnesses or injuries associated with exposure to fipronil were identified from DPR's Pesticide Illness Surveillance Program (PISP), the adverse human health effects reports submitted by fipronil registrants, the Sentinel Event Notification System for Occupational Risk (SENSOR) Pesticides program, published case studies on patients with purposeful or accidental fipronil ingestion, and human population-based studies. Overall, effects observed were consistent between data sources and were similar to effects seen in laboratory animals. Clinical signs in humans included red and irritated eyes and skin, vomiting, diarrhea and gastrointestinal upset, rash, vertigo, headache, disorientation, coughing, throat irritation, dizziness, tremors, and spinal pain. Two out of approximately 6800 adverse human health effects reports submitted to DPR by registrants identified people who died following fipronil exposure. In one case, the person died from allergic reaction to a pet spray product. Details were not available for the other case. Kidney and liver effects, seizures, and death were reported in published case studies on hospitalized patients.

Population-based studies monitoring human exposure to pesticides showed the main fipronil metabolite, fipronil-sulfone, in blood specimens in workers at a fipronil veterinary product factory, in neonate-parent groups, and in volunteers with no known pesticide exposure. The study on workers did not find a correlation between fipronil levels and levels of thyroid hormone (T4) in the blood. The study on newborns and their parents reported that increased fipronil-sulfone levels were associated with decreased triiodothyronine (T3) levels and neonate Apgar scores (measurement of well-being of the newborn). Two studies looked at potential exposure to fipronil in farm communities; in one, fipronil-sulfide was the most frequently detected pesticide in a wrist-band-based passive-sampling study of dermal and inhalation exposure of 1500 different chemicals. In the other study, no significant health risks due to inhalation of pesticides during spraying activities was found to rice farmers.

II.A.2. Toxicokinetics

Limited toxicokinetic data for humans were available. Oral ingestion showed variability as high as 46-fold in toxicokinetic parameters such as maximum concentration and time to maximum concentration (C_{max} and T_{max})(Mohamed *et al.*, 2004). C_{max} is the maximum or peak serum concentration that a xenobiotic achieves after dosing or exposure and the related pharmacokinetic parameter T_{max} is the time it takes to achieve C_{max}. Oral absorption in rats was assumed to be over 80%. Fipronil was extensively metabolized and distributed throughout the body. No unmetabolized fipronil was detected in the tissues or urine. The highest levels were found in the fat at 7 days after dosing. In both rats and humans, fipronil was metabolized mainly to its lipophilic sulfone-form. The feces were the major route of elimination for fipronil and its metabolites. About 20% of the radioactivity was excreted in feces within the first 24 hours of treatment as both unchanged fipronil and some metabolites, whereas only metabolites were seen

at later time points. Bile cannulation experiments confirmed that a significant portion of the metabolites were eliminated in the bile enterohepatic circulation. In rats, the relatively slow elimination of fipronil and its metabolites (elimination half-life, $T_{1/2}$ of > 6 days) was proposed to be due to a partitioning onto fat. Dermal absorption was estimated to be 4.3%, based on studies conducted in rats. This value was also used to estimate dermal absorption in humans.

II.A.3. Acute Toxicity

Exposure to high levels of fipronil result in seizures, dizziness, sweating, nausea and vomiting, agitation, high blood pressure, rash, tearing eyes, vertigo, headache, disorientation, coughing, throat irritation, burning eyes and lips, dizziness, tremors and spinal pain. The neurological symptomology is consistent with GABA receptor antagonism in the central nervous system (CNS). Published case studies showed acute kidney injury (retention of urine, high serum creatinine, kidney shutdown) and abnormal liver function following fipronil ingestion.

Fipronil also cause clinical signs suggestive of excessive CNS stimulation (convulsions, seizures and tremors), which is consistent with fipronil being classified as a convulsant. Decreased coordination, gait abnormalities, hypoactivity, lethargy, diarrhea, decreased respiratory rate and prostration were also observed. Acute oral doses of fipronil which were lethal to 50% of the experimental animals (LD_{50}) ranged from 92–103 mg/kg. Acute inhalation exposures resulting in 50% mortality in rats (LC_{50}) ranged from 0.36 to 0.68 mg/L (equivalent to 41 to 53 mg/kg). Acute dermal LD_{50} values were > 2000 mg/kg in rats and 354 mg/kg in rabbits. Short-term effects in rats (i.e., effects occurring after 1–7 days of exposure) included reduced weight gain, decreased triiodothyronine and thyroxine (T3 and T4) levels, and increased thyroid stimulating hormone (TSH) levels.

II.A.4. Subchronic Toxicity

Fipronil elicited a variety of responses under subchronic scenarios, including neurological disturbances, reductions in body weight, decreased litter survival, decreased pup viability, liver effects (increased liver weight, hypertrophy and fat deposition), thyroid effects (increased thyroid weight, hypertrophy and hyperplasia, decreased serum T4 and increased serum TSH) and delayed developmental signs (incisor eruption, vaginal patency and preputial separation). Increased oxidative stress also occurred under subchronic exposure conditions. Two human-based population studies showed altered thyroid-related hormones in humans.

II.A.5. Chronic Toxicity

Effects of chronic exposure included neurological disturbances, convulsions and tremors, mortality, changes in thyroid hormone levels, reductions in body weight, progressive senile nephropathy, and liver periacinar vacuolation.

II.A.6. Oncogenicity

In chronic toxicity/oncogenicity studies, fipronil induced thyroid follicular adenomas and carcinomas in male and female rats at the high dose. The weight of evidence indicates that the formation of thyroid follicular cell tumors in rat occurred by an anti-thyroidal mode of action via a process that disrupts the hypothalamo-pituitary-thyroid axis. Sustained elevations in TSH levels in turn cause follicular cell hypertrophy, hyperplasia and neoplasia. This mode of action is a well-recognized rat-specific mechanism for thyroid neoplasms and is not relevant to humans because humans have more efficient serum thyroid buffering.

II.A.7. Genotoxicity

Six genotoxicity studies were submitted to DPR to fulfill pesticide registration data requirements. Five of the studies were negative for genotoxicity under the experimental conditions used and concentrations tested. The remaining study, an *in vitro* study, showed damage to chromosomes along with cytotoxicity. Given the relationship between induction of chromosomal damage and cytotoxicity, the former appears to be part of the process of cell toxicity leading to cell death, as opposed to indicating a significant genotoxic potential. Three studies published in the open literature showed genotoxic effects in association with apoptosis, cytotoxicity or oxidative stress, but had methodological deficiencies that limited their reliability.

II.A.8. Developmental and Reproductive Toxicity

No developmental effects were attributed to treatment with fipronil in rats or in rabbits in standardized developmental toxicity studies. In a rat comparative thyroid assay (CTA), developmental toxicity was characterized by decreased T4 levels in fetuses and increased liver weight in pups. Reported effects of fipronil on rat reproduction included decreased body weights and convulsions in pups, decreased mating and fertility, reduced pup viability, decreased litter size, and pup developmental delays. Developmental neurotoxicity study in rats revealed dead/missing pups, decreases in body weight, decreased startle response, and developmental delays in male pups characterized by delays in preputial separation. Additional studies in the open literature indicate fipronil has other developmental and reproductive effects in rats and changes to sperm motility and morphology.

II.A.9. Thyroid Endocrinology

Repeated exposure to fipronil caused decreased T4 levels, increased thyroid weight and histopathology in adult and developing rats, and thyroid tumors at sufficiently high doses. Fipronil did not act directly on the thyroid. Rather, fipronil induced hepatic (liver) metabolism, which led to an increase in elimination of T4. Sustained T4 elimination is the required first step for all subsequent thyroid pathologies observed in rats. In humans, decreased T4 levels are unlikely to be deleterious to adults in the short-term. In the young, however, thyroid hormone deficiencies of even a short duration during development can lead to irreversible brain damage.

II.A.10. Neurotoxicity

Fipronil neurotoxic potential was measured under acute and subchronic exposure durations in rats. The principal effects included convulsions, tremors, gait abnormalities, absence of response to stimuli, decreased rearing and grip strength, and impaired righting reflex.

II.A.11. Metabolite and Photodegradate Toxicity

The toxicological properties of fipronil depend on at least three chemical species: the parent compound, its sulfone form (the major metabolite in both vertebrates and insects) and the desulfinyl photoproduct. Fipronil itself is a toxicant for mammals even without oxidation to the sulfone. The available database for the sulfone form showed that its lethal potency is in the same range as the parent compound. Fipronil-desulfinyl is the principal photoproduct in plants and soils. Fipronil-desulfinyl had a lower oral LD_{50} in rats than fipronil, but other available data indicate that fipronil-desulfinyl has similar levels of toxicity compared to the parent. Fipronil-desulfinyl did not cause thyroid tumors following chronic administration. As such, all critical points of departure (POD) used in this assessment were established using the parent compound fipronil.

II.A.12. Toxicity Forecaster™ (ToxCast™)

The ToxCast™ analysis for fipronil was conducted in 1175 out of 2205 high-throughput screening assays. Positive hits occurred in fourteen ToxCast™ intended target families, with assays related to cytochrome P450 enzymes (CYP), neuroactivity, nuclear receptor, neurodevelopment, cell cycle, DNA binding, and transferase target families (3–7 assays each).

II.B. Hazard Identification

II.B.1. Acute Toxicity

An acute oral point of departure (POD) of 0.77 mg/kg/day from an acute neurotoxicity study in rats was used to estimate the risk from the acute exposures to fipronil (PODs are described in Table 1). This POD was calculated with the Benchmark Dose (BMD) modeling. It represents the threshold dose $BMDL_{10}$, which caused 10% reduction in hindlimb splay in the rats at the BMD of 2.09 mg/kg. The critical acute oral POD of 0.77 mg/kg was used to evaluate the acute dermal and inhalation risk to humans.

II.B.2. Subchronic Toxicity

The subchronic oral POD of 0.02 mg/kg/day was selected to characterize the risk of subchronic oral exposure of humans to fipronil. It was the no observed effect level (NOEL) in a chronic toxicity study in rats in which decreases in T4, convulsions, and mortality were seen at the lowest observed effect level (LOEL) of 0.06 mg/kg/day within one year of exposure. The critical POD of 0.02 mg/kg/day also was used to characterize human risks from subchronic dermal and inhalation exposures to fipronil.

II.B.3. Chronic Toxicity

The chronic oral POD of 0.02 mg/kg/day was selected to characterize the risk of chronic oral exposure of humans to fipronil. It was the NOEL in the chronic toxicity study in rats. Effects at the LOEL of 0.06 mg/kg/day included decreases in serum T4 in males at week 1 through week 50, convulsions, mortality, and progressive senile nephropathy. The critical POD of 0.02 mg/kg/day also was used to characterize human risks from chronic dermal and inhalation exposures to fipronil.

II.B.4. Oncogenic Risk

Cancer potency was not calculated for fipronil by linear extrapolation. Instead, cancer risk was assessed by a threshold approach using an upstream marker (T4) of the relevant oncogenic process.

Table 1. Critical Points of Departure (PODs) for Fipronil

Duration	Route	Critical Endpoint	POD (mg/kg/day)
Acute	Oral, Dermal and Inhalation	Decreased hindlimb splay in an acute oral neurotoxicity study in rats	0.77[a]
Subchronic	Oral, Dermal and Inhalation	Convulsions and mortality associated with convulsions and sustained decreases in T4 after subchronic oral exposure in a chronic study in rats	0.02[b]
Chronic	Oral, Dermal and Inhalation	Convulsions and mortality, sustained decreases in T4, and increased progressive senile nephropathy in a chronic oral study in rats	0.02[c]

T4: thyroxine thyroid hormone. POD: point of departure.

[a]Benchmark Dose Software (BMDS, version 3.2) of hindlimb splay data from acute neurotoxicity study in rat (Hughes, 1997).

[b]Combined chronic and oncogenic study in rat (Aughton, 1993). The critical subchronic POD was supported by POD established in three additional studies in rodents following subchronic exposure: 0.03 mg/kg/day based on presence of urine in the FOB observation area and exaggerated tail pinch response in rats (Driscoll and Hurley, 1993); 0.05 mg/kg/day based on delayed development and decreased startle response in rat pups (Broadmeadow, 1991; Aughton, 1993; Mandella, 1995); 0.05 mg/kg/day based on liver periacinar hypertrophy in mice (Mandella, 1995).

[c]Combined chronic and oncogenic study in rat (Aughton, 1993).

II.C. Exposure Assessment

II.C.1. Occupational Handler, Home User and Residential Post-application Exposures

Occupational handler, home user and residential post-application exposure assessments for fipronil were prepared as a separate document that included a complete description of the methods used (e.g., input data, formulae, assumptions, etc.). The exposure assessment includes estimates for short-term, seasonal, annual, and lifetime exposures for fipronil handler scenarios. Residential exposure was calculated for home users and for adults and children post-application (Jiang, 2022).

II.C.2. Exposure from Diet and Drinking Water

Dietary exposure estimates to fipronil residues in food and in drinking water were calculated for the US population and sensitive subpopulations including infants, children 1–2 years old, and women of childbearing age (13–49 years) using a deterministic point estimate approach. Dietary exposure estimates were based on residues from monitoring databases or the maximum allowed residue level (tolerance).

Acute dietary exposure estimates

At the 95th exposure percentile, the estimated acute exposures to fipronil ranged from 0.068 µg/kg/day to 0.352 µg/kg/day. Children 1–2 years old were exposed to the highest dietary levels of fipronil when compared to other subpopulations. Milk the main contributor to the dietary exposure of children 1–2 years old (71% of the total exposure). Fipronil residues in drinking water were not a major contributor (≥ 5%) to dietary exposure except for nursing infants (11.4%).

Chronic dietary exposure estimates

Estimates for chronic exposure to fipronil from diet ranged from 0.007 µg/kg/day for nursing infants to 0.070 µg/kg/day for children aged 1–2 years.

II.D. Risk Characterization

Reference doses (RfDs) for fipronil were developed for the oral and dermal routes following DPR guidelines. RfDs were calculated using critical points of departure (PODs) for fipronil based on effects observed in oral toxicity studies in rats and mice divided by the total UF of 100 (10x for interspecies extrapolation, UF_A and 10x for intrahuman variability, UF_H). Reference concentrations were not calculated because inhalation exposure was minor compared to exposure by the non-dietary incidental oral and dermal route.

The acute oral RfD was 0.008 mg/kg. The acute dermal RfD was 0.18 mg/kg (adjusted for 4.3% absorption). The subchronic and chronic oral RfDs were 0.0002 mg/kg/day. The subchronic and chronic dermal RfDs were 0.005 mg/kg/day (adjusted for absorption).

The potential for non-oncogenic health effects resulting from exposure to fipronil was expressed as the margin of exposure (MOE). An MOE is the ratio of the critical POD derived from the definitive acute, subchronic, or chronic studies to the estimated human exposure. The resulting value is compared to the acceptable or target MOE which, for purposes of this risk assessment, is equivalent to the total uncertainty factor (UF_{TOTAL}) of 100 that accounts in differences between lab animals and humans and between different humans. Calculated MOEs that are lower than the target MOE indicate a potential health concern.

$$\text{Margin of Exposure (MOE)} = \text{POD} / \text{Exposure Concentration}$$

Risks were calculated for occupational handlers, home users, and for adult and child residents from single and multiple exposure routes (e.g., aggregate exposure). Risks from dietary (food and drink water) exposure were calculated for the general population, including sensitive sub-populations.

Risks from Single-Route Exposure Scenarios

Occupational handler exposure scenarios included applications of structural dust, structural liquid concentrate, turf granules and structural bait gel uses, as well as pet spray and spot-on treatments applied by professional pet groomers. Risk was estimated separately for dermal and inhalation exposures. Residential user and bystander exposure scenarios included adult applications of pet products (home users) and post-application exposures to adults and children following residential structural and turf applications and at-home use of flea and tick products per label requirements. Exposure calculations are described in the Exposure Assessment Document (Jiang, 2022).

Using the equation above and the estimated inhalation, dermal and oral exposure estimates (Appendix VII), this assessment found numerous single-route exposure scenarios that result in either occupational or bystander risk depending on duration of exposure (see Summary Table 2 and Tables 40–43 further in this document). If analyzed as single-route exposure scenarios, occupational risks are found from professional use of pet products and to a lesser extent by subchronic and/or chronic exposures to liquid concentrates and turf granules. Risks to adults who are either home users or exposed post-application are found largely from pet products. Risks to children are found following application of turf granules (acute non-dietary oral exposure) and pet spray products (subchronic oral and dermal exposure).

Aggregate Occupational Risk

DPR estimates risk based on aggregate exposures when it is appropriate to do so. Aggregate exposures include a summation of all potential exposure pathways for a single pesticide, and provides a conservative, health-protective approach to evaluating risk. This methodology assumes that a single receptor will be exposed to single pesticidal active ingredient (a.i.) through all possible exposure pathways based on legal uses.

Risk was characterized for each occupational exposure scenario. Aggregate MOEs that combined simultaneous dermal and inhalation exposure are shown in (Table 2). The aggregate risk was calculated using the hazard index approach:

$$MOE_{aggregate} = 1/(\sum_{\mathbb{M}=1}^{MM} \frac{1}{MMMMMMMM} + \cdots + \frac{1}{MMMMMMMM})$$

Acute MOEs for occupational exposure to pet spray was lower than the target of 100, indicating a risk. All subchronic MOEs for occupational handlers, with the exception of the MOE for structural dust handlers, were below the target MOE of 100. All chronic MOEs except those for turf granule and structural dust handlers were also below the acceptable risk target. Dermal

exposure was the predominant route of exposure for occupational handlers. Exposure from diet and drinking water were not included in the aggregate occupational MOE calculations because the dietary exposure estimates had little impact on total exposure or on the overall aggregate MOE calculations.

Table 2. Aggregate Margins of Exposure for Occupational Handlers

Product	Scenario	Acute MOE	Subchronic MOE	Chronic MOE
Pet spot-on	groomer	183	**28**	**28**
Pet spray	groomer	**14**	**1**	**1**
Structural dust	handler	7196	1170	1786
Structural LC	no overhead	399	**29**	**51**
Structural LC	overhead	119	**15**	**26**
Turf granules	handler	1088	**78**	156

MOE: margin of exposure. LC: Liquid concentrate. **Bold** text indicates MOE below target of 100.

Aggregate Home User Risk. Risk was characterized for adults who use fipronil at home. As with occupational risk calculations, aggregate MOEs that combined simultaneous dermal and inhalation exposures were calculated (Table 3). Exposure from diet and drinking water were not included in the aggregate MOE calculations because the dietary exposure estimates had little impact on total exposure or on the overall aggregate MOE calculations. The aggregate risk was calculated using the hazard index approach, above.

Acute aggregate MOEs for home user exposure to pet spray were lower than the target of 100, indicating a risk. Subchronic and chronic aggregate risk was not calculated for home users, as exposure was not anticipated. The predominant route of exposure for home users was dermal.

Table 3. Aggregate Margins of Exposure for Home Users

Product	Scenario	Acute MOE	Subchronic MOE	Chronic MOE
Pet spot-on	adult pet owner	513	--	--
Pet spray	adult pet owner	**40**	--	--

MOE: margin of exposure. Note: Seasonal and annual exposure of residents to pet products is calculated in the post-application exposure scenarios. **Bold** text indicates MOE below target of 100.

Aggregate Residential Post-Exposure Risk. Aggregate risk calculations for adult and child residential exposures following application (i.e., post-application) are presented in Table 4. Aggregate values include exposure estimates for inhalation and dermal exposures (adults) and inhalation, dermal and non-dietary incidental oral exposure (child).

Table 4. Aggregate Margins of Exposure for Post-Application Exposure to Residents

Product	Scenario	Acute MOE	Subchronic MOE
Pet spot-on	adult	550	222
Pet spot-on	child	241	**95**
Pet spray	adult	321	**39**

Table 4. Aggregate Margins of Exposure for Post-Application Exposure to Residents

Product	Scenario	Acute MOE	Subchronic MOE
Pet spray	child	135	**17**
Structural bait gel	adult	> 100,000	> 100,000
Structural bait gel	child	> 100,000	45662
Structural LC	adult	15714	2564
Structural LC	child	5540	1010
Turf granules	adult	> 100,000	NA
Turf granules	child	**33**	NA

LC: liquid concentrate. **Bold** text indicates MOE below target of 100. NA = calculation not applicable.

The only acute aggregate MOE below the target of 100 was for child exposure to turf granules. In that case, the risk value was driven by incidental oral (hand-to-mouth) exposure. Subchronic aggregate MOEs for exposure of adults and children to pet spray and for exposure of children to pet spot-on products were all less than the target of 100, indicating a risk. The predominant route of exposure for residents following application or use of fipronil-containing products was by the dermal and oral routes. Longer term exposures to turf granules were not anticipated if the products were applied according to the label and because of the very limited allowable use in California. Therefore, subchronic risk for turf granules for post-application scenarios involving either adult or child residents were not calculated.

Dietary Risk. MOE were calculated for the combined exposures from fipronil residues in food and drinking water. The acute dietary MOEs at the 95th exposure percentile were all above the target MOE of 100. Similarly, all chronic dietary MOE were above the target of 100. Therefore, neither acute nor chronic dietary and drinking water exposure was considered a risk for any subpopulation.

II.E. Risk Appraisal

The main uncertainties with the toxicity of fipronil were associated with (*i*) the use of animal data to evaluate the toxic effects in humans (*ii*) the use of oral PODs to characterize dermal and inhalation risk, (*iii*) the critical acute POD was based on time to peak effect of 7 hours for convulsions and tremors that may not capture the actual peak effect for hindlimb splay, and (*iv*) the confidence in the thyroid hormone measurements in the comparative thyroid assay (CTA) was reduced due to uncertainties with non-guideline analytical quality control metrics.

The exposure assessment for fipronil used the best information available to evaluate exposure. However, defaults were used for some scenarios due to a lack of data. There were also several data gaps identified during the exposure assessment. These gaps and the discussion of how they were addressed is found in the Exposure Assessment Document (Jiang, 2022).

Uncertainties in the dietary (food and drinking water) exposure assessment were introduced with the use of analytical limits of detection or tolerances used as surrogates for pesticide monitoring residue concentrations.

III. INTRODUCTION

Fipronil is a broad-spectrum insecticide that is toxic to numerous insects and arachnids including cockroaches, locusts, wire-worms, ticks and fleas (Aajoud *et al.*, 2003). It is a phenylpyrazole insecticide that blocks gamma-aminobutyric acid-gated (GABA-gated) chloride channels, leading to central nervous system excitation and to death at sufficient doses.

The first fipronil-containing products were registered in the US in 1996 for use on golf course and commercial turf. Fipronil was first registered for use in California in 1997. As of June 2022, 137 fipronil-containing products are registered in California. In California, fipronil is registered for non-food uses such as to control structural pests with public-health impacts. Fipronil is also used in to control ticks and fleas on companion animals in veterinary practices, pet grooming business, and in residential homes. Limited geographical and seasonal use is allowed on turf to control fire ants. Only licensed applicators can use turf granules, structural liquid concentrates, and structural dust or power products. None of these products are licensed in California for home consumer use, however residents can purchase bait gel and station products in retail stores to treat structural pests. There are no food uses of fipronil allowed in the state.

Fipronil was first prioritized for risk assessment by DPR in 2009 because:

- Acute neurotoxicity in rats (convulsions)
- Low acute, subchronic, and chronic NOELs in mammals compared to most pesticides
- Convulsions and neurological disturbances in chronic toxicity studies in rats and dogs
- Oncogenicity in rat thyroid
- Possible oncogenicity in mouse liver, and
- Thousands of adverse human health effects reports submitted to DPR detailing potential toxicity in human following fipronil exposure (adverse effects were first reported in 1999).

In 2013, DPR requested an independent peer review of its risk assessment practices by the National Research Council, an external committee of the National Academy of Sciences (NAS). As part of that review and audit, NRC recommended several improvements, including focusing on California-specific data, identifying relevant US EPA evaluations of the same pesticidal active ingredient, and identifying important sources of uncertainty and variability in the data. Fipronil is the first pesticide to enter risk assessment using the revised approach and this document is designed to incorporate and be responsive to the NRC recommendations.

This assessment evaluates potential human health risks associated with exposure to fipronil. The studies evaluated in this document include guideline studies submitted to fulfill pesticide registration data requirements and studies required under the California Birth Defect Prevention Act of 1984 (SB 950). DPR also reviewed relevant reports and risk assessments conducted by other regulatory agencies including US EPA, the National Toxicology Program (NTP), the European Food Safety Authority (EFSA), and the European Commission (EC). Studies available in the open literature were also reviewed using the National Center for Biotechnology

Information (NCBI) electronic database. The most recent NCBI search was conducted in June 2022.

III.A. Chemical Identification

III.A.1. Fipronil and Phenylpyrazoles

Fipronil is a phenylpyrazole insecticide discovered in 1987 by scientists at Rhône-Poulenc Agro laboratories (now Bayer CropScience) following experimental alteration of the core phenylpyrazole structure (Figure 1) (Moffat, 1993; Perrior, 1993). Phenylpyrazoles operate principally as herbicides. However, fipronil acts as an insecticide following contact or ingestion. It contains a unique trifluoromethylsulfinyl functional group not present in any other pesticide, which may contribute to its greater toxicity to insects over mammals (Hainzl and Casida, 1996).

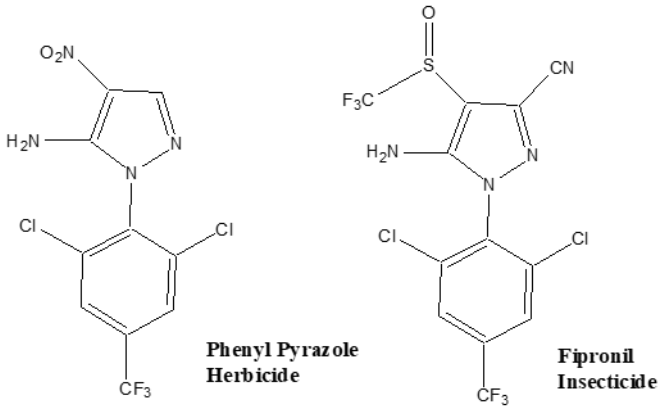

Figure 1. Chemical Structure of Fipronil and Phenyl Pyrazoles

III.A.2. GABA Receptor as Primary Fipronil Target

Fipronil (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfinyl]-1H-pyrazole-3-carbonitrile) is a phenylpyrazole insecticide that blocks gamma-aminobutyric acid (GABA)-gated chloride channels, leading to excitation of the central nervous system (CNS) and to death at sufficient doses (US EPA, 2011b; Simon-Delso *et al.*, 2015). Fipronil represents the second generation of insecticidal chloride channel blockers. The selective toxicity for insects relative to mammals is more favorable for fipronil than for most of the first generation chloride channel blockers (chlorinated cyclodienes and polychlorinated cycloalkanes) (Hainzl and Casida, 1996). In addition, insects resistant to pyrethroid, cyclodiene, organophosphate and carbamate insecticides are not cross-resistant to fipronil (Simon-Delso *et al.*, 2015).

Initial functional studies showed that toxic signs elicited by fipronil in house flies and mice are similar to those of trioxabicyclooctanes, which block GABA-gated chloride channels (Cole *et al.*, 1993). GABA is a major inhibitory neurotransmitter in both vertebrates and invertebrates. It increases chloride conductance into cells, which results in decreased release of excitatory neurotransmitters and rapid inhibition of synaptic CNS signal transmission (McKernan and Whiting, 1996; Raymond and Sattelle, 2002). Compounds such as fipronil, which act as

antagonists, stabilize non-conducting conformations of the chloride channel, reduce neuronal inhibition, and lead to hyper-excitation of the CNS, which can cause convulsions and death (Bloomquist, 2003).

Vertebrate GABA Receptor

GABA receptors are transmembrane glycoproteins consisting of five subunits that form a central pore. Many therapeutic drugs such as barbiturates, benzodiazepines and alcohols act as agonists or activators of the GABA receptor, and induce neuronal inhibition, sedation, anesthesia or coma (Eldefrawi and Eldefrawi, 1987; Kurata *et al.*, 1993; McKernan and Whiting, 1996). Electrophysiological and ligand binding studies confirm that fipronil reversibly and noncompetitively blocks passage of chloride ions through GABA receptors in the CNS (Millar *et al.*, 1994). Humans exposed to fipronil show symptoms such as headache, nausea and seizures, which are associated with antagonism of GABA receptors in the brain (Mohamed *et al.*, 2004).

Much of the knowledge about fipronil action as a chloride channel blocker comes from binding studies with radioligands, computational molecular modeling, site-mutation studies, and electrophysiological assays (Cole *et al.*, 1993; Kurata *et al.*, 1993; Hainzl *et al.*, 1998; Ikeda *et al.*, 2001; Ratra and Casida, 2001; Ikeda *et al.*, 2004). According to a proposed binding site model, fipronil and other antagonists interact at a single site in the GABA-gated chloride channel (Ratra and Casida, 2001; Chen *et al.*, 2006).

Insect GABA Receptor

Structure and operation of the GABA receptor and the GABA binding domain in insects are not well understood and subunit composition of the native GABA receptor is not known (Comitani *et al.*, 2014). The 'Resistant to Dieldrin' subunit (RDL) is used as an experimental model for the native receptor because it forms a functional GABA-gate chloride channel and exhibits many of the same toxicological properties as the native insect GABA receptor (Buckingham *et al.*, 2005). The *Drosophila* RDL subunit has high sequence homology to that of the human β_3 subunit (Ffrench-Constant *et al.*, 1991; Ffrench-Constant *et al.*, 1993; Sirisoma *et al.*, 2001).

The insect RDL GABA receptor mediates inhibitory neurotransmission (Ashby *et al.*, 2012; Comitani *et al.*, 2014). Blocking by fipronil occurs in the Cl^- channel, not at the GABA recognition site (Inoue and Akaike, 1988; Zhang *et al.*, 2016). This allosteric binding site is also known as the picrotoxin or noncompetitive blocking site (Sirisoma *et al.*, 2001). The binding site is best characterized in *Drosophila* (fruit fly), *Musca* (housefly) and *Periplaneta* (cockroach) (Ashby *et al.*, 2012; Comitani *et al.*, 2014). Evidence indicates that allosteric binding occurs with high affinity and selectivity (Comitani *et al.*, 2014).

III.A.4. Glutamate Receptors as Secondary Target for Fipronil in Insects

L-glutamate-activated chloride channels are a secondary fipronil target in invertebrates (Horoszok *et al.*, 2001; Ikeda *et al.*, 2003; Zhao *et al.*, 2004). Unlike mammals, where glutamate is an excitatory neurotransmitter, glutamate serves as an inhibitory neurotransmitter in

invertebrates (Wolstenholme, 2012). They are closely related to GABA and glycine receptors, and to date have been found in invertebrate nerve and muscle cells (Cully *et al.*, 1996; Horoszok *et al.*, 2001; Raymond and Sattelle, 2002; Eguchi *et al.*, 2006). The selective toxicity of fipronil in insects over mammals could be influenced by its action on multiple target sites in insects.

III.B. Regulatory History

1995: US EPA established a temporary combined tolerance for fipronil and its sulfide metabolite on corn grain.

1996: Fipronil was registered in the US to control mole crickets on golf course and commercial turf (US EPA, 1996; US EPA, 2011a).

1997: US EPA established tolerances for combined residues of fipronil and its sulfone and sulfide metabolites on corn grain, stover and forage, eggs, milk, and animal fat, meat, organs and byproducts.

1997: Fipronil first registered in California by DPR.

1998: Fipronil spray and spot-on products first registered by the US EPA. The fipronil photo degradate, fipronil-desulfinyl, was added to the combined fipronil tolerances. Tolerances for fipronil on rice grain and straw were established.

1999: US EPA issued an Experimental Use Permit for fipronil on tuftgrass to evaluate the control of imported fire ants.

2001: US EPA approved fipronil use on wood structures to control Formosan termites.

2002: US EPA approved specific uses of fipronil to control fire ants and authorized limited use of fipronil in rodent bait boxes to control ticks.

2005: BASF petitioned US EPA to establish combined fipronil tolerances for raw agricultural commodity corm vegetables (potato, sweet potato), and indirect and inadvertent residues on wheat grain, forage, hay and straw.

2007: US EPA issued an amended human health risk assessment including an analysis of aggregate exposure to fipronil in workers, the US population, and for infants and children. That assessment included an updated dietary assessment included modeled values for fipronil levels in water. Tolerances were established for wheat forage, grain, hay and stray, potato and potato wet peel.

2009: BASF petitioned US EPA to reestablish combined tolerances for fipronil on rice. Use for fipronil for corn-in-furrow was cancelled.

2009: Fipronil enters the risk assessment process at DPR in California.

2010. US EPA released a review of the large number of incident reports for pets (cats and dogs) treated with flea and tick spot-on products including two formulations containing fipronil.

2013: DPR starts monitoring fipronil in fresh produce sold in California through the California Pesticide Residue Monitoring Program.

2014: US EPA waived the requirement for an immunotoxicity study for fipronil because the weight-of-evidence indicated that potential immunology-related effects occurred only at doses above those used for points of departure (US EPA, 2014). US EPA also changed the critical short-term incidental oral study from the rabbit developmental study to the developmental neurotoxicity study in rat.

2015: US EPA requested a comparative thyroid assay be completed by the registrants because a determination that thyroid effects observed in adult rats were not measured in developing rats.

2015: DPR selects fipronil as pilot pesticide to incorporate recommendations by the National Academy of Sciences (NAS) National Research Council (NRC) for improving DPR's risk assessment process. In 2013, DPR contracted with NAS to conduct an independent peer review of DPR's risk assessment practices. An external committee of NAS, NRC completed its review and issued its report in April 2015.

2017: DPR released its Problem Formulation Document for fipronil. As part of the NAS NRC report mentioned above, NRC recommended that DPR conduct a Problem Formulation/Scoping phase prior to drafting the risk assessment. During this phase, risk managers and risk assessors met and discussed the scope of the risk assessment to determine scope. The problem formulation/scoping discussions resulted in a Problem Formulation Document and a diagram of exposure pathways, which were presented to the Pesticide Registration and Evaluation Committee and posted to DPR's website for public comment (DPR, 2017b).

2020: US EPA released a Draft Human Health Risk assessment which included among other things a comparative thyroid assay, revised points of departure, a reduction in the Food Quality Protection Act (FQPA) safety factor, and a reduction of the interspecies extrapolation factor from 10x to 3x when the POD was based solely on thyroid effects.

IV. TOXICOLOGICAL PROFILE

The database used to develop the fipronil toxicological profile consisted of registrant-submitted studies, other regulatory documents, and scientific publications in the open literature. A systematic literature review was conducted to identify relevant studies in the open literature. The database search was most recently conducted in June 2022 using the common name as the key word ("fipronil"). The resulting studies (1641) were screened for relevancy to this assessment. Results of the systematic literature review are included in Appendix I.

IV.A. Human Illness Reports and Epidemiology

Documented human exposure to fipronil has resulted in central nervous system effects (dizziness, sweating, vomiting, seizures), irritation at the contact site, agitation, high blood pressure, pneumonia, and death. Effects possibly caused by fipronil include hives, renal failure and allergic reaction. Fipronil metabolites were detected in serum in limited biomonitoring studies. Experiments in human cell lines showed increased cellular metabolism and oxidative stress. Humans exposed to fipronil show symptoms such as headache, nausea and seizures, which are associated with antagonism of GABA receptors in the brain (Mohamed *et al.*, 2004).

IV.A.1. Acute Effects Observed in Humans

Five case studies were available concerning for patients who ingested fipronil and one was available for accidental dermal and inhalation exposure. The first study, which details a number of patients in Sri Lanka who ingested fipronil as a method of self-poisoning, is described in detail in the Toxicokinetics section of this RCD (Mohamed *et al.,* 2004). To summarize, severe toxicity including CNS effects and death was observed in some of the patients. In contrast, the authors note that there were no obvious toxic signs in other patients who ingested similar doses. Body weights were not reported. Assuming a default adult weight of 71.8 kg, the dose of fipronil ingested by patients with severe symptoms (Patients 2 and 8) would be 70 mg/kg. Patients with little to no symptoms (Patients 3 and 7) would have ingested 35 mg/kg.

The other published case studies are briefly described below.

A 77-year-old woman in Hong Kong accidentally ingested a commercial ant bait (less than 0.14 mg fipronil ingested) (Fung *et al.*, 2003). Thirty minutes later she experienced mild impairment of senses. After admitting to the hospital, she had no other symptoms. At a follow up two weeks later she had raised serum creatinine of 93 mmol/L (reference range 58 to 91 mmol/L).

A 50-year-old male in Poland was admitted to a medical clinic after 5 hours of spraying his field with fipronil without protective equipment (Chodorowski and Anand, 2004). He experienced headache, nausea, vertigo and weakness, and went to the medical clinic. Physical examinations and biochemical results were normal, and all symptoms resolved spontaneously after about 5 hours. At follow-up appointments, he was asymptomatic.

A 25-year-old male was brought to the emergency department for generalized tonic-clonic seizures and altered sensory faculties (Bharathraj *et al.*, 2015). Case study authors state that he had an "alleged history of fipronil compound consumption" and refer to the episode as "acute". The patient continued to have seizures, was unconscious for 10 hours, and was delirious for 3 days. He also had coarse tremors and incoordination and some retention of urine. All other tests were normal, and he was discharged on the 8th day.

A 45-year old female patient in India accidentally consumed 100 ml fipronil (Yadla *et al.*, 2017). The patient had four seizures and severe metabolic acidosis on the first day, followed on the second day by a shutdown of urine production. Her serum creatinine and urea levels, both measures of kidney function, were 4–8 times the normal range on the first and second days. Renal biopsy revealed acute tubular necrosis, vacuolation of tubular epithelial cells, and intratubular hemorrhages. After 10 days, fipronil levels were measured with mass spectrophotometry/ liquid chromatography at the National Institute of Nutrition, Hyderabad, India. Levels were described by the investigators as being 40 times the upper limit of quantification for screening methods used on produce. After 5 weeks, the patient's urine output and creatinine levels had improved. The report did not comment further on her general health.

A 32-year-old male in India consumed 150 mL of a 5% fipronil solution to self-harm (Gutta *et al.*, 2019). After two weeks of symptoms (altered sensory faculties with recurrent seizures, jaundice and pallor) the patient presented to the hospital. He had occurrences of generalized tonic–clonic seizures while at the hospital, and liver function tests were abnormal. His liver and neurological function resolved by the third week of managed care, and case study authors report that he was doing well at follow-up visits. The observed effects were consistent with fipronil-induced hepatic dysfunction and neurotoxicity.

IV.A.2. Illness Reports

Illnesses or injuries associated with exposure to fipronil were identified from three main sources: DPR's Pesticide Illness Surveillance Program (PISP), the adverse human health effects reports submitted by fipronil registrants,[3] and the Sentinel Event Notification System for Occupational Risk (SENSOR) Pesticides program.

PISP maintains a database of pesticide-related illnesses and injuries reported in California. Case reports are received from physicians and workers' compensation records. The DPR database indicated that 78 cases of illness were linked to fipronil from 1999 to 2019 (the latest date data were available when this analysis was performed) (DPR, 2014; DPR, 2018; DPR, 2020). The health effects attributed to exposure to fipronil alone or in combination with other pesticides

[3] 40 CFR § 159.152(b). Section 152.50(f)(3) requires applicants to submit, as part of an application for registration, any factual information of which [they are] aware regarding unreasonable adverse effects of the pesticide on humans or the environment, which would be required to be reported under section 6(a)(2) if the product were registered.

were rated as definite (3 cases), probable (25 cases) or possible (50 cases). Illnesses identified as "definite" included:

- A six-year-old female child who experienced red and irritated eyes after accidentally squirting a dog spot-on treatment into her eyes.
- A 60-year old woman who sustained eye irritation and pain after accidentally getting dog flea treatment in her eyes.
- A 49-year old male professional pesticide applicator who touched his face with a glove contaminated with pesticide mixture and experienced itching and burning face and eyes.

Illnesses defined as "probably" due to fipronil exposure included reports on pest control operators and residents exposed at work or home to bait gels (5), termite or insect products (12), or pet products (9). Illness reports categorized as "possibly" due to fipronil exposure came from the use of pet products (5), ant or roach gel or bait (8), and structural liquid, dust, suspension or spray (36).

Clinical signs included vomiting, diarrhea and gastrointestinal upset, rash, tearing eyes, vertigo, headache, disorientation, coughing, throat irritation, burning eyes and lips, dizziness, tremors, and spinal pain. The number of illness reports has generally increased over time. A large number of incidents occurred in 2013 (10 incidents for up to 20 people) at a retirement community in Santa Barbara, California (Figure 2).

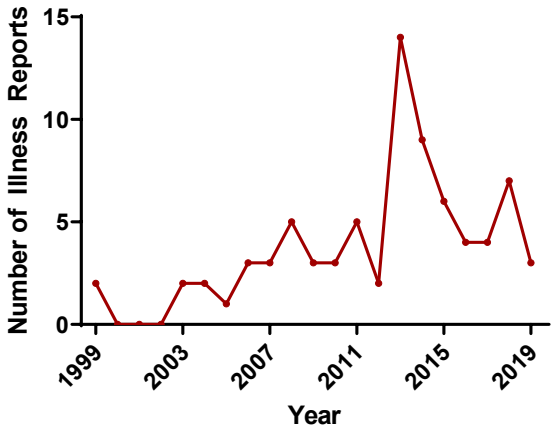

Figure 2. Yearly Incidence in California of Illness Reportedly Caused by Fipronil

IV.A.3. Adverse Human Health Effects Reports

Adverse Human Health Effects Reports are submitted to DPR by fipronil registrants as mandated under Section 12825.5 of the California Food and Agricultural Code. These include but are not limited to information required under Section 6(a)(2) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The effects are mostly self-reported, and are graded as human fatality, major, moderate, minor or unspecified. The exposure level or duration is generally unknown, and

the connection between exposure and symptoms is usually not verified. These reports are from across the entire US and are not California-specific; actual location of the event is usually not available.

DPR's databases for fipronil illness reports include cases submitted between 2009 and 2022. Earlier reports were also available from the US EPA. These reports were reviewed and summarized based on severity of effect and fipronil product type (Table 5).

Table 5. Adverse Effects Reports on Fipronil

Database	**US EPA (2011)[a]**	**DPR 2009 to 2015[b]**	**DPR 2016 to 2022**
Years	2002 to 2010	2009 to 2015	2016 to 2022
Number of incidents	4243 total	1483 total*	1032 total
	- 1 death	- 0 deaths	- 1 death
	- 10 major	- 11 major	- 5 major
	- 377 moderate	- 207 moderate	- 121 moderate
	- 3815 minor	- 1236 minor	- 772 minor
	- 40 unspecified	- 29 unspecified	- 127 unspecified
			- 6 other (no severity grading)
Types of products reported[c]	Pet products, termite and insect products, ant and roach bait, fire ant control.	Pet products, roach bait, fire ant control.	Pet products, termite and insect products, roach bait, fire ant control, and area foggers.
Notes	75% of major and moderate incidents, including one death, due to pet products.	95% of major and moderate incidents, due to use of pet products; 5% from unknown formulations;	One death from termite product. 80% of all reports from use of pet products (89% spot-on, 11% spray); 10% from ant and roach bait; 11% from termite and insect products.

[a]Data published in (US EPA, 2011d) includes Adverse Effects Reports as well as incidents from other federal and state health and environmental agencies and individual consumers.
[b]These reports (and the reports in the DPR 2016 to 2022 database) are from incidents across the United States and are not California-specific. Because the timing of US EPA (2011d) and DPR (2008 to 2015) had overlap, it is possible some incidents were included twice.
[c]All databases included incidents where the specific fipronil product was not described (unknowns).
*Database included reports of effects in animals and wildlife and reports in which no species was mentioned. The total presented here represents incidents that clearly occurred in humans; reports where species was unclear were removed. This number could therefore omit human incidents.

Comprehensive data for fipronil reports prior to 2008 are only available in a publication (Fipronil: Review of Human Incidents) by the US EPA (US EPA, 2011d). That publication summarizes human health effects reported following fipronil use from January 1, 2002 to November 30, 2010.

Starting in 2009, DPR began electronically documenting health effects for both humans and animals. Two databases are available, the first with reports from 2005–2015 (though a limited

number of reports exist prior to 2009), and the second with reports from 2016 until August 2022. These databases contain human health effects reports and reports on effects in pets, wildlife and plants for all pesticides registered in California. Both databases include registrant name, US EPA pesticide registration number, date the report was received, and US EPA chemical code. Some reports include the affected species and specific reported effects. Reports are for single incidents of exposure or aggregate reports with more than one incident.

Following review of the three databases, DPR identified 6758 human health effects reported following fipronil use since 2002. Of these, 2 were deaths, 26 were major, 705 were moderate, 5832 were minor, 196 were unspecified, and 6 had no severity grading. In the first case of lethality, a 64-year-old female in Texas died following an allergic reaction to a pet spray product. In the second case, a person died in 2017 following exposure to a termiticide; additional details on the person or effects were not available.

Incidents with effects graded as major or moderate included neurological and cardiovascular effects, slurred speech, lethargy, allergic edema, neuropathy, breathing trouble, fatigue, coma, swelling, seizures and renal failure. The minor, unspecified and ungraded incidents generally do not include specific effects, but may indicate a pattern of, or potential for, exposure to fipronil.

Of the 1032 adverse effect reports submitted to DPR from 2016–2022, 994 included a US EPA registration number. Therefore, information on product type and approved label uses could be extracted. Of these, 52% were for dog spot-on treatments, 19% were for cat spot-on products, 9% for pet spray, and 11% for termite control, 7% for roach bait, and 3% for ant bait. Taken together, pet products accounted for ~80% of all adverse effects reports submitted to DPR.

In addition to effects in humans, tens of thousands of incidents were also reported in domestic animals following the use of fipronil in veterinary products.

IV.A.4. Sentinel Event Notification System for Occupational Risk (SENSOR) Pesticides Program

SENSOR, run by the National Institute for Occupational Safety and Health, collects pesticide illness surveillance data from 11 states and health departments. Data for 2001–2007 were obtained from Lee *et al.,* 2010. The authors reported a total of 103 cases, of which the SENSOR Pesticides Program reported 92 cases of illness from fipronil exposure, and an additional 11 cases were provided by DPR (Lee *et al.*, 2010). Most cases were identified through the National Poison Control System. The majority involved exposure to a single product containing fipronil (as opposed to several products). Factors contributing to exposure included inadvertent splash, spray/spill, inadequate ventilation, and failure to leave a treated area during application. Two pest control operators experienced high-severity illnesses or seizure. Most cases were related to residential exposures. Nine cases were labeled as moderate severity and 92 were labeled as low severity. The most common symptoms were neurological, including dizziness, paresthesia and headache. Between 2008 and 2019, SENSOR Pesticides Program reported 160 cases of illness from fipronil exposure. After 2008, the severity of cases was not indicated.

IV.A.5. Human Incidence Data from Other Countries

In their 2005 risk assessment, the French Food Health and Environmental Health Safety Agencies included data showing 458 cases of human effects resulting from exposure to veterinary products (307 cases), home insecticide use (95 cases) and professional application (53 cases) (Agence Francaise de Securite Sanitaire des Aliments, 2005). Ninety-five percent of the human cases were acute poisoning cases. Symptoms included erythema, persistent rash skin irritation, burning lips, blisters at the point of contact, hives, and edema of the eyelids. An additional 112 cases (105 accidental exposures and 7 cases of deliberate ingestion) were reported from January 1995 through March 2004. The report also included 8 cases over 3 years from the National Poison Control Center of the Netherlands (ingestion or dermal exposure in children) as well as 138 cases described by the World Health Organization from 1995 to May 2004. Symptoms include nausea, vomiting, transient dizziness and respiratory effects from oral exposure, and irritation following eye contact.

In 2003, the Australian Pesticide and Veterinary Medicine Authority (APVMA) initiated a risk assessment on fipronil due to "adverse experiences in humans and animals" involving fipronil veterinary products. During the time from initial registration in 1997 until 2006, 73 adverse experiences were reported. These included skin reactions in animals and humans, neurological signs, and death in pets and non-target animals (rabbits in particular). The APVMA preliminary review recommends revision to safety directions associated with some fipronil products and new re-handling intervals for fipronil veterinary spray products (APVMA, 2011).

Unintentional insecticide poisoning that occurred in young children (<5 years) in Queensland, Australia was assessed to determine mechanisms of acute poisoning and whether age affected poisoning patterns (English *et al.*, 2016). Fipronil was involved in 4.6% of calls for which medical attention was recommended for a young child. These involved ingestion of baits, ant liquids and veterinary products. Authors found a peak of exposure in one-year-olds, explained by mouthing (hand-to-mouth) behaviors.

IV.A.6. Population-Based Studies

Several population-based studies sought to characterize exposure to pesticides and the resulting effects by quantifying biomarkers of exposure and effect.

In the first study, fipronil, fipronil-sulfone and thyroid hormones levels were measured in the blood specimens of 159 workers in a fipronil veterinary product manufacturing factory in France (Herin *et al.*, 2011). Blood fipronil concentrations averaged 7.79 ± 7.65 µg/L. Fipronil-sulfone levels increased with increasing employment time. There was a negative association between fipronil-sulfone levels and levels of TSH; free and total T4 levels had no association with fipronil levels. More explanation of the perturbation of thyroid hormones in animals is described in the Hazard Identification and Risk Appraisal sections of this document.

In the second study, levels of fipronil and fipronil-sulfone were measured in the blood specimens of 59 newborn infant-mother pairs and 51 fathers in South Korea (Kim *et al.*, 2019). Thyroid

parameters were also measured. Fipronil-sulfone was detected in all patients. Fipronil was detected in one father only. The average fipronil-sulfone concentration in blood was 525 ± 240 µg/L in neonates, 744 ± 426 µg/L in mothers and 1163 ± 797 µg/L in fathers. These concentrations are similar to the median values in the Mohamed (2004) study. While T3 levels were considered to be within a normal range, infant cord blood T3 and free T3 levels were inversely associated with fipronil-sulfone levels. Fipronil-sulfone levels were also associated with decreased five-min Apgar scores[4] of newborn infants. This study showed placental transfer of fipronil-sulfone *in utero* and potential neurological effects on human development. Exposure is assumed to be subchronic or chronic duration, although sources of exposure and amount of exposure are unknown.

In another study, blood specimens from 96 human volunteers living in the Raleigh-Durham, North Carolina area were collected by the National Institute for Environmental Health Sciences between April and June 2001 (McMahen *et al.*, 2015). Fipronil-sulfone was detected in 25% of the samples, with concentrations ranging from 0.1 to 3.9 ng/mL. These levels were lower than those from other studies, and study subjects had no known pesticide exposure.

In a different study, levels of fipronil and fipronil degradants were measured in human serum, plasma and blood cells and paired urine samples from a general population of people in China. Fipronil sulfone was detected in all blood samples and accounted for 86-95% of the total fipronil (Shi *et al.*, 2021). Fipronil desulfinyl was detected in 97% of blood samples and accounted for 5–14% of total fipronil. The parent fipronil was detected in 2.6% of blood samples and represented less than 1% of total fipronil. Fipronil sulfone levels ranged from 0.01–1.53 ng/mL and total fipronil levels ranged from 0.04–1.56 ng/mL in plasma, blood cells and serum. Fipronil sulfone was detected in 10% of urine samples, and neither parent fipronil nor fipronil desulfinyl were detected in urine. There was no correlation between blood and urine levels of fipronil or its metabolites. Neither fipronil amide nor fipronil sulfide was detected in any sample. Similar results regarding fipronil and its metabolites were obtained in a larger scale study using blood serum samples only. Authors suggest that the total fipronil levels increase with city size and urbanization scale (Shi *et al.*, 2021).

Levels of fipronil and the sulfone metabolite were measured postmortem in human liver and adipose tissues from patients with and without Alzheimer's disease (N=12, Arizona, US) (Manivannan *et al.*, 2019). Only fipronil, not the sulfone, was detected. Fipronil accumulated in both the liver and the adipose tissue. There was no evidence of tissue burden association with Alzheimer's disease.

In a study conducted in Latina females aged 14–26 living in an agricultural community (Salinas, California), fipronil-sulfide was the most frequently detected pesticide in a wrist-band-based passive-sampling study of dermal and inhalation exposure of 1500 different chemicals (Harley *et*

[4] The Apgar test is conducted at 1 and 5 minutes after birth to assess the well-being of a newborn. A low Apgar score can indicate that the neonate requires medical attention and is considered a marker for developmental vulnerability.

al., 2019). Fipronil-sulfide was measured in 87% of the 97 study participants. Measurements, reported as ng fipronil per gram silicone bracelet per day, averaged 34.5 ng/g/day with a median of 12.9 ng/g/day and a 95th percentile exposure level of 145 ng/g/day. Sulfide levels were significantly lower ($p < 0.05$) in homes with door mats and were higher in homes where a professional exterminator spray had been used in or around the home within the last six months (OR = 7.0; 95% CI: 1.4–35.7; $p < 0.05$). While this study did not measure fipronil levels in blood, it did establish the prevalence of fipronil exposure in a community in California.

In a related study, 3 to 5-year-old children (n=125) from 33 childcare centers in San Francisco and San Joaquin Valley, CA, US, wore two silicone wristbands to monitor exposure to 13 different pesticides including fipronil (Alkon *et al.*, 2022). Each child wore two bands: one at the childcare center only for 30 weekday hours, and the other continuously (at childcare and at home) for 7 days and 7 nights. Fipronil was detected in > 20% of samples (Alkon *et al.*, 2022). Concentration on wristbands was associated with pesticide use data. An increase in pesticide concentration was observed if chemicals were not stored in original containers, at centers with less frequent handwashing, and when pests were not observed at centers, and when floors were not cleaned daily at home.

Inhalation exposure was measured for 83 rice paddy farmers handling fipronil in Malaysia (Hamsan *et al.*, 2017). Authors found no significant health risks due to inhalation of pesticides during spraying activities.

In a study conducted in Shijiazhuang, North China, concentrations of fipronil and metabolites were measured in seminal fluid from 200 men (Xu *et al.*, 2022). The total fipronil concentrations ranged from 0.003 to 0.180 ng/mL with a median: 0.043 ng/mL, with fipronil sulfone present in 100% of samples. Other chemical species include fipronil desulfinyl (62.5%), fipronil (10.0%), fipronil amide, (4.50%) and fipronil sulfide (0.50%). Higher levels of fipronil were observed in overweight or obese men. Men with a higher education level group (college and above) had higher level of fipronil desulfinyl compared to the low education group (less than high school). No significant association was found between the concentrations of fipronils in seminal plasma and impaired semen quality parameters (concentration, sperm count, and motility).

In a monitoring study conducted in North Carolina, US, 30 pairs of people and their household dogs wore silicone wristbands and collar tags, respectively, for 5 days to assess exposure to several pesticides (Wise *et al.*, 2022). Urine was collected on days 1, 3, 5 and pooled. Fipronil detected in 100% of silicone wristbands and dog tags, and there were significant correlations between human wristbands and dog tags (r=0.67). An increased concentration of fipronil in human bands was correlated with a reported use of fipronil-containing products.

In two related studies, fiproles (parent, sulfone, desulfinyl and sulfide) were measured in human breast milk samples. In the first study, fipronil was detected in 100% of samples, with a mean of 941 ng/L, median of 665 ng/L and maximum of 9009 ng/L (Liu *et al.*, 2022a). In this study, 109 breast milk samples were collected from one urban site (HuiNong, n = 49) and two rural sites (ShaPiTou, n = 30; YongNing, n = 30) from mothers within 3−8 weeks of delivery. In the second

study, fipronil was detected in 100% of samples, with a mean of 1097 ng/L, median of 921 ng/L and maximum of 2947 ng/L (Liu *et al.*, 2022b). Authors estimated the average daily intake of infants exposed to total fipronil through breast milk to be 209 ng/kg/day (Liu *et al.*, 2022b).

IV.B. Toxicokinetics

Limited data were available for fipronil toxicokinetics in humans, including one hospital-based study. Several *in vitro* studies investigated changes in enzymatic activity or expression in human cell lines. In addition, 11 studies on metabolism and toxicokinetics in laboratory animals were submitted to DPR by registrants. These included 7 oral studies (4 in rats and one each in laying hens, lactating goats and lactating cows), 2 comparative toxicokinetic studies and a whole body autoradiography study in rabbits, rats and mice, and 1 dermal study with rats. An *in vitro* dermal study was also available that measured the absorption of fipronil through human, rabbit and rat skin.

IV.B.1. Human Toxicokinetics

Case reports were described for seven patients treated in hospitals in Sri Lanka in 2002 following intentional self-harm using fipronil (Mohamed *et al.*, 2004). The study also included a retrospective case review for one patient (Patient 8) who died 17 days following fipronil ingestion. All patients ingested Regent 50 SC® formulation (4.95% fipronil in propylene glycol). The estimated time from fipronil ingestion to hospital admission varied from 45 min to 18 hours (Table 6). Patient symptoms ranged from none to dizziness, sweating, nausea, agitation, seizures, high blood pressure, vomiting, mouth ulcers, pneumonia and death (Patient 8).

Blood specimens were taken from six patients on admission. Additional specimens were taken from five patients at later time points. The time from fipronil ingestion to the first blood sample varied from 75 min to 3.5 hours. Time between ingestion, admission and first blood sample is important when calculating half-life ($T_{1/2}$) estimates. The blood was analyzed for total fipronil with enzyme-linked immunosorbent assays (ELISA) or for fipronil and its sulfone and desulfinyl metabolites with liquid chromatography mass spectrometry (LC-MS). Toxicokinetic analysis was conducted when data were deemed sufficient by the investigators. Patient 3, who ingested 2.5 g fipronil, was the only patient with an increase in blood concentration over time; the C_{max} of 1040 µg/l was measured at an approximate time of 4.6 hours [DPR used GetData Graph Digitizer 2.26 to extract time from Figure 2 in (Mohamed *et al.*, 2004)]. Based on the C_{max} value, the authors note that Patient 3 likely ingested a larger amount of fipronil compared to Patients 2 and 4. The estimated C_{max} for Patients 2, 4 and 7 was based on the first and highest concentration measured after admittance. For these patients, fipronil levels in blood were similar, and leveled off after about 15 hours post ingestion. Patient 7 reportedly ingested the same amount of fipronil as Patient 3 (2.5 g), but his C_{max} of 82 µg/l at 1.3 hours was 46-fold lower than the other patients, and he did not show clinical signs.

The blood concentration for total fipronil (parent and metabolites) remained constant after 15 to 20 hours (the last time points tested), although the patients no longer exhibited symptoms. Study

authors determined that for two of the patients (4 and 7), the fipronil-sulfone concentration increased, while there was a reduction in levels of the parent compound. The elimination $T_{1/2}$ of fipronil was estimated to be 36 hours (Patient 4) and 47 hours (Patient 7). However, the authors state that these times may not be conclusive as they were based on three measurements with no further follow up. In patients 2, 3 and 7, total fipronil concentration in blood started to increase over time after an initial decrease.

Table 6. Summary of Toxicokinetic Data for Humans with Fipronil Self-Harm

	Patient 1	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**	**Patient 6**	**Patient 7**	**Patient 8**
Age, gender	17, male	31, male	44, male	31, male	21, male	34, female	30, male	23, male
Other poisons	OP, imi	none	none	none	OP	none	none	none
Fipronil (g)	unknown	5	2.5	unknown	unknown	unknown	2.5	5
Admission (min)	1100	100	225	190	180	150	45	180
Time to first sample (min)	NA[b]	200	170	210	210	180	75	NA
Peak (µg/L)	NA	1600	1040	3744	7	20	82	NA
$T_{1/2}$ elim (hr)	NA	> 70*	NA	36	NA	NA	47	NA
Symptoms	none	dizziness, sweating, nausea, vomiting, agitation, seizures	high blood pressure	vomiting, seizures, mouth ulcers	none	nausea, vomiting, heart burn	none	unconscious, seizures, pneumonia
Outcome after 12 hr	asympt	asympt	asympt	asympt	asympt	asympt	asympt	died (day 17)

OP (organophosphate): dimethoate, fenthion; NA not available; Imi: imidacloprid; Peak: peak total fipronil in blood; Asympt = asymptomatic.
*Initial elimination half-life ($T_{1/2}$) estimate based on increases in total fipronil in blood over time; final measured time point was 70 hours. Data from Mohamed (2004).

IV.B.2. *In Vitro* Metabolism

Published studies with recombinant human isozymes of CYP450 and human liver microsomes suggest that CYP3A4 is the major isoform that oxidizes fipronil to fipronil-sulfone, the major metabolite (Tang *et al.*, 2004; Carrao *et al.*, 2019a). This was confirmed in inhibition experiments using the irreversible, non-competitive inhibitor chlorpyrifos (Joo *et al.*, 2007). Chlorpyrifos, an organophosphorus pesticide that contains a P=S moiety, inhibited CYP3A4 and resulted in a 70-80% decrease in the production of fipronil-sulfone (Joo *et al.*, 2007).

Testosterone, a CYP3A4 substrate, activated fipronil metabolism by human liver microsomes, while fipronil inhibited testosterone metabolism (Tang *et al.*, 2004). These activation/inhibition interactions were in agreement with a previously proposed multisite kinetics model for CYP3A4 for the metabolism of testosterone (Tang *et al.*, 2004). These studies suggest that fipronil may interact with endogenous chemicals that are CYP3A4 substrates (Tang *et al.*, 2004; Joo *et al.*, 2007; Carrao *et al.*, 2019a).

Fipronil was a potent inducer of CYP isoforms in cultured human hepatocytes (Das *et al.*, 2006; Hodgson and Rose, 2007; Hodgson and Rose, 2008). Among 23 tested environmental chemicals, fipronil was the strongest inducer of CYP1A1 and CYP3A4 mRNA at concentrations between 0.5 µM -1 µM. Induction of CYP3A4 mRNA varied from 10-27-fold following 1 µM fipronil exposure. This induction level was similar to the level observed for a well-known CYP3A4 inducer, rifampicin (20 fold, n=3) (Das *et al.*, 2006). Changes were seen in CYP enzyme levels in human primary hepatocytes following fipronil exposure *in vitro* (Mitchell *et al.*, 2016). Exposure to 10 uM fipronil for 72 hours resulted in upregulation of mRNA (measured with qPCR) for CYPs 3A4, 3A5, 2A6, 1A1, 3A7, 2B6, 2B7 and 2C9. mRNAs for CYP4A11 and 4A22 were both down-regulated. In a different study, fipronil inhibited CYP3A1, CYP2E1 and CYP2D6 enzyme activity in rat liver microsomes (Zhang *et al.*, 2021). Finally, one study showed that fipronil and fipronil sulfone inhibit CYP2D6 in human microsomes (Carrao *et al.*, 2019b).

Fipronil was shown to bind *in vitro* and *in silico* to human serum albumin and competitively inhibited the binding of a heme-Fe(III) complex (Ascenzi *et al.*, 2018). This could reduce the direct toxicity of fipronil and/or impair binding of endogenous molecules such as T4, which could increase hepatic clearance of T4.

The concentrations of fipronil in egg whites and yolks were investigated following *in vitro* simulated digestion and cooking (Kim and Hur, 2018; Kim *et al.*, 2020). Fipronil levels did not change after cooking at 100 ºC (Kim and Hur, 2018). *In vitro* models including all steps for human digestion (passage through the mouth, stomach, small intestine, and large intestine with enteric bacteria) suggested that fipronil concentration did not change until digestion in the small intestinal. The conditions of large intestinal digestion with *L. sakei* reduced fipronil concentration by ~10%, although degradation products were not analyzed (Kim and Hur, 2018; Kim *et al.*, 2020).

IV.B.3. Rodent Toxicokinetics

Powels, 1992

To investigate the oral absorption of fipronil, Crl:CD(SD)BR rats (5/sex/dose) received a single oral dose of 4 mg/kg or 150 mg/kg ^{14}C-fipronil (ring-labeled, 44.8 µCi/mg) (Powles, 1992). The radiolabeled fipronil was dissolved in 0.5% methylcellulose with 0.01% Tween 80. A parallel group included rats that were pre-administered non-radiolabeled fipronil for 14 days (4 mg/kg) before receiving a single oral dose of 4 mg/kg ^{14}C-fipronil. Whole blood was collected from the lateral tail vein at various intervals for up to one week after treatment to determine the

concentration of radioactivity. Greater than 95% of the administered radioactivity was recovered at termination of the study at 168 hours (7 days) post-dose. No toxic signs were reported at 4 mg/kg, which is about 4% of the rat oral LD_{50} (92–103 mg/kg). Toxicity at 150 mg/kg, above the LD_{50}, included flushed and stressed faces, red staining, arched back while walking, and fluid around the nose. There were no deaths in this group up to 7 days following treatment.

Single dose of 4 mg/kg of [^{14}C]-fipronil. The plasma reached a maximum concentration (C_{max}) of 0.60-0.68 µg/g at 4–6 hours (T_{max}; Table 7). Adjusted for 100% mass balance, the average radioactivity recovered in the urine within 168 hours was 6% for both females and males. In the first 24 hours, 10–13% of the parent compound was detected in feces. Approximately 20% of the administered radioactivity was excreted in the feces within 24 hours with 3 to 6% of the dose recovered in feces at each remaining time point. An average of 46% radioactivity was recovered in the feces for both sexes within 168 hours. A similar portion of the dose, 46% (approximately 2 mg), was found in tissues (liver, adrenals, skin, pancreas, kidney, abdominal fat, ovaries, uterus and muscle) and in the carcass in both sexes at termination at 168 hours. The highest concentrations were measured in the abdominal fat (14.7–18.9 µg equivalents of ^{14}C-fipronil/g). The elimination $T_{1/2}$ was 149–200 hours. The authors concluded that absorption was relatively rapid, followed by slower elimination.

Multiple doses of 4 mg/kg of [^{14}C]-fipronil. Pretreatment with non-radiolabeled doses of fipronil for 14 days (14 doses) resulted in a 2.5-fold greater renal excretion of labeled residues (14%-16% of total administered radioactivity) following a single dose of ^{14}C-fipronil. The 168-hour recovery of radioactivity in feces was 56% for males and 61% for females (~10% higher than in the single dose group), and ~20% radioactivity was measured in the tissues and carcass (about half that of the single dose group). The authors note body compartment saturation with fipronil or an increase in metabolism and excretion.

Single dose of 150 mg/kg [^{14}C]-fipronil. The absorption of fipronil at 150 mg/kg (T_{max} between 48–72 hr) was much slower than at the low dose (T_{max} 4 - 6 hr). The C_{max} for the high dose was 19.6-–9.7 µg/g, about 30-fold higher than at the low dose (Table 7). The average radioactivity recovered in the urine within 168 hours was 22% for females and 29% for males. In feces, 67% and 75% was recovered in males and females, respectively. Between 2–5% (3–7.5 g) of the radioactivity was found in tissues and carcass at termination (1.5–4 times higher than the low dose exposure). The elimination $T_{1/2}$ at this dose (150 mg/kg) was 51–54 hours.

Intact fipronil was not detected in urine; the excreted radioactive compounds were mainly N-glucuronide conjugates. After deconjugation, up to 14 urinary metabolites were identified including fipronil (MB 46030), the sulfide metabolite (MB 45950), sulfone product (MB 46136), and fipronil-sulfone with the sulfonic acid removed (MB 45897), the amide metabolite RPA200766, and two pyrazole ring-opened metabolites (Figure 3). Based on the finding that conjugates, but not intact fipronil, were excreted in urine, the authors speculated that fipronil was largely metabolized prior to entering the systemic circulation.

At 24 hours, metabolites in the feces included the parent fipronil (MB 46030, 6–14%), the sulfide MB 45950 (1–3%), the sulfone MB 46136 (1–1.5%) and trace amounts of conjugates of the amide product RPA 200766 (< 1%). At later time points (73-120 h), the major metabolite was fipronil-sulfone (2–8%). Biliary excretion of fipronil was suggested based on the presence of metabolites in feces but was not directly demonstrated.

Lipophilic metabolites were preferentially distributed and accumulated in tissues. Repeated low dosing or a single high dose of 150 mg/kg resulted in an overall decrease in the proportion of radioactivity in tissues, likely due to saturation (Powles, 1992). The only metabolite identified in all tissue samples was fipronil-sulfone. Trace radioactivity (< 0.25%) was recovered in expired air.

The study authors conclude that toxicokinetics of the 150 mg/kg dose was different from the 4 mg/kg dose. At 4 mg/kg, the T_{max} occurred within of 4–6 hours, compared to 48–72 hours at the high dose. The main route of excretion for both doses was the feces, although a higher percentage of the administered dose was excreted via the urine following the high-dose exposure. In the first 24 hours, 1–13% of the parent compound was detected in feces following the low-dose exposure, while 8–14% of the parent was detected following the high-dose exposure. Only metabolites were excreted after 72 hours. A lower limit of oral absorption in rats was assumed to be 80%.

Totis and Fisher, 1994

A separate laboratory performed a second study on the kinetics of fipronil in rat (Totis and Fisher, 1994). In this study, Charles River CD rats (5/dose/sex) received [^{14}C-phenyl]-fipronil as a single oral dose of 4 or 40 mg/kg. Blood from the tail vein was collected at various intervals up to 2 weeks after treatment.

At 4 mg/kg, blood levels reached a maximum of 0.39–0.52 µg/g (C_{max}) at 4.8–6.2 hours (T_{max}). This was followed by a relatively slow elimination (135–171 hours). The C_{max}, T_{max} and $T_{1/2}$ were similar to values reported by Powles at the same dose (Powles, 1992). At 40 mg/kg, the T_{max} was 34–38 hours and the C_{max} was 6.7–7.6 µg/g, approximately 18-fold higher than the low-dose exposure. The elimination $T_{1/2}$ was 183–245 hours. However, the actual $T_{1/2}$ may exceed the study duration of 7 days, as radioactivity levels remained high at the last measured time point. DPR considered the absorption profile of the 40 mg/kg dose to be similar to that of the 150 mg/kg dose in the study by Powles (1992), but the elimination profile was more similar to that of the 4 mg/kg dose in Powles (1992).

Kemp, 1999

Biliary reabsorption of ^{14}C-fipronil was studied in male Sprague Dawley CD rats with cannulated bile ducts treated orally with 3.26 mg/kg (^{14}C) fipronil (Kemp, 1999). Absorption was 75%, with 3% of the administered dose recovered from urine, 12.7% from bile and 59% from the tissues and carcass at 72 hours post-dose. The fat and adrenal glands had the highest fipronil

concentration. The pancreas, liver and thyroid gland represented a second tier of tissues with high fipronil concentrations. A second group of rats were treated with the bile product from the first exposed group. In this group, 73% of the administered radiolabel was recaptured in the urine, bile, and skin and carcass up to 96 hours after dosing. Approximately 38% of this dose was reintroduced via enterohepatic circulation.

Bile excretion was also studied in the rat (Totis, 1995). The predominant site of recovery 72 hours post-dose was in the tissues.

Cheng, 1995

In a dermal absorption study with ^{14}C-fipronil, a formulation containing 79% fipronil suspended in 1% aqueous carboxymethylcellulose was applied to the shaved backs of 4 male Crl:CDBR rats per dose at doses of 0.876 mg/rat (0.07 mg/cm^2), 8.35 mg/rat (0.67 mg/cm^2) and 48.5 mg/rat (3.9 mg/cm^2) (Cheng, 1995). Two control animals were treated with the vehicle alone. The site was covered with filter paper after fipronil application. The "directly" absorbed radioactivity was estimated from residues recovered in blood, carcass, cage wash and cage wipes and urine and feces at 0.5, 1, 2, 4, 10, and 24 hours after treatment. The amount of radioactivity "indirectly absorbed" (left in or on the skin after skin washing) was determined at the same time points. Over 94% of the applied radioactivity was washed off the rat skin. Absorption of the applied dose was linear at the low and intermediate doses but reached saturation at the highest dose.

Published Studies in Rodents

In an oral study in rats with ^{14}C-fipronil and fipronil-sulfone, a single oral dose of 10 mg/kg was eliminated via feces (4.2 ± 2.6% of administered dose) and urine (4.1 ± 0.8%) within 72 hours. After terminal sacrifice at 72 hours, fipronil was found in the adipose tissue, adrenals, intestine and liver, with the highest levels present in adipose and adrenals. A small amount of fipronil (0.16 ± 0.01% of administered dose) was detected in the brain, demonstrating that fipronil and/or its metabolites were able to cross the blood–brain barrier. Fipronil sulfone was the major (>90%) metabolite in all tissues including brain (Cravedi *et al.*, 2013).

In another study, pharmacokinetics and transplacental transfer experiments were conducted in Sprague-Dawley rats (Chang and Tsai, 2020). Transplacental transfer of fipronil and fipronil sulfone occurred in pregnant rats following IV administration *in vivo* (Chang and Tsai, 2020). Both fipronil and fipronil sulfone partially penetrated the blood-placental barrier to reach the fetus. Biodistributions of fipronil and fipronil sulfone for placental transfer were approximately 2 and 4 hours, respectively. This study also demonstrated that fipronil is rapidly metabolized to fipronil sulfone following oral administration and that the transformation rate increases with dose. Study authors suggested that elimination of fipronil sulfone may be slow compared to the parent.

In a study with mice, one dermal dose (50 mg/kg) was applied topically to the back of the neck. After application, fipronil and fipronil sulfone levels were measured over five days. Fipronil reached its highest concentration in all organs on the first day. The authors reported higher

fipronil accumulation in adipose tissue, liver, adrenal gland, and brain samples, and fipronil was persistent in adipose tissue and brain. Fipronil sulfone reached the highest concentration in the adipose tissue on the first day, but in all other organs it reached the peak on the third day. The time for maximum concentration of fipronil was 10 ± 0.0 hours, $T_{1/2}$: 41 ± 6.8 hours, and mean residence time of fipronil: 56 ± 7.0 hours. For fipronil sulfone the time for maximum concentration was 45 ± 5.2 hours, and mean residence time of fipronil sulfone was 200 ± 35 hours (Suzuki T et al, 2021). Note: while study authors did not report the percent purity, it appears to be > 99% based on manufacturer.

IV.B.4. Comparative Toxicokinetics

In a comparative toxicokinetic study, female rabbits, rats and mice dosed orally by gavage for 14 days showed differences in the fipronil $T_{1/2}$ and T_{max} (Brockelsby *et al.*, 1991). Rabbits received 0.4 or 1.2 mg/kg/day while rats and mice received 0.4 or 4.0 mg/kg/day.

In a second comparative study, the percent recovery from a single dose of 5 mg/kg was determined (Lowden and Savage, 1999). In rats and mice, fipronil was not present in blood after 6 hours, whereas fipronil (sulfone) levels peaked on day 15 in rabbits. The maximum concentration was found in fat in all animals. Significant levels were also found in rabbit liver and thyroid. Fipronil-sulfone was the predominant metabolite for all species. The estimated elimination $T_{1/2}$ was longer in rabbit (11 and 10 days for blood and fat compared to 5 to 7 days in rodent). The $T_{1/2}$ in the thyroid of rodents was 5 days; data were too variable to calculate thyroid $T_{1/2}$ for rabbits.

The absorption of ^{14}C-fipronil through human, rabbit and rat skin was measured *in vitro* in horizontal glass diffusion cells (Walters and Brain, 1990). Rat and rabbit skin was obtained from dorsal and flank regions of euthanized animals. Human female abdominal skin was obtained from autopsy. Absorption rates were estimated for fipronil applied at doses of 0.2, 4 and 200 g/l in an aqueous formulation base (EP60145A). Fipronil at 4 g/l penetrated through the skin of all three species more slowly than either testosterone (a fast penetrant) or hydrocortisone (a slow penetrant). Based on this result, fipronil was considered a slow penetrant. The extent of fipronil absorption at 0.2 g/l was similar for human and rat skin (0.9–1.0%) at 8 hours and was greater for rabbit skin (13.9%).

Whole body autoradiography performed in rat, mouse and rabbit following oral administration (5 mg/kg) of ^{14}C-fipronil showed rapid dispersion throughout the body with the highest level residing in the fat (Whitby, 1991). Moderate levels were in the adrenal and pituitary glands, mucosa of the gastrointestinal tract, kidneys, liver, lungs, pancreas and skin. The radioactivity was present in the central nervous system in the mouse only (Whitby, 1991).

IV.B.5. Metabolism

One major metabolic pathway was proposed in the rat based on metabolite profiles (Figure 3) (Powles, 1992). The Phase I metabolites found in rats (fipronil-sulfone; fipronil-sulfide and the amide product RPA200766) were also identified in goats (Stewart, 1994a). The fipronil

metabolic profile (excretion and accumulation) in rats was similar to lactating goats, laying hens and lactating cows (Byrd, 1994; Stewart, 1994b). Sulfone was the predominant moiety detected in tissues of all animals tested.

Toxicologically, the formation of fipronil-desulfinyl (MB 46513) in mammals was of particular interest because desulfinyl is 9-times more potent than fipronil at the vertebrate GABA receptor (Hainzl *et al.*, 1998) and 2-fold more acutely toxic to mice (Cole *et al.*, 1993). However, fipronil-desulfinyl was not detected in tested animals.

Rats administered 3 mg/kg/day fipronil by the oral route for 14 days exhibited an increase in elimination of antipyrine and glucuronidation of 4-nitrophenol, indicating increased cytochrome P450 (CYP) activity and increased UDP-glucuronosyltransferase (UGT) activity, respectively (Leghait *et al.*, 2009). Plasma concentrations of fipronil-sulfone were at least 20 times higher than concentrations of the parent compound, suggesting the metabolite may contribute to toxic effects.

A microarray mRNA study conducted on 2-3 month old female Wistar rats receiving 3 mg/kg/day oral fipronil (95.6% purity) for 14 days showed a significant increase in expression of hepatic enzyme mRNAs linked to the metabolism and transport of xenobiotics, hormones and endogenous compounds (Roques *et al.*, 2013). These included Phase I enzymes (CYP2b1, CYP2b2, CYP3a1), Phase II transporters (UGT1a1, SULT1b1), and Phase III ATP-binding cassette transporters (ABCc2, ABCc3, ABCg5, and ABCg8). CYP3a4, identified in other studies as the main enzyme responsible for fipronil metabolism, was not reported in this study. Plasma concentrations of fipronil-sulfone were 700-fold higher than the parent compound. In the same study, fipronil altered hepatic expression of genes involved in metabolism and excretion in male C57BL/6J mice administered 3 mg/kg/day fipronil orally for 14 days (Roques *et al.*, 2013).

A separate study conducted in the same laboratory measured increased UGT, Sulfotransferase enzyme (SULT) and CYP expression and activity, and a decreased T4 C_{max}, as well as evidence of increased hepatic metabolism in thyroidectomized female Wistar rats (Roques *et al.*, 2012).

Fipronil also induced CYP activity in male rats after 6 days of exposure (the only time point tested) at 5 mg/kg/day, but not at 1 mg/kg/day (Caballero *et al.*, 2015).

In a study conducted with rat and human liver microsomes, the rat K_m and rat V_{max} were determined to be as 19.9 µM and 0.39 nmol/mg protein min, respectively (Tang *et al.*, 2004). CYP3A4 was identified as the major CYP isoform, and data suggested a higher intrinsic clearance of fipronil in rats than in humans.

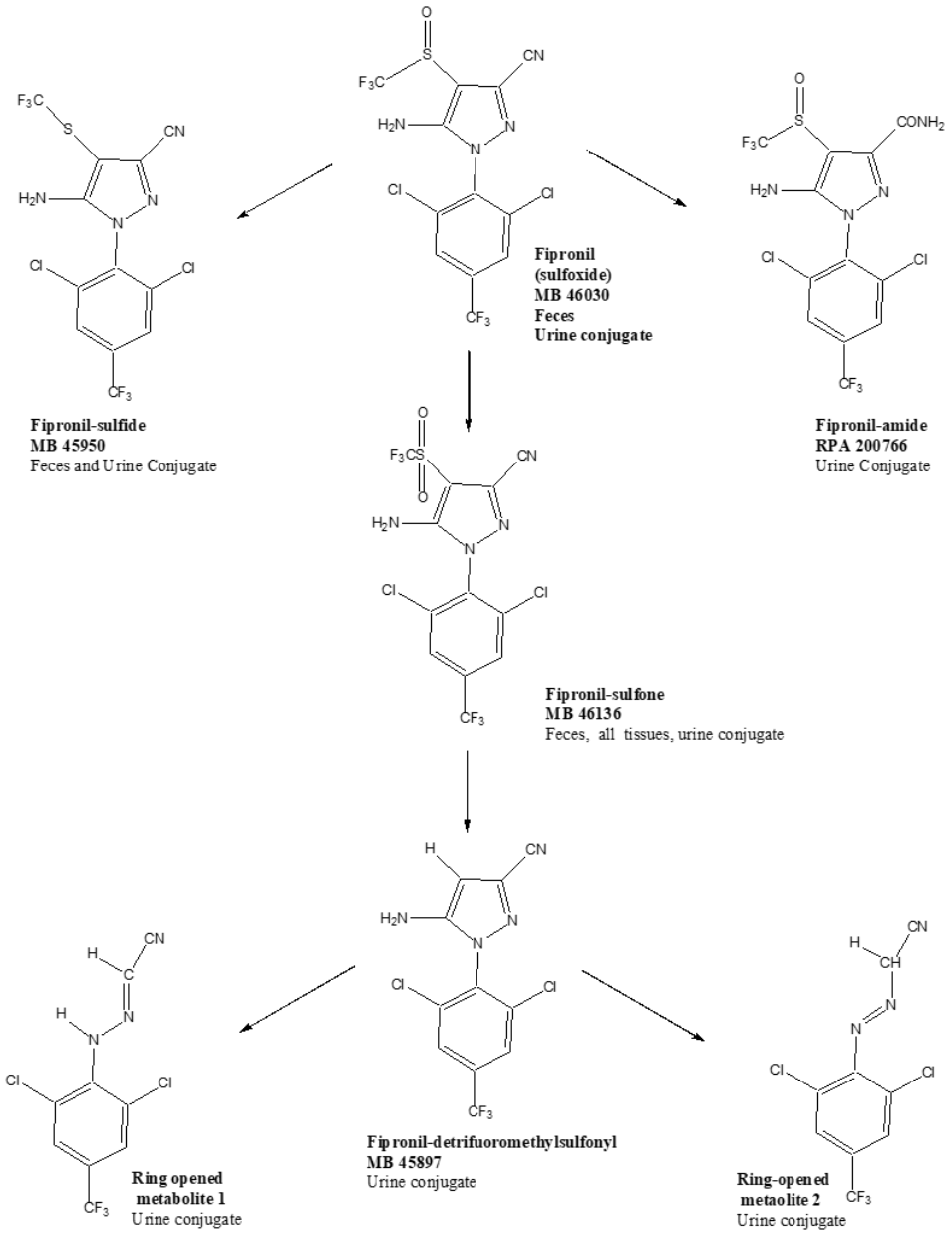

Figure 3. Biotransformation Pathways for Fipronil (sulfoxide) from Powell (1992).

IV.B.6. Toxicokinetic and Metabolism Summary

Based on oral studies in rats, the oral absorption was assumed to be over 80% of the administered dose. For purposes of internal dose calculation, DPR considers oral absorption of > 80% to be complete (100%). Fipronil was extensively metabolized and distributed throughout the body. The parent compound was not detected in any tissues or in urine. At 7 days post dosing, fat tissue contained the highest metabolite levels. In both rats and humans, fipronil was metabolized primarily to the lipophilic sulfone metabolite. Other metabolites identified in rats included fipronil-sulfide and the amide product RPA 200766. The same or similar metabolites

were found in goats and hens. Fipronil and its metabolites were eliminated mainly through the feces. In rats, the relatively slow elimination rate ($T_{1/2}$ > 6 days) was likely due to partitioning into fat and to enterohepatic recirculation.

The available toxicokinetic data for humans and rats was compared in Table 7. The data show that humans dosed at 35 mg/kg achieve peak blood concentrations of fipronil sooner (T_{max} of 1.3–4.6 hr) than rats dosed at 40 mg/kg (T_{max} 34-38 hr). The maximum blood concentration was 6 to 95-fold lower in humans. Elimination half-lives were calculated for rats and humans based on total fipronil concentrations in the blood (Table 7). However, substantial uncertainties were associated with these estimates. In rats, $T_{1/2}$ values were calculated to be 135-200 hours for a 4 mg/kg dose. For humans, an elimination $T_{1/2}$ of 47 hours was estimated for one individual (Patient 7, Table 6) who reportedly ingested 2.5 g of fipronil (estimated dose of 35 mg/kg using a default body weight of 71.8 kg). However, this $T_{1/2}$ estimate was based on only three time points with no measurements beyond 40 hours. A $T_{1/2}$ of 36 hours (also based on three time points) was estimated for Patient 4. Estimates for Patient 2, who ingested 5 g of fipronil (estimated dose of 70 mg/kg using a default body weight of 71.8 kg) indicate the $T_{1/2}$ in humans could be longer than 70 hours.

Additional toxicology data and toxicokinetics for the major fipronil metabolites and its environmental degradate are found in Appendix II.

Table 7. Comparison of Toxicokinetic Parameters for Fipronil in Rats and Humans

Exposure	Dose (mg/kg)	T_{max} (hours)	C_{max} (mg/ml)	$T_{1/2}$ (hours)
rats[a]	4	4.0–6.0	0.60–0.68	149–200
rats[b]	4	4.8–6.2	0.39–0.52	135–171
rats[b]	40	34–38	6.68–7.58	183–245
rats[a]	150	48–72	19.60–19.70	51–54
Patient 3[c]	35[d]	4.6	> 1.04	N/A
Patient 7[c]	35[d]	< 1.3	0.08	47
Patient 4[c]	UK	< 3.5	> 3.7	36
Patient 2[c]	70[d]	< 3.3	> 1.6	> 70[e]

C_{max}: maximum concentration in blood; T_{max}: time of C_{max} (absorption); $T_{1/2}$: elimination half-life.
[a]Powles (1992). [b]Totis (1994). [c]Mohamed (2004).
[d]Estimated dose using a default body weight of 71.8 kg.
[e]Last measured time point used as $T_{1/2}$.

IV.C. Acute Toxicity

Acute toxicological studies with fipronil were conducted in rats, mice and rabbits to establish median lethal doses (LD_{50}) or concentrations (LC_{50}) necessary for determining the toxicity category of technical grade pesticides and formulations. In these studies, fipronil induced clinical signs suggestive of excessive CNS stimulation (convulsions, seizures and tremors). Other symptoms included incoordination and gait abnormalities, hypoactivity, lethargy, diarrhea,

decreased respiratory rate and prostration. Fipronil caused eye irritation in rabbits but did not produce skin irritation in rabbits or dermal sensitization in guinea pigs.

IV.C.1. Registrant-Submitted Acute Studies

One oral study was available in rat, with a median lethal dose (LD_{50}) of 92 mg/kg for males and 103 mg/kg for females (96.7% pesticidal active ingredient, a.i.; Table 8) (Gardner, 1988b). The LOEL for systemic effects was 50 mg/kg, the lowest dose tested, based on clinical signs (piloerection, hunched posture, gait abnormalities, and diarrhea; Table 9).

Two acute inhalation studies were available in the rat. In the first, fipronil was assessed in Sprague Dawley rats (Nachreiner, 1995) by nose-only exposure to fipronil dust (96.7% a.i.) for 4 hours. The LC_{50} was 0.36 mg/l for males and 0.42 mg/l for females (Table 8). The LOEL of 0.33 mg/l (lowest dose tested) was based on clinical signs and death (Table 9). This concentration was converted to a LOEL[5] of 53 mg/kg using a rat default breathing rate of 0.96 m^3/kg.

In the second acute inhalation study, fipronil (95.4% a.i.) was tested in CD rats via nose-only exposure for 4 hours (Cracknell, 1991). The LC_{50} for male and female rats, calculated with a log probit model, was 0.682 mg/l. The LOEL was 0.259 mg/l (lowest dose tested) or 41.4 mg/kg, based on mortality in females. Because particle size did not meet the US EPA requirements of 4 µm median mass aerodynamic diameter (MMAD), the registrant-submitted supplemental information regarding particle size (Cracknell, 1994).

Acute dermal toxicity studies were conducted in rat and in rabbit. In CD rats, fipronil (93% a.i.) in distilled water was applied to a shaved area on the back to 5/sex at a dose of 2000 mg/kg (Gardner, 1988a). The treatment sites were covered during the 24-hour exposure period. The pathological evaluation did not reveal treatment-related changes. The acute dermal LD_{50} of fipronil in rats was therefore > 2000 mg/kg (Table 8).

Fipronil (96.7% a.i.) was also tested for acute dermal toxicity (wetted in corn oil at 100, 250, 500, 1000 and 2000 mg/kg) in New Zealand White rabbits (Myers and Christopher, 1992), resulting in an acute dermal LD_{50} of 354 mg/kg for both sexes (Table 8). The LOEL for acute dermal toxicity in rabbits was 100 mg/kg (lowest dose tested) based on hyperactivity in one male on days 5–7 and diarrhea in 2 females on days 1–2.

A primary dermal irritation study in New Zealand White rabbits resulted in a dermal Toxicity Category IV designation (Table 8) (Myers and Christopher, 1993b). A primary eye irritation study in New Zealand White rabbits resulted in a Category III designation (Myers and Christopher, 1993a). Delayed contact hypersensitivity was tested in guinea pigs (Smith, 1990).

[5] Equivalent 1-day doses were calculated using the rat default breathing rate of 0.96 m^3/kg/day in the following equations: $\text{Dose}\left(\frac{\text{mg}}{\text{kg.day}}\right) = \text{Concentration}\left(\frac{\text{mg}}{\text{m}^3}\right) \text{x} \frac{0.96\ \text{m}^3}{\text{kg.day}}\ \text{x}\ \frac{4\text{hours}}{24\ \text{hours}}$

Based on results, the technical grade fipronil was regarded by DPR as “not a dermal sensitizer” in guinea pigs.

Acute oral, dermal and inhalation studies were also available for a 0.25% fipronil Frontline formulation (Clouzeau, 1994b; Clouzeau, 1994a; Robinson, 1994).

IV.C.2. Acute Studies from Published Literature

Two intraperitoneal studies in mice were available from the published literature. The studies were performed by the same group and utilized similar protocols (Cole *et al.*, 1993; Hainzl and Casida, 1996). The LD_{50} for fipronil was reported in Cole *et al.* to be 32 mg/kg and in Hainzl *et al.* to be 41 mg/kg. The LD_{50} for fipronil-desulfinyl was 23 mg/kg (Hainzl and Casida, 1996), i.e., slightly more toxic than the parent compound. The LD_{50} was 80 mg/kg for fipronil-sulfone (Cole *et al.*, 1993). The IC_{50} at the GABA receptor was also measured. For fipronil-desulfinyl, the IC_{50} was less than one-tenth that of the parent compound, indicating a greater inhibitory potential at the mouse receptor (IC_{50} = 1010 nM for fipronil and 97 nM for fipronil-desulfinyl). One study reported an LD_{50} of 669.1 mg/kg for an Australian mammal, the striped-faced dunnart (Story *et al.*, 2022). This study used the Up-and-Down Procedure with addition of two hypothetical animals.

Acute toxicology, median lethality and irritation studies conducted with fipronil are summarized below and in Table 8. NOELs and LOELs from the appropriate studies are summarized in Table 9. NOELs and LOELs were based on clinical findings and were determined only from studies that employed a sufficient number of animals (e.g., at least 5 per dose group), included a range of doses, and provided experimental details on treatment protocol and experimental observations. Acute studies from the published literature were also reviewed. Studies relevant to the critical acute NOEL designation are described in the Hazard Identification section of this RCD.

Table 8. Acute Lethal Doses (LD_{50}, mg/kg) and Lethal Concentrations (LC_{50}, mg/l) for Fipronil

Chemical	Animal Species	Route of Exposure	Males	Females	Toxicity Category	References
Fipronil technical (93-97%)	Rat – Crl:CD (SD)BR	Oral	92	103	II	Gardner (1988)
Fipronil technical (93-97%)	Rat – Sprague Dawley	Inhalation 4 hr dust	0.36[a,b]	0.42	II	Nachreiner (1995)
Fipronil technical (93-97%)	Rat – CD	Inhalation 4 hr dust	0.682[b]	0.682	--	Cracknell (1991)
Fipronil technical (93-97%)	Rat – CD	Dermal	>2000	>2000	III	Gardner (1988)
Fipronil technical (not described)	Mouse – Swiss-Webster	Intraperitoneal	32-41	--	--	Cole (1993), Hainzl (1996)
Fipronil technical (93-97%)	Rabbit – NZW	Dermal	354	354	II	Myers (1992)
Fipronil technical (93-97%)	Rabbit – NZW	Dermal Irritation	Not a dermal irritant, Category IV			Myers (1993)
Fipronil technical (93-97%)	Rabbit – NZW	Eye Irritation	Mild eye irritant[c], Category III			Myers (1993)
Fipronil technical (93-97%)	Guinea Pig	Dermal Sensitization	Not a dermal sensitizer			Smith (1990)
Fipronil-desulfinyl (MB 46513, 98.6%)	Rat	Oral	18	15	I	(Dange, 1993b)*
Fipronil-Desulfinyl (MB 46513, 98.6%)	Rat	Dermal	>2000	>2000	III	(Dange, 1993a)
Fipronil-Desulfinyl (MB 46513)	Mouse	Intraperitoneal	23	-	I	(Hainzl and Casida, 1996)
Fipronil-Sulfide (MB 45950)	Rat	Oral	83	83	II	(Dange, 1994)*
Fipronil-Sulfone (MB 46136)	Mouse	Intraperitoneal	80	-	II	(Cole *et al.*, 1993)
Fipronil formulation (0.25%)	Rat – Sprague Dawley	Oral[d]	>5000	>5000	--	Clouzeau (1994a)
Fipronil formulation (0.25%)	Rat – Sprague Dawley	Dermal[d]	>2000	>2000	--	Clouzeau (1994b)
Fipronil formulation (0.25%)	Rat – Sprague Dawley	Inhalation[d]	>5.0[b]	>5.0	--	Robinson (1994)

Toxicity category designation from US EPA (2020).

[a]More than 97% of the particles had an aerodynamic droplet size (MMAD) < 3 μm.

[b]The equivalent dose is calculated using the rat default breathing rate of 0.96 m^3/kg/day.

[c]Eye irritation cleared in 7 days.

Table 9. NOELs and LOELs Derived from Acute Exposure Studies of Fipronil

Species, Strain	Study Type and Exposure	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
Rat, Wistar	oral acute LD_{50}, gavage	abnormal gait, hunched posture, diarrhea, piloerection	< 50	50	Gardner (1988)
Rat, Sprague Dawley	oral acute neurotox, gavage	reduced hindlimb splay, rearing	0.5	5	Gill (1993)
Rat, Crl:CD BR	oral acute neurotox, gavage	reduced hindlimb splay	2.5	7.5	Hughes (1997)
Rabbit, New Zealand White	dermal acute LD_{50}, 24-hr	hyperactivity, diarrhea, bloody kidney	< 100	100	Myers and Christopher (1992)
Rat, CD	inhalation acute LC_{50}, 4 hour nose-only	mortality in females	< 41.4[a]	41.4	Cracknell (1994)
Rat, Sprague Dawley	inhalation acute LC_{50}, 4 hour nose-only	death, clinical signs, gross pathology of stomach, lungs and brain	< 53 [a]	53	Nachreiner (1995)

[a]Oral equivalent dose; calculated internal absorbed dose from external air concentrations by using a default rat breathing rate of 0.96 m^3/kg/day and 100% inhalation absorption.

IV.D. Subchronic Toxicity

Eight registrant-submitted subchronic toxicity studies were available in laboratory animals. These included oral studies in rat, mouse and dog, an inhalation study in rat, dermal studies in rat and rabbit, and an oral neurotoxicity study in rat.

The thyroid and the liver were the principal targets following subchronic exposure. Effects included decreased bodyweight and body weight gain; increased liver weight with enlarged hepatocytes, fat deposition and periacinar hypertrophy; changes in blood serum proteins indicative of liver injury; increased thyroid weights with follicular cell hypertrophy; clinical signs (including hyperactivity, convulsions, and body tremors); and death. Subchronic fipronil studies and NOELs are summarized below and in Table 12.

Additional subchronic studies were identified in the published literature, which monitored endpoints such as inflammation, genotoxicity or reproduction. These studies contributed to understanding mechanistic issues and are described in the Hazard Identification and Appraisal sections of this RCD, when appropriate.

IV.D.1. Subchronic Oral Studies

Rat, 4 week (Peters, 1996)

Crl:DC (SD) BR rats (5/sex/dose) were exposed to fipronil (93%) over a 4-week period at dietary levels of 0, 25, 50, 100, 200 and 400 parts per million (ppm). These concentrations corresponded to doses of to 0, 3.4, 6.9, 12.6, 24.5 and 45.3 mg/kg/day (males) and 0, 3.5, 6.7, 12.9, 24.9 and 54.9 mg/kg/day (females) (Peters, 1996).

One female from the 400-ppm group died after 4 days of treatment, with no evident clinical signs or pathological findings. While hair loss occurred in all exposure groups, all four of the surviving high-dose females displayed hair loss at termination, compared to 1/5 control animals.

Exposure to 100, 200 and 400 ppm caused a decrease in food consumption in both sexes (12-54% compared to control) during the first week of treatment. During this period, body weights were reduced up to 20% and remained 6–9% lower than controls until the end of the study. Body weight gain was significantly reduced compared to control during the first week ($p < 0.01$) but was not different for the remainder of the study, indicating that decreases in body weights were caused by reduction of food consumption. Food utilization was also reduced during the first week of exposure.

The absolute and relative liver weights were increased in all exposed groups in a dose-dependent manner. The increase in absolute liver weights ranged from 10 to 65% and was significant in males at 200 ppm and higher and in females at all treated doses ($p < 0.05$). The increase in relative liver weights ranged from 8 to 81%.

Pathology examination revealed thyroid follicular hypertrophy in all treated groups. The severity of the effect was graded as minimal to moderate, and, in males, severity increased with dose. Histopathologic examination revealed hepatocyte enlargement in animals exposed to fipronil at doses higher than 50 ppm in males and at doses higher than 100 ppm in females. Enlarged livers were observed in males at the high dose (5/5 compared to 0/5 in controls) and in 3/5 females at the high dose (compared to 0.5 controls). Fat deposition was observed in the liver in 2 of 4 females examined in the 400-ppm group.

Changes in liver function in all treated groups were evident in the increased levels of globulin and total protein in serum (11–34%, $p < 0.01$). Particularly drastic was the increase in serum cholesterol, from 37% in the females at 25 ppm to 128% in the females at 400 ppm ($p < 0.01$). A decrease in aspartate aminotransferase was observed in both sexes above 50 ppm ($p < 0.05$).

The LOEL was set at the lowest tested dose of 25 ppm (3.4 mg/kg/day) based on increased absolute and relative liver weight in females, and liver and thyroid hypertrophy. A NOEL was not established.

Rat, 13 week (Holmes, 1991a)

Fipronil (95.4% a.i.) was administered through the diet to 10 CD rats/sex/group at 0, 1, 5, 30 and 300 ppm (Holmes, 1991a). This corresponded to average daily doses of 0, 0.07, 0.3, 1.9 or 20 mg/kg/day for males and 0, 0.07, 0.4, 2.3 and 24 mg/kg/day for females. The main effects after 13 weeks of treatment included increased absolute and relative thyroid and liver weights, thyroid hypertrophy, and fat in the liver.

Mortality and clinical signs were not evident at any dietary level. Body weights were significantly reduced 15% in males at 300 ppm ($p \leq 0.01$) and 5% in females at 300 ppm ($p \leq 0.001$) during the first week of treatment; reductions in bodyweight persisted for three additional weeks. There was a 26% and 10% decrease in food intake in males during the first and second weeks of dosing, respectively, and a decrease of 14% in females during the first week. Authors note that the food utilization in males and females at the 300-ppm dose group were lower during week 1, indicating animals required more food to generate body mass.

The absolute weight of the thyroid was increased by 68% and 100% ($p < 0.01$) in the females and males, respectively, at 300 ppm. When expressed as thyroid/body weight ratio the increase was 75% for the females and 107% for the males ($p < 0.01$). The drastic increase in thyroid weight was likely due to both increased follicular cell size as well as to increased number of follicular cells. Hypertrophy of follicular cells was seen in all 10 females and in 8/10 males in the 300-ppm group. For comparison, only 1 female and 3 males in the control group had hypertrophic follicular cells. Six of 10 males and 2/10 females had follicular cell hyperplasia, whereas none of the control females and only 2 of the control males showed increased number of follicular cells.

Absolute liver weight was increased by 54% and 42% ($p < 0.01$) in females and males, respectively, at 300 ppm. The increase in relative (to bodyweight) weight was of similar magnitude (58% for females and 43% for the males, $p < 0.01$). Analysis of the serum chemistry of these animals revealed 15–30% ($p < 0.01$) increase in urea, glucose and α1-globulin in the blood, which could indicate changes in liver synthetic/metabolic function. Accumulation of fat in the liver was noted in both sexes at 300 ppm (20 mg/kg/day). The highest incidence was detected in liver sections of males (7/10 at 300 ppm versus 0/10 controls). Higher absolute and relative liver weight (14–24%, $p < 0.01$) was reported for the rats in the 30-ppm group.

The investigators proposed that fipronil affected the hypothalamic-pituitary-thyroid-liver axis. However, they did not measure thyroid hormone levels, pituitary hormones, or liver metabolic activity. The subchronic oral NOEL was 30 ppm (1.9 mg/kg/day). Effects at the LOEL of 300 ppm (20 mg/kg/day) included marked increases in the absolute and relative thyroid and liver weights, thyroid hypertrophy, fat accumulation the liver and altered serum proteins. DPR considered the increased relative liver weight in males and increased blood glucose in females seen at the NOEL not sufficiently robust for LOEL designation.

Mouse, 13 week (Broadmeadow, 1991)

The toxicity of fipronil (95.4%) was evaluated in CD-1 mice (12/sex/dose) for a period of 13 weeks. The dietary levels of 0, 1, 3, 10 and 25 ppm corresponded to internal doses of 0, 0.13, 0.38, 1.27 and 3.2 mg/kg/day (males) and 0.17, 0.57, 1.72 and 4.53 mg/kg/day (females) (Broadmeadow, 1991). Hematology, clinical chemistry and ophthalmology were not evaluated. Thyroids were collected but not analyzed.

The liver was the principal target organ. The absolute weight of the liver was increased by 24% ($p < 0.01$) in males treated with 3.2 mg/kg/day fipronil (Table 10). Liver weights were also increased 5–14% in males at 1.27 mg/kg/day and in females at 1.72 mg/kg/day and higher, though statistical significance was not achieved. Relative to body weight, increases of up to 33% were significant in both sexes at the high dose ($p < 0.05$) and were increased up to 12% in the mid-high group in both sexes (not significant).

The increase in liver weight was in part due to increased hepatocyte size. Periacinar hypertrophy was reported for all male treatment groups, but not in controls. At the lowest dose, 2 of 12 males had liver hypertrophy. At 3 ppm, 3 of 12 males had hypertrophy. This effect became significant at 10 and 25 ppm fipronil (6/12 and 10/12 males, respectively). One male treated at the highest tested dose had focal necrosis of liver cells. While liver hypertrophy was not detected in the females, fatty vacuolation occurred in hepatocytes in 2 female mice from the mid-high dose group and 1 from the high dose group.

The LOEL for this study was set at the lowest tested dose (0.13 mg/kg/day) based on the appearance of periacinar hypertrophy in the livers of male mice. The absence of statistical significance at 0.13 and 0.38 mg/kg/day is noted by DPR.

Table 10. Toxicity in CD-1 Mice Following 13-Weeks of Fipronil Dietary Exposure

	Males					**Females**				
ppm	**0**	**1**	**3**	**10**	**25**	**0**	**1**	**3**	**10**	**25**
mg/kg/day	**0**	**0.13**	**0.38**	**1.27**	**3.2**	**0**	**0.17**	**0.57**	**1.72**	**4.53**
N	12	12	12	12	12	12	12	12	12	12
BW gain (g)	14.6 ± 3.5	12.2 ± 4.9	13.9 ± 4.8	13.2 ± 4.2	11.4 ± 5.2	6.8 ± 3.1	5.8 ± 3.2	4.8 ± 2.3	7.6 ± 1.5	4.3 ± 3.0*
% Control	100	84	95	90	78	100	85	71	112	63
Liver - Abs. Weight (g)	2.2 ± 0.3	2.2 ± 0.4	2.2 ± 0.4	2.4 ± 0.4	2.8 ± 0.3**	1.7 ± 0.2	1.6 ± 0.2	1.6 ± 0.3	0.9 ± 0.2	1.75 ± 0.2
Liver - Rel. Weight	5.0 ± 0.7	5.1 ± 0.4	4.9 ± 0.5	5.6 ± 0.5	6.6 ± 0.8**	5.6 ± 0.7	5.5 ± 0.6	5.8 ± 0.4	6.1 ± 0.6	6.3 ± 0.5**
Periacinar Hypertrophy	0	2	3	6*	10***	0	0	0	0	0

Table 10. Toxicity in CD-1 Mice Following 13-Weeks of Fipronil Dietary Exposure

	Males					Females				
ppm	**0**	**1**	**3**	**10**	**25**	**0**	**1**	**3**	**10**	**25**
mg/kg/day	**0**	**0.13**	**0.38**	**1.27**	**3.2**	**0**	**0.17**	**0.57**	**1.72**	**4.53**
Fatty Vacuolation	0	0	0	0	0	0	0	0	2	1

*, ** and *** indicate p-values < 0.05, 0.01 and 0.001, respectively. BW: body weight; Abs: absolute weight; Rel: relative weight.
Data from Broadmeadow (1991)

Dog, 13 week (Holmes, 1991b)

The subchronic toxicity of fipronil (95.4%) was examined in Beagle dogs by daily gelatin capsule administration for a period of 13 weeks (Holmes, 1991b). The study included four groups of dogs, each containing four males and four females at doses of 0, 0.5, 2.0 and 10.0 mg/kg/day. Three females and one male in the 10 mg/kg/day dose group were sacrificed during the second week of treatment due to poor health. The subchronic oral NOEL of 2 mg/kg/day was based on severe toxicity (mortality, weight loss, clinical signs and neurological disturbances) at the LOEL of 10 mg/kg/day. However, reduced food intake (up to 11%) was reported for three of the females from the 2 mg/kg/day (NOEL) group during weeks 2–9.

IV.D.2. Subchronic Inhalation Studies

Rat, 4 week (Adamo-Trigiani, 1999)

Groups of 15 Sprague Dawley CD (Crl®(SD)BR) rats/sex/group were exposed by nose-only inhalation to micronized fipronil (95% purity) for 4 hours per day on 28 consecutive days (Adamo-Trigiani, 1999). Air concentrations were 0, 0.001, 0.005, or 0.03 mg/L fipronil, (MMAD 2.1 to 2.4 μm ± GSD 1.7 to 1.8). Oral equivalent doses were calculated using a rat default breathing rate[6].

Six females and 1 male at the high dose exhibited ungroomed fur on day 2. The male also showed decreased activity, and two of the females showed salivation and decreased activity on day 2 (Table 11). Two additional high dose females displayed sustained convulsions (in one case, with handling) on day 2 (Table 11). Mortality was not observed.

Decreased body weight in males (9%, $p < 0.01$) and decreased food consumption in both sexes were seen at day 8. Body weights remained significantly decreased in high dose males until the end of the study. In females at the mid- and high-dose groups, body weights were significantly reduced (~6%) from day 22 to day 28.

[6] Rat default breathing rate of 0.96 m^3/kg/day:

$$\text{Dose}\left(\frac{\text{mg}}{\text{kg.day}}\right) = \text{Concentration}\left(\frac{\text{mg}}{\text{m}^3}\right) \text{x} \frac{0.96\ \text{m}^3}{\text{kg.day}}\ \text{x}\ \frac{4\text{hours}}{24\ \text{hours}}\ \text{(1 day exposure)}$$

Altered liver function was indicated by the clinical chemistry data, including: significantly elevated globulin in the mid- and high-dose males and the high-dose females; decreased bilirubin in males at all doses and in females at the mid- and high-doses; increased cholesterol in females at the mid- and high-dose; and increased prothrombin (PT) times ($p < 0.001$) and activated partial thromboplastin time in males at the mid- and high-doses (Table 11).

At study termination, absolute and relative liver weights were elevated by 11–22% in mid- and high-dose males, and 16–24% in high dose females.

The subchronic NOEL was 0.001 mg/L, based on decreased body weights in females, increased relative liver weights in both sexes, increased absolute liver weights in males, and changes in blood chemistry parameters in males and females at the LOEL of 0.005 mg/L (Table 11). Oral equivalent doses were calculated using a rat default breathing rate, making the subchronic NOEL and LOEL equal to 0.16 and 0.8 mg/kg/day, respectively.

Although this was a repeated exposure study, clinical signs were noted on day 2 in the rats at the high dose (0.03 mg/L), indicating an acute response. These animals also had significant decreases in body weight and food consumption after 1 week of exposure. Therefore, the NOEL of 0.005 mg/L (0.8 mg/kg/day) for these effects can be used to characterize acute and short-term inhalation exposures to fipronil.

Table 11. Toxicity in Sprague Dawley Rats following 28-days of Inhalation Exposure to Fipronil

	Males				Females			
Dose (mg/l)	**0**	**0.001**	**0.005**	**0.03**	**0**	**0.001**	**0.005**	**0.03**
Dose (mg/kg/day)	**0**	**0.16**	**0.8**	**4.8**	**0**	**0.16**	**0.8**	**4.8**
N	15	15	15	15	15	15	15	15
Day 1 BW	197 ± 10.5	202 ± 9.0	201 ± 8.5	196 ± 10.0	149 ± 7.1	151 ± 5.3	151 ± 5.9	150 ± 5.7
% Control	100%	103%	102%	99%	100%	101%	101%	101%
Day 8 BW	243 ± 14.0	238 ± 14.5	242 ± 14.6	221 ± 14.2**	171 ± 9.1	170 ± 8.3	167 ± 9.7	166 ± 9.2
% Control	100%	98%	100%	91%	100%	99%	98%	97%
Day 22 BW	320 ± 18.7	303 ± 22.5	312 ± 26.6	290 ± 20.3**	212 ± 12.4	202 ± 13.4	200 ± 12.8*	199 ± 12.5*
% Control	100%	95%	98%	91%	100%	95%	94%	94%
Day 28 BW	341 ± 18.1	331 ± 29.0	332 ± 32.3	311 ± 22.7**	228 ± 14.2	221 ± 15.5	213 ± 14.1*	215 ± 13.2*
% Control	100%	97%	97%	91%	100%	97%	93%	94%
Fur, ungroomed - day 2	0	0	0	1	0	0	0	6
Slight salivation - day 2	0	0	0	0	0	0	0	1
Moderate salivation - day 2	0	0	0	0	0	0	0	1
Activity, decreased - day 2	0	0	0	1	0	0	0	2

Table 11. Toxicity in Sprague Dawley Rats following 28-days of Inhalation Exposure to Fipronil

	Males				Females			
Dose (mg/l)	**0**	**0.001**	**0.005**	**0.03**	**0**	**0.001**	**0.005**	**0.03**
Dose (mg/kg/day)	**0**	**0.16**	**0.8**	**4.8**	**0**	**0.16**	**0.8**	**4.8**
Convulsions - day 2[a]	0	0	0	0	0	0	0	1
Sustained convulsions	0	0	0	0	0	0	0	1
Absolute Liver (g)	8.6 ± 0.8	8.7 ± 1.0	9.63 ± 1.3*	9.57 ± 0.7*	6.32 ± 0.6	6.36 ± 0.7	6.31 ± 0.6	7.34 ± 0.6**
% Control	100%	101%	112%	111%	100%	101%	100%	116%
Relative Liver (g)	2.8 ± 0.1	2.95 ± 0.2	3.32 ± 0.5***	3.42 ± 0.1***	3.14 ± 0.2	3.25 ± 0.2	3.34 ± 0.2*	3.90 ± 0.3**
% Control	100%	105%	119%	122%	100%	104%	106%	124%
Cholesterol (mg/dl)	52.6 ± 9.3	55.1 ± 12.7	56.0 ± 5.8	62.0 ± 11.9	58.9 ± 12.2	62.7 ± 11.6	72.1 ± 9.1*	90.6 ± 16.0**
% Control	100%	105%	106%	118%	100%	106%	122%	154%
Globulin (g/dl)	2.8 ± 0.2	2.9 ± 0.2	3.1 ± 0.2**	3.1 ± 0.2**	2.9 ± 0.2	2.9 ± 0.2	2.9 ± 0.1	3.1 ± 0.2**
% Control	100%	104%	111%	111%	100%	100%	100%	107%
Total Bilirubin (mg/dl)	0.15 ± 0.03	0.13 ± 0.03*	0.11 ± 0.02**	0.10 ± 0.02**	0.16 ± 0.03	0.14 ± 0.04	0.11 ± 0.03**	0.11 ± 0.03**
% Control	100%	87%	73%	67%	100%	88%	69%	69%
Prothrombin Time (sec)	15.2 ± 0.5	15.9 ± 1.4	16.3 ± 1.8*	17.4 ± 2.5***	15.2 ± 0.6	14.8 ± 0.8	15.1 ± 0.5	14.9 ± 0.5
% Control	100%	105%	107%	114%	100%	97%	99%	98%
Activated Partial Thromboplastin Time (sec)	18.7 ± 1.9	20.7 ± 1.8	21.9 ± 2.3**	25.2 ± 4.7***	16.8 ± 1.5	16.6 ± 2.4	16.6 ± 2.2	16.7 ± 2.0
% Control	100%	111%	117%	135%	100%	99%	99%	99%

[a]Sustained convulsions when handling - day 2

*, ** and *** indicate p-values < 0.05, 0.01 and 0.001, respectively. BW: body weight.

Data from Adamo-Trigiani (1999)

IV.D.3. Subchronic Dermal Studies

Rat, 4 week (Henwood, 1997)

Fipronil (94.95% a.i.) mixed with 1% methylcellulose in reverse osmosis water was applied to a porous gauze dressing and administered to the clipped, intact dorsal skin on the trunk of 10 Crl:CD(SD)BR rats/sex/dose. Doses were 0, 100, 500, or 1000 mg/kg/day (Henwood, 1997). Animals were exposed for 6 to 7 hours per day, 5 days per week, for 4 consecutive weeks.

One male at the high dose displayed excessive salivation on day 22 of treatment. No other clinical signs were observed. The incidence of erythema was sporadically increased in the 500 and 1000 mg/kg/day dose groups after 18 to 29 days (males) and 18 to 31 days (females).

Body weight gains of males were reduced by 20–36% in the 500 and 1000 mg/kg/day dose groups from days 1-8 (significant in high-dose only, $p < 0.05$). Food consumption was significant decreased by 14% in females at the high dose during days 1-8.

Increased total protein levels were observed in all exposed male groups (up to 7%, $p < 0.05$) and in the two higher doses in females (7%, $p < 0.05$). Globulin levels were increased by at least 8% in all treated groups; the increase of 17% in the high-dose males and 20% in the mid- and high-dose females was significant ($p < 0.05$). The albumin:globulin ratio was decreased in all exposed males by 10–15% and females by 5–15%. The decrease was significant in the high-dose males (15%, $p < 0.05$) and in females at the mid- and high-doses (15%, $p < 0.05$).

Increased absolute liver weights were observed in all treated groups (14–22% in males, 9–20% in females). The increases were significant in the mid- and high-dose females only ($p < 0.05$). The relative liver weights were also increased in all treated groups in both sexes (10–22% in males and 7–20% in females). Relative liver weights were significantly increased at the mid- and high doses in both sexes ($p < 0.05$).

The dermal LOEL was 100 mg/kg/day (lowest dose tested) based on altered clinical chemistry and increased relative liver weights.

Rabbit, 3 week (Hermansky and Wagner, 1993)

In a 21-day dermal toxicity study, fipronil (96.7%) was applied as a paste under a semi-occlusive cover to a shaved area of the back of 6 New Zealand white rabbits/sex/dose at doses. Doses were 0, 0.5, 1.0, 5.0 or 10.0 mg/kg (Hermansky and Wagner, 1993) in 0.5% carboxymethylcellulose in water (vehicle). Treatment was for 6 hours/day, 5 days/week over 3 weeks (15 applications).

Toxic effects were reported for animals at the 10 mg/kg/day dose. These included “extreme” hyperactivity in 1 male and 1 female on days 20–21, decreased food consumption (44% in males and 21% in females) during the last week of treatment that was significant in males only ($p < 0.05$), decreased bodyweight gain in from days 1 to 15 and 1 to 22, which was significant at day 21 ($p < 0.01$), and weight loss in males at the end of the study (7%, $p < 0.05$).

The subchronic NOEL for fipronil was 5 mg/kg/day, based on “extreme” hyperactivity, reduced body weight gain and decreased food consumption at the LOEL of 10 mg/kg/day.

Table 12. NOELs and LOELs Derived from Subchronic Exposure Studies

Species, Strain	Study Type and Exposure	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
Rat, Crl:CD(SD)BR	oral, dietary, 4 weeks	increased liver weight, thyroid hypertrophy	< 3.4	3.4	Peters (1996)
Rat, CD	oral, dietary, 13 weeks	increased relative and absolute liver and thyroid weights, thyroid	1.9	20	Holmes (1991)

Table 12. NOELs and LOELs Derived from Subchronic Exposure Studies

Species, Strain	Study Type and Exposure	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
		hypertrophy, and fat accumulation in liver			
Rat, Sprague Dawley	oral, neurotox, dietary, 13 weeks	autonomic nervous system effects	0.03	0.3	Driscoll and Hurley(1993)
Mouse, CD-1	oral, dietary, 13 weeks	hepatocyte periacinar hypertrophy	< 0.13	0.13	Broadmeadow (1991)
Dog, Beagle	oral, gelatin capsule, 13 weeks	mortality, weight loss, clinical signs and neurological disturbances	2	10	Holmes (1991)
Rabbit, New Zealand White	dermal, 3 wk, 5 day/wk, 6 hr/day	extreme hyperactivity, reduced BW gain and food consumption	5	10	Hermansky and Wagner (1993)
Rat, Crl:CD(SD)BR	dermal, 4 wk, 5 day/wk, 6-7 hr/day	increased total protein and globulin levels and increased relative liver weights	< 100	100	Henwood (1997)
Rat, Sprague Dawley	inhalation, 4 weeks, 4 h/day, nose-only	decreased BW and food consumption, increased relative liver weights in males, changes in blood chemistry	0.16[a]	0.8	Adamo-Trigiani (1999)

[a]Oral equivalent dose; calculated internal absorbed dose from external air concentrations by using a default rat breathing rate of 0.96 m^3/kg/day and 100% inhalation absorption.

IV.E. Chronic Toxicity

Four chronic toxicity studies were available to characterize fipronil's chronic effects and potential to cause cancer in laboratory animals. These included two-year dietary studies in rats and mice and two one-year oral studies in dogs, Studies are summarized in Table 15.

The most common effects following chronic exposure were convulsions and tremors in rats and dogs. Morphologic effects included liver and kidney lesions in rats and mice and thyroid lesions in rats. Thyroid disruption in rats was suggested by increased thyroid weights and changes in serum thyroid hormones (decreased T4 and increased TSH). Liver effects in mice included hypertrophy and periacinar vacuolation. Progressive senile nephropathy was seen in rats. In addition, thyroid follicular cell tumors were reported in rats at levels that caused clinical signs and death. Fipronil action on an extrathyroidal site was suggested as the cause of thyroid neoplasia but was not directly demonstrated. An extrathyroidal cancer model was proposed based on evidence that fipronil does not interfere with the incorporation of iodide in the thyroid but does increase the clearance of T4 from the blood. The chronic exposure studies are summarized below.

IV.E.1. Chronic Oral – Rat (Aughton, 1993)

In a chronic toxicity/oncogenicity study, fipronil (95.4% a.i.) was administered to CD rats at dietary levels of 0, 0.5, 1.5, 30 and 300 ppm (Aughton, 1993). The study was divided into three phases. The Oncogenicity Phase consisted of 50 rats/sex/dose that received fipronil for 89-91 weeks. In the Toxicity Phase, an additional 15 rats/sex/dose were examined for effects after 53 weeks of treatment. In the Reversibility Phase, 15 more rats/sex/dose received fipronil for 52 weeks, then given untreated food for 13 weeks to assess the reversibility of any toxicity. The average daily doses were 0.02, 0.06, 1.3 and 13 mg/kg/day for males and 0.03, 0.08, 1.6 and 17 mg/kg/day for females. The Oncogenicity Phase was shortened from 104 weeks to 89 weeks (males) or 91 weeks (females) due to poor survival of both controls and treated rats.

At the conclusion of the study, no dose-dependent difference in mortality were observed. However, death in males treated with fipronil was observed sooner than the control males. The first control male died at week 42. In contrast, the first male in the 0.5, 1.5, 30 and 300 ppm groups died at weeks 27, 23, 25 and 1, respectively (Table 13). Convulsions lasting up to 25 min were reported in 3 males exposed to 1.5 ppm fipronil and in a total of 24 animals from both sexes in the 30- and 300-ppm groups (Table 13). Convulsions were frequently observed during the first few weeks of treatment and sporadically throughout the entire exposure duration in both sexes at the high dose. Convulsions occurred at the LOEL (1.5 ppm, 0.06 mg/kg/day) during weeks 23, 61 and 69 in males and at 30 ppm (1.6 mg/kg/day) in females from weeks 1 through 55. One out of 3 males from the 1.5-ppm group and 8 out of 24 animals from the 30- and 300-ppm groups died following convulsions. Other clinical signs in rats treated with 1.5 ppm fipronil and higher included irritability, vocalization, salivation, aggressive behavior, grinding teeth and a higher number of thin animals.

Chronic exposure to 300 ppm fipronil resulted in a substantial reduction in body weights (up to 18% for the males and 25% for the females, $p \leq 0.01$, one-way ANOVA by DPR) at all times. Decreases in body weight of same magnitude (15–22% ($p \leq 0.05$) was also observed in males and females at the 30 ppm dietary level toward the end of the treatment (weeks 68 – 88 of the study). The reduction in the body weight was clearly treatment-related because food intake of these animals was similar to the control. Body weight data indicate that the maximum tolerated dose was reached or exceeded in this study at the high dose. Body weights of the animals receiving 30 and 300 ppm fipronil did not recover to the control level during the 13-week reversibility period (Table 13). Other effects occurring from exposure at or above 1.5 ppm included decreased hemoglobin concentrations and erythrocyte counts, increased platelet numbers, increased plasma cholesterol and calcium concentrations, increased alpha- and beta-globulins and decreased albumin levels, and shorter prothrombin time.

Thyroid and pituitary function were affected by altered serum levels of thyroid hormones. Triiodothyronine (T3) was significantly reduced (13%) in males from the 300-ppm group after 1 week of treatment ($p < 0.05$). It was sporadically decreased and increased at other doses and time points. Thyroxine (T4) was markedly lower in all treated male groups at most time points (week 1 to week 50) and was not detectable in the serum of male or female rats exposed at 300 ppm

after 1 week of treatment ($p < 0.001$). At week 1, a dose-dependent and significant decrease in T4 occurred in males at 1.5 ppm and higher (24–100% decrease, $p < 0.05$). From weeks 4–12, a significant and dose-dependent decrease occurred in all exposed males (14–88% decrease, $p < 0.05$). T4 levels were decreased in females by 46 and 100% at the mid- and high-dose at week one ($p < 0.01$) and at the high-dose were by 49–74% for the duration of the study ($p < 0.001$). The T4 levels in females were significantly decreased at lower doses at various times. Correspondingly, TSH was 292 to 441% of controls ($p < 0.001$) for males and 197 to 265% of controls for females treated at 300 ppm ($p < 0.001$). TSH was also increased (205 to 251% of control) in males at 30 ppm at week 1, 4 and 50 ($p < 0.05$). After 11 weeks of recovery, the TSH level remained at 221% of controls in the 300 ppm males, ($p < 0.01$, Table 13). At the time, TSH was 21–121% higher in all exposed male groups and was sporadically 15% higher in the high-dose females.

The absolute and relative-to-body weights of several organs from rats exposed to 1.5-300 ppm fipronil were consistently higher than controls at 52 and 88–91 weeks of treatment. These organs included liver (up to a 43% and 58% increase in absolute and relative weights, respectively, $p < 0.05$), thyroid (up to a 123% and 163% increase in absolute and relative weights, respectively, $p < 0.05$); adrenals (up to a 48% and 55% increase in absolute and relative weights, respectively, $p < 0.05$) and kidney (up to a 56% and 84% increase in absolute and relative weights, respectively, $p < 0.05$). The absolute weight of the thyroid gland increased significantly in all male treatment groups at terminal examination (88 weeks, Table 13).

Pathological examination revealed an increase in incidence and severity of progressive senile nephropathy (PSN) that was not reversible at the high dose in females. PNS in rats is characterized by enlarged and pale kidneys with cystic spaces caused by thickening and dysfunction of the basement membrane, possibly due to increased content of collagen-like polypeptides (Greaves and Faccini, 1984; Abrass, 2000). PSN was seen as early as 23 weeks in treated males and 52 weeks in treated females, compared to 62 weeks (male) and 66 weeks (female) for controls. In animals that survived until the end of treatment, the effect was significant for males in all treatment groups (Table 13). Changes were most dramatic in males treated with 30 and 300 ppm exposed for 89 weeks. When decedents are included, incidence was significantly increased in the 30 ppm females and 300 ppm males and females. Also, there was an increase in PSN in animals at the interim (52 week) sacrifice, which was significant in high dose females. Decreased albumin and increased α-globulins and cholesterol have been described in rats with PSN (Barthold, 1979), and were observed in this study in rats at 30 and 300 ppm. PSN is common in aging rats, but fipronil increased the rate at which PSN occurred.

The chronic NOEL was 0.5 ppm (0.02 mg/kg/day) based on convulsions, clinical signs, increased incidence of progressive senile nephropathy, and thyroid hormone alterations at the LOEL of 1.5 ppm (0.06 mg/kg/day). Some of the effects at the LOEL were first seen within the first week of treatment (altered T4 levels) or within six months (convulsions) and continued until the end of the study. Thyroid tumors observed in males and females at the high dose are described in the oncogenicity section, below.

Table 13. Toxicity in CD Rats following Chronic Dietary Exposure to Fipronil

	Males					Females				
Dose (ppm)	**0**	**0.5**	**1.5**	**30**	**300**	**0**	**0.5**	**1.5**	**30**	**300**
Dose (mg/kg/day)	**0**	**0.02**	**0.06**	**1.3**	**13**	**0**	**0.03**	**0.08**	**1.6**	**17**
N[a]	80	80	80	80	80	80	80	80	80	80
Total mortality (weeks 88-91)	30	36	28	30	36	28	29	28	37	28
Week of first mortality	42	26	23	25	1	44	37	58	42	1
Convulsions[b]	0	0	3	1	8	0	0	0	3	12
Death after convulsions	0	0	1	0	4	0	0	0	1	3
BW (g) week 1	253 ± 22	253 ± 21	250 ± 20	249 ± 19	216 ± 19†††	190 ± 16	187 ± 16	186 ± 15	183 ± 14†	171 ± 12††††
% Control	100%	100%	99%	98%	85%	100%	98%	98%	96%	90%
BW (g) week 52	904 ± 164	918 ± 145	896 ± 119	861 ± 145	792 ± 114††††	500 ± 97	508 ± 100	498 ± 95	488 ± 86	435 ± 76
% Control	100%	102%	99%	95%	88%	100%	102%	100%	98%	87%
BW (g) week 13 reverse[c]	880 ± 243	855 ± 168	965 ± 142	853 ± 116	703 ± 115†	546 ± 144	537 ± 124	507 ± 81	547 ± 118	428 ± 64††††
% Control	100%	97%	110%	97%	80%	100%	98%	93%	100%	78%
BW (g) week 88-91	881 ± 138	964 ± 206	963 ± 138	836 ± 222	758 ± 76	612 ± 126	565 ± 135	599 ± 117	480 ± 135††	486 ± 96††††
% Control	100%	109%	109%	95%	86%	100%	92%	98%	78%	79%
N (hormone measurements)	10	10	10	10	10	10	10	10	10	10
T4 week 1 (µg/dL)	2.93 ± 0.5	3.02 ± 0.6	2.23 ± 0.74*	1.16 ± 0.7***	0.00 ± 0***	2.23 ± 1.0	1.86 ± 0.5	2.58 ± 1.15	1.26 ± 0.6**	0.0 ± 0***
% Control	100%	103%	76%	40%	0%	100%	83%	116%	57%	0%
T4 week 50 (µg/dL)	5.95 ± 1.1	5.51 ± 1.0	4.83 ± 0.6**	3.90 ± 0.7***	2.07 ± 0.4***	3.31 ± 1.1	3.46 ± 0.7	3.0 ± 0.6	2.06 ± 0.6***	1.38 ± 0.4***
% Control	100%	93%	81%	66%	35%	100%	105%	91%	62%	42%
T4 week 11 reverse (µg/dL)[c]	3.7 ± 0.8	3.58 ± 0.7	3.25 ± 0.6	3.29 ± 0.6	3.52 ± 0.6	2.95 ± 0.6	3.6 ± 0.7	3.27 ± 0.4	3.65 ± 0.7*	3.09 ± 1.0
% Control	100%	97%	88%	89%	95%	100%	122%	111%	124%	105%
T3 week 1 (ng/mL)	0.61 ± 0.09	0.59 ± 0.07	0.61 ± 0.10	0.58 ± 0.06	0.53 ± 0.06*	0.78 ± 0.16	0.75 ± 0.14	0.77 ± 0.12	0.75 ± 0.13	0.8 ± 0.23
% Control	100%	83%	100%	83%	83%	100%	100%	100%	100%	114%

Table 13. Toxicity in CD Rats following Chronic Dietary Exposure to Fipronil

	Males					Females				
Dose (ppm)	**0**	**0.5**	**1.5**	**30**	**300**	**0**	**0.5**	**1.5**	**30**	**300**
Dose (mg/kg/day)	**0**	**0.02**	**0.06**	**1.3**	**13**	**0**	**0.03**	**0.08**	**1.6**	**17**
T3 week 50 (ng/mL)	0.7 ± 0.13	0.67 ± 0.12	0.84 ± 0.14*	0.82 ± 0.13*	0.69 ± 0.14	0.87 ± 0.12	0.88 ± 0.12	0.83 ± 0.21	0.84 ± 0.08	0.87 ± 0.21
% Control	100%	86%	114%	114%	86%	100%	100%	100%	100%	100%
T3 week 11 reverse (ng/mL)[c]	0.56 ± 0.15	0.52 ± 0.09	0.59 ± 0.10	0.62 ± 0.10	0.64 ± 0.43	0.75 ± 0.14	0.81 ± 0.12	0.83 ± 0.06	0.95 ± 0.18**	1.11 ± 0.21***
% Control	100%	93%	105%	111%	114%	100%	108%	111%	127%	148%
TSH week 1 (ng/mL)	4.7 ± 0.9	7.1 ± 2.7	6.2 ± 2.5	11.8 ± 5.6***	20.3 ± 7.4***	3.5 ± 1.1	3.5 ± 0.7	3.2 ± 1.2	3.6 ± 0.9	7.6 ± 4.2***
% Control	100%	151%	132%	251%	432%	100%	100%	91%	103%	217%
TSH week 50 (ng/mL)	13.0 ± 7.2	17.1 ± 5.6	12.4 ± 5.5	26.6 ± 18.9*	57.3 ± 21.7***	6.2 ± 1.4	8.0 ± 4.0	5.5 ± 2.1	6.1 ± 1.3	13.5 ± 5.0***
% Control	100%	132%	95%	205%	441%	100%	129%	89%	98%	218%
TSH week 11 reverse (ng/mL)c	3.8 ± 1.4	4.6 ± 2.3	4.6 ± 1.2	5.1 ± 2.5	8.4 ± 6.0**	2.7 ± 0.5	3.1 ± 1.2	2.9 ± 0.8	2.7 ± 0.5	3.1 ± 1.0
% Control	100%	121%	121%	134%	221%	100%	115%	107%	100%	115%
N (52 weeks)	12	14	14	15	12	14	14	14	14	13
52 week absolute liver wt (g)	29.5 ± 5.5	30.0 ± 7.8	29.1 ± 6.2	34.3 ± 8.0	40.5 ± 5.2**	16.4 ± 3.6	17.3 ± 4.3	17.1 ± 3.1	19.1 ± 4.3	23.4 ± 4.6**
% Control	100%	102%	99%	116%	137%	100%	105%	104%	116%	143%
52 week relative liver wt	3.53 ± 0.55	3.44 ± 0.37	3.40 ± 0.35	4.32 ± 0.61**	5.26 ± 0.40**	3.56 ± 0.51	3.62 ± 0.60	3.7 ± 0.51	4.31 ± 0.53**	5.49 ± 0.68**
% Control	100%	97%	96%	122%	149%	100%	102%	104%	121%	154%
52 week absolute thyroid wt (mg)	39 ± 7	35 ± 12	42 ± 8	47 ± 15	56 ± 11**	27 ± 9	31 ± 7	30 ± 6	32 ± 7	45 ± 11**
% Control	100%	90%	108%	121%	144%	100%	115%	111%	119%	167%
52 week relative thyroid wt[d]	4.7 ± 1.1	4.0 ± 1.0	5.0 ± 0.6	5.8 ± 1.4*	7.3 ± 1.5**	5.9 ± 1.7	6.4 ± 0.8	6.5 ± 1.3	7.3 ± 1.6*	10.7 ± 3.1**
% Control	100%	85%	106%	123%	155%	100%	108%	110%	124%	181%

Table 13. Toxicity in CD Rats following Chronic Dietary Exposure to Fipronil

	Males					Females				
Dose (ppm)	**0**	**0.5**	**1.5**	**30**	**300**	**0**	**0.5**	**1.5**	**30**	**300**
Dose (mg/kg/day)	**0**	**0.02**	**0.06**	**1.3**	**13**	**0**	**0.03**	**0.08**	**1.6**	**17**
N (13 week reverse)[c]	13	15	13	13	10	13	13	11	15	10
13 week reverse absolute liver wt (g)[c]	30.3 ± 7.0	30.3 ± 10.2	32.9 ± 6.4	30.9 ± 5.2	30.6 ± 5.9	18.5 ± 4.8	19.4 ± 5.2	17.8 ± 3.8	21.0 ± 5.7	19.7 ± 4.6
% Control	100%	100%	109%	102%	101%	100%	105%	96%	114%	106%
13 week reverse relative liver wt[c,d]	3.5 ± 0.7	3.7 ± 1.2	3.4 ± 0.5	3.7 ± 0.4	4.6 ± 1.1*	3.4 ± 0.3	3.6 ± 0.4	3.5 ± 0.3	3.8 ± 0.5*	4.7 ± 1.2**
% Control	100%	106%	97%	106%	131%	100%	106%	103%	112%	138%
13 week reverse absolute thyroid wt (mg)[c]	38 ± 13	39 ± 7	43 ± 11	45 ± 11	45 ± 6	31 ± 5	31 ± 8	30 ± 5	34 ± 10	35 ± 6
% Control	100%	103%	113%	118%	118%	100%	100%	97%	110%	113%
13 week reverse relative thyroid wt[c,d]	4.5 ± 1.3	4.7 ± 0.6	4.5 ± 1.0	5.4 ± 1.5	6.7 ± 1.7*	5.9 ± 1.1	5.8 ± 1.2	6.1 ± 0.9	6.3 ± 1.1	8.0 ± 0.7**
% Control	100%	104%	100%	120%	149%	100%	98%	103%	107%	136%
N (89–91 weeks)	20	14	22	20	12	22	22	21	13	22
90 week absolute liver wt (g)	28.3 ± 10.6	32.4 ± 6.2	32.1 ± 4.9	33.9 ± 6.4	39.4 ± 5.3**	23.0 ± 6.8	22.0 ± 5.4	21.5 ± 5.0	25.0 ± 6.4	27.9 ± 5.0*
% Control	100%	114%	113%	120%	139%	100%	96%	93%	109%	121%
90 week relative liver wt	3.30 ± 1.18	3.24 ± 0.52	3.48 ± 0.89	4.4 ± 1.14**	5.41 ± 0.64**	3.88 ± 0.90	3.98 ± 0.67	3.82 ± 0.65	5.12 ± 1.08**	6.14 ± 1.06**
% Control	100%	98%	105%	133%	164%	100%	103%	98%	132%	158%

Table 13. Toxicity in CD Rats following Chronic Dietary Exposure to Fipronil

	Males					Females				
Dose (ppm)	**0**	**0.5**	**1.5**	**30**	**300**	**0**	**0.5**	**1.5**	**30**	**300**
Dose (mg/kg/day)	**0**	**0.02**	**0.06**	**1.3**	**13**	**0**	**0.03**	**0.08**	**1.6**	**17**
90 week absolute thyroid wt (mg)	42 ± 13	51 ± 10*	53 ± 13*	63 ± 20**	94 ± 24**	36 ± 11	38 ± 17	36 ± 8	44 ± 14	72 ± 79*
% Control	100%	121%	126%	150%	224%	100%	106%	100%	122%	200%
90-week relative thyroid wt[d]	4.9 ± 1.4	5.2 ± 1.2	5.6 ± 1.5	8.2 ± 2.8**	12.9 ± 2.9**	6.0 ± 1.9	7.0 ± 2.9	6.5 ± 1.4	9.0 ± 2.8**	15.6 ± 16.2*
% of con	100%	106%	114%	167%	263%	100%	117%	108%	150%	260%
% PSN at 52 weeks -- scheduled[e]	42	21	36	47	75	29	36	21	43	54
% PSN at 52 weeks -- all	40	20	33	47	73	27	40	27	40	53
% PSN at 13-week reversibility	54	47	54	62	60	38	31	45	73	90*
% PSN at 89-91 weeks -- scheduled	40	79*	77*	85**	92**	26	57	24	69*	55
% PSN at 89-91 weeks -- all	52	56	64	84	88*	28	42	34	62*	48**

BW: body weight; wt: weight; PSN: progressive senile nephropathy.

[a]Animals included 50 rats/sex/dose from the Oncogenicity Phase, 15 rats/sex/dose from the Toxicity Phase and 15 rats/sex/dose from the Reversibility Phase.

[b]Animals from all phases were examined for convulsions.

[c]After fipronil administration for 52 weeks, animals were fed diet without fipronil for 13 weeks to assess the reversibility of fipronil-induced toxicity. Thyroid hormones were measured after 11 weeks on the fipronil-free diet (study week 63).

[d]Relative thyroid weight multiplied by 1000.

[e]"Scheduled" includes animals that survived to schedule sacrifice date; "all" includes animals that died prior to the scheduled sacrifice date.

†, ††, ††† Significantly different from controls at $p \leq 0.05$, 0.01 or 0.001 (calculated by DPR with one-way ANOVA and Dunnett's test for multiple comparisons in GraphPad Prism 7).

*, **, *** Significantly different from controls at $p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$ (one-tailed Fisher's Exact test, Mann Whitney U Test, Behrens-Fisher test or Dunnet's test for pairwise comparisons with Bartlett's test for variance homogeneity performed by study authors)

Data from Aughton (1993).

IV.E.2. Chronic Oral – Mouse (Broadmeadow, 1993)

In a chronic toxicity/oncogenicity study, fipronil (95.4% a.i.) was administered in the diet to CD-1 mice (Broadmeadow, 1993). Animals in the Oncogenicity Phase (52/sex/dose) received fipronil for 78 weeks. Animals in the Toxicity Phase (20/sex/dose) received fipronil for 53 weeks. The dietary levels of fipronil were 0, 0.1, 0.5, 10, 30 or 60 ppm. However, due to marked toxicity at 60 ppm, all survivors from this dose group were sacrificed without necropsy at week 10 and will not be further discussed. The highest tested dose was, therefore, 30 ppm, with reported average daily doses of 0, 0.011, 0.055, 1.181 and 3.430 mg/kg/day for males and 0, 0.012, 0.063, 1.230 and 3.616 mg/kg/day for females. Control animals received untreated diet.

Convulsions in 3 males and a marked decline in weight gain and food intake in both sexes were noted at 60 ppm. Fourteen 60-ppm males and 7 females died during the first 9 weeks of treatment (19% of the males and 10% of the females), indicating that the maximum tolerated dose was exceeded. The decedents had higher relative liver weights at necropsy.

Effects at 10 and 30 ppm consisted of lower body weight gains (14–19%, $p < 0.05$) and reduced body weight (for 7–13%) while the food consumption was not significantly affected by the treatment.

Examination at 53 and 78 weeks revealed that liver weight was increased up to 36% ($p < 0.01$, absolute weight) and up to 51% ($p < 0.01$ relative weight) in the mice exposed to 10 and 30 ppm fipronil. This effect was more pronounced in the males (Table 14).

Histopathology findings at 53 and 78 weeks included an increased incidence of microvesicular periacinar vacuolation of hepatocytes in the liver. Males from all treated groups had vacuolation of hepatocytes: 2/15 in the 0.1-ppm group, 2/19 in the 0.5-ppm group, with the effect becoming significant at 10 ppm and 30 ppm (incidence of 7/16 and 12/18, respectively; 0/14 for control) at both durations. In females, the effect was significant at 30 ppm. The severity of this effect was graded as slight to marked (Table 14). This effect was also seen in mice following subchronic (13-week) dietary exposure (Broadmeadow, 1991).

The chronic NOEL for non-oncogenic effects in mice was 0.5 ppm (0.06 mg/kg/day), based on significantly increased liver weights (absolute and relative) and liver pathology (periacinar vacuolation) at the LOEL of 10 ppm (1.18 mg/kg/day). Possible oncogenic effects are described in the Oncogenicity section of this RCD, below.

Table 14. Effects in CD-1 Mice after Chronic Dietary Exposure to Fipronil

	Males					**Females**				
Dose (ppm)	**0**	**0.1**	**0.5**	**10**	**30**	**0**	**0.1**	**0.5**	**10**	**30**
Dose (mg/kg/day)	**0**	**0.01**	**0.06**	**1.18**	**3.43**	**0**	**0.01**	**0.06**	**1.23**	**3.62**
N (week 78)	24	31	26	26	26	33	35	27	41	38
Bodyweight gain (g) week 0-78	23.1 ± 5.9	21.9 ± 5.7	23.7 ± 4.9	21.3 ± 6.6	19.9 ± 4.8	25.4 ± 10.0	21.7 ± 8.1	25.8 ± 11.2	22.1 ± 8.6	20.6 ± 6.6*

Table 14. Effects in CD-1 Mice after Chronic Dietary Exposure to Fipronil

	Males					Females				
Dose (ppm)	**0**	**0.1**	**0.5**	**10**	**30**	**0**	**0.1**	**0.5**	**10**	**30**
Dose (mg/kg/day)	**0**	**0.01**	**0.06**	**1.18**	**3.43**	**0**	**0.01**	**0.06**	**1.23**	**3.62**
% Control	100%	95%	103%	92%	86%	100%	85%	102%	87%	81%
N (livers, week 53)	14	15	19	16	18	18	19	15	17	13
Abs. liver wt (g) week 53	2.6 ± 0.4	2.6 ± 0.5	2.7 ± 0.7	3.0 ± 0.8	3.4 ± 0.6**	1.7 ± 0.2	1.8 ± 0.4	1.9 ± 0.2	1.8 ± 0.2*	2.0 ± 0.4**
% Control	100%	100%	104%	115%	131%	100%	106%	112%	106%	118%
Rel. liver wt week 53	5.1 ± 0.5	5.6 ± 1.1	5.7 ± 1.1*	6.1 ± 1.5*	7.7 ± 1.4**	4.4 ± 0.8	4.4 ± 0.6	4.5 ± 0.7	4.7 ± 0.6	5.4 ± 0.9**
% Control	100%	110%	112%	120%	151%	100%	100%	102%	107%	123%
N (livers, week 78)	24	31	26	26	26	32	32	26	37	38
Abs. liver wt (g) week 78	2.8 ± 0.7	2.8 ± 1.0	2.9 ± 1.6	3.3 ± 1.5	3.8 ± 1.1**	2.0 ± 0.3	1.9 ± 0.4	2.0 ± 0.5	2.0 ± 0.3	2.1 ± 0.4
% Control	100%	100%	104%	118%	136%	100%	95%	100%	100%	105%
Rel. liver wt week 78	5.6 ± 1.1	5.7 ± 1.9	6.0 ± 3.4	7.0 ± 2.8*	8.3 ± 2.5**	4.5 ± 0.9	4.6 ± 1.0	4.5 ± 0.8	4.8 ± 0.8	5.3 ± 0.9**
% Control	100%	102%	107%	125%	148%	100%	102%	100%	107%	118%
Periacinar vacuolation week 53	0/14	2/15	2/19	7/16**	12/18***	1/18	1/19	4/15	1/17	4/13
% Periacinar vacuolation week 53	0	13%	11%	44%	67%	6%	5%	27%	6%	31%
Periacinar vacuolation week 78	5/24	7/31	7/26	13/26*	13/26*	0/32	0/32	4/26*	3/37	7/38*
% Periacinar vacuolation week 78	21%	23%	27%	50%	50%	0%	0%	15%	8%	18%

Abs: absolute; Rel: relative; wt: weight; Vacuol: vacuolation.
*, **, *** Significantly different from controls (one-tailed Fisher's Exact test, performed by study authors) at $p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$.
Data from Broadmeadow (1993).

IV.E.3. Chronic Oral – Dog

Two chronic toxicity studies with fipronil in Beagle dogs were submitted to DPR. Both studies were performed in the Pharmaco-Life Science Research Laboratory for a period of 52 weeks. In the first study, fipronil was administered to the dogs in gelatin capsule (Holmes, 1992). In the second study, fipronil was incorporated into ground diet (Holmes, 1993).

Capsule (Holmes, 1992)

A chronic study in Beagle dogs was conducted with fipronil (95.4%) administered in gelatin capsules (Holmes, 1992). Six dogs/sex/dose were given 0, 0.2, 2.0 or 5.0 mg/kg/day for 52 weeks. The principal treatment-related effects were neurological. All animals treated at 5 mg/kg/day, as well as most at 2 mg/kg/day, exhibited tremors, muscular twitching and leg

rigidity, nervous behavior, gait abnormalities, and excessive salivation. The most serious neurological sign was convulsions, with 1 male and 1 female from the 2 mg/kg/day group and 2 males and 1 female from the 5 mg/kg/day dose group showing this sign during weeks 2–51. Convulsions typically lasted from 30 sec to 2 min and occurred as soon as 15 min after dosing. Most of the dogs had a series of convulsive episodes, which were graded from severe to epileptiform petit-mal seizures. Following the convulsions, the dogs appeared dazed, unsteady and unaware of their surroundings. They were also sensitive to sudden noise or touch and had rigid legs. One male in the 2 mg/kg/day group and 2 males in the 5 mg/kg/day group were sacrificed during weeks 11, 31 and 34, respectively, due to health deterioration following convulsions. Other signs included blindness, weight loss, severe ataxia and blood stained saliva around the mouth. The females receiving the lowest dose of fipronil (0.2 mg/kg/day) showed a higher incidence of salivation (2 out of 6 dogs) on several occasions during weeks 34–51 compared to controls (0/30). Females treated at the mid-dose also showed a higher incidence of salivation (3/6), while females at the high dose did not (1/6). There were also sporadic effects on hemoglobin, platelet and plasma protein levels.

Based on severe toxicity (convulsions and other neurological effects) observed at the LOEL of 2 mg/kg/day, the chronic oral NOEL for dogs was 0.2 mg/kg/day.

Dietary (Holmes, 1993)

A second chronic study in Beagle dogs was performed with fipronil (95.4%) incorporated into the diet (Holmes, 1993). This study included 5 dogs/sex/dose fed at target doses of 0, 0.075, 0.3, 1.0 or 3.0 mg/kg/day for 52 weeks. Achieved doses were 0.074, 0.299, 0.998 and 1.992 mg/kg/day for males and 0.074, 0.295, 0.996 and 1.995 mg/kg/day for females. The highest tested dose (3 mg/kg/day) was given to the dogs for 38 days but was reduced thereafter to 2 mg/kg/day due to significant toxicity (death); 3 mg/kg/day therefore exceeded the maximum tolerated dose for dogs.

The main effects of fipronil exposure occurred in the central nervous system. One high-dose male suffered convulsions during weeks 6 and 45, and one female had severe convulsions during week 5. Convulsions lasted from 30 sec to 10 min and were usually followed by tremors and whole body twitching. Convulsive dogs showed excessive chewing behavior, extensor limb rigidity, head nodding and urine stains on limbs and tail. After the convulsive episodes in week 5, the female dog was sacrificed *in extremis*. Rigidity in limbs occurred in both sexes at 3 mg/kg/day and in 1 female at 1 mg/kg/day. It also occurred in 1 male at 0.3 mg/kg/day but did not occur in males at the next higher dose. Tremors were observed in three males at the high dose during week 4, weeks 4–6 and, for one male, at numerous times during the first 10 weeks of treatment. Tremors and convulsions were observed in one female at the high dose (sacrificed on day 32 due to poor health), and twitching was observed in one female at 1 mg/kg/day during week 13. Higher absolute and relative spleen weight were noted in high dose males, along with higher incidence of swollen or large spleens and hyperplasia of the red pulp in the spleen under microscopic examination.

The chronic oral NOEL for dogs receiving fipronil in the diet was 0.3 mg/kg/day based on CNS effects (twitching of muscles and rigidity of limbs) at the LOEL of 1 mg/kg/day.

Table 15. Chronic No-Observed-Effect Levels (NOELs) and Lowest-Observed-Effect Levels (LOELs) for Fipronil

Species, Strain	Study Type and Exposure	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
Rat, CD	combined chronic/onco, dietary, 91 weeks	convulsions and mortality in males, decreases in thyroid hormones weeks 1 to 50, progressive senile nephropathy	0.02	0.06	Aughton (1993)
Mouse, CD-1	combined chronic/onco, dietary, 78 weeks	increased relative liver weights and periacinar vacuolation	0.06	1.18	Broadmeadow (1993)
Dog, Beagle	gelatin capsule, 53 weeks	convulsions, neurological effects	0.2	2	Holmes (1992)
Dog, Beagle	dietary, 52 weeks	muscle rigidity and tremors	0.3	1	Holmes (1993)

IV.F. Oncogenicity

IV.F.1. Rat (Aughton, 1993)

In a chronic toxicity/oncogenicity study, fipronil (95.4% a.i.) was administered to CD rats at dietary levels of 0, 0.5, 1.5, 30 and 300 ppm (Aughton, 1993). The Oncogenicity Phase consisted of 50 rats/sex/dose that received fipronil for 89-91 weeks. The average daily doses were 0.02, 0.06, 1.3 and 13 mg/kg/day for males and 0.03, 0.08, 1.6 and 17 mg/kg/day for females. The Oncogenicity Phase was shortened from 104 weeks to 89 weeks (males) or 91 weeks (females) due to poor survival of both controls and treated rats.

Various types of tumors were reported in a total of 28 organ or tissue sites. There was an increase in rat liver neoplasms in the high dose male group only. Notably, thyroid follicular cell tumors were found in fipronil-treated rats, but not in controls (Table 16). These included follicular cell adenomas in male rats treated with 1.5 to 300 ppm and in females at 300 ppm, and carcinomas in males at 300 ppm and females at 30 and 300 ppm. The first tumor was detected at week 42 in the high dose group. Historical control data from the conducting laboratory (Pharmaco-LSR Ltd) between 1989 and 1992 showed 26 of 359 (7.2%) control males and 10 of 365 (2.7%) control females had follicular cell tumors. Based on these data, the incidence of thyroid follicular tumors at the high dose of 300 ppm (17/50 males and 10/50 females) was outside the historical control ranges. However, the incidence of adenomas in males at 0.06 and 1.3 mg/kg/day did not suggest a dose response and could not clearly be attributed to fipronil.

Cochran-Armitage trend and Fisher-Exact tests were performed on thyroid tumor poly-3 survival-adjusted incidence data in male rats (Portier and Bailer, 1989; Bieler and Williams,

1993). Both on- and two-tailed Cochran-Armitage trend tests were significant ($p < 0.001$). Additionally, pair-wise Fisher's tests (one-tailed) showed that tumor incidence in the 0.06 mg/kg/day group and 13 mg/kg/day (high-dose) group were significantly ($p < 0.05$) increased compared to controls. However, tumor incidence in the 0.02 and 1.3 mg/kg/day groups was not significantly different from controls, thus indicating no clear dose-response. See Appendix III for more details on these tests.

Table 16. Thyroid Tumor Incidence in CD Rats Exposed to Fipronil for 89 to 91 Weeks via Diet and Historical Control Data

	Males					Females				
Dose (ppm)	**0**	**0.5**	**1.5**	**30**	**300**	**0**	**0.5**	**1.5**	**30**	**300**
Dose (mg/kg/day)	**0**	**0.02**	**0.06**	**1.3**	**13**	**0**	**0.03**	**0.08**	**1.6**	**17**
N (thyroids)	49	48	50	50	50	50	50	50	50	50
Follicular Cell Adenoma	0	1	5*	3	12***	0	0	0	0	8**
Follicular Cell Carcinoma	0	0	0	0	5*	0	1	0	1	2
Adenoma + Carcinoma	0	1	5*	3	17***	0	1	0	1	10***
Percent Occurrence (%)	0%	2%	10%	6%	34%	0%	2%	0%	2%	20%
N (historical control)	359					365				
Adenoma + Carcinoma	26					10				
Percent Occurrence (range)	7.2 (3.6-16)%					2.7 (0-12)%				
N (liver)	49	48	50	50	50	50	50	50	50	50
Hepatocellular Carcinoma	2	2	0	1	0	0	0	0	0	0
Hepatocellular Adenoma	1	0	2	0	4	0	0	1	0	0

Historical control data from the conducting laboratory were supplied in the combined chronic and oncogenic study data volume. Controls included 7 combined chronic toxicity and oncogenicity or oncogenicity studies conducted in the four years prior to the present study. Statistics performed by study authors.
Data from Aughton (1993).

IV.F.2. Mouse (Broadmeadow, 1993)

In a chronic toxicity/oncogenicity study, fipronil (95.4% a.i.) was administered in the diet to CD-1 mice (Broadmeadow, 1993). Animals in the Oncogenicity Phase (52/sex/dose) received fipronil for 78 weeks at 0, 0.1, 0.5, 10, 30 or 60 ppm. However, due to marked toxicity at 60 ppm, all survivors from this dose group were sacrificed without necropsy at week 10 and will not be further discussed. The highest tested dose was, therefore, 30 ppm, with reported average daily doses of 0, 0.011, 0.055, 1.181 and 3.430 mg/kg/day in males and 0, 0.012, 0.063, 1.230 and 3.616 mg/kg/day in females. Control animals received untreated diet.

Tumors were reported in 17 organ/tissue sites. Liver carcinomas in males were possibly treatment related at the high dose, although the Fisher's exact comparison showed no significant differences from controls (5/47 at 30 ppm versus 1/47 in controls; see next paragraph) (Table 17). The data for liver adenomas were difficult to interpret because of the relatively high incidence in control animals or low incidence in the 0.1 and 0.5 ppm treatment groups. All liver tumor incidence rates fell within historical control ranges, that is control rates recorded in studies

conducted in the same species and strain by the same laboratory within the 2 years bracketing this study (Doi, 2008). (See Table 18.) Tumor incidence reported in published literature also fell within historical controls rates, except for carcinoma incidence at the high dose (12%) in Doi, 2008, which was equal to the maximum historical control rate (11.5%) (Carmichael *et al.*, 1997; Giknis and Clifford, 2005). The 46% survival rate in concurrent controls was low compared to the 61–66% rate documented for historical controls (Carmichael *et al.*, 1997), a factor which could have affected the tumor incidence rate.

Table 17. Liver Tumor Incidence in CD-1 Mice Exposed to Fipronil for 78 Weeks via Diet

	Males					Females				
Dose (ppm)	**0**	**0.1**	**0.5**	**10**	**30**	**0**	**0.1**	**0.5**	**10**	**30**
Dose mg/kg/day	0	0.01	0.06	1.18	3.43	0	0.01	0.06	1.23	3.62
Animals at start of the study	52	52	52	52	52	52	52	52	52	52
Animals at-risk for adenoma on day 317	47	50	44	40	47	--	--	--	--	--
Adenomas	10	3	2	6	6[a]	0	0	0	0	1
% adenoma	21	6	5	15	13	0	0	0	0	2
Animals at-risk for carcinoma on day 409	41	39	34	32	42	--	--	--	--	--
Carcinomas	1	1	2	1	5	0	0	0	0	0
%carcinoma	2	3	6	3	12	0	0	0	0	0
Adenomas + carcinomas	11	4	4	7	10[a]	0	0	0	0	1
% adenoma + carcinoma[b]	23	8	9	18	21	0	0	0	0	2

[a]One male in the 30-ppm group developed both an adenoma and a carcinoma. Both are reported here.
[b]Denominators for adenomas + carcinomas are the number of at-risk animals on day 317.
Data from Broadmeadow (1993).
-- at risk animals were not determined due to lack of tumors in the females

DPR performed Fisher's Exact comparisons and Cochran-Armitage trend tests on the incidence data in males for adenomas, carcinomas, and adenomas + carcinomas. Trend tests were performed using two sets of n values: (1) those adjusted for at-risk animals based on the first detection of mice with adenoma or carcinoma, and (2) those derived from a Poly-3 survival-adjusted quantal-response test (Portier and Bailer, 1989; Bieler and Williams, 1993). While none of the Cochran-Armitage trend tests on adenomas or on carcinomas + adenomas achieved

statistical significance, those on carcinomas alone did ($p < 0.05$), with and without continuity correction, with one-tail or two-tails, and when the denominators were adjusted for at-risk animals or weighted for survival using poly-3 determinations. Despite this, all pair-wise Fisher's comparisons between control and treatment groups were negative. Detailed discussion and results of the statistical analyses appear in Appendix III.

Table 18. Historical Control Liver Tumor Data for CD-1 Mice

	Males	Females
N	212[a]	--
Percent adenoma (range)	4.7 (0 - 17.2)	--
Percent carcinoma (range)	10.4 (2.9 - 25)	--
Percent adenoma + carcinoma (range)	15.7 (2.9 - 25)	--
N	426[b]	425
Percent adenoma (range)	11.27 (0 - 23.1)	0.24 (0 - 2)
Percent carcinoma (range)	7 (2 - 11.5)	0.47 (0 - 2)
Percent adenoma + carcinoma (range)	18.27 (2 - 34.6)	0.71 (0 - 4)

[a]Published historical control data for CD-1 mice (Carmichael *et al.*, 1997; Giknis and Clifford, 2005). Includes data for all animals (including decedents); oral gavage and dietary administration.
[b]Historical male tumor incidence data from in the same lab during the two years bracketing the fipronil study (Doi, 2008).

IV.G. Genotoxicity

Six genotoxicity studies were submitted to DPR to fulfill the pesticide registration data requirements for the State of California (Table 19). These included 2 *in vitro* point mutation studies and four chromosome aberration studies (two *in vivo* and two *in vitro*) at fipronil concentrations up to its toxicity or solubility limits. In five of these studies, fipronil (95-97.2%) was negative for gene mutations or cytogenetic damage under the experimental conditions of these tests. The remaining study, an *in vitro* study, showed damage to chromosomes along with cytotoxicity. Given the relationship between induction of chromosomal damage and cytotoxicity, the former appears to be part of the process of cell toxicity leading to cell death, as opposed to indicating a significant genotoxic potential.

DPR identified 20 studies with genotoxic endpoints published in open literature (latest systematic literature review conducted on June 16, 2022). Of these, 17 had experimental, design or reporting issues that would preclude their use in human health risk assessment (Appendix V). Three of the remaining publications (two *in vivo* and one *in vitro*) met DPR's minimum data acceptance criteria. These studies showed genotoxicity results in association with apoptosis, cytotoxicity, or indirect evidence of oxidative stress (Khan *et al.*, 2015; Badgujar *et al.*, 2016b; Quesnot *et al.*, 2016). In no case was mutagenicity directly implicated. However, DPR noted methodological deficiencies in these studies that limited the reliability of their overall conclusions.

Khan et al. (2015). A decrease in sperm density, motility, viability, and acrosome integrity and a change in sperm morphology was reported in rats after 28 consecutive days of fipronil administration via gavage. Given that almost all of the assays were done on spermatozoa, this study was not designed to test fipronil's genotoxic potential to somatic cells in the testis. However, it may provide evidence of reproductive toxicity (see section Reproductive Studies Published in Literature). The investigators proposed that fipronil caused male reproductive toxicity through oxidative stress-induced DNA damage and apoptosis in spermatozoa. This was based on assays showing formation of ROS and lipid peroxidation, and Annexin V binding as a measure of apoptosis in spermatozoa. DPR has concerns about the study as follows. First, body weights suggest the animals may have been sexually immature (<10 weeks old) when dosing started. Additionally, it appears that the entire epididymis was used, meaning that comingled spermatozoa in all stages of epidydimal maturation were used for the assays, potentially affecting the endpoints. Testicular histology did not appear to follow standard protocols (US EPA or OECD). A commercial kit was used for the sperm comet assay, but assay details were not provided, including the pH used for electrophoresis and lack of a positive control. "Apoptotic" sperm were identified by Annexin V binding to phosphatidylserine, although such binding is seen with healthy sperm; furthermore, recognition of sperm phosphatidylserine by receptors on oocytes may facilitate sperm-oocyte fusion. Finally, it should be noted that given the quick onset of hypothyroidism induced by fipronil in male rats (Aughton, 1993), some sperm changes reported in Khan et al. (2015) may stem from decreased thyroid-hormone support for steroidogenesis and spermiogenesis.

Badgujar et al. (2017). Rats and mice were exposed once by gavage to fipronil in corn oil and sacrificed 24 h later (Badgujar *et al.*, 2016b). Fipronil was genotoxic *in vivo* in a dose-related manner in both sexes in three assays. In mice, fipronil increased micronucleus formation in bone marrow erythrocytes while in rats, fipronil induced chromosome aberrations in bone marrow cells and caused DNA breakage in peripheral blood nucleated cells as measured by the alkaline Comet assay. The effects in mice occurred in the absence of significant decreases in the ratio of polychromatic to normochromatic erythrocytes (a metric for bone marrow toxicity) while in rats, bone marrow toxicity was not assessed. The study authors attributed the positive results in the three assays to fipronil-induced formation of reactive oxygen species (ROS). This was based on the finding that pretreatment of animals with vitamin E reduced the genotoxicity in each of the assays.

DPR has concerns with the study. First, mice were gavaged with corn-oil suspensions using volumes of 1 mL/kg BW or about 20-30 µL per animal, which calls into question whether fipronil doses were reliably delivered. Second, despite indicating that OECD Guideline TG475 was followed in the rat study, the mitotic index was not determined for bone marrow cells and chromosomal aberration data were only provided graphically in a pooled form and not by category, which limited a more informative analysis of individual results. Third, in contrast to the positive mouse micronucleus test in Badgujar et al. (2017) at 12.5 mg/kg and 25 mg/kg fipronil, the two other available *in vivo* mouse micronucleus assays were negative up to 50 mg/kg (Edwards, 1993; Edwards, 1995). The latter studies were sponsored by the registrant and

are summarized in Table 19 below. The lack of agreement occurred even though the three studies were similar in experimental design: outbred mouse strains, both sexes; single gavage dosing, with sacrifice 24 h later; 25 mg/kg among the doses tested; and vehicle and positive controls.

Based on the presently existing genotoxicity data, DPR has more confidence in the registrant-submitted studies by Edwards (1993, 1995) than in Badgujar et al. (2017) for the following reasons. 1) In the registrant-submitted studies, slides containing bone marrow cells were coded to prevent scoring bias, 2) The negative micronucleus findings at 25 mg/kg were reproducible in both registrant-sponsored studies, 3) Micronucleus testing was negative at 50 mg/kg (Edwards, 1995) even though clinical signs (hunched posture, piloerection, convulsions) were induced in some of the animals, and 4) The registrant-submitted studies included negative results following fipronil dosing both at 25 mg/kg (Edwards, 1993) and at 50 mg/kg (Edwards, 1995), with sacrifice at 48 and 72 h postdosing.

Quesnot et al. (2016). The investigators tested fipronil in an *in vitro* genotoxicity assay based on *in situ* detection of the histone γH2AX phosphorylation in human HepaRG hepatoma cells (Quesnot *et al.*, 2016). γH2AX induction was measured after 1-day exposure to 5–25 µM fipronil and 7-day and 14-day exposures to 5-20 µM fipronil. A second genotoxicity test, based on induction of micronuclei, was used for comparison. Evaluations were performed after 1 or 7 days of treatment at fipronil concentrations of 15 to 25 µM. Authors considered the HepaRG cells relevant for hepatic genotoxicity because the cells express drug metabolizing enzymes and have a wild type p53 (Quesnot *et al.*, 2016).

In the γH2AX assay, 20 µM fipronil was positive for genotoxicity after 7 days of treatment, but not after 1 or 14 days of exposure. In the micronucleus assay, fipronil was only positive after 7 days of treatment but the effects seen at 15 and 20 µM were marginal and did not increase with dose. An MTT assay was performed to evaluate cytotoxicity. The authors indicated an enhanced cytotoxic effect between 24 hours and 7 days of treatment, but the poor quality of the graphed data in the supplemental materials precluded the exact determination of cell viability. In addition, during both genotoxicity tests cells were exposed to culture media containing 1% DMSO, which can cause epigenetic changes by itself.

Table 19. Registrant-Submitted Genotoxicity Studies Conducted with Fipronil

Test Type, System	Species or Culture	Exposure Regime	S9[a]	Results	Cytotoxicity	Reference
Ames Test *in vitro* mutagenicity reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	0 (DMSO), 0.8, 2, 20, 100, 400 or 500 µg/plate, 48 hr	±	Negative	No	(Clare, 1988a)
HGPRT *in vitro* mutagenicity 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)

Table 19. Registrant-Submitted Genotoxicity Studies Conducted with Fipronil

In vitro chromosome aberration	Human lymphocytes	0 (DMSO), 75, 150 or 300 µg/ml, 3 hr	±	Negative	Decreased cell viability	(Marshall, 1988a)
In vitro chromosome aberration	Chinese hamster lung cell line CHL	0 (DMSO), 30, 45 or 60 µg/ml for 6 hr	±	Positive at 45 and 60 µg/ml, no S9	Cell count decreased to 76% (45 µg/ml) and 44% (60 µg/ml)	(Wright, 1995)[b]
In vivo clastogenicity 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)
In vivo clastogenicity bone marrow micronucleus test	CD-1 mouse bone marrow erythrocytes	0 (0.5% methyl cellulose), 12.5, 25 or 50 mg/kg, gavage, single dose	n/a	Negative	No	(Edwards, 1995)

[a]S9 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. MN: Micronucleus assay; ROS: reactive oxygen species.

[b]In (Wright, 1995), cells were exposed for 6, 24 or 48 h. After 6-h exposures, cells were incubated for 18 h in fipronil-free media before cell harvesting. For 24 and 48 h exposures, cells were exposed continuously prior to cell harvesting. Only a 6 h exposure included the use of S9. Highest test concentrations were based on cytotoxicity and were 60, 30 and 22.5 µg/mL for exposures lasting 6, 24 and 48 h, respectively. No increase in chromosomal aberrations occurred after 24 or 48 h exposures. Since the positive control data showed that the incidence of chromosomal aberrations after 48 h (59%) was twice that observed after 24 h (29%), the test methods were sufficient for assessing clastogenic potential.

IV.H. Developmental Toxicity

IV.H.1. Developmental Toxicity – Rat

Fipronil (93% a.i.) was administered daily by gavage to mated female CRL:CD(SD)BR VAF/Plus rats from gestation day (GD) 6 through 15 (Brooker and John, 1991). Each dose group consisted of 25 rats. The doses were 0, 1, 4 or 20 mg/kg/day. Control animals were treated with vehicle (0.5% methylcellulose). On GD 20, fetuses were delivered by cesarean section and examined for developmental abnormalities.

Maternal effects at 20 mg/kg/day included reduced body weight (up to 8%) and reduced body weight gain (up to 61%) from the second day of treatment until study termination. Food consumption also decreased up to 24% during GD 6–11, with significant decreases from GD 8–9 ($p \leq 0.001$, one-way ANOVA by DPR).

The maternal NOEL of 4 mg/kg/day was based on reduced body weight at the LOEL of 20 mg/kg/day. The developmental NOEL was 20 mg/kg/day, based on no effects detected at the high dose.

IV.H.2. Developmental Toxicity – Rabbit

The developmental toxicity of fipronil was examined in the rabbit. Artificially inseminated New Zealand White rabbits (22/dose level) were treated by gavage for a total of 14 days, from GD 6 through GD 19, with daily doses of 0.1, 0.2, 0.5 or 1 mg/kg/day (King, 1990). Cesarean section and examination of the does and fetuses were performed on GD 29. Histological examination was performed on reproductive organs from the controls and high dose group animals and on the thyroid glands and liver from all adult animals in all groups.

No developmental effects were seen in the fetuses at any dose level. Food consumption in does treated with 0.5 and 1 mg/kg/day was significantly reduced by up to 33% ($p \leq 0.01$) compared to controls during the treatment period (Table 20). In turn, the mean body weight gain of these rabbits was decreased by 50–70% ($p < 0.01$) from the second day of treatment (GD 8) until the end of the study at GD 24 (e.g., five days after the last dose). After two doses, the body weight gain of the does at and above 0.2 mg/kg/day was reduced by 50% ($p < 0.01$) on GD 8. The decreased body weight gain of these does was significant until the end of the treatment (GD 19). The rabbits in the lowest tested group (0.1 mg/kg/day) exhibited significant reductions (54%, $p < 0.01$) on GD 10; however, a dose-response was not observed as the reduction in body weight gain at 0.2, 0.5 and 1 mg/kg/day was 36%, 45% and 45%, respectively.

The NOEL for maternal toxicity was 0.1 mg/kg/day, based on reduced weight gain at the LOEL of 0.2 mg/kg/day.

Table 20. Toxicity Observed in Rabbit Does following Oral Fipronil Exposure in a Developmental Toxicity Study

Dose (mg/kg/day)	**0**	**0.1**	**0.2**	**0.5**	**1**
# Females inseminated	22	22	22	22	22
# Females pregnant	20	21	21	18	19
# Females surviving treatment	19	21	21	18	19
Total litter loss	1	1	0	0	1
Food consumption (%) GD 1-5	100	91	98	100	96
Food consumption (%) GD 6-12	100	91	90	96	88**
Food consumption (%) GD 13-19	100	85	90	79*	67**
BW gain (g) GD 6-8	60 ± 40	40 ± 40	30 ± 40*	30 ± 40**	30 ± 30*
% Control	100%	67%	50%*	50%**	50%*
BW gain (g) GD 6-10	110 ± 50	50 ± 40***	70 ± 60*	60 ± 50***	60 ± 50**
% Control	100%	45%***	64%*	55%***	55%**
BW gain (g) GD 6-12	150 ± 60	100 ± 70	70 ± 150	90 ± 70*	80 ± 60*
% Control	100%	67%	47%	60%*	53%*
BW gain (g) GD 6-18	300 ± 70	240 ± 100	210 ± 190*	170 ± 140*	120 ± 150***
% Control	100%	80%	70%*	57%*	40%***
BW gain (g) GD 6-20	300 ± 100	220 ± 120	220 ± 230	150 ± 150**	90 ± 170***

Table 20. Toxicity Observed in Rabbit Does following Oral Fipronil Exposure in a Developmental Toxicity Study

Dose (mg/kg/day)	0	0.1	0.2	0.5	1
% Control	100%	73%	73%	50%**	30%***
BW gain (g) GD 6-24	370 ± 90	290 ± 130	310 ± 190	250 ± 150*	170 ± 120***
% Control	100%	78%	84%	68%*	46%***

GD: gestation day; BW: body weight. Data from King (1990).
*, **, *** Significantly different from controls at $p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$ (determined by study authors).

IV.H.3. Developmental Studies Published in the Open Literature

The ability of fipronil to impair the development and quality of mouse preimplantation embryos was assessed *in vitro* and *in vivo* in ICR (CD-1 IGS) mice (Sefcikova *et al.,* 2018). Dams were exposed *in vivo* by gavage to 0, 0.9 or 0.009 mg/kg/day from GD 1 to GD 3. Controls received the vehicle (water with 0.01% DMSO). A decrease in the number of isolated cells, slower transition from oviduct to uterus, increased degraded embryos, and a decrease in the number of blastocysts were observed at 0.9 mg/kg/day. A tendency toward decreased average embryo number (not significant) and increased number of blastocysts with dead cells was seen at 0.009 mg/kg/day. However, this dose did not affect the ability of embryos to reach the blastocyst state. The biological meaning of these data is unclear, as the animals were not followed through birth or into postnatal development.

Table 21. Developmental NOELs and LOELs for Fipronil

Species, Strain	Study Type and Exposure	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
Rat, CRL:CD(SD)BR VAF/Plus	gavage, GD 6 - GD 15	maternal: reduced bodyweight	4	20	(Brooker and John, 1991)[a]
		developmental: no effects at high dose	20	> 20	
Rabbit, New Zealand White	gavage, GD 6 - GD 19	maternal: decreased BW gain	0.1	0.2	(King, 1990)[a]
		developmental: no effects at high dose	1	> 1	
Rat, Sprague Dawley, female	DNT, dietary, GD 6 - LD 10	maternal: death, alopecia, dead/missing pups, decreased BW, BW gain and food consumption	0.9	15	(Mandella, 1995)[a]
		developmental: delayed preputial separation in males, decreased maximum startle response, and decreased BW during lactation	0.05	0.9	
Rat, Sprague Dawley, female	CTA, dietary, GD 6 - LD 21	maternal: decreased T4, increased TSH, histopathology	1	3	(Coder, 2019)[a]
		developmental: decreased T4, increased liver weight	0.3	1	
Rat, CD	gavage, GD 6 - GD 15	maternal: decreased BW	0.2	1	(Foulon, 1997)[b]

Table 21. Developmental NOELs and LOELs for Fipronil

	fipronil-desulfinyl	developmental: decreased BW and delayed bone ossification	1	2.5	
Mouse, ICR (CD-1 IGS), female	gavage, GD 1 - GD 3	decreased average embryo number (not significant) and increased number of blastocysts with dead cells	< 0.009	0.009	(Sefcikova *et al.*, 2018)

[a]Registrant-submitted study.
[b]Registrant-submitted study with fipronil-desulfinyl.
GD: gestation day; LD: lactation day; BW: body weight; DNT: developmental neurotoxicity study; CTA: comparative thyroid assay.

IV.I. Reproductive Toxicity

IV.I.1. Two-Generation Study (King, 1992)

The effects of fipronil (95.4%) on reproduction and development were examined in a two-generation study (2 litters/generation) in CD rats (30/sex/dose in the parental generation, F0) (King, 1992). Animals received dietary doses containing 0, 3, 30 or 300 ppm fipronil. The F0 generation was exposed for 10 weeks prior to mating and then during mating, gestation and lactation for both the F1A and F1B litters. The F0 animals were sacrificed after the F1B animals were weaned. For the second generation, F1A parental animals (30/sex/dose) were treated similarly to the F0 animals. Clinical signs and mortality were monitored daily. Animals were weighed at least weekly for the duration of the study. Mating and gestational time, litter size, mortality, sex ratio, body weight, physical development, macroscopic pathology and histology were recorded. Fipronil intake was determined based on food consumption during each week of the 10-week pre-mating period. Food intake was lower in the high-dose group than in controls for both sexes. Animals generally had a decreased fipronil intake over time; the intake of fipronil was lowest for both sexes and both generations at week 10 of treatment. The ranges of daily fipronil intake during the 10-week treatment period prior to mating were as follows:

Fipronil intake was determined based on food consumption during each week of the 10-week pre-mating period. Food intake was lower in the high-dose group than in controls for both sexes. Animals generally had a decreased fipronil intake over time; the intake of fipronil was lowest for both sexes and both generations at week 10 of treatment. The ranges of daily fipronil intake during the 10-week treatment period prior to mating were as follows:

- F0 generation: 3 ppm: 0.16–0.46 mg/kg/day (males) and 0.20–0.47 mg/kg/day (females); 30 ppm: 1.68–4.48 mg/kg/day (males) and 2.00–4.56 mg/kg/day (females); 300 ppm: 16.97–33.96 (males) and 20.76–36.39 mg/kg/day (females).
- F1 generation: 3 ppm: 0.16–0.40 mg/kg/day (males) and 0.20–0.39 mg/kg/day (females); 30: ppm 1.69–4.14 mg/kg/day (males) and 2.09–4.06 mg/kg/day (females); 300: ppm 17.72–45.60 (males) and 22.92–44.94 mg/kg/day (females).

A total of 9 rats died or were sacrificed due to ill health during weeks 1–24 of treatment in the parental generation (F0). This included one male from the 30-ppm group, 2 males and 5 females

from the 300-ppm group, and 1 control female. Two females treated with 300 ppm had convulsions but survived until the end of the study. The major clinical signs were convulsions lasting 15 to 20 minutes, loss of muscular control, limited use of limbs, irregular breathing, pupil dilation, and blood and urine stains.

Compared to controls, the F0 females treated with 300 ppm fipronil had a substantial reduction in body weight during gestation (up to 10%, $p < 0.001$) and during lactation (up to 13%, $p \leq 0.001$). Decreased food consumption (29–32%, $p < 0.001$) and premating body weights (up to 16%, $p \leq 0.001$) were reported for the F0 and F1 males and females.

The thyroid and the liver were the principal target organs in the F0–F1 rats with treated with 30 ppm and 300 ppm fipronil. The liver weight was increased up to 9% ($p < 0.05$, absolute and relative weight) in the rats exposed to 30 ppm fipronil and up to 37% (absolute weight, $p < 0.01$) and 49% (relative $p < 0.01$) in the rats from the highest dose group (300 ppm). Pathology examination revealed fatty vacuolation of hepatocytes in the 300 ppm females (19 out of 26; 0/30 for control). Investigators graded the severity of this effect as slight to marked.

The absolute weight of the thyroid gland was increased from 15% to 51% ($p < 0.01$) in the females and males in the 30 and 300 ppm dose groups. When expressed as thyroid/body weight ratio the increase was about 20% ($p < 0.01$) for both sexes treated with 30 ppm fipronil, and up to 60% ($p < 0.01$) for the 300-ppm group. Pathology examinations revealed thyroid follicular hypertrophy in 30-ppm males (2/29 versus 0/30 controls). In the 300-ppm group follicular hypertrophy rose to 10/ 29 males ($p < 0.001$) and 6/26 ($p < 0.01$) females. Investigators graded the severity of this effect as minimal to slight.

Fipronil at 300 ppm affected the mating, fertility, pre- and postnatal viability, litter size, body weight and the development of the F1 and F2 generations.

Following pairing, only 25 of 30 F1 animals from the 300-ppm group mated (83%, $p < 0.01$), compared to 30/30 controls. The fertility index[7] of these rats was reduced (80% for the 300-ppm group versus 90% for the control). The live birth index[8] was 83% ($p < 0.01$) for the F1 offspring and 78% ($p < 0.001$) for the F2 offspring in the 300 ppm pups; this index was 98%for the controls. The F1 and F2 pup viability index[9] were 89% ($p < 0.05$) and 73% ($p < 0.001$), respectively, in the 300-ppm group, compared to 97-98% for the control pups. The F1 and F2 mean litter size was significantly lower in the 300-ppm group, with 10 F1 pups/litter and 10.5 F2 pups/litter on postnatal day (PND) 1, respectively. For comparison, the mean control litter sizes on PND 1 were 14.5 F1 pups/litter and 13.6 F2 pups/litter. F1 pups litter size at 300 ppm

[7] Fertility index: (number of animals that achieved a pregnancy)/(animals paired) x 100
[8] Live birth index[8]: (number of live offspring on day 1 *postpartum*/total number of offspring on day 1 *postpartum*) x 100.
[9] Pup viability index was calculated as: (total pups alive on day 4 before culling/total pups born alive on day 1 *postpartum*) x 100.

remained decreased until litter size adjustment on PND 4 (a mean of 9.6 pups/litter compared to 14 pups/litter in the control, $p < 0.001$).

The offspring of the F0 and F1 dams treated with 300 ppm fipronil had a markedly lower body weight (up to 22%, $p \leq 0.001$) compared to controls from PND 1–25. Thirteen F1 pups from 9 litters had convulsions between PND 14–20 in the 300-ppm group. Four F2 pups in 3 litters had convulsions on PND 15 and 18 in the 300-ppm group. F1 and F2 pups from the 300-ppm groups exhibited slower development compared to the control, as evidenced by the delays in tooth eruption (0.7 days, $p < 0.05$; F1 pups) and pinna unfolding (0.5 days delay, F2 offspring).

In summary, toxicity was observed at doses of 30 ppm fipronil and higher. The main effects included mortality, convulsions, decreased body weight, thyroid hypertrophy, fat accumulation in the liver, decreased mating and fertility, decreased offspring viability, decreased pup body weight and delays in pup development. The parental NOEL was 3 ppm (0.16 mg/kg/day), based on mortality, increased absolute and relative weights of liver and thyroid, and hypertrophy of thyroid follicular cells at the LOEL of 30 ppm. The reproductive and developmental NOELs were 30 ppm (1.7 mg/kg/day), based on decreased body weights and convulsions in F0–F1 adults and F1–F2 pups, decreased mating and fertility of F0–F1 adults, reduced pup viability, decreased litter size, and developmental delays of pups of both generations at 300 ppm.

IV.I.2. Reproductive Studies Published in Literature

Few reproductive and/or developmental studies with rats were available in the published literature. Endpoints assayed included sperm quality, reproduction, estrus cycling, sperm structural integrity and DNA damage. Effects occurred at similar doses to those seen in the registrant-submitted data.

In a 14-day oral (gavage) study, Wistar rat dams were exposed to 0, 0.03, 0.3 or 3 mg/kg/day fipronil from GD 15 to PND 7 (i.e., between the final week of pregnancy and the first week of lactation) (de Barros *et al.*, 2016b). Study authors report a decrease in absolute and relative pituitary weights in Wistar rat dams. While the results were significant at lower doses, DPR notes that dose-dependence was not evident. Exposure to the highest dose of fipronil (3 mg/kg) delayed vaginal opening and first estrus. The duration of the estrus cycle was increased at 0.3 mg/kg/day (but not at other doses). At PND 80, a significant decrease in absolute and relative weight of the thyroid was seen in high dose animals. DPR established the NOEL for this study as 0.3 mg/kg/day based on delayed vaginal opening and time to first estrus at the LOEL of 3 mg/kg/day.

The same investigators found a significant decrease in the number of spermatozoa from pubertal males exhibiting a mobile progressive path at all doses ($p < 0.05$) (de Barros *et al.*, 2016a). Results also indicated an increase in non-mobile spermatozoa at the highest dose of 3 mg/kg/day ($p < 0.05$). Thyroid weights, fertility, and time of preputial separation were not affected. The lack of dose-response for the number of mobile spermatozoa data over the 100-fold dose range combined with small sample sizes make these findings unsuitable for human hazard assessment.

Another study examined the effects of fipronil (98%) on adult male Wistar rat reproductive health (Khan *et al.*, 2015). Fipronil doses of 0, 2.5, 5 or 10 mg/kg/day were administered by oral gavage (N = 8 per group) for 28 consecutive days. After 4 weeks, there was a decrease in sperm density, motility, viability and acrosome integrity at 5 and 10 mg/kg/day ($p < 0.05$) as well as a change in sperm morphology at 2.5 mg/kg/day and higher. An increase in reactive oxygen species, DNA fragmentation (sperm chromatin structure assay), and loss of sperm mitochondrial membrane potential and apoptosis occurred at 5 mg/kg/day and higher. DNA damage as shown by comet assay and lipid peroxidation occurred at all doses. The investigators proposed that fipronil causes male reproductive toxicity through oxidative stress-induced DNA damage and apoptosis in spermatozoa. A LOEL was set at the lowest dose of 2.5 mg/kg/day, based on changes in sperm morphology, DNA damage and lipid peroxidation.

In another study that looked at the effects of fipronil on sperm, male Wistar rats were given 5 mg/kg fipronil by gastric gavage for 14 days (Mazzo *et al.*, 2018). Significant reductions were noted in the number of epididymal sperm as well as GSH concentration and catalase activity in the testis homogenate. Malondialdehyde concentration and glutathione peroxidase activity concentration in the testis homogenate were significantly elevated in treated versus control animals.

One published study described in the Population-Based Studies section of this RCD showed no significant association between the concentration of fipronil or its metabolites and degradates detected in seminal plasma and changes to sperm concentration, sperm count, or motility in human males (Xu *et al.*, 2022).

Table 22. Reproductive NOELs and LOELs for Fipronil

Species, Strain	Study Type and Exposure	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
Rat, CD	2-generation, 2-litter repro, dietary, 10 weeks prior to mating through lactation for both liters	paternal: mortality, increased absolute and relative liver and thyroid weights, hypertrophy of thyroid follicular cells	0.16	1.7	(King, 1992)[a]
		developmental: convulsions, decreased bodyweights, mating and fertility, pup viability and litter size and developmental delays	1.7	17.7	
Rat, Wistar, female	gavage, GD 15 to PND 7	female pups: delay in vaginal opening and first estrus, increased duration of estrus, decreased pituitary weight all doses that was not dose-dependent	0.3	3	(de Barros *et al.*, 2016b)
	gavage, GD 15 to PND 7	male pups: increase in non-mobile sperm	0.3	3	(de Barros *et al.*, 2016a)

Table 22. Reproductive NOELs and LOELs for Fipronil

Species, Strain	Study Type and Exposure	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
Rat, Wistar, male	gavage, 28-day	changes in sperm morphology, DNA damage, lipid peroxidation, loss of spermatozoa mitochondria membrane potential and increased cell death	< 2.5	2.5	(Khan *et al.*, 2015)

[a]Registrant-submitted study.
GD: gestation day; PND: postnatal day. Repro: reproduction

IV.J. Thyroid Endocrinology

IV.J.1. Rat Thyroid-Pituitary Function

Two studies were submitted to DPR that investigated mechanisms that may contribute to fipronil's effect on thyroid-pituitary function. These were submitted because thyroid hormone levels were altered following fipronil exposure in the chronic study in rat, described above (Aughton, 1993).

Perchlorate Discharge (Peters et al., 1991)

The first study utilized the Perchlorate Discharge Test, designed to detect chemicals that act directly on the thyroid gland (Peters *et al.*, 1991a). Perchlorate is an ion that inhibits the transport of iodide into the thyroid, causing free, non-organified cytoplasmic iodide to "discharge" back into the bloodstream. If a compound interferes with the incorporation of iodide into thyroglobulin (iodide organification), the perchlorate discharge test detects a loss of radioactive iodine from the thyroid and an increase of radioactive iodide in the blood. In this study, male Crl:CD (SD) BR rats were exposed for 14 days to fipronil (10 mg/kg/day), propylthiouracil (200 mg/kg/day) or Noxyflex (50 mg/kg/day). Propylthiouracil blocks the incorporation of iodide to thyroglobulin by inhibiting the enzyme thyroperoxidase. Noxyflex inhibits iodide organification *in vitro*. Both of these compounds represent positive controls. On day 15, all animals were injected intraperitoneally (IP) with ^{125}I. Six hours later, all animals received 10 or 25 mg/kg potassium perchlorate.

Rats receiving fipronil for 14 days did not show increased ^{125}I efflux into blood following potassium perchlorate treatment when compared to the control. This indicated that fipronil did not inhibit iodide organification. However, fipronil treatment increased accumulation of radioactive iodine in the thyroid and increased thyroid weight (8%), indicating increased thyroid follicular cell stimulation. Noxyflex increased accumulation of ^{125}I and increased thyroid weight similar to fipronil, suggesting Noxyflex stimulated thyroid follicular cells but did not block iodide organification *in vivo*. In contrast, propylthiouracil acted as a potent inhibitor of iodide organification, evidenced by a 75% increase in radioactive iodine in whole blood compared to controls, a drastic increase in thyroid weight (174% of control), and a decrease in the ratio of

radioactivity found in the thyroid versus the blood (12% of control). To conclude, fipronil increased thyroid follicular cell stimulation but did not affect iodide organification.

Thyroxine Clearance (Peters et al., 1991b)

The second study investigated whether fipronil causes thyroid-pituitary disruption by altering thyroid hormone clearance from the blood. Fipronil (10 mg/kg/day) was given via gavage to two groups of 6 male Crl:CD (SD) BR rats for 1 or 14 days. Four hours after dosing, the rats received ^{125}I-thyroxine (Peters *et al.*, 1991b). The radioactivity was monitored in whole blood for 30 hours to calculate thyroxine (T4) half-life ($T_{1/2}$), clearance, and volume of distribution. Phenobarbital, known to enhance T4 clearance via induction of liver microsomal enzymes, was used as a reference compound.

After one day of treatment, the fipronil-treated group showed no significant effects on toxicokinetic parameters compared to controls. The phenobarbital-treated group, however, showed significantly decreased thyroxine clearance (18%, $p < 0.01$) and increases in the volume of distribution (9%) and clearance (32%). Rats treated with fipronil for 14 days showed significant changes: the T4 $T_{1/2}$ was decreased by 48%, the clearance was increased by 161% (261% of control) and the volume of distribution was increased by 37% (137% of control) ($p < 0.01$). Phenobarbital (80 mg/kg/day) treatment for 14 days resulted in similar changes but was less potent than fipronil (T4 $T_{1/2}$ was decreased by 31%, clearance was increased by 84%, and volume of distribution increased 25%, $p < 0.01$).

In conclusion, fipronil decreased the T4 $T_{1/2}$ and increased clearance of T4 from the blood in rats. The study authors proposed a mechanism for thyroid neoplasia in which the decreased level of serum T4 leads to increased secretion of thyroid stimulating hormone (TSH), consequently stimulating the thyroid gland and increasing thyroid weight. This proposal is discussed further in the Risk Appraisal section below.

IV.J.2. Comparative Thyroid Assay

The potential for impacts on thyroid function in pregnant adult female Sprague-Dawley Crl:CD (SD) rats and their offspring was assessed prenatally and postnatally during gestation and lactation (Coder, 2019). Pregnant adult females (45 per dose group) were exposed daily to fipronil (99.9%) via the diet from implantation (GD 6) through weaning of the offspring (PND 21). The pups were indirectly exposed to fipronil for a total of 35 days (15 days *in utero* and 20 days via lactation). Doses were 0, 0.1, 0.3, 1 or 3 mg/kg/day. Study authors reported that the dietary formulations contained 82.6% to 113% of the target test substance concentration, which was within the protocol-specified range of 85% to 115%. Dietary administration of 6-propyl-2-thiouracil in a separate group acted as a positive control, which demonstrated the ability of the assay system to detect changes in thyroid weight and thyroid hormone levels. Animals in the negative control group were exposed to acetone (carrier).

Dams were monitored for viability, BW, food consumption, compound consumption, and parturition (ease of birth, mean litter survival, survival index, etc.). One cohort of F0 dams (21

per dose group) was terminated on GD 20. Blood samples were taken from the jugular vein of the dams and from the umbilical vein of each fetus on that day for thyroid hormone analysis. Fetal blood was pooled by litter, regardless of sex. F0 animals were sacrificed for necropsy, tissue collection, organ weights (liver and thyroid), and thyroid histology / histopathology. The same parameters, including thyroid hormones, were measured at necropsy on the remaining dams on lactation day (LD) 21. The F1 litters were observed for viability, clinical signs, and gender and body weight. Pup blood was collected by cardiac puncture for thyroid hormone determinations on PND 4 and PND 21. Litters (N = 20–24 per group) were euthanized and endpoints were measured on PND 21.

Fipronil exposure at these doses did not affect gestation length or parturition. Lower food consumption was recorded at the high dose for GD 6–9 (18 g/day versus20 g/day in controls, $p < 0.05$). This corresponded to a decrease in BW gain noted for the same period at the same dose.

In dams, significant increases in absolute and relative liver weight (11%) were seen on GD 20 at 3 mg/kg/day (Table 23). On LD 21, relative liver weights were marginally increased (7%, $p \leq 0.05$) at 1 and 3 mg/kg/day. On LD 21, histopathology of the thyroid showed an increase in follicular cell height and a decrease in colloid area in dams at the high dose. Follicular cell height measurements were scored as Grade 1–5, with higher grades indicating a higher follicular cell height. Controls included 4 dams at Grade 1 and 18 dams at Grade 2. In the 3 mg/kg/day group, one dam was at Grade 1, 12 dams were at Grade 2, and 11 dams were at Grade 3. Colloid area measurements were scored from Grade 1–5, with higher grades indicating a larger colloid area. Control dams included one dam at Grade 3, 18 dams at Grade 4, and 5 dams at Grade 5. In the 3 mg/kg/day group, 11 dams were Grade 3, 12 dams were Grade 4, and one dam was Grade 5. Colloid area decreased with dose. On GD 20, there was a 28% decrease in T4 levels in dams ($p \leq 0.01$), with a corresponding 44% increase in TSH at 3 mg/kg/day ($p \leq 0.05$, Table 23).

On PND 4, male pups showed significant increases in absolute liver weights at 1 mg/kg/day (20%) and 3 mg/kg/day (13%) (Table 24). On PND 21, increased absolute liver weights were seen in males and females at 0.3, 1 and 3 mg/kg/day. The increases ranged from 11 to 16% and were significant in all male treatment groups and in the 1 mg/kg/day female treatment group. Relative-to-body liver weights in fetuses and pups (calculated by DPR) were increased by 13–20% at 3 mg/kg/day, 9–20% at 1 mg/kg/day and 5–11% at 0.3 mg/kg/day on PND 4 and PND 21. Thyroid follicular cell height and colloid area were affected in male and female pups at 3 mg/kg/day. In GD 20 fetuses, significantly reduced T4 levels (19–30%) were observed at 1 and 3 mg/kg/day (Table 24). On PND 4, reduced T3 and T4 (26–33%) were observed in male and female pups at 3 mg/kg/day ($p < 0.01$). In PND 21 pups, there were no clear effects on thyroid hormones.

The maternal NOEL of 1 mg/kg/day was based on reduced T4, increased TSH, increased thyroid follicular cell height and decreased colloid area, and increased liver weight on GD 20–21 at the LOEL of 3 mg/kg/day. The developmental NOEL of 0.3 mg/kg/day was based on decreased T4 in fetuses on GD 20 and increased absolute liver weights in males on PND 4 and in both sexes on PND 21 at the LOEL of 1 mg/kg/day. Effects seen at the study NOEL (increased absolute

liver weights in pups at 0.3 mg/kg/day on PND 21 and increased relative liver weight in dams at 1 mg/kg/day on PND 21) were not considered sufficiently robust for LOEL determination because they were relatively small and not associated with liver histopathology.

Table 23. Effects in Rat Dams following Fipronil Exposure in Comparative Thyroid Assay

	0 (acetone)	positive control[a]	Dose (mg/kg/day)			
			0.1	0.3	1	3
GD 20 number of Animals	21	21	21	21	21	21
GD 20 liver wt (abs, g)	15.7 ± 2.0	16.0 ± 1.5	16.0 ± 1.5	16.6 ± 1.2	16.8 ± 1.4	17.6 ± 1.8**
%	100	102	101	105	106	111
GD 20 liver wt (rel x 100)	4.3 ± 0.30	4.4 ± 0.33	4.4 ± 0.32	4.5 ± 0.35	4.6 ± 0.35*	4.8 ± 0.34**
%	100	103	102	104	107	112
LD 21 number of animals	24	22	23	23	23	24
LD 21 liver wt (abs, g)	16.7 ± 1.9	17.7 ± 2.0	17.0 ± 1.9	17.4 ± 2.4	17.9 ± 2.4	17.8 ± 1.9
%	100%	106	102	104	107	106
LD 21 liver wt (rel x 100)	5.1 ± 0.4	5.5 ± 0.4**	5.2 ± 0.4	5.4 ± 0.5	5.5 ± 0.5*	5.4 ± 0.4*
%	100	108	103	106	107	107
GD 20 number of animals	21	21	21	21	21	21
T4, GD 20 (pg/ml)	19407±6527	8337±2756	17236±4790	21448±4733	17625±5353	14046±3678**
%	100%	43%	89%	111%	91%	72%
TSH, GD 20 (pg/ml)	3383.4±1224	7085.3±2249**	3878.4±1450	3837.4±1450	4038.7±1576	4869.7±2068*
%	100%	209%	115%	113%	119%	144%
LD 21 number of animals	24	22	23	23	23	24
TSH, LD 21 (pg/ml)	3758.6±3219	8765.3±3970**	4419.1±2416	3190.9±1257	4608.0±2865	4934.9±4435
%	100%	233%	118%	85%	123%	131%

[a]Positive control 6-propyl-2-thiouracil (6-PTU) was administered at 3 ppm, which equaled 0.23 mg/kg/day during pregnancy and 0.5 mg/kg/day during lactation.

*, **: Significantly different from controls (determined by study authors) at $p \leq 0.05$ or $p \leq 0.01$, respectively.

GD: gestation day; wt: weight; abs: absolute; rel: relative; wt: weight; LD: lactation day; T4: thyroxine; TSH: thyroid stimulating hormone.

Data from Coder (2019).

Table 24. Effects in Rat Fetuses and Pups following Fipronil Exposure in Comparative Thyroid Assay

	0 (acetone)	positive control[a]	Dose (mg/kg/day)			
			0.1	0.3	1	3
PND 4 male numbers	15	17	17	16	15	20
PND 4 male liver wt (abs, g)	0.37 ± 0.05	0.36 ± 0.04	0.38 ± 0.05	0.40 ± 0.03	0.440 ± 0.04**	0.41 ± 0.04*
%	100	99	103	109	120	113
PND 4 male liver wt (rel)	3.27	3.24	3.55	3.57	3.93	3.94
%	100	99	109	109	120	120
PND 4 female numbers	17	18	18	15	16	13
PND 4 female liver wt (abs, g)	0.38 ± 0.05	0.35 ± 0.04*	0.37 ± 0.04	0.39 ± 0.05	0.41 ± 0.05	0.41 ± 0.06
%	100	92	95	103	108	106
PND 4 female liver wt (rel)	3.49	3.37	3.66	3.64	3.8	4.1
%	100	97	105	105	109	118
PND 21 male numbers	24	22	23	23	23	24
PND 21 male liver wt (abs, g)	2.42 ± 0.4	2.23 ± 0.3	2.45 ± 0.4	2.77 ± 0.3*	2.77 ± 0.5*	2.79 ± 0.5**
%	100	93	101	115	115	116
PND 21 male liver wt (rel)	4.61	4.39	4.71	5.09	5.11	5.36
%	100	95	102	111	111	116
PND 21 female numbers	24	22	23	23	23	23
PND 21 female liver wt (abs, g)	2.38 ± 0.4	2.13 ± 0.3**	2.42 ± 0.5	2.68 ± 0.4	2.74 ± 0.4*	2.65 ± 0.5
%	100	89	102	113	115	111
PND 21 female liver wt (rel)	4.64	4.41	4.75	5.05	5.06	5.22
%	100	95	102	109	109	113
GD 20 fetus numbers	19	21	21	21	21	21
T4, GD 20 fetuses (pg/ml)	5064 ± 1613	1337 ± 599**	4233 ± 893	4658 ± 1309	4122 ± 988*	3569 ± 855**
%	100%	26%	84%	92%	81%	70%
PND 4 male numbers	15	17	17	16	14	19
T3, PND 4 males (pg/ml)	172.9 ± 57	212.7 ± 77	204.0 ± 61	187.9 ± 30	163.4 ± 44	116.6 ± 33**
%	100%	123%	119%	109%	95%	67%
T4, PND 4 males (pg/ml)	20653 ± 4600	10814 ± 2776**	24265 ± 5656	22781 ± 2222	18979 ± 4376	14706 ± 3962**
%	100%	52%	117%	110%	92%	71%
PND 4 female numbers	17	16	18	15	16	11
T3, PND 4 females (pg/ml)	186.2 ± 51	202.1 ± 87	208.3 ± 58	187.1 ± 37	176.3 ± 50	133.2 ± 48*
%	100%	109%	112%	101%	95%	72%
T4, PND 4 females (pg/ml)	21600 ± 4065	10517 ± 2666**	23822 ± 5794	23553 ± 3664	19544 ± 3214	15945 ± 3473**
%	100%	49%	110%	109%	90%	74%

[a]Positive control 6-propyl-2-thiouracil (6-PTU) was administered at 3 ppm, which equaled 0.23 mg/kg/day during pregnancy and 0.5 mg/kg/day during lactation.

*, **: Significantly different from controls (determined by study authors) at $p \leq 0.05$ or $p \leq 0.01$, respectively.

PND: postnatal day; GD: gestation day; g: grams; rel: relative weight; abs: absolute weight; T4: thyroxine; T3: triiodothyronine; TSH: thyroid stimulating hormone.

Data from Coder (2019).

IV.K. Neurotoxicity

The potential for fipronil to result in neurotoxicity under acute and subchronic exposure durations was addressed in three registrant-submitted studies conducted in rats. The principal effects documented in these studies included convulsions, tremors, gait abnormalities, absence of response to stimuli, decreased rearing and grip strength, decreased hindlimb splay and motor activity, impaired righting reflex, exaggerated tail pinch and startle responses and increased urine in the observation arena.

IV.K.1. Acute Neurotoxicity

Gill et al., 1993

Fipronil (96.7%) was administered as a single gavage dose to Sprague-Dawley rats, 15/sex/dose at 0 (corn oil) at 0.5, 5 or 50 mg/kg/day (Gill *et al.*, 1993). Neurobehavioral signs were evaluated using the functional observational battery (FOB). As part of the study protocol, authors determined the time to peak effect (TOPE) in a dose range-finding probe study. An abbreviated FOB was performed every hour for 7 hours and 24 hours after dosing. The TOPE of 7 hours was based on the highest incidence of convulsions and tremors in the preliminary study. In the current study, the FOB evaluations were performed pretest, at the TOPE (7 hours after treatment), and on days 7 and 14. Changes in motor activity were assessed pretest, at 8 hours post-treatment, and on days 7 and 14 using an automated recording apparatus. Ten rats/sex/group were subjected to pathological evaluation of brain, spinal cord, peripheral nerves (sciatic, peroneal, sural and tibial), dorsal root, and gasserian ganglia.

The high dose (50 mg/kg/day) produced severe toxicity including death. Five males and 1 female died within 2–6 days of treatment (Table 25). Brain hemorrhage was detected at necropsy in the five animals found dead within 2 days of dosing. Major clinical signs included convulsions and tremors. Convulsions were clonic or clonic and tonic[10], and were seen in 4 males and 1 female within the first day of treatment. Three of these animals died within 24 hours of the convulsive episodes. Other clinical signs within 2–14 days of treatment at 50 mg/kg included dehydration, emaciation, unkempt appearance, urine stains, perioral encrustation, and cold extremities. Decreased body weights in males (up to 10%, $p < 0.01$) were observed at the 7- and 14-day evaluations.

The FOB findings at the TOPE from the 50 mg/kg/day group were indicative of CNS stimulation (convulsions, tremors, head bobbing, myoclonic movements, and a decrease in landing hindlimb splay by up to 29%, $p \leq 0.01$). In contrast, neurological depression was evident in the decreased rearing (95–97%, $p \leq 0.01$), decreased arousal, tail-pinch and approach responses, and decreased muscle tone. Some of the acute effects on neuromuscular function were persistent, as they were

[10] Clonic convulsions involve myoclonic jerks, running fits or explosive jumps; tonic convulsions involve rigorous extension of the head and body; clonic and tonic convulsions alternate convulsion types.

also seen in FOB conducted on days 7 and 14 (e.g., ~20% decreased hindlimb landing splay in females). Both males and females treated with 50 mg/kg fipronil showed 90–93% decreases in motor activity at the TOPE ($p \leq 0.01$).

FOB effects at the TOPE at the intermediate dose (5 mg/kg/day) included decreased hindlimb splay in both sexes (12–13% compared to controls, $p \leq 0.05$), reduction in the number of rears in females (42%, $p \leq 0.01$), and absence of an approach response (2/15 males). Significant decreases in rearing at the TOPE (50%, $p \leq 0.01$) were also reported for the females treated with 0.5 mg/kg/day.

Fipronil caused acute neurotoxicity in rats ranging from neuromuscular effects at 5 mg/kg to convulsions and mortalities at 50 mg/kg. The NOEL of 0.5 mg/kg/day was based on neurobehavioral signs (decreased hindlimb splay, rearing and approach response) at the LOEL of 5 mg/kg/day. At the NOEL of 0.5 mg/kg, there was a significant but not dose-dependent decrease in rearing in females (50%) and a non-significant decrease in hindlimb splay (9%) in males.

Table 25. Neurological Effects in Sprague-Dawley Rats following a Single Gavage Dose with Fipronil

	Males				**Females**			
Dose (mg/kg)	**0**	**0.5**	**5**	**50**	**0**	**0.5**	**5**	**50**
Mortality	**0/15**	**0/15**	**0/15**	**5/15**	**0/15**	**0/15**	**0/15**	**1/15**
Clonic convulsions	0/15	0/15	0/15	4/15	0/15	0/15	0/15	1/15
Tonic convulsions	0/15	0/15	0/15	2/15	0/15	0/15	0/15	0/15
Dehydration	0/15	0/15	0/15	6/15	0/15	0/15	0/15	6/15
Urine stains	0/15	0/15	0/15	4/15	0/15	0/15	0/15	6/15
Perioral encrustation	0/15	0/15	0/15	13/15	0/15	0/15	0/15	9/15
Body weight (% of control)								
7-hr post treatment	100	99	100	99	100	100	101	99
7-day post treatment	100	99	99	90**	100	101	102	97
14-day post treatment	100	99	98	94**	100	100	103	99
FOB observations at 7-hr post tx								
Fine tremors	0/15	0/15	0/15	6/15*	0/15	0/15	0/15	6/15*
Coarse tremors	0/15	0/15	0/15	5/15*	0/15	0/15	0/15	1/15
Altered gait	0/15	0/15	0/15	10/15*	0/15	0/15	0/15	8/15
Palpebral closure (drooping)	0/15	0/15	0/15	4/15	0/15	0/15	0/15	3/15
Tail pinch (no response)	2/15	1/15	1/15	6/15	0/15	0/15	0/15	3/15
Pupil size (decreased)	1/15	0/15	0/15	9/15**	5/15	4/15	2/15	8/15
Muscle tone (decreased)	0/15	0/15	0/15	5/15*	1/15	0/15	1/15	10/15**
Air righting reflex (uncoordinated)	1/15	0/15	0/15	8/15*	0/15	0/15	0/15	0/15
Approach (no response)	0/15	0/15	2/15	6/15*	0/15	0/15	0/15	0/15
Hindlimb splay (% Control)	100	91	88*	72**	100	101	87*	71**

Table 25. Neurological Effects in Sprague-Dawley Rats following a Single Gavage Dose with Fipronil

	Males				Females			
Dose (mg/kg)	**0**	**0.5**	**5**	**50**	**0**	**0.5**	**5**	**50**
Urination								
7-hr post treatment	0/15	0/15	1/15	8/15**	2/15	1/15	2/15	6/15
7-day post treatment	0/15	0/15	1/15	3/10	0/15	2/15	1/15	1/14
14-day post treatment	0/15	2/15	2/15	6/10**	1/15	2/15	2/15	1/14
Rearing (% of control)								
7-hr post treatment	100	178	107	5.4*	100	50**	58**	3**
7-day post treatment	100	89	187	289**	100	72	87	104
14-day post treatment	100	105	153	123	100	66	84	89

FOB: functional observational battery; TOPE: time of peak effect (7 hours)*, ** Significantly different from controls at $p < 0.05$ or $p < 0.01$. Study authors analyzed data variance and used ANOVA (heterogeneous variance) or t-test (unequal variance).
Data from Gill (1993).

Hughes et al., 1997

Fipronil (97.9%) was administered by gavage in a single dose to 10 Crl:CD BR rats/sex/dose at 0 (corn oil), 2.5, 7.5 or 25 mg/kg (Hughes, 1997). This study was intended to provide a more accurate NOEL for acute neurotoxicity, as the earlier study by Gill 1993 had a 10-fold difference between the LOEL and the NOEL (5 and 0.5 mg/kg/day, respectively). The rats were evaluated for neurotoxicity using FOB testing. A time to peak effect (TOPE) of 7 hours was based on neurobehavioral changes (the highest incidences in rats having convulsions, chewing, licking and wet anogenital region) observed in a preliminary study conducted by study authors. FOB evaluations were performed pretest at the TOPE and on days 7 and 14. Changes in motor activity were assessed at the same time points using an activity monitoring system equipped with an infrared detector to determine time spent in no movement, locomotor activity, and non-locomotor activity. Histopathological evaluations of brain, spinal cord, peripheral nerves (sciatic, peroneal, sural and tibial), dorsal root, and ganglia were conducted in 5 rats/sex/dose.

Males and females in the 25 mg/kg/day group displayed reduced body temperature on day 0 ($p < 0.05$). Clinical signs at the high dose that were not seen in the controls included staining of head regions in males (5/10) and females (6/10) and soiled angiogenital region in males (3/10). There were no deaths during the study. Rats treated with 7.5 and 25 mg/kg/day fipronil had significantly decreased body weight gains (47–75%, $p < 0.01$) and decreased food consumption (7–26%, $p < 0.01$) during the first week after exposure (Table 26). Markedly impaired food conversion (grams food consumed per week per kilogram body weight gain) was observed after one week in females at 7.5 and 25 mg/kg and in males at the high dose.

FOB findings at the TOPE for the 25 mg/kg/day group included increased forelimb grip strength in males (21%, $p < 0.01$) and decreased landing hindlimb splay in both sexes (21–28%, p <

0.01). Significantly decreased hindlimb landing foot splay (up to 23%, $p < 0.01$) was also reported for males treated with 7.5 mg/kg/day fipronil. Motor activity at 25 mg/kg was reduced by 42–51% compared to control levels ($p < 0.01$).

The study NOEL of 2.5 mg/kg/day was based on neurobehavioral signs (decreased hindlimb splay) in males and decreased body weight gain and decreased food consumption in females at the LOEL of 7.5 mg/kg/day. There was a non-significant decrease in hindlimb splay (10%) in males at the NOEL.

Table 26. Neurotoxicity in Crl:CD BR Rats Following a Single Gavage Dose

	Males				Females			
Dose (mg/kg)	**0**	**2.5**	**7.5**	**25**	**0**	**2.5**	**7.5**	**25**
Body temperature Day 0	37.9	38	37.9	37.6*	38.2	38.1	38.2	37.6**
Unusual behavior/posture	0/10	0/10	0/10	1/10	0/10	0/10	0/10	1/10
Food consump. week 1 (% control)	100	95	93	74**	100	92	84**	76**
Food consump. week 2 (% control)	100	97	93*	90**	100	97	90	99
BW gain week 1 (% control)	100	88	84	53**	100	94	44**	25**
BW gain week 2 (% control)	100	89	74	94	100	106	71	112
BW 7 hours post-treatment (% control)	100	98	98	96	100	98	98	99
BW 7 days post-treatment (% control)	100	97	97	92	100	98	94	93
BW 14 days post-treatment (% control)	100	96	95	92	100	99	92	95
Food conversion, week 1	7	7.4	7.7	9.7	10.2	9.9	20.2	30.8
% Control	100	106	110	139	100	97	198	302
Food conversion, week 2	6.2	6.8	7.8	6	9.1	8.3	11.3	8
% Control	100	110	126	97	100	91	124	88
Hindlimb splay at 7 hours (% control)	100	90	77**	72**	100	100	86	79*
Forelimb grip strength 7 hours (% control)	100	108	106	122**	100	100	104	108
Motor activity 8 hours (% control)	100	94	92	49**	100	104	94	58**

Food consumpt: food consumption; BW: body weight

*, ** Significantly different from controls at $p < 0.05$ or $p < 0.01$. Data were analyzed by study authors with Bartlett's test for variance, one-way ANOVA or Student's t-test, and William's test for dose-response.

Data from Hughes (1997).

III.K.2. Subchronic Neurotoxicity

Driscoll and Hurley, 1993

In a subchronic neurotoxicity study, fipronil (96.7% a.i.) was administered to Sprague-Dawley rats (15/sex/dose) at dietary levels of 0, 0.5, 5 or 150 ppm for 13 weeks (Driscoll and Hurley, 1993). The average daily doses based on food consumption corresponded to 0, 0.03, 0.3 and 9 mg/kg/day for males and 0, 0.03 0.4 and 11 mg/kg/day for females. Animals were examined for mortality and clinical signs twice per day, 7 days per week. The FOB and motor activity tests were performed 1 week pretest and at weeks 4, 9 and 13 of treatment. Six control and six high-dose rats/sex were subjected to pathological evaluation of the brain, spinal cord, trigeminal

nerve, dorsal root, and gasserian ganglion. Mortality was not observed in this study. Periocular encrustation (2/15) was observed in high-dose males.

During the first week of exposure, fipronil caused body weight reduction in both sexes at 150 ppm (up to 6% in females and 8% in males, $p \leq 0.05$) and decreased body weight gain of up to 70% (Table 27). Effects persisted in male rats through the second week ($p < 0.01$) and may have resulted, at least partially, from decreased food consumption (23–24%) during week 1. By the end of the study, body weights in males were significantly higher than controls in the 5- and 150-ppm groups (7–8% higher, $p < 0.01$). Absolute brain weights were also increased in high-dose males (5%, $p < 0.05$), but relative brain weights were not.

In FOB tests, males treated with 5 and 150 ppm showed increased incidence of urine in the observation area during weeks 4 and 9 (7 to 10/10 in treated groups versus 5/10 in controls); the increase was significant in the high high-dose group during week 4 ($p < 0.05$). Males in the 150-ppm group also showed exaggerated tail pinch response during weeks 4 and 9 (2 to 4/10 versus 0/10 in controls) and exaggerated startle response at weeks 4, 9 and 13 (2 to 4/10 versus 0/10 in controls). One female showed "unusual behavior" according to the investigators during week 4.

At study termination, one of 15 high-dose males showed scar or abscess on the skin, a dilated kidney pelvis, or calculus in the kidney. One of 15 high-dose females showed oral malocclusion, alopecia, scabbing of skin, or nose fracture. Microscopic analysis showed minimal myelin sheath swelling in one of 6 high dose males and two of 6 high dose females (compared to 0/6 controls). Tissues from the 0.5- and 5-ppm groups were not assayed.

The NOEL for the subchronic neurotoxicity of fipronil in rats was 0.5 ppm (0.03 mg/kg/day), based on the LOEL of 5 ppm (0.3 mg/kg/day) for effects on the autonomic nervous system (presence of urine in the observation area and exaggerated tail pinch response).

Table 27. Effects seen in subchronic neurotoxicity study following oral fipronil exposure in rats

	Males				**Females**			
Dose (ppm)	**0**	**0.5**	**5**	**150**	**0**	**0.5**	**5**	**150**
Dose (mg/kg/day)	**0**	**0.03**	**0.3**	**9**	**0**	**0.03**	**0.4**	**11**
N	15	15	15	15	15	15	15	15
BW (g) week 1	295.4 ± 9.4	300.9 ± 7.8	296.9 ± 12.0	277.4 ± 10.5**	191.3 ± 7.6	193.1 ± 8.0	192.5 ±7.5	179.9 ± 7.5**
% Control	100	102	101	94	100	101	101	94
BW gain (g) week 1	28.9 ± 5.2	32.8 ± 4.7	30.4 ± 5.0	11 ± 5.1**	14.2 ± 4.0	16.2 ± 5.1	14.3 ± 4.6	4.2 ± 4.3**
% Control	100	113	105	38	100	114	101	30
BW (g) week 2	315.5 ± 12	325.2 ± 10.8	319.2 ± 15.4	307.3 ± 12.2	203.2 ± 9.8	204.1 ± 9.0	204.8 ± 11.9	197.7 ± 10.7
% Control	100	103	101	97	100	100	101	97
BW gain (g) week 2	49.1 ± 7.3	57.1 ± 7.5	52.6 ± 9.1	40.9 ± 6.3**	26 ± 8.7	27.2 ± 5.9	26.7 ± 5.5	22.1 ± 6.5
% Control	100	116	107	83	100	105	103	85

Table 27. Effects seen in subchronic neurotoxicity study following oral fipronil exposure in rats

	Males				Females			
Dose (ppm)	**0**	**0.5**	**5**	**150**	**0**	**0.5**	**5**	**150**
Dose (mg/kg/day)	**0**	**0.03**	**0.3**	**9**	**0**	**0.03**	**0.4**	**11**
Final BW (g)	426.6 ± 19.9	441.5 ± 21.5	454.6 ± 22.3**	458.6 ± 22.4**	265.1 12.9±	263.9 ± 14.9	264.3 ± 10.6	266.7 ± 23.7
% Control	100	103	107	108	100	100	100	101
food consum.[a] week 1	22.4 ± 1.3	22.7 ± 1.4	22.6 ± 1.1	17.2 ± 1.2**	16.5 ± 0.9	16.8 ± 1.2	16.1 ± 1.3	12.5 ± 1.2**
% Control	100	101	101	77	100	102	98	76
food consum. week 2	22.1 ± 1.4	22.5 ± 1.6	22.5 ± 1.2	22.8 ± 1.1	16.7 ± 1.2	16.4 ± 1.2	16.3 ± 1.8	17.1 ± 1.3
% Control	100	102	102	103	100	98	98	102
brain weight (g)	2.1 ± 0.07	2.1 ± 0.08	2.1 ± 0.1	2.2 ± 0.07*	1.9 ± 0.08	1.9 ± 0.07	1.9 ± 0.07	2 ± 0.1
% Control	100	100	100	105	100	100	100	105
unusual behavior week 4	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
Urine in Observation Arena								
week 4	5/10	6/10	9/10	10/10*	4/10	6/10	0/10	4/10
week 9	5/10	4/10	7/10	9/10	2/10	6/10	5/10	6/10
week 13	5/10	5/10	4/10	9/10	7/10	8/10	7/10	6/10
Exaggerated Startle Response								
week 4	0/10	0/10	0/10	4/10	0/10	0/10	0/10	0/10
week 9	0/10	0/10	1/10	2/10	0/10	0/10	0/10	0/10
week 13	0/10	0/10	0/10	2/10	0/10	0/10	0/10	0/10
Exaggerated Tail Pinch Response								
week 4	0/10	0/10	0/10	4/10	1/10	2/10	0/10	1/10
week 9	0/10	0/10	1/10	2/10	0/10	0/10	0/10	0/10
week 13	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10

BW: body weight; Food consum: food consumption (g/animal/day)

*,** Significantly different from controls at $p < 0.05$ or $p < 0.01$

Data from Driscoll and Hurley (1993).

IV.K.3. Neurotoxicity Studies in Published Literature

Several neurotoxicity studies were available in the published literature. A single oral (gavage) exposure of 25 or 50 mg/kg fipronil caused a significant change in electroencephalography readings assessing the CNS in Long-Evans rats (Freeborn *et al.*, 2015). Repeated administration for 14 days in the same study reported audiogenic seizures in the presence of loud laboratory equipment (Freeborn *et al.*, 2015). Motor activity and behavioral evaluations were conducted in C57BL/6NCrSlc male mice (14 or 15 mice/dose) exposed to a single oral (gavage) dose at 0.05, 0.5 or 5 mg fipronil/kg (Maeda *et al.*, 2021). The authors reported significant increases in

locomotor activity and time the mice spent in the central zone in the open field test at 5 mg/kg/day, which reflected anxiety-like behavior. In another study, a single IP injection of 40 mg/kg resulted in facial clonus and head twitching in male albino Swiss Webster mice (Kamijima and Casida, 2000). Oral gavage of 10 mg/kg/day for 21 days resulted in significant reductions in dopamine and serotonin in male Sprague Dawley rat brain regions, particularly the striatumin (Bharatiya *et al.*, 2020). Fipronil at 10 mg/kg/day for 45 days via oral gavage led to an increase in oxidative stress in all tested brain regions in rats; authors suggested apoptosis induction (Awad *et al.*, 2021; Awad *et al.*, 2022). Fipronil at 10 mg/kg once a week for 48 weeks resulted in hyperactivity under moderate stress conditions in the open-field test (Koslowski *et al.*, 2020). A single dermal exposure (50 mg/kg) increased the time mice spent in an open arm plus-maze test and number of entries into maze, and altered neurotransmitter levels in the brain (Suzuki *et al.*, 2021).

A variety of effects associated with neurotoxicity were also observed *in vitro*. These included:

- A reduction in the amount of neurofilament heavy chain, shortened neurite outgrowth, apoptosis, mitochondrial dysfunction, increased intracellular ROS, and reduced levels of dopamine in human neuroblastoma SH-SY5Y cell line (Lee *et al.*, 2011; Ruangjaroon *et al.*, 2017; Kanat Ö and Selmanoğlu, 2020)
- Increased oxidative stress and decreased ATP in mitochondria isolated from fresh rat brain and rat primary immortalized N27 mesencephalic dopaminergic cells (Seydi *et al.*, 2021; Souders *et al.*, 2021);
- Reductions in axon-like processes in mouse N2a neuroblastoma cells (Sidiropoulou *et al.*, 2011);
- Induction of two neurotoxic amyloid peptides in mouse neural crest-derived cells and CHO cells (Cam *et al.*, 2018);
- Promoted glial phenotype and suppressed neuronal phenotype in rat primary neural stem cells (Slotkin *et al.*, 2016);
- Decreased or biphasic changes in mean firing rate in rat neurons (Mack *et al.*, 2014; Alloisio *et al.*, 2015; Wallace *et al.*, 2015).

IV.L. Developmental Neurotoxicity

IV.L.1. Mandella *et al.*, 1995

Developmental neurotoxicity of fipronil (96.1%) was evaluated in a developmental neurotoxicity study in which 30 mated female Sprague Dawley rats were administered fipronil in the diet (Mandella, 1995). The dams were treated from gestation day (GD) 6 through lactation day (LD) 10 at doses of 0, 0.5, 10 and 200 ppm fipronil in acetone. The average daily intake of fipronil calculated from food consumption was 0, 0.05, 0.9 and 15 mg/kg/day. Twenty-seven control rats and 29 rats in each of the three treatment groups produced litters. The pups were indirectly exposed to fipronil for a total of 25 days (15 days *in utero* and 10 days via lactation). The dams and pups (4 males and 4 females/litter after culling on LD 4) were observed for signs of general toxicity throughout the treatment period. All dams were sacrificed on LD 21, following weaning.

One male and 1 female/litter were evaluated for developmental toxicity until ~65 days of age. Parameters included timing of developmental landmarks such as pinna detachment, incisor (tooth) eruption, eye opening, vaginal patency, and preputial separation. Motor activity was assessed on 4 separate occasions between PND 13-60. Auditory startle habituation was tested on PND 22 and 60. Swimming ability was examined on 5 different occasions between PND 6 and 14. Learning and memory (short and long-term) were evaluated in using a water "Y maze" in 6 tests between PND 24 and 65. Brain tissues, spinal cord and peripheral nerves (sciatic, sural and tibial) from PND 60 rats (six/sex/group) were examined for neuropathology.

Dams

Two females in the 200-ppm group died on lactation days 6 and 9. Both decedents showed discolored lungs at necropsy. Compared to controls, fewer high-dose dams were described as "normal within limits" (17/30 at GD 21 and 11/27 at LD 21 for the 200 ppm dams versus 25/30 and 21/27 for controls). The investigators based "normal within limits" on physical observations during the gestation and lactation period (though the examined parameters were not provided in the study). The high-dose dams had a total of 41 dead/missing pups from LD 1 through the end of the lactation period; 4 litters lost all pups (Table 28). Control dams had no dead or missing pups. In addition, the high-dose dams showed a significantly higher alopecia incidence through the treatment period (13/30 in the 200-ppm group versus 4/30 for controls on GD 21, $p < 0.05$, Fisher's exact test performed by DPR). Other abnormal findings for the 200 ppm dams during the physical observations included broken/maloccluded incisors and scabs.

Offspring

Twenty-seven control rats and 29 rats in each of the three treatment groups produced litters. All 27 control litters and all 29 litters in groups treated at 0.5 and 10 ppm survived until weaning. In contrast, 4 of 29 litters from the 200-ppm group lost all of the pups by LD 4 (85.2% survival). Pup viability was significantly reduced in the 200-ppm group ($p < 0.01$). The number of pups in the 200-ppm group dying or missing between LD 0 and LD 21 was 170 (out of 428 born alive; 40%). For comparison, 35 control pups were dead or missing by LD 21 (out of 398 born alive; 9%). Most of the pups died between LD 0–4. The viability index on day 4 (total pups born alive) was 75.5% in the 200-ppm group versus 98.9% for the control pups. There were no significant pathological findings for the dead pups.

Male and female pups in the 10- and 200- ppm groups exhibited reduced body weights at all examinations during lactation (7–41%, $p \leq 0.01$, Table 29). Difference in weight at the high dose persisted even when fipronil was removed from the dam's diet (LD 10). At the end of the study, the body weight of these rats was significantly reduced compared to controls (12–14%, $p \leq 0.01$). Absolute brain weights were significantly decreased for both sexes (7–20 %, $p < 0.01$) at the two evaluations on postnatal day (PND) 11 and PND 60. This effect may be, at least in part, due to the reduction of 12–41% of the body weights of these animals.

Males and females treated with 200 ppm fipronil had slower development compared to the control, evidenced by the significant delays in pinna detachment (0.5 days delay) and delays in upper and lower incisor eruption (0.6–1.5 day delay, Table 30). In addition, vaginal patency, a landmark for female puberty, was delayed by 1.5 days. The number of days to preputial separation, an indicator of male puberty, was significantly increased by 1.4 and 4.8 days at 10 and 200 ppm, respectively, compared to concurrent controls. The mean days to preputial separation (range) were: 44.0 (40.7–46.7); 44.7 (41.7–53.5); 45.4 (42.0–53.3, $p < 0.01$); and 48.8 (45.0–54.0, $p < 0.001$) for ascending doses, respectively. Furthermore, the mean values for the 10-ppm and 200-ppm groups exceeded historical control means in the same rat strain from 4 studies conducted in the same laboratory between 1989 and 1995 (Mandella and Rodwell, 2005): 43.6 (40.0–47.0); 42.8 (39.0–46.90); 43.6 (41.0–48.0); and 45.0 (41.8–49.7).

Pups in the 200-ppm dose group showed decreased maximum response (23–27%; $p \leq 0.0001$) and decreased average response (25–26%; $p < 0.001$) in auditory startle evaluations on PND 22. This could be due to a general depression of the nervous system or a loss of hearing. A significant decrease in maximal response was also seen in males at 10 ppm (13%; $p \leq 0.05$) (Table 31).

On PND 6, a significantly higher number of pups in the 200-ppm group were unable to stay afloat or swim in straight line compared to controls. By PND 14, nearly all control pups could maintain their ears, nose, and tops of heads above water, whereas 62–63% ($p < 0.04$) of the 200 ppm pups could not hold their heads above the surface. The 200 ppm pups also had a significant delay in swimming development on PND 22 (Table 31).

The maternal NOEL was 10 ppm (0.9 mg/kg/day) based on death, alopecia, dead/missing pups, and decreased body weight, body weight gain and food consumption at the LOEL of 200 ppm fipronil (15 mg/kg/day). The NOEL for developmental neurotoxicity was 0.5 ppm (0.05 mg/kg/day) based on significant delays in preputial separation in male pups, decreased maximum startle response in male pups, and decreases in body weight in both sexes at the LOEL of 10 ppm (0.9 mg/kg/day).

Table 28. Body Weight and Litter Effects on Sprague Dawley Rat Dams

Dose (ppm)	**0**	**0.5**	**10**	**200**
Dose (mg/kg/day)	**0**	**0.05**	**0.9**	**15**
Dam BW GD 0-6 (g)	40 ± 8	43 ± 8	39 ± 9	40 ± 8
Dam BW GD 6-10 (g)	19 ± 7	19 ± 3	18 ± 8	-29 ± 10**
Dam BW GD 10-15 (g)	35 ± 6	33 ± 6	35 ± 5	49 ± 9**
Total Number of Litters on PND 0	27	29	29	29
Surviving Litters on PND 21	27	29	29	23**
Pup Live Birth Index[a] (%)	99.1	98.9	98.4	93
Pup Viability Index[b] (%)	98.9	95.7	98.3	75.5**

Number of dams: 26-28 per dose group; 23-29 pups per dose group.

[a]Pup Live Birth Index: (total pups born alive/total pups born) x 100

[b]Pup Viability Index: (total pups alive on PND $4_{pre\text{-}cull}$ /total pups born alive) x 100
*, ** Significant different from controls at $p < 0.05$ or $p < 0.01$, respectively
BW: body weight; GD: gestation day. PND: postnatal day.
Data from Mandella (1995)

Table 29. Body Weight of Sprague Dawley Rat Pup Litters Exposed During Gestation and Lactation

	Male Pups				Female Pups			
Dose (ppm)	**0**	**0.5**	**10**	**200**	**0**	**0.5**	**10**	**200**
Dose (mg/kg/day)	**0**	**0.05**	**0.9**	**15**	**0**	**0.05**	**0.9**	**15**
PND 0	100	100	97	91**	100	98	95*	92**
PND 4 post cull	100	96	93*	73**	100	94*	91**	73**
PND 11	100	96	93	66**	100	95	92*	66**
PND 17	100	99	93*	75**	100	97	91**	73**
PND 60	100	96	96	88**	100	102	99	87**

Numbers are percent of control. PND: postnatal day. Data from Mandella (1995).
*, **, and **** are equal to $p \leq 0.05$, 0.01, and 0.0001, determined by DPR with one-way ANOVA and Dunnett's multiple comparison test post hoc.

Table 30. Developmental Landmarks in Sprague Dawley Rat Pups Exposed During Gestation and Lactation

Dose (ppm)	**0**	**0.5**	**10**	**200**
Dose (mg/kg/day)	**0**	**0.05**	**0.9**	**15**
N (all animals)	394	426	436	323
Pinna (ear) attachment	2.5 (0.6)	2.6 (0.6)*	2.5 (0.6)	3.0 (1)****
Lower incisor eruption	11.4 (0.9)	11.6 (0.9)*	11.4 (0.9)	12.1 (0.1)****
Upper incisor eruption	10.3 (0.9)	10.5 (0.9)	10.4 (1.0)	10.9 (1.2)****
N (females)	96	96	100	78
Vaginal patency (days)	31.4 (1.1)	32.0 (1.4)**	31.6 (1.3)	32.9 (1.7)****
N (males)	89	103	100	70
Preputial separation (days)	44 (2.5)	44.7 (1.03)	45.4 (2.9)**	48.8 (3.3)****

Data presented as mean (standard deviation).
*, **, and **** are equal to $p \leq 0.05$, 0.01, and 0.0001. Significance determined by DPR with one-way ANOVA and Dunnett's multiple comparison test post hoc.
Data from Mandella (1995).

Table 31. Neurological Effects on Sprague Dawley Rat Pups Exposed during Gestation and Lactation

	Males				Females			
Dose (ppm)	**0**	**0.5**	**10**	**200**	**0**	**0.5**	**10**	**200**
Dose (mg/kg/day)	**0**	**0.05**	**0.9**	**15**	**0**	**0.05**	**0.9**	**15**
N	26	28	29	20	26	27	29	21
Maximum Startle Response (% Control)	100	106	87*	73****	100	108	99	77****

Table 31. Neurological Effects on Sprague Dawley Rat Pups Exposed during Gestation and Lactation

Average Startle Response (% Control)	100	111	92	74***	100	107	97	75***
Unable to Stay Afloat PND 6	12%	7%	7%	50%**	8%	7%	4%	35%
Unable to Swim in Straight Line PND 6	50%	32%	41%	73%**	39%	37%	25%	57%
Unable to Keep Head out of Water PND 6	64%	82%	79%	96%	77%	63%	64%	91%*
Unable to Keep Head out of Water PND 14	8%	18%	28%	63%	8%	30%	18%	46%

PND: postnatal day.
*, ***, ** and **** are equal to $p \leq 0.05$, 0.01, 0.001 or 0.0001 by DPR with one-way ANOVA and Dunnett's multiple comparison test post hoc.
Number of pups ranged from 19 to 28 per sex, per exposure group, per time point.
Startle response was measured on PND 22 and PND 60. Data are from PND 22.
Data from Mandella (1995).

IV.L.2. Lassiter *et al.,* 2009

The *in vitro* neurotoxicity of fipronil were assayed in undifferentiated and differentiating PC12 cells (Lassiter *et al.*, 2009). Authors also investigated the effects of chlorpyrifos, an insecticide that is neurotoxic to mammals, as well as a mixture of fipronil and chlorpyrifos. Endpoints included cell replication, cell number, differentiation, and viability after short-term (1 hour) and long-term (6 day) exposures. Fipronil elicited a greater effect during replication and differentiation, was more potent and caused more widespread effects than chlorpyrifos. Effects were seen as low as 1 μM. These included decreased cell number, reduction in DNA synthesis, impairment of protein synthesis, and increased oxidative stress without decreased cell viability, all suggesting neuro-developmental alterations. Fipronil was also more potent on differentiating cells, inducing a switch in transmitter phenotype that can cause mis-wiring of neural circuits. The investigators concluded that fipronil is inherently a more potent disruptor of neuronal cell development than is chlorpyrifos in this system. The authors concluded that neurodevelopmental effects were not dependent on GABAα antagonist properties as PC12 cells lack that receptor.

IV.M. Toxicity of the Metabolites and Photodegradate of Fipronil

Fipronil, its sulfone and sulfide metabolites, and the desulfinyl environmental degradate (a product of UV decomposition) all block GABA-gated chloride channels to greater or lesser degrees. Fipronil-sulfone is the major metabolite in both vertebrates and insects, while fipronil-desulfinyl is the principal photoproduct on plants and soils (Hamon *et al.*, 1996). Registrant-submitted studies were available for fipronil-desulfinyl fipronil-sulfide and fipronil-sulfone. Published studies included limited evaluations of other fipronil metabolites (detrifluoromethylsulfonyl-fipronil and the amide RPA200766). DPR considers critical values for the parent fipronil protective for the other moieties. The toxicity of the metabolites and the environmental photodegradate are more fully described in Appendix II.

V. RISK ASSESSMENT

V.A. Hazard Identification

For purposes of this risk assessment, points of departure (PODs) are internal doses that did not produce toxicologically significant effects following oral, dermal or inhalation exposure. Critical PODs are the most toxicologically-robust study PODs established for particular exposure durations and routes, and represent the threshold doses for non-oncogenic effects. Cancer risk was assessed by a threshold approach using an upstream marker of the relevant oncogenic process, instead of by linear extrapolation (see discussion below). For reasons delineated in the following paragraphs, the critical PODs by the dermal and inhalation routes were set at the same oral values for the corresponding exposure duration.

Data from toxicity studies submitted by the registrant were used to establish critical PODs. None of the studies identified through a systematic review of the published literature produced a critical POD. The PODs were either experimentally determined (i.e., NOELs) or data-derived. Data-derived POD values for fipronil were calculated using dose extrapolation factors. Benchmark dose (BMD) modeling was used to derive PODs from datasets that were amenable to modeling. BMD modeling, also called low-dose extrapolation, was performed using US EPA's Benchmark Dose Software (BMDS; version 3.2). In this approach, the dose-response data for each endpoint were analyzed to generate a family of mathematically-based models (US EPA, 2012a). Each model resulted in the generation of a BMD and a corresponding BMDL value for a predetermined benchmark response level. A BMDL represents the 95% lower bound of a BMD and is considered equivalent to the POD for the observed effect. BMD analyses are available in Appendix IV. A detailed description of the hierarchical examination DPR conducts for BMDL goodness-of-fit is described in Appendix IV.

The critical PODs were used to estimate the risks posed by fipronil exposures. PODs were evaluated as critical based on their corresponding route and duration, relative value, and toxicological considerations. All critical PODs used in this assessment were based on the parent compound fipronil and the effects observed in laboratory animals exposed to technical grade fipronil (93–99.9%) (summarized in Table 32).

Table 32. Summary of Critical Points of Departure for Fipronil

Duration	Route	Critical Endpoint	POD (mg/kg/day)
Acute	Oral, Dermal and Inhalation	Decreased hindlimb splay in an acute oral neurotoxicity study in rats	0.77[a]
Subchronic	Oral, Dermal and Inhalation	Convulsions and mortality associated with convulsions and sustained decreases in T4 after subchronic oral exposure in a chronic study in rats	0.02[b]
Chronic	Oral, Dermal and Inhalation	Convulsions and mortality, sustained decreases in T4, and increased progressive senile nephropathy in a chronic oral study in rats	0.02[c]

T4: thyroxine thyroid hormone. POD: point of departure.
[a]Benchmark Dose Software (BMDS, version 3.2) of hindlimb splay data from acute neurotoxicity study in rat (Hughes, 1997).
[b]Combined chronic and oncogenic study in rat (Aughton, 1993). The critical subchronic POD was supported by POD established in three additional studies in rodents following subchronic exposure: 0.03 mg/kg/day based on presence of urine in the FOB observation area and exaggerated tail pinch response in rats (Driscoll and Hurley, 1993); 0.05 mg/kg/day based on delayed development and decreased startle response in rat pups (Mandella, 1995); 0.05 mg/kg/day based on liver periacinar hypertrophy in mice (Broadmeadow, 1991).
[c]Combined chronic and oncogenic study in rat (Aughton, 1993).

V.A.1. Points of Departure for Acute Toxicity

Six registrant-submitted acute studies were evaluated for acute POD determination. These included one oral LD_{50} study in rats, two oral neurotoxicity studies in rats, one dermal study in rabbits, and two inhalation studies in rats. Studies that employed repeated exposures were also considered for acute POD determinations if the effects were observed during the first week of treatment (e.g., with short-term exposure duration). Studies with acute and short-term exposure durations were also available in the published literature. The critical acute PODs and relevant studies and endpoints are described in Table 33. Acute and short-term symptoms included neurological disturbances, changes in serum thyroid hormone concentrations, decreased maternal and offspring body weights, and delayed development. Acute intraperitoneal injection caused hepatocyte vacuolation, hypertrophy and lipid accumulation, changes in plasma membrane morphology, and increased oxidative stress in rodents. However, this atypical dosing route limited the utility of this study for establishing a critical NOEL.

Acute Oral Toxicity

The lowest acute oral NOEL was established in an acute neurotoxicity study in rats (Gill *et al.*, 1993). In this study, the rats received a single dose of fipronil (0, 0.5, 5 or 50 mg/kg/day) by gavage. The NOEL of 0.5 mg/kg/day was based on neurobehavioral signs (decreased hindlimb landing splay, rearing, and approach response) at the LOEL of 5 mg/kg/day. There was a significant but non-dose-dependent decrease (50%) in rearing in females and a decrease in hindlimb splay (9%, not significant) in males at the study NOEL. Attempts to model the

hindlimb spay and rearing data using the BMDS approach were unsuccessful as the models did not pass goodness-of-fit tests. Moreover, decrease in rearing could have been due to biological variation. The likelihood that rearing was a true response to fipronil was minimized by the absence of such an effect at a similar dose range in the Hughes (1997) study (described below). Because there was a 10-fold difference between the LOEL and NOEL doses in this study, the registrant conducted a second acute neurotoxicity study in rats designed to provide a more precise POD for acute neurotoxicity (Hughes, 1997).

In contrast to Gill *et al*., Hughes (1997) used more widely spaced doses (0, 2.5, 7.5 and 25 mg/kg/day) (Hughes, 1997). A significant dose-dependent reduction in hindlimb splay was observed 7 hours after gavage administration of 7.5 mg/kg (LOEL), resulting in a study NOEL of 2.5 mg/kg/day. A non-significant 10% decrease in hindlimb splay was also observed at the study NOEL of 2.5 mg/kg/day. It should be noted that a similar decrease (9%) was also observed at the NOEL of 0.5 mg/kg/day in the earlier acute neurotoxicity study (Gill *et al.*, 1993). Because this effect did not increase in magnitude or reach significance over a 5-fold dose increase (0.5 to 2.5 mg/kg/day), DPR considered it to be within the observed range of variation. Therefore, DPR selected a 10% benchmark response level (BMR) to model hindlimb splay. BMDS modeling provides a statistically rigorous approach to the dataset and a better characterization of the dose-response relation at the low-end of the observable dose range. Application of BMDS to this dataset resulted in a $BMDL_{10}$ of 0.77 mg/kg/day (BMD = 2.09 mg/kg/day) (Exponential 4 model). The $BMDL_{10}$ of 0.77 mg/kg/day was therefore selected as the critical POD to characterize the acute risk due to oral exposure. BMDS outputs are provided in Appendix IV.

Published studies identified acute or short-term NOELs in the range of 1–5 mg/kg/day. Effects in rodents included neurotoxicity (Freeborn *et al.*, 2015; Maeda *et al.*, 2021), increased hepatic metabolism (Caballero *et al.*, 2015), and decreased T3 and T4 and altered metabolic profiles (Moser *et al.*, 2015). A 3-day study in mice showed an increased number of blastocysts containing dead cells in embryos extracted from dams exposed to 0.009 mg/kg/day (Sefcikova *et al.*, 2018). This observation was difficult to interpret, as noted in the Appraisal section.

Acute Inhalation Toxicity

Three registrant-submitted inhalation toxicity studies with acute or short-term endpoints were available for fipronil. These included two acute LC_{50} studies and one subchronic study, all in rats. Of these, the lowest NOEL was established in the subchronic inhalation study for effects seen as early as 2 days of exposure (Adamo-Trigiani, 1999). In this study, animals were subjected to nose-only exposure at 0, 0.001, 0.005, or 0.03 mg/L aerosolized fipronil for 4 hours per day, 7 days per week, for 4 weeks. Clinical signs (salivation, decreased activity, and convulsions) in females on day 2, decreased body weight in males, and decreased food consumption in both sexes were observed at week 1 at the LOEL of 0.005 mg/L. The NOEL was 0.001 mg/L. Oral equivalent doses were calculated using a rat default breathing rate, making the acute inhalation NOEL and LOEL equal to 0.8 mg/kg/day and 0.16 mg/kg/day, respectively.

In both acute inhalation LC_{50} studies, rats were exposed for 4 hours (nose-only). In the first study, mortality in females occurred at the LOEL of 0.259 mg/L (oral equivalent dose of 41.4 mg/mg/day) (Cracknell, 1994). In the second study, death, clinical signs, and gross pathology of stomach, lungs and brain were seen at the LOEL of 0.33 mg/l, with an oral equivalent dose of 53 mg/kg/day (Nachreiner, 1995).

The lowest acute inhalation POD of 0.8 mg/kg/day was based on clinical signs and decreased body weight and food consumption in rats. This value was very similar to the critical acute oral POD of 0.77 mg/kg/day, which was based on decreased hindlimb splay in rats. A route-specific inhalation POD is generally preferable for characterizing risks to humans from inhalation exposure. However, this assessment used the critical oral POD for risk calculation. There is less uncertainty was associated with the oral value because it was obtained through BMD modeling, in contrast to a calculated absorbed internal dose from an air concentration value in the inhalation study, which requires use of a default value for a normalized rat inhalation rate (in mg/kg-BW/day). In addition, the inhalation studies did not provide toxicokinetic data nor FOB outcomes. Therefore, the acute inhalation POD of 0.77 mg/kg/day was based on significant dose-dependent reduction in hindlimb splay observed in rats 7 hours after oral (gavage) administration of fipronil in an acute neurotoxicity study (Hughes, 1997).

Acute Dermal Toxicity

Two registrant-submitted dermal studies, an acute LD_{50} dermal study in rabbits and a subchronic (4-week) dermal study in rats, were available for analysis. In the acute study, rabbits were treated with a single dermal dose of fipronil wetted in corn oil at 100, 250, 500, 1000 or 2000 mg/kg/day for 24 hours (Myers and Christopher, 1992). Hyperactivity in one male on days 5–7, diarrhea in two females on days 1–2, and a dark purple kidney in one female at necropsy were observed at the LOEL of 100 mg/kg/day.

One acute dermal study was available in the published literature (Suzuki *et al.*, 2021). Effects observed in mice at the single tested dose (50 mg/kg) included neurotoxicity (increased time spent in an open arm plus-maze test and increased number of entries into maze) and increased levels of dopamine and 5-hydroxytryptophan in the striatum and hippocampus, respectively. This study is described in more detail in the Toxicokinetics section.

In the subchronic dermal study, fipronil was applied to the skin on the trunks of rats for 6–7 hours per day, 5 days per week, for 4 consecutive weeks at doses of 0, 100, 500, or 1000 mg/kg/day (Henwood, 1997). Body weight and food consumption were significantly decreased by 5% and 14%, respectively, at the LOEL of 1000 mg/kg/day during days 1–8. The acute/short-term dermal NOEL was 500 mg/kg/day.

The lowest acute dermal LOEL was 100 mg/kg/day for hyperactivity, diarrhea, and bloody kidney in rabbits (NOEL not determined). However, hindlimb splay, the most sensitive endpoint seen following acute oral fipronil exposure, was not measured in either dermal study. Therefore,

the critical acute oral POD of 0.77 mg/kg was used to evaluate the acute dermal risk to humans (Hughes, 1997).

DPR used a dermal absorption study in rat (Cheng, 1995) to estimate a dermal fipronil absorption rate of 4.3% (DPR, 1999a).

Table 33. Summary of Acute Points of Departure (PODs) for Fipronil

Species, Strain	Study Type and Exposure	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
Rat, Wistar	oral acute LD_{50}, gavage	abnormal gait, hunched posture, diarrhea, piloerection	< 50	50	Gardner (1988)
Rat, Crl:CD BR	**oral acute neurotox, gavage**	**reduced hindlimb splay**	**0.77[a]**	**7.5**	**Hughes (1997)**
Rat, Sprague Dawley	oral acute neurotox, gavage	reduced hindlimb splay, rearing	0.5	5	Gill (1993)
Rat, Wistar	single dose, gavage, 6 days	increased hepatic metabolism	1	5	Caballero (2015)†
Rat, Long-Evans	single dose, gavage	decreased T3 and T4 and altered metabolic profiles	5	10	Moser (2015)†
Rabbit, New Zealand White	dermal acute LD_{50}, 24-hr	hyperactivity, diarrhea, bloody kidney	< 100	100	Myers and Christopher (1992)
Rat, Crl:CD(SD)BR	dermal subchronic, 4 wk, 5 day/wk, 6–7 hr/day	decreased BW, decreased food consumption within one week	500	1000	Henwood (1997)
Rat, Sprague Dawley	inhalation subchronic, 4 weeks, 4 hr/day, nose-only	clinical signs on day 2, decreased BW and decreased food consumption during week 1	0.8[b]	4.8	Adamo-Trigiani (1999)
Rat, CD	inhalation acute LC_{50}, 4 hr nose-only	mortality in females	< 41.4[b]	41.4	Cracknell (1994)
Rat, Sprague Dawley	inhalation acute LC_{50}, 4 hr nose-only	death, clinical signs, gross pathology of stomach, lungs and brain	< 53[b]	53	Nachreiner (1995)

Note: The shaded and bolded row indicates the critical acute study and POD (Hughes, 1997).

† Published study

[a]Benchmark Dose Software (BMDS, version 3.2) modeling – derived NOEL (BMDL).

[b]Oral equivalent dose; calculated internal absorbed dose from external air concentrations by using a default rat breathing rate of 0.96 m^3/kg/day and 100% inhalation absorption.

V.A.2. Points of Departure for Subchronic Toxicity

Subchronic toxicity studies were available via the oral, inhalation and dermal routes. Subchronic exposure to fipronil elicited a variety of responses including neurological disturbances, autonomic dysregulation, reductions in body weight, decreased litter survival and pup viability, liver effects (increased liver weight, hypertrophy and hepatocytic fat deposition), thyroid effects (decreased T4, increased thyroid weight, hypertrophy and hyperplasia) and delayed developmental signs (incisor eruption, decreased vaginal patency and delayed preputial separation).

Subchronic Oral Toxicity

Subchronic oral toxicity endpoints for fipronil were analyzed in registrant-submitted studies in rat, mouse, rabbit, and dog, and in studies obtained from the published literature. The subchronic oral POD of 0.02 mg/kg/day was based on decreases in T4, convulsions, and mortality in rats within one year in a combined chronic and oncogenic study (Aughton, 1993). A significant dose-dependent decrease in T4 was observed in male rats from week 1 through week 50 at the LOEL of 0.6 mg/kg/day. In addition, one male had convulsions and died at 23 weeks at the LOEL.

Several studies showed effects at similar dose levels, resulting in subchronic PODs ranging from 0.01 to 0.06 mg/kg/day. The PODs were based on the following effects:

1. Significant delays in preputial separation and altered startle response in rat pups at the LOEL of 0.9 mg/kg/day with a NOEL of 0.05 mg/kg/day in a developmental neurotoxicity study (Mandella, 1995).

2. Autonomic dysregulation in rats evidenced by presence of urine in the FOB observation area and exaggerated tail pinch response at the LOEL of 0.3 mg/kg/day with a NOEL of 0.03 mg/kg/day in a subchronic neurotoxicity study (Driscoll and Hurley, 1993).

3. A significant and dose-dependent increase in the incidence of hepatocyte periacinar hypertrophy in male mice at the LOEL and lowest dose tested (0.13 mg/kg/day) in a 13-week subchronic oral toxicity study in CD-1 mice (Broadmeadow, 1991). Benchmark dose modeling resulted in a $BMDL_{10}$ of 0.05 mg/kg/day (10% effect, Hill model).

The oral POD of 0.02 mg/kg/day, based on decreases in T4, convulsions and mortality (Aughton, 1993), was selected as the critical value to characterize the human risk from subchronic oral exposure to fipronil.

Additional published studies with subchronic exposure durations were identified through systematic literature review. Effects described in studies that passed DPR's quality screening criteria included induction of oxidative stress, neurotoxicity, decreased thyroid hormone levels, and reproductive effects at doses as low as 1.5 mg/kg/day (Leghait *et al.*, 2009; Leghait *et al.*, 2010; Roques *et al.*, 2012; Roques *et al.*, 2013; Badgujar *et al.*, 2015; Freeborn *et al.*, 2015; Khan *et al.*, 2015; Moser *et al.*, 2015; Badgujar *et al.*, 2016a; de Barros *et al.*, 2016a; de Barros *et al.*, 2016b; Mazzo *et al.*, 2018; Bharatiya *et al.*, 2020; Koslowski *et al.*, 2020; Martin *et al.*, 2020; Aldayel *et al.*, 2021; Awad *et al.*, 2021; Seif *et al.*, 2021; Awad *et al.*, 2022). While these studies support weight-of-evidence analyses, they did not provide critical PODs.

Subchronic Inhalation Toxicity

One registrant-submitted study in rats was available to evaluate subchronic inhalation toxicity (Adamo-Trigiani, 1999). Animals were subjected to aerosolized fipronil (0, 0.001, 0.005, or 0.03 mg/L) in nose-only chambers for four hours per day, seven days per week, for four weeks. Decreased body weight and food consumption in females, increased relative and absolute liver

weights in males, and altered blood chemistry parameters indicating liver effects in both sexes were observed at the LOEL of 0.005 mg/l. The NOEL was 0.001 mg/l, or 0.16 mg/kg/day when converted to an oral equivalent dose using a rat default breathing rate. Because thyroid hormones and development were not monitored in this study, the critical subchronic oral POD of 0.02 mg/kg/day was used to characterize human risks from subchronic inhalation exposures to fipronil.

Subchronic Dermal Toxicity

Two registrant-submitted studies were available to evaluate the potential for fipronil to results in subchronic dermal toxicity (Table 34). Of these, the lower subchronic dermal NOEL of 5 mg/kg/day came from a study in rabbits (Hermansky and Wagner, 1993). Animals were administered dermal doses for six hours per day, five days per week, for three weeks. Extreme hyperactivity in both sexes, reduced body weight gain (days 1–22) and decreased food consumption (days 15–21) in males were observed at the LOEL of 10 mg/kg/day. Because thyroid-related and developmental endpoints were not monitored in either subchronic dermal study, the subchronic dermal risk evaluation was based on the critical subchronic oral POD of 0.02 mg/kg/day.

Table 34. Summary of Subchronic Points of Departure (PODs) for Fipronil

Species, Strain	Study Type and Exposure	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
Rat, Crl:CD(SD)BR	oral, dietary, 4 weeks	increased liver weight, thyroid hypertrophy	< 3.4	3.4	Peters (1996)
Rat, CD	oral, dietary, 13 weeks	increased relative and absolute liver and thyroid weights, thyroid hypertrophy, and fat accumulation in liver	1.9	20	Holmes (1991)
Rat, Sprague Dawley, female	oral, DNT, dietary, GD 6 - LD 10	maternal: death, alopecia, dead/missing pups and decreased BW, weight gain and food consumption	0.9	15	Mandella (1995)

Table 34. Summary of Subchronic Points of Departure (PODs) for Fipronil

Species, Strain	Study Type and Exposure	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
		<u>developmental:</u> delayed preputial separation and decreased startle response	0.05	0.9	
Rat, Sprague Dawley	oral, neurotox, dietary, 13 weeks	reduced BW, autonomic nervous system effects	0.03	0.3	Driscoll and Hurley (1993)
Rat, CD	**oral, combined chronic, dietary, 52-91 weeks**	**convulsions, death and decreased thyroid hormones**	**0.02**	**0.06**	**Aughton (1993)**
Mouse, CD-1	oral, dietary, 13 weeks	hepatocyte periacinar hypertrophy	0.05[a]	0.13	Broadmeadow (1991)
Dog, Beagle	oral, gelatin capsule, 13 weeks	mortality, weight loss, clinical signs and neurological disturbances	2	10	Holmes (1991)
Rabbit, New Zealand White	dermal, 3 wk, 5 day/wk, 6 hr/day	extreme hyperactivity, reduced BW gain and food consumption	5	10	Hermansky and Wagner (1993)
Rat, Crl:CD(SD)BR	dermal, 4 wk, 5 day/wk, 6-7 hr/day	increased total protein and globulin levels and increased relative liver weights	< 100	100	Henwood (1997)
Rat, Sprague Dawley	inhalation, 4 weeks, 4 h/day, nose-only	decreased BW and food consumption, increased relative liver weights in males, changes in blood chemistry	0.16[b]	0.8	Adamo-Trigiani (1999)

Table 34. Summary of Subchronic Points of Departure (PODs) for Fipronil

Species, Strain	Study Type and Exposure	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
Rat, CD	2-generation, 2-litter repro, dietary, 10 weeks prior to mating through lactation for both liters	paternal: mortality, increased absolute and relative liver and thyroid weights, hypertrophy of thyroid follicular cells	0.16	1.7[c]	King (1992)
		developmental: convulsions, decreased bodyweights, mating and fertility, pup viability and litter size and developmental delays	1.7	17.7	
Rat, CRL:CD(SD)BR VAF/Plus	developmental, gavage, GD 6 - GD 15	maternal: reduced bodyweight	4	20	Brooker (1991)
		developmental: no effects at high dose	20	> 20	
Rabbit, New Zealand White	developmental, gavage, GD 6 - GD 19	maternal: decreased BW gain	0.1	0.2	King (1990)
		developmental: no effects at high dose	1	> 1	
Rat, Sprague Dawley, female	DNT, dietary, GD 6 - LD 10	maternal: death, alopecia, dead/missing pups, decreased BW, BW gain and food consumption	0.9	15	Mandella (1995)

Table 34. Summary of Subchronic Points of Departure (PODs) for Fipronil

Species, Strain	Study Type and Exposure	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
		developmental: delayed preputial separation in males, decreased maximum startle response, and decreased BW during lactation	0.05	0.9	
Rat, Sprague Dawley, female	CTA, dietary, GD 6 - LD 21	maternal: decreased T4, increased TSH, histopathology	1	3	Coder (2018)
		developmental: decreased T4, increased liver weight	0.3	1	
Rat, CD	developmental, gavage, GD 6 - GD 15	maternal: decreased BW	0.2	1	Foulon (1997)
	fipronil-desulfinyl	developmental: decreased BW and delayed bone ossification	1	2.5	

Note: The shaded and bolded row indicates the critical subchronic study and POD (Aughton, 1993).
* While numerous published studies with subchronic exposure durations passed DPR's screening criteria, effects in those studies were observed at doses 50 to 100-fold higher than those used to derive and support the subchronic POD, and many of the studies tested a single dose of fipronil only. The published studies are therefore not included in the above table.
[a] Benchmark Dose Software (BMDS, version 3.2) modeling of data resulted in a $BMDL_{10}$ of 0.05 mg/kg/day and BMD of 0.11 mg/kg/day with the Dichotomous Hill model.
[b] Oral equivalent dose; calculated internal absorbed dose from external air concentrations by using a default rat breathing rate of 0.96 m^3/kg/day and 100% inhalation absorption.

V.A.3. Points of Departure for Chronic Toxicity

Chronic toxicity studies on fipronil were available by the oral route in rat, mouse, and dog. Effects of chronic exposure to fipronil included neurological disturbances, convulsions and tremors, mortality, changes in thyroid hormone levels, reductions in body weight, progressive senile nephropathy in rats, and liver periacinar vacuolation in mice. Chronic studies are summarized in Table 35.

The chronic oral POD of 0.02 mg/kg/day was established in chronic toxicity study in rats, which was the lowest chronic oral NOEL (Aughton, 1993). Effects at the LOEL of 0.06 mg/kg/day included a significant dose-dependent decrease in serum T4 in males at week 1 that was sustained through week 50, an increase in convulsions and mortality compared to controls, and a significant increase in progressive senile nephropathy. Support for the critical chronic POD came from a chronic dietary study in mice administered fipronil for 78 weeks (Broadmeadow, 1993). The NOEL of 0.06 mg/kg/day was based on increased relative liver weights in males and females and increased liver periacinar vacuolation in males at 52 and 78 weeks at the LOEL of 1.18 mg/kg/day.

In the absence of a chronic inhalation or dermal toxicity study, risk by both routes was evaluated using the chronic oral POD of 0.02 mg/kg/day.

Table 35. Summary of Chronic Points of Departure (PODs) for Fipronil

Species, Strain	Study Type and Exposure	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
Rat, CD	**combined chronic/onco, dietary, 52-91 weeks**	**convulsions and mortality, decreases in thyroid hormones weeks 1 to 50, progressive senile nephropathy**	**0.02**	**0.06**	**Aughton (1993)**
Mouse, CD-1	combined chronic/onco, dietary 53-78 weeks	increased relative liver weights and periacinar vacuolation	0.06	1.18	Broadmeadow (1993)
Dog, Beagle	gelatin capsule, 53 weeks	convulsions, neurological effects	0.2	2	Holmes (1992)
Dog, Beagle	dietary, 52 weeks	muscle rigidity and tremors	0.3	1	Holmes (1993)

Note: The shaded and bolded row indicates the critical subchronic study and POD (Aughton, 1993).

V.A.4. Oncogenicity

Oncogenicity studies conducted with fipronil were available in rats and mice. A significant increase in thyroid follicular cell adenomas and carcinomas occurred in male and female rats at the highest administered doses (13 and 17 mg/kg/day, respectively) after 89 and 91 weeks of daily dietary exposure (Table 36) (Aughton, 1993). These incidence rates exceeded historical control rates established in 7 studies from the same laboratory conducted during the 4 years preceding this study. Thyroid follicular cell oncogenesis in rats following exposure to xenobiotics is based on a well-characterized, non-mutagenic extra-thyroidal mechanism involving disruption of the hypothalamus-pituitary-thyroid axis. As such, it is not relevant to humans for reasons outlined in the Risk Appraisal section this document (US EPA, 1998; International Agency for Research on Cancer, 1999).

Table 36. Thyroid Tumor Incidence in CD Rats Exposed to Fipronil for 89 to 91 Weeks via Diet and Historical Control Data

	Males					Females				
Dose (ppm)	**0**	**0.5**	**1.5**	**30**	**300**	**0**	**0.5**	**1.5**	**30**	**300**
Dose (mg/kg/day)	**0**	**0.02**	**0.06**	**1.3**	**13**	**0**	**0.03**	**0.08**	**1.6**	**17**
N (thyroids)	49	48	50	50	50	50	50	50	50	50
Follicular Cell Adenoma	0	1	5*	3	12***	0	0	0	0	8**
Follicular Cell Carcinoma	0	0	0	0	5*	0	1	0	1	2
Adenoma + Carcinoma	0	1	5*	3	17***	0	1	0	1	10***
Percent Occurrence (%)	0%	2%	10%	6%	34%	0%	2%	0%	2%	20%
N (historical control)	359					365				
Adenoma + Carcinoma	26					10				
Percent Occurrence (range)	7.2% (3.6–16%)					2.7% (0–12%)				

Data from Aughton (1993).

*, **, *** Significantly different from controls (one-tailed Fisher's Exact test, performed by study authors) at $p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$.

In addition to neoplasms in rats, chronic exposure to fipronil may have induced liver carcinomas in male CD-1 mice at the high dose of 3.43 mg/kg/day (Broadmeadow, 1993).

The carcinoma incidence rates, expressed in relation to the effective animal number (the number of animals alive on study day 409, the time of first detection) were 1/41, 1/39, 2/34, 1/32, and 5/42 at 0, 0.01, 0.06, 1.18 and 3.43 mg/kg/day, respectively. Incidence of adenomas in relation to the effective animal number (animals alive on day 317) did not increase with treatment: 10/47, 3/50, 2/44, 6/40, and 6/47. Combination of the carcinoma and adenoma incidence data (using day 317 to determine the effective animal number) yielded similarly unremarkable incidence rates: 11/47, 4/50, 4/44, 7/40, and 10/47. Fisher's Exact comparisons did not indicate statistical significance compared to controls for carcinomas or adenomas at any dose. However, Cochran-Armitage trend tests were positive for carcinoma incidence.

While the mouse liver carcinoma data were consistent with a fipronil-induced increase at the high dose, quantitative cancer potency analysis using multistage linear modeling was not conducted. As noted above, the multistage approach assumes that the lesions were generated by mutagenic mechanisms (US EPA, 2005). However, the overall evaluation of registrant-submitted and published genotoxicity studies, as well as the ToxCast^{TM} database for fipronil, did not show evidence for mutagenic activity.

V.A.5. Genotoxicity

Six genotoxicity studies were submitted to DPR to fulfill pesticide registration data requirements. Overall, these studies were negative for genotoxicity under experimental conditions and concentrations that did not cause cytotoxicity. Three studies published in the open literature showed genotoxic effects in association with apoptosis, cytotoxicity or oxidative stress, but had methodological deficiencies that limited their reliability. In no case was mutagenicity directly implicated.

V.A.6. Toxicity Forecaster (ToxCast™)

Introduction

Fipronil was included in the US EPA ToxCast™/Tox 21 program, which leverages high-throughput screening technologies to produce a growing database consisting of *in vitro* toxicity and *in vivo* zebrafish developmental data, and generate predictive models for thousands of chemicals in the environment (US EPA, 2016). The results from ToxCast™ assays are used to support chemical screening, hazard prioritization and characterization, and to develop integrated approaches to testing and assessment (Bajard *et al.*, 2022). The output of a particular assay endpoint is an AC_{50}, the concentration of a chemical that results in 50% of maximal activity. AC_{50} values that are classified as active (also referred to as "hits") suggest that a chemical is active against a particular biological target or process (Williams *et al.*, 2017).

This ToxCast™ analysis was performed using the CompTox website (https://comptox.epa.gov/dashboard) in January 2023.

Fipronil was tested in 1175 out of 2205 ToxCast™ assays (accessed 10 January 2023). It was active in 353 of those assays (US EPA, 2022). The resultant AC_{50} values ranged from 0.012 µM to 369 µM. However, many active assays had AC_{50} values higher than the experimentally determined cytotoxicity level for fipronil of 7.1 µM (lower bound). Chemical-assay combinations with AC_{50} values greater than the cytotoxicity value can be due to cell death, protein denaturation, low affinity binding, or reflect dysregulation of cellular machinery at higher exposure concentrations (Judson *et al.*, 2016).

Results

There were 44 assays comprising 14 intended target families that had AC_{50} values below the fipronil cytotoxicity level of 7.1 µM. These target families included CYP and neuroactivity target families (each with 7 hits), nuclear receptor families (6 hits), neurodevelopment families (5 hits), and cell cycle, DNA binding and transferase target families (each with 3 hits). DNA binding refers to assays that measure the binding of a specific protein or transcription factor to DNA to initiate, stimulate, or terminate RNA transcription. The other target families included cell morphology, channel 2, cytokine, deiodinase kinase, malformation, and transporters.

The most sensitive assay was the Tanguay_ZF_120hpf_YSE_up assay which presented an AC_{50} of 0.012 µM. This test examines the morphology of fertilized zebrafish embryos to identify potential developmental defects (Truong *et al.*, 2014; US EPA, 2022).

The second most sensitive assay was the CLD_SULT2A_48hr, showing an AC_{50} of 0.193 µM. This assay, conducted in primary human hepatocytes, measured levels of sulfotransferase-2 enzyme mRNA after a 48-hour exposure. Upregulation was also observed at 24 hours (AC_{50} of 3.25 µM). The SULT enzyme family adds a sulfonate moiety to a compound, thus increasing its water solubility and decreasing its biological activity (Gamage *et al.*, 2006). Increased SULT2 activity is associated with fatty liver disease (Hardwick *et al.*, 2013). Other active hits and

markers of fatty liver included NVS_NR_hCAR_Antagonist (AC_{50} of 0.491) and LTEA_HepaRG_ADK_dn assay (AC_{50} of 4.82).

Several positive assays were related to neuroactivity, including CCTE_Shafer_MEA_acute_burst_percentage_mean (AC_{50} of 0.212), CCTE_Shafer_MEA_acute_per_network_burst_spike_number_mean (AC_{50} of 0.392), and CCTE_Shafer_MEA_acute_per_burst_spike_number_mean_up (AC_{50} of 0.621) indicated that fipronil stimulated spontaneous neural activity.

Fipronil was positive in a total of 7 CYP assays, including NVS_ADME_hCYP2C19 (AC_{50} of 0.698) and CLD_CYP2B6_24hr (AC_{50} of 0.775), related to inhibited CYP2C19 activity and increased CYP2B6 expression, respectively.

Another sensitive hit for fipronil was the CCTE_GLTED_hDIO3_dn assay (AC_{50} value of 1.16 µM). This biochemical assay tests the ability of a chemical to inhibit human iodothyronine deiodinase type 3 (DIO3) activity.

Fipronil was not active in any of the 6 GABA receptor-specific assays, as discussed in the Discussion section below.

As part of the ToxCast™ assay suite, the Endocrine Disruption Screening Program for the 21st Century (EDSP) assays are specific for interactions with the thyroid, androgen and estrogen receptors and with steroidogenesis pathways. Related ToxCast™ assays are also grouped into the Collaborative Estrogen Receptor Activity Prediction Project (CERAPP) and the Collaborative Modeling Project for Androgen Receptor Activity (COMPARA) to screen for chemicals that could affect corresponding endocrine signal pathways. Fipronil was positive in 5 of 18 ER assays and 7 of 14 AR assays, however CERAPP and COMPARA modeling did not predict binding or agonist/antagonist activity toward estrogen or androgen receptors.

To summarize, a total of 14 target families were impacted by exposure to fipronil. The highest frequency of active hits occurred in the, CYP, neuroactivity, nuclear receptor neurodevelopment and malformation target families.

Discussion

In zebrafish, fipronil caused a significant increase in the yolk sac fluid, which was potentially indicative of a developmental effect, aryl hydrocarbon receptor activation, or oxidative stress (Knecht *et al.*, 2013; Truong *et al.*, 2014). This assay belongs to the malformation target family. The result is notable because of its sensitivity and because it represents a whole-animal response. ToxCast™ data on developmental effects in zebrafish are commonly used for cross-species comparisons, specifically with data for pregnant rats and rabbits (Padilla *et al.*, 2012; Boyd *et al.*, 2016). However, it is unknown if the biological pathways affected by fipronil and leading to yolk sac defects in zebrafish are in any way connected to effects seen in the rodent developmental studies (e.g., dead/missing pups, decreases in body weight, developmental delays, decreases in T4 and increases in liver weights).

Fipronil caused upregulation of sulfotransferase-2 (SULT2) after incubations of 24 and 48 hours. SULT2 enzymes are found in the liver and are involved in steroid and xenobiotic metabolism (Assem *et al.*, 2004). Up-regulation of SULT2 increases hepatic excretion of substrates into bile and feces. Expression of SULT2 is regulated by the constitutive androstane receptor (CAR) nuclear receptor. Biologically, the results of this assay concord with *in vivo* toxicity data that show that fipronil increases the metabolism and excretion of thyroid hormones through the CAR nuclear receptor in both rats and mice (Roques *et al.*, 2013).

Fipronil antagonized the binding of nuclear receptor NR1I3 by competitively binding to the ligand-binding domain of this molecule, thus preventing its binding to its cofactor. NR1I3 (also known as CAR) binds to DNA and regulates the transcription of target genes involved in drug metabolism and bilirubin clearance such as cytochrome P450 family members. Fipronil also decreased expression of adenosine kinase (ADK), a marker of steatosis. These results are consistent with evidence that fipronil increases the incidence of fatty liver and related pathologies in rodents (Ferreira *et al.*, 2012a; Roques *et al.*, 2013; de Medeiros *et al.*, 2015; Tavares *et al.*, 2015; Badgujar *et al.*, 2016a; Abdel-Daim *et al.*, 2018).

The results of the CYP assays are consistent with the published fipronil database. Altered CYP enzyme levels and activities were observed in rodents and in rat and human liver microsomes following exposure to fipronil (Peters *et al.*, 1991b; Tang *et al.*, 2004; Das *et al.*, 2006; Joo *et al.*, 2007; Leghait *et al.*, 2009; Roques *et al.*, 2012; Roques *et al.*, 2013; Caballero *et al.*, 2015; Badgujar *et al.*, 2016a; Carrao *et al.*, 2019a). As CYP enzymes are well known mediators of xenobiotic metabolism, the evidence from ToxCast™ suggest that they underlie the fipronil-induced increases in thyroid hormone clearance in the rat.

Increases in SULT2 and CYP activities and expression in human cells support the potential for increased hepatic metabolism and clearance of thyroid hormones *in vivo.* The ToxCast™ data are consistent with effects seen in the published literature, including increased clearance of thyroid hormones *in vivo* in rats and sheep following acute or subchronic oral or acute intraperitoneal administration (Leghait *et al.*, 2009; Leghait *et al.*, 2010; Ferreira *et al.*, 2012b; Roques *et al.*, 2012; Roques *et al.*, 2013; Moser *et al.*, 2015; Ehsan *et al.*, 2016). Also, decreased thyroid hormones were observed in two registrant-submitted toxicity studies (Aughton, 1993; Coder, 2019). Two additional studies conducted by registrants to elucidate possible metabolic pathways showed that the decrease in thyroid hormones was caused by an increase in hepatic metabolism, as opposed to thyroid-specific effects or effects on iodine organification (Peters *et al.*, 1991a; Peters *et al.*, 1991b).

Increased CYP and SULT enzyme expression and activity can also lead to inflammation and oxidative stress, observed in published genotoxicity and oncogenicity studies, (Peters *et al.*, 1991b) as well as increased hepatic metabolism and increased oxidative stress that were observed in animal tissues and in human cell cultures (Marshall, 1988a; Quesnot *et al.*, 2016).

Five of the assays with the most potent response following fipronil exposure were related to regulation of the constitutive androstane (CAR) nuclear receptor, which is important in hepatic

metabolism (Assem *et al.*, 2004; Roques *et al.*, 2013). These results are in concordance with liver effects seen in laboratory animals.

Another ToxCast^{TM} observation that may be relevant to *in vivo* fipronil toxicity is the inhibition of the human iodothyronine deiodinase type 3 (DIO3) enzyme. The DIO3 is a thyroid hormone-inactivating enzyme which depletes sources of active hormone by inner ring deiodination of both T4 and T3. The enzyme is thought to regulate circulating fetal thyroid hormone concentrations, prevent premature exposure of fetal tissues to adult levels of thyroid hormones. As such, it plays a critical role in mammalian development.

While concordances between *in vitro* hits and *in vivo* toxicity have been noted, associations to toxicity pathways have not been validated with respect to *in vivo* apical endpoints or a definitive AOP. For example, at present there is no validated AOP relating SULT2 and changes in thyroid hormone hepatic clearance. A potentially related AOP for altered amphibian metamorphosis as an adverse outcome is under development (AOP-Wiki). Its molecular initiating event involves the activation of hepatic nuclear receptors, leading to increased hepatic thyroid hormone uptake/transport, followed by increased clearance and decrease of T4 from serum (AOP-Wiki). However, no similar AOP has yet been proposed for mammals.

Fipronil was negative in all of the 6 available GABA receptor assays in ToxCast^{TM}, failing to predict fipronil interactions with the $GABA_A$ receptor, its best understood target for neurotoxicity. This is likely due to the selection of the binding target in ToxCast^{TM} that in all assays includes subunits containing the GABA binding site. Fipronil, similar to other insecticidal channel blockers, acts at an allosteric inhibitor by binding to the picrotoxin site of the ionotropic $GABA_A$ receptor. The picrotoxin site is located on a different subunit than the GABA site (Ratra and Casida, 2001; Zhang *et al.*, 2016). Without appropriate assays that include the correct receptor subunits or intact $GABA_A$ receptor, fipronil and many other insecticidal chloride channel blockers are likely to be inactive within the GABA/ToxCast^{TM} activity data system.

An AOP exists for fipronil neurotoxicity depicting key molecular events starting from its binding to the GABA receptor and leading ultimately to epileptic seizures (AOP-Wiki, 2021a). In this AOP, the molecular initiating event is fipronil binding to the picrotoxin binding site situated at or near the central pore of the ionotropic GABA receptor complex leading to blockage of the ion channel. The first key event (KE) is a decrease in inward chloride conductance through the ligand-gated ion channel. This leads to the second KE, a reduction in postsynaptic inhibition, manifested as reduced frequency and amplitude of spontaneous inhibitory postsynaptic currents (sIPSCs) and inhibition of GABA-induced firing in GABAergic neuronal membranes. As a result, the resistance of excitatory neurons to fire is decreased, thereby generating a large excitatory postsynaptic potential (EPSP). Intracellular Ca2+ levels rise in the affected region, the result of a large group of excitatory neurons firing in an abnormal, excessive, and synchronized manner. This excitatory firing causes voltage-gated Na+ channels to open, leading to seizures.

In conclusion, the ToxCast^{TM} data were consistent with several types of interaction between fipronil and molecular and tissue-level target sites already suggested by *in vivo* experiments.

V.B. Exposure Assessment

V.B.1. Pesticide Handler, Home User and Residential Post-Application Exposures

Fipronil is the active ingredient of many products used for structural pest control, pet flea/tick control, and lawn maintenance. Currently, no fipronil product is registered for food use in California. The exposure assessment evaluated a total of 26 exposure scenarios based the label-approved uses of the above products. Of these 26 scenarios, low human exposure is expected for eight scenarios. Fipronil exposure was estimated for the remaining 18 scenarios over short-term, seasonal, annual and life-time durations.

For professional handlers, exposure may occur during mixing and loading of liquid concentrate and granular products as well as application of all products (all formulations). Fipronil has a low vapor pressure (2.8×10^{-9} mmHg at 25°C), so risk of inhalation is expected to be low from liquid concentrate or application of pet spot-on products. However, inhalation exposure from aerosolized pet spray products may occur at the time of application, largely because of the application method. This is reflected in Tables 17 and 18 in the exposure assessment (Jiang, 2022). There may also be minimal exposure to aerosols from mixing or applying granular products, and from structural dust products and this is reflected in Tables 29 and 34 (Jiang, 2022).

Exposures for home users are largely driven by the dermal route for pet spot-on and spray products, although there is some potential for inhalation exposure to the spray products during application, much like for the professional handlers (see Table 19) (Jiang, 2022). Exposures for post-application scenarios for residential adults and children were evaluated for bait products, turf granules, structural liquid concentrate, and the pet products. For adults, exposures were highest via the dermal route for pet products. For children, exposures were largest via the dermal route for pet products and incidental oral routes for both turf granules and pet products. All exposure estimates for scenarios involving occupational handlers, home users, and adult and child residents are detailed the Exposure Assessment Document (EAD) (Jiang, 2022). The exposure estimates are also used in the calculation of the margins of exposure in the Risk Characterization section, below.

V.B.2. Dietary and Drinking Water Exposure

The dietary exposure from food and drinking water was estimated from residues of the parent fipronil. Monitoring data on food commodities were available for the metabolites fipronil-sulfide and fipronil-sulfone but not for photodegradate fipronil-desulfinyl. Monitoring data for residues in drinking water are discussed in the EAD (Jiang, 2022).

Introduction

DPR conducts acute and chronic dietary exposure assessments to evaluate the potential risk of human exposure to pesticide residues on food (Bronzan and Jones, 1989). These analyses are performed per DPR guidance for the total dietary exposure based on residues on all commodities

with established fipronil tolerances, as well as residues in drinking water (DPR, 2009). A tolerance is the legal maximum residue concentration of a pesticide, which may exist in or on a raw agricultural commodity or processed food. Tolerances are set by US EPA and are published in the Code of Federal Regulations (Title 40, part 180).

Dietary exposure is a product of food consumption and the corresponding residue concentration of a given pesticide. The total dietary exposure for an individual during a defined period of time (e.g., a day) is the sum of dietary exposures for all foods (in various forms and as ingredients in food items) consumed within that time-period:

$$\text{Exposure} = \sum_{i=1}^{n}(\text{residue}_i \text{ x } \text{consumption}_i)$$

where *n* is the number of food items in the diet.

Data on the amount of the pesticide residue on food and food consumption provide dietary exposure estimates for various population subgroups based on age, gender, ethnicity, season, and pregnancy/lactation status. In this document, exposure estimates were calculated for the US population and sensitive subpopulations including infants, children aged 1–2, and women of childbearing age (13–49 years old).

For estimating acute exposure, the highest residue values at or below the tolerance, or distribution of residues are considered. Acute exposure is calculated on a per-user basis including in the distribution of exposures only the days of survey that at least one commodity with potential pesticide residues is consumed. Chronic exposure to pesticides is calculated using per-capita, mean consumption estimates that include the entire population (DPR, 2009).

The acute and chronic dietary exposure assessments for fipronil were conducted for all combined food uses and included drinking water. As of June 2022, US EPA has established tolerances for the combined residues of the parent fipronil and its metabolites fipronil-sulfone and fipronil-sulfide, and its photodegradate fipronil-desulfinyl. The published tolerances for fipronil are listed in 40 CFR 180.517 and are expressed for plant and animal commodities as follows: milk fat (1.5 ppm), milk (0.05 ppm), animal fat (0.05–0.4 ppm), meat (0.01–0.04 ppm), meat byproducts (0.01–0.04 ppm), liver (0.02–0.1 ppm), eggs (0.03 ppm), wheat (0.005 ppm), corn (0.02 ppm), rice (0.04 ppm), and potato (0.03 ppm) (Code of Federal Regulations, 2015).

On the date of publication of this document, no products containing fipronil were registered for use on agricultural commodities in California. However, as legal uses exist at the federal level, the possibility of dietary exposure to fipronil was considered. Corn was not included in this analysis because fipronil is only registered for use on seed corn for export. Wheat and rice were also excluded, as there was no reported use of fipronil on wheat or rice in the US in the last 10 years (US EPA, 2018).

Consumption Data and Dietary Exposure

The US EPA's Dietary Exposure Evaluation Model-Food Commodity Intake Database (DEEM-FCID™, version 4.02)[11] was used to calculate the dietary exposure and risk. DEEM-FCID™ incorporates food consumption data from the National Health and Nutrition Examination Survey)/"What We Eat in America" (NHANES/WWEIA) dietary survey for the years 2005–2010[12]. The WWEIA survey collected food consumption data from 24,673 respondents. Each WWEIA respondent provided two days of food consumption data, which leads to a total of 49,346 food diaries. WWEIA-FCID translates food consumption as reported eaten in WWEIA (2005–2010 survey cycles) into consumption of DEEM-FCID™-defined food commodities.

Acute exposure was calculated on a per-user basis (i.e., the days included in the exposure distribution were those in which a specific commodity was consumed). Chronic exposure to pesticides was calculated using per-capita mean consumption estimates to include the entire population. The estimates for both acute and chronic exposure were expressed as a dosage in µg/kg/day.

Residue Data Sources

The residue data for fipronil used in the current risk assessment were based on the USDA Pesticide Data Program (PDP). The PDP (www.ams.usda.gov/science/pdp/download.htm) is the most representative monitoring residue database because it is designed to obtain pesticide residue data for risk assessments. The PDP samples are collected in ten states, including California.

PDP monitoring data for fipronil were available for potato, beef muscle and adipose, egg, butter, infant formula, milk, pork muscle and adipose, and chicken meat. There was a total of 59,972 samples screened for fipronil from 2003 through 2019. Standard protocol dictates the use of data spanning the last five years. Because fipronil detections in food were rare (92 detects out of 59,972 samples, 0.12% detection rate), additional years (data from 2003 to 2019) were used to increase the sample number and incidence of detects. The latest available PDP residue data (2020) were not used because they did not include foods with fipronil tolerances.

PDP data also included monitoring of fipronil-sulfone (19,393 samples since 2016) and fipronil-sulfide (315 samples, all in 2017), for which there were no detections. The range of the limit of detection (LOD) for sulfone was 0.0025 to 0.05 ppm; the sulfide LOD was 0.01 ppm. No data were available for fipronil-desulfinyl, the photodegradate.

Residue data on fipronil were also available from other databases such as the US Food and Drug Administration (FDA) Total Diet Study[13], DPR's California Pesticide Residue Monitoring Program (California Food and Agricultural Code §12532)[14] and registrant-submitted field trail

[11] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/deem-fcidcalendex-software-installer
[12] http://fcid.foodrisk.org/
[13] https://www.fda.gov/Food/FoodScienceResearch/TotalDietStudy/default.htm
[14] https://www.cdpr.ca.gov/docs/enforce/residue/rsmonmnu.htm

data. These databases did not monitor commodities that were not screened by PDP. Fipronil residues in drinking water were evaluated based on surface water monitoring data collected by DPR and other state and federal agencies (Jiang, 2022). The residues used in acute and chronic dietary exposure assessments are shown in Table 37.

Acute Dietary Exposure Assessment

DPR estimates the acute dietary exposure to a pesticide using the tiered approach in the selection of the appropriate pesticide residue values (DPR, 2009). The tiered approach begins with the point estimate (deterministic) analyses (Tiers 1 and 2) that employ the tolerance or the upper bound value or the mean residue value. The Monte Carlo probabilistic approach (Tier 3) can subsequently be used to refine the assessment by taking into account the occurrence and distribution of residue levels and providing the probability distribution of exposure.

DPR uses two thresholds to indicate if the next tier of assessment is needed. The thresholds are based on the dietary risk expressed as margin of exposure (MOE, defined as the POD divided by the estimated exposure). The two possible screening thresholds are:

(1) the MOE at the 99th percentile exposure is within 5-fold of the target MOE, or
(2) the MOE at the 95th percentile exposure is within 10-fold of the target MOE.

For fipronil, the target MOE is 100 (described in the Risk Characterization section of this RCD). Therefore, the screening threshold for the next tier of assessment would be an MOE of 500 at the 99th percentile or an MOE of 1000 at the 95th percentile. If the MOE do not reach the threshold (e.g., are lower than the target), the next tier of exposure assessment may be needed. These thresholds provide room for exposures from other potential routes during exposure aggregation; the 5-and 10-fold distance from the target MOE ensures the likelihood that dietary exposure will not be a major contributor to the aggregate risk.

Tier 1 Acute Deterministic (Point Estimate) Dietary Exposure Assessment

The acute dietary exposure to fipronil of the US population and various population subgroups was assessed using the deterministic approach (Tiers 1 and 2). In this approach, a single value (referred to as a point estimate) was selected to represent the concentration of fipronil on each of the registered commodities.

This assessment assumed that all foods consumed in a given day contain pesticide residues at the tolerance level. For fipronil, this analysis produced exposures that resulted in MOEs below the thresholds of 1000 and 500 at the 95th or 99th percentiles, respectively, for all of the population subgroups. Therefore, the Tier 2 point estimate assessment was used to refine the dietary exposure calculation.

Table 37. Fipronil Residues used for Acute and Chronic Dietary Exposure Assessment

Commodity	Source of Data	Year	Samples (N)	Fipronil Detects (N)	Detected Residues (ppm)	Range LOD (ppm)	Acute Point Estimate (ppm)	Chronic Average Residue (ppm)
Beef, goat, pork and sheep fat and baby food fat and skin	Beef PDP LOD (adipose)	2008-2009	292	0	NDR	0.0012	0.0012	0.006
Beef, goat, horse, and sheep meat, meat byproducts, kidney, and baby food meat and meat byproducts[a]	Beef PDP LOD (muscle)	2008-2009	292	0	NDR	0.0012	0.0012	0.006
Pork, meat, meat byproducts, kidney, and baby food meat and meat byproducts	Pork PDP LOD (muscle)	2005	352	0	NDR	0.0006	0.0006	0.0003
Pork, fat and baby food fat and skin	Pork PDP LOD (adipose)	2005	352	0	NDR	0.0021	0.0021	0.00105
Beef, liver, baby food	Tolerance	2015	--	--	--	--	0.1	0.05
Pork, liver	Tolerance	2015	--	--	--	--	0.02	0.01
Sheep, liver	Tolerance	2015	--	--	--	--	0.1	0.05
Poultry, chicken and turkey fat, skin and baby food fat and skin	Tolerance (poultry fat)	2015	--	--	--	--	0.05	0.025
Poultry, chicken and turkey meat, meat byproducts, and baby food meat and meat byproducts	PDP residue (poultry meat)	2006	1309	3	0.001-0.0012	0.0006	0.0012	0.0003
Egg white, whole, yolk and baby food white (solids), whole and yolk	PDP LOD (egg)	2010-2016	1036	0	NDR	0.003-0.04	0.003	0.0015
Milk fat and baby food/infant formula fat	PDP LOD (butter)	2003-2012	736	0	NDR	0.0027-0.02	0.02	0.00238
Milk, nonfat, water and solids	CA-specific PDP LOD (milk)	2015 to 2019	598	0	NDR	0.0025	0.0025	0.00125
Milk, sugar, nonfat, water and solids-baby food and formula	PDP LOD (infant formula)	2013-2014	705	0	NDR	0.0018	0.0018	0.0009
Potato tuber with and without peel, flour, dried and chips and baby food	PDP residue	2015-2016	1723	36	0.003-0.0096	0.0018-0.005	0.0096	0.00419
Water, direct and indirect	SURF	2002 to 2017	3371	303*	0.0017-0.275 ppb	n/a	0.000275	0.000033

LOD: Limit of detection; PDP: Pesticide Data Program (USDA); NDR no detectable residues; SURF: Surface Water Database at DPR; ppb: part per billion.

[a]adjustment factor of 1.92 used for dried beef.

*only includes positive fipronil detections from waters that could reasonably be used as drinking water.

Tier 2 Acute Deterministic (Point Estimate) Dietary Exposure Assessment

The typical assumptions in Tier 2 assessments are: (*i*) all consumed foods contain the highest reported residue at or below the tolerance, (*ii*) pesticide residues below the LOD are equal to that limit, (*iii*) all crops are treated with the pesticide, and (*iv*) residue concentrations do not vary from the time of sampling to the time of consumption (DPR, 2009).

For the fipronil acute Tier 2 analysis, the highest detected residue or the highest LOD from PDP were used for potato, beef muscle and adipose, egg, butter, infant formula, milk, pork muscle and adipose, and chicken meat. For milk, no residues were detected from 2003 to 2019. The California-specific range of LODs for the last five years (2015 to 2019) were selected to refine the fipronil residue level for this commodity. For egg, the lowest LOD was used as the highest LOD was higher than the tolerance. PDP data were not available for horse, goat, or sheep. For these commodities, residues measured by PDP on beef meat and fat were used as a surrogate. Beef meat was also used as a surrogate for meat byproducts, which lacked monitoring data, when the byproduct and meat tolerances were the same. In cases where the byproduct tolerance was different than the meat tolerance, the byproduct tolerance was used. For drinking water, the residue was the highest reasonable detection (e.g., in water that people might reasonably be expected to consume).

The acute Tier 2 dietary exposure assessment resulted in a 95th percentile of user-day exposures that ranged from 0.068 µg/kg/day for women of childbearing age (13–49 years old) to 0.352 µg/kg/day for children ages 1–2 (Table 38). Exposure at the 99th percentile level ranged from 0.105 µg/kg/day (women of childbearing age) to 0.484 µg/kg/day (children 1–2). Because additional exposure data for fipronil were not available, Tier 3 refinement was not performed.

Table 38. Acute Tier 2 Dietary Exposure Estimates for Fipronil

Population Subgroup (Users)	95th Percentile	99th Percentile
	µg/kg/day	µg/kg/day
Total US Population	0.117	0.229
Nursing Infants	0.111	0.234
Non-Nursing Infants	0.263	0.402
All Infants	0.242	0.394
Children 1–2 years old	**0.352**	**0.484**
Women of childbearing age (13–49 years old)	0.068	0.105

DEEM-FCID™, version 4.02 was used for the analysis.
The acute point estimate dietary exposure from all commodities with fipronil registrations was calculated at the 95th and 99th percentiles of user-days for all population subgroups. The highest exposures are indicated in bold.
95th and 99th percentile exposure estimates are in µg fipronil/kg body weight/day.

A critical exposure contribution analysis was performed using DEEM-FCID to determine which food commodities contributed most to the total dietary exposure. Milk contributed to 71% of the estimated dietary exposure for children aged 1–2. Potato, chicken fat, milk and water were the high contributors for other population subgroups. Exposure estimates for water and potato were

based on the single highest detect, while estimates for the other commodities were derived from the analytical LOD or from the tolerance, not from actual measured values.

Chronic Dietary Exposure Assessment

The fipronil chronic dietary exposure analysis was assessed using a deterministic approach. In this approach, a single value (referred to as a point estimate) was selected to represent the concentration of fipronil on each registered commodity. The value is either the arithmetic mean of measured residue values from monitoring data or ½ the LOD, when available. For commodities for which monitoring data were not available, the chronic residue concentration was set equal to ½ of the tolerance (Table 37).

The chronic dietary exposure assessment resulted in exposures estimates that ranged from 0.007 µg/kg/day for nursing infants to 0.070 µg/kg/day for children aged 1–2 (Table 39).

Table 39. Tier 2 Chronic Dietary Exposure Analysis

Population Subgroup	Exposure (µg/kg/day)
Total US Population	0.015
Nursing Infants	0.007
Non-Nursing Infants	0.025
All Infants	0.019
Children 1–2	**0.070**
Women of childbearing age (13–49 years old)	0.011

DEEM-FCID™, version 4.02 was used for the analysis. Exposure estimates are in µg fipronil/kg body weight/day.

A critical exposure contribution analysis performed in DEEM-FCID determined that milk (fat, sugar, solids and water), potato, chicken fat and skin, and indirect sources of water contributed the most to chronic exposure through diet. Milk contributed to 78% of the estimated dietary exposure for children aged 1–2, and to 45–54% of the dietary exposure for the other subpopulations. With the exception of potato and water, exposure estimates for high-contributor crop groups were derived from the tolerance (chicken skin and fat) or from the LOD values (milk products).

V.C. Risk Characterization

The margin of exposure (MOE) is a quantitative tool used by DPR to determine the potential risk arising from exposure to a pesticidal active ingredient. The potential for non-oncogenic health effects resulting from fipronil exposure was estimated from critical POD for a specific exposure duration and route divided by the estimate of human exposure for that duration and route:

$$MOE = \frac{POD}{Exposure\ Dosage}$$

The resulting value is compared to the acceptable or target MOE which, for purposes of this risk assessment, is equivalent to the total uncertainty factor (UF_{TOTAL}) of 100. Values at or above the target MOE are generally considered protective against the toxicity of fipronil when the POD is derived from an animal study. This reflects the default assumption that (1) humans are 10-fold more sensitive than animals, and (2) a 10-fold range of sensitivity exists within the human population (Dourson *et al.*, 2002).

The critical acute, subchronic, and chronic PODs for fipronil are summarized in the Hazard Identification section in Table 32. DPR used these values to calculate risks from short-term, seasonal, and annual exposures to pesticide handlers (professional and home users) and residents following pesticide applications. Handler and residential exposure estimates are detailed in the EAD (Jiang, 2022). Dietary exposure is described in the Exposure Assessment section of this document. As a reminder, fipronil is not licensed for agricultural crop production in California, so no agricultural handler exposure is anticipated. Dietary exposure can occur from food grown outside of California where fipronil crop uses are legal.

This assessment estimated risks by route-specific pathways and by aggregating exposures from all routes. Aggregate exposures are defined as exposures to a single chemical (i.e., a pesticide) through multiple pathways and routes (US EPA, 2001). When using an aggregate exposure approach, DPR assumes that a pesticide can enter the human body from multiple pathways including those related to occupational applications and through dermal exposure, air, food, and drinking water. Exposures by different routes are then combined (aggregated) when exposure durations and toxic effects of the pesticide correspond. For fipronil, the predominant routes of exposure are incidental oral (children) and dermal (children and adults). DPR analyzed inhalation-only exposure scenarios and aggregate exposures which included the inhalation route and found that risk from fipronil inhalation exposure is minimal.

Risk was estimated for the following population subgroups:

(a) Occupational Handlers: Includes those who are licensed or certified to apply fipronil and professional pet groomers. Risk was estimated for separately for dermal and inhalation scenarios and for aggregated exposures where both dermal and inhalation exposures were expected to occur simultaneously.

(b) Home Users: Individuals who apply pet products at home. Risks were estimated for persons 18 years and older, as these products are restricted to adult-use only. Risk was estimated separately for dermal and inhalation scenarios, and for aggregated exposures where both dermal and inhalation exposure were expected to occur simultaneously.

(c) Residential Post-Application Exposure, Adult: Adults who are potentially exposed to fipronil following professional residential treatments (such as bait gels and lawn treatments) or applications of pet products at home. Risk was estimated separately for dermal and inhalation scenarios, and for aggregated exposures where both dermal and inhalation exposure were expected to occur simultaneously.

(d) Residential Post-Application Exposure, Child: Children (1–2 years old) who are potentially exposed to fipronil following professional residential treatment or applications of pet products at home. Risk was estimated separately for incidental oral, dermal, inhalation scenarios, and for aggregated exposures where each route of exposure is expected to occur simultaneously.

Aggregate MOEs were estimated by using a hazard index approach, that is:

- Aggregate MOE for Occupational Handlers = $1/(1/MOE_D + 1/MOE_I)$
- Aggregate MOE for Home Users = $1/(1/MOE_D + 1/MOE_I)$
- Aggregate MOE for Adults Post-Application = $1/(1/MOE_D + 1/MOE_I)$
- Aggregate MOE for Children Post-Application = $1/(1/MOE_D + 1/MOE_O + 1/MOE_I)$

Where,

D: dermal exposure (i.e., short-term absorbed daily dose, STADD)
I: inhalation exposure
O: non-dietary incidental (hand-to-mouth) oral exposure

MOE aggregation was conducted in a sequential, additive manner in order to obtain information on the relative contribution of each exposure scenario to overall risk. Oral exposures were not anticipated in handler or home user scenarios. Dietary and drinking water values were not included in calculating the aggregate MOE values because they did not impact the final risk values (see below).

V.C.1. Margins of Exposures for Occupational Handlers

Dermal and inhalation MOEs for occupational exposure to fipronil are summarized in Table 40. The exposure estimates used to calculate MOEs are found in the EAD (Jiang, 2022) and are duplicated in Appendix VII of this document for ease of reference. Acute inhalation MOEs for all occupational categories were greater than 100, and therefore not considered a risk. Acute dermal MOEs for professional groomers applying pet spray were less than the target value of 100, indicating a concern. The only aggregated acute MOE for handler exposure that was below the target value of 100 was for professional groomers applying pet spray (aggregate MOE of 14). In this case, risk was driven by dermal exposure which, alone, was less than the target MOE.

The subchronic dermal MOEs for all handler categories were below 100, which indicate a concern. The exception was handlers of structural dust products, which resulted in a dermal MOE of 1429, largely because of the label-required personal protective equipment. The professional groomer subchronic inhalation MOE for pet spray was also below 100 (MOE of 51). Aggregated subchronic MOEs were all below 100, driven by dermal exposure. The exception being the aggregate MOE for structural dust handlers.

The chronic dermal MOEs for all occupational settings were below 100, except for handlers of turf granules and structural dust. The chronic inhalation MOE for professional groomers applying pet spray-on products was also below 100. Aggregated chronic MOEs were below the target of 100 (driven by dermal exposure) for all occupational exposure scenarios except for handlers of structural dust and turf granules.

Table 40. Risk Estimates for Occupational Handlers Exposed to Fipronil

Product	Scenario	Acute MOE[a]			Subchronic MOE[b]			Chronic MOE[c]		
		Dermal	Inhal.	Agg.[d]	Dermal	Inhal.	Agg.	Dermal	Inhal.	Agg.
pet spot-on	groomer	183	NA	183	**28**	NA	**28**	**28**	NA	**28**
pet spray	groomer	**15**	592	**14**	**2**	**51**	**1**	**2**	**51**	**1**
structural dust	handler	9059	35000	7196	1429	6452	1170	2198	9524	1786
structural LC	no overhead	550	1453	399	**41**	105	**29**	**71**	182	**51**
structural LC	overhead	122	4529	119	**15**	313	**15**	**27**	541	**26**
turf granules	handler	1167	16042	1088	**83**	1176	**78**	167	2353	156

MOE: margin of exposure (POD/exposure). Acute POD was 0.77 mg/kg/day (Hughes, 1997); subchronic and chronic POD were 0.02 mg/kg/day (Aughton, 1993). NA: Inhalation exposure is negligible considering the product formulation and the application method (US EPA, 2012c); LC: liquid concentrate; Inhal: inhalation; Agg: aggregate. Exposure estimates from Exposure Assessment Document (EAD) (Jiang, 2022).

[a]Acute risk calculated with short-term absorbed daily dose (STADD). The STADD = exposure (µg/lb) × fipronil handled (lb/d) × absorption rate (0.043 for dermal and 1 for inhalation) ÷ body weight (70kg).

[b]Subchronic risk calculated with the seasonal average daily dose (SADD). SADD = exposure (µg/lb) × fipronil handled (lb/d) × absorption rate ÷ body weight.

[c]Chronic risk calculated with the annual average daily dose (AADD). Annual Average Daily Dose = AADD × (annual use months ÷ 12).

[d]Aggregate risk = $1(1/MOE_D + 1/MOE_I)$ where D = dermal and I = inhalation exposures.

Bold text indicates MOE below the target of 100 and is considered a concern for human health.

V.C.2. Margins of Exposure for Home Users

Acute dermal and inhalation MOEs were calculated for home users exposed to fipronil through pet spot-on or spray formulations (Table 41). Non-dietary incidental oral (hand-to-mouth) MOEs were not calculated for home users, as oral exposure was not anticipated. Only acute exposure scenarios were considered because of the once-monthly restrictions on use of these products.

Seasonal and annual exposures were not anticipated for home users by any route. As noted in the EAD, gloves are required for the home use of spray products but not for the spot-on treatments. Even with the protection factor of gloves accounted for, spray products resulted in acute dermal and acute aggregate MOEs of concern because of the high level of exposure to the spray to the forearm, upper arm and chest areas of the user (Jiang, 2022).

Table 41. Risk Estimates for Home Users Exposed to Fipronil

Product	Scenario	Acute[a] Dermal MOE	Acute Inhalation MOE	Acute Aggregate MOE
pet spot-on	adult pet owner	513	NA	513
pet spray	adult pet owner	**41**	1638	**40**

MOE: margin of exposure (POD/exposure). Acute POD was 0.77 mg/kg/day (Hughes, 1997). NA: Inhalation exposure is not anticipated with pet spot-on products; Inhal: inhalation; Agg: aggregate. Exposure estimates from EAD (Jiang, 2022).
[a]Acute risk calculated with short-term absorbed daily dose (STADD). The STADD = exposure (µg/lb) × fipronil handled (lb/d) × absorption rate (0.043 for dermal and 1 for inhalation) ÷ body weight (70kg).
[d]Aggregate risk = $1(1/MOE_D + 1/MOE_I)$ where D = dermal and I = inhalation exposures.
Bold text indicates MOE below the target of 100.

V.C.3. Post-Application Margins of Exposure in Residential Settings

Adults

MOEs for residential post-application exposure of adults appear in Table 42. Non-dietary incidental (hand-to-mouth) oral MOEs were not calculated for adults, nor were chronic MOEs for any exposure scenarios. Acute dermal MOEs were calculated for five exposure scenarios. Acute inhalation MOEs were calculate for one exposure scenario. All acute MOEs (singular and aggregated) were above the target MOE of 100.

Subchronic MOEs were calculated for four dermal exposure scenarios and one inhalation exposure scenario. Subchronic dermal and aggregate MOE calculations for pet spray users were less than the target of 100, indicating a risk. Aggregate risk was driven by dermal exposure, and the lowest MOE was for pet spray.

Table 42. Risk Estimates for Adult Residential Post-Application Exposure to Fipronil

Product	Acute[a] MOE			Subchronic[b] MOE		
	Dermal	Inhal.	Agg.	Dermal	Inhal.	Agg.
pet spot-on	550	NA	550	222	NA	222
pet spray	321	NA	321	**39**	NA	**39**
structural bait gel	>100,000	NA	>100,000	>100,000	NA	>100,000
structural LC	29615	33478	15714	9091	3571	2564
turf granules	>100,000	NA	>100,000	NA	NA	NA

MOE: margin of exposure (POD/exposure). Acute POD was 0.77 mg/kg/day (Hughes, 1997); subchronic chronic POD was 0.02 mg/kg/day (Aughton, 1993). NA: Inhalation exposure is not anticipated with pet products, bait gel or turf granules; subchronic dermal exposure not anticipated for turf granules; LC: liquid concentrate; Inhal: inhalation; Agg: aggregate. Exposure estimates from EAD (Jiang, 2022).

[a]Acute risk calculated with short-term absorbed daily dose (STADD). The STADD = exposure (µg/lb) × fipronil handled (lb/d) × absorption rate (0.043 for dermal and 1 for inhalation) ÷ body weight (70kg).
[b]Subchronic risk calculated with the seasonal average daily dose (SADD). SADD = exposure (µg/lb) × fipronil handled (lb/d) × absorption rate ÷ body weight.
[d]Aggregate risk = $1(1/MOE_D + 1/MOE_I)$ where D = dermal and I = inhalation exposures.
Bold text indicates MOE below the target of 100.

Children

MOEs for residential post-application exposure of children (age 1–2) to fipronil appear in Table 43. Annual MOEs were not calculated for these scenarios. Unlike with adults, post-application exposure to children included non-dietary incidental (hand-to-mouth) oral exposures for some scenarios.

Acute oral MOEs were calculated for five exposure scenarios. The only acute MOE below the target were those for oral and aggregate exposure resulting from usage of turf granules. Risk was driven by the oral route.

Subchronic MOEs were calculated for four subchronic exposure scenarios. Oral and dermal exposure to pet spray products resulted in MOEs lower than the target for both dermal and inhalation exposure. Aggregate MOEs for oral and dermal exposure to pet spot-on and spray products scenarios were also below the target value of 100 (driven primarily by the dermal route), thus indicating a risk.

Table 43. Risk Estimates for Child Residential Post-Application Exposure to Fipronil

Product	Acute[a] MOE				Subchronic[b] MOE			
	Oral	**Dermal**	**Inhal.**	**Agg.**	**Oral**	**Dermal**	**Inhal.**	**Agg.**
pet spot-on	1283	296	NA	241	500	118	NA	**95**
pet spray	700	167	NA	135	**91**	**21**	NA	**17**
structural bait gel	>100,000	>100,000	NA	>100,000	57143	>100,000	NA	45662
structural LC	10548	42778	16042	5540	3226	12500	1667	1010
turf granules	**33**	77778	NA	**33**	NA	NA	NA	NA

MOE: margin of exposure (POD/exposure). Acute POD was 0.77 mg/kg/day (Hughes, 1997); subchronic POD was 0.02 mg/kg/day (Aughton, 1993). NA: Inhalation exposure is not anticipated with pet products, bait gel or turf granules; subchronic dermal exposure not anticipated for turf granules; LC: liquid concentrate; Inhal: inhalation; Agg: aggregate. Exposure estimates from EAD (Jiang, 2022).
[a]Acute risk calculated with short-term absorbed daily dose (STADD). The STADD = exposure (µg/lb) × fipronil handled (lb/d) × absorption rate (0.043 for dermal and 1 for inhalation) ÷ body weight (70kg).
[b]Subchronic risk calculated with the seasonal average daily dose (SADD). SADD = exposure (µg/lb) × fipronil handled (lb/d) × absorption rate ÷ body weight.
[c]Chronic risk calculated with the annual average daily dose (AADD). Annual Average Daily Dose = AADD × (annual use months ÷ 12).
[d]Aggregate risk = $1(1/MOE_D + 1/MOE_I + 1/MOE_O)$ where D = dermal, I = inhalation and O = incidental oral exposures.
Bold text indicates MOE below the target of 100.

V.C.4. Diet and Drinking Water Margins of Exposure

The MOEs for the combined exposures from fipronil residues in food and drinking water exposures in various subpopulations are summarized in Table 44. The acute MOEs were calculated using the critical oral POD of 0.77 mg/kg, based on decreased hindlimb splay in rats following a single dietary dose of fipronil (Hughes, 1997). The chronic dietary MOEs were calculated using the critical oral POD of 0.02 mg/kg/day, based on sustained decreases in T4 in male rats, convulsions and death, and progressive senile nephropathy in male rats (Aughton, 1993). Exposure values from diet and drinking water were calculated as described above in the Dietary and Drinking Water Exposure section. Acute (Tier 2) MOEs at the 95th exposure percentile were all above the target MOE of 100, thus not of potential health concern. Similarly, all chronic (Tier 2) MOEs were above the target of 100.

Table 44. Risk Estimates for Exposure from Fipronil Residues in Food and Drinking Water

Population Subgroup	Acute MOE 95th Percentile	Chronic MOE
Total US Population	6564	1325
Nursing Infants	6954	2757
Non-Nursing Infants	2923	800
All Infants	3179	1030
Children 1–2 years old	2190	287
Females 13–49 years old	11332	1894

DEEM-FCID™, version 4.02 was used for the analysis. Acute and chronic point estimate dietary exposures were calculated from residues in all commodities with fipronil registrations and in drinking water.
MOE: margin of exposure = point of departure/exposure. Acute POD was 0.77 mg/kg/day (Hughes, 1997); chronic POD was 0.02 mg/kg/day (Aughton, 1993).
Acute (Tier 2) dietary exposure was calculated at the 95th and 99th percentiles of user-days for all population subgroups.

VI. REFERENCE DOSES

Reference doses (RfDs) are estimates of the concentration or dose of a substance to which a human population can be exposed (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (US EPA, 2011f). RfDs are calculated by dividing the critical POD by the total uncertainty factor (UF_{TOTAL}). DPR uses a default UF_{TOTAL} of 100, which reflects the default assumption that (1) humans are 10-fold more sensitive than animals (UF_A of 10), and (2) a 10-fold range of sensitivity exists within the human population (UF_H of 10). The UF_A and UF_H both consist of pharmacokinetic and pharmacodynamic variables.

RfDs were calculated for acute, subchronic, and chronic oral and dermal exposure to fipronil. Oral and dermal RfDs were calculated because significant exposure and risk was identified for adults and children through these routes. While inhalation exposure and risk were calculated for fipronil products, DPR found that the inhalation risk was minimal compared to the dermal risk for adults and compared to the dermal and incidental oral risk for children. In addition, inhalation exposure always occurred simultaneously with dermal exposure. Therefore, a separate RfC was not calculated for inhalation.

Dermal and oral RfDs both considered the percent absorbed and the concentration of active ingredient. The concentration of the technical grade fipronil was > 95% by volume. DPR estimated the percent absorbed orally as > 80%, which DPR considers complete (100%). The percent absorbed through the skin was estimated to be 4.3% in rats and in humans (Thongsinthusak and Ross, 1999). As the most sensitive endpoints were not measured in the available dermal studies, the acute, subchronic and chronic critical dermal RfDs were derived from the oral PODs.

$$RfD = (NOEL/ \% \text{ absorbed})/UF_{TOTAL}$$

VI.A. Acute Reference Doses

The acute oral POD of 0.77 mg/kg was derived from an acute neurotoxicity study in rat (Hughes, 1997). The acute dermal POD was derived from the same study. Acute oral and dermal RfDs are listed in Table 45.

Table 45. Acute Oral and Dermal Reference Doses Derived with Default Uncertainty Factors

Route	POD (mg/kg)	% Abs.	UF	UF_{TOTAL}	RfD (mg/kg)
Oral	0.77[a]	100	10_A	100	0.008
			10_H		
Dermal	0.77[a]	4.3	10_A	100	0.18
			10_H		

POD: Point of Departure; % Abs: percent absorption; UF: Uncertainty factors: UF_{TOTAL}: total UF, includes 10x for intrahuman variability (UF_H) and 10x for interspecies extrapolation from animal to human (UF_A); RfD: reference dose.

[a]Benchmark Dose Software (BMDS, version 3.2) of hindlimb splay data from acute neurotoxicity study in rat (Hughes, 1997).

VI.B. Subchronic and Chronic Reference Doses

The subchronic POD of 0.02 mg/kg/day was based on convulsion-associated mortality and a significant dose-dependent decrease in T4 in males at week 1 through week 50 (Aughton, 1993).

The chronic POD of 0.02 mg/kg/day was based on sustained decreases in T4, increased incidence of convulsions, convulsion-associated mortality and mortality, and progressive senile nephropathy in rats in the combined chronic / oncogenicity study (Aughton, 1993).

Subchronic and chronic oral and dermal RfDs are listed in Table 46.

Table 46. Subchronic and Chronic Oral and Dermal Reference Doses Derived with Default Uncertainty Factors

Duration	Route	POD (mg/kg)	% Abs.	UF	UF_{TOTAL}	RfD (mg/kg)
Subchronic and Chronic	Oral	0.02	NA	10_A / 10_H	100	0.0002
Subchronic and Chronic	Dermal	0.02	4.3	10_A / 10_H	100	0.005

POD: Point of Departure; % Abs: percent absorption (dermal route only)
Uncertainty factors: UF_{TOTAL}: total UF, includes 10x for intrahuman variability (UF_H) and 10x for interspecies extrapolation from animal to human (UF_A); RfD: reference dose
Subchronic POD based on effects seen following subchronic exposure in chronic study in rat (Aughton, 1993).
Chronic POD from combined chronic and oncogenic study in rat (Aughton, 1993).

VII. RISK APPRAISAL

Risk assessment is the process by which the toxicity of a chemical is compared to its potential for human exposure under specific conditions in order to estimate the risk to human health. This assessment evaluated the health risks associated with handler, residential, dietary, and aggregate exposures to fipronil. All risk assessments have limitations in the basic assumptions and the data on which they are based. These limitations contribute to uncertainties in the hazard identification, dose-response assessment, and exposure assessment processes, resulting ultimately in uncertainty in the final the estimation of human risk. Qualitatively, risk assessments for all chemicals have similar uncertainties. However, their magnitude varies with the availability and quality of the relevant data and with the types of exposure scenarios assessed. Prominent areas of risk assessment uncertainty involve assumptions about inter- and intraspecies sensitivity, the effectiveness of dose and route extrapolations, and the quality of the available experimental data. Specific uncertainties associated with this fipronil risk assessment are detailed in the following paragraphs.

VII.A. Uncertainties Associated with Fipronil Toxicity and Critical Points of Departure

VII.A.1. Acute Oral Point of Departure

The critical acute oral POD of 0.77 mg/kg/day was based on neurotoxic effects observed in adult rats

The critical acute oral POD of 0.77 mg/kg/day was based on a significant, dose-dependent reduction in hindlimb splay in rats measured 7 hours following gavage administration in an acute oral neurotoxicity study (Hughes, 1997). It was calculated using Benchmark Dose (BMD) modeling with a benchmark response level (BMR) of 10%. The study NOEL for this effect was 2.5 mg/kg/day and the LOEL was 7.5 mg/kg/day. A non-significant 10% decrease in hindlimb splay was also observed at the study NOEL of 2.5 mg/kg/day. BMD modeling incorporated the entire data set, including variability and uncertainty due to sampling and measurement error (US EPA, 2012a). For the BMD modeling, DPR chose the BMR of 10% as the lower limit of a biologically detectable response for designation as the acute BMDL. The statistical and biological considerations for setting the 10% BMR include: 1) 10% reduction in hindlimb splay compared to background control levels, 2) a 10% reduction can be reliably measured for the two available acute neurotoxicity rat studies that reported reduction in hindlimb splay, and 3) 10% is at or near the limit of sensitivity of these measurements for observing a statistically significant decrease in hindlimb spay. The use of the $BMDL_{10}$ of 0.77 mg/kg as the critical acute POD is supported by several lines of evidence, as described below.

- Decreased hindlimb splay is consistent with a CNS effect, as would be expected with a GABA receptor antagonist.
- An earlier acute neurotoxicity study (Gill *et al.*, 1993) reported reduced hindlimb splay at the LOEL of 5 mg/kg/day, which is similar to the LOEL of 7.5 mg/kg/day in the critical acute study (Hughes, 1997).

- While the data from (Gill *et al.*, 1993) were not amenable to BMD modeling, the study supported the finding that reduced hindlimb splay was the most sensitive acute effect caused by fipronil.
- The critical acute oral POD was supported by the subchronic inhalation toxicity study by Adamo-Trigiani (1999). Clinical signs noted on day 2 (ungroomed fur, decreased activity, salivation and convulsions) and decreased body weight and food consumption during week 1 at the LOEL of 4.8 mg/kg/day resulted in a short-term study NOEL of 0.8 mg/kg/day. The proximity of this value to the critical oral value of 0.77 mg/kg/day is noteworthy in that the experimental data confirm the model output.
- BMD modeling also gives a more precise estimate of POD than route-to-route extrapolation, which requires conversion from an air concentration (0.005 mg/L) to an internal dose (0.8 mg/kg/day) using a default rat breathing rate. There is also a higher degree of confidence in the modeled value because it evaluated all data in the observed dose range.

The only remaining uncertainty with the critical acute oral POD is the time at which the hindlimb splay measurements were made during the acute neurotoxicity study (Hughes, 1997). The measurements were conducted 7 hours post dosing, which was experimentally determined as the time-to-peak effect (TOPE). The TOPE for fipronil was defined as the time after dosing when the highest incidence of neurobehavioral changes (convulsions, chewing, licking and wet anogenital region) were measured in the rats. However, the TOPE for hindlimb splay could be different than that for convulsions.

Additional empirical support for the critical acute oral POD of 0.77 mg/kg/day

Four repeated dose studies in rats identified PODs lower than the critical acute POD of 0.77 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 as explained below

A developmental neurotoxicity study (DNT) showed delayed preputial separation in pups after 25 doses *in utero* and via lactation (LOEL of 0.9 mg/kg/day and NOEL of 0.05 mg/kg/day) (Mandella, 1995). Although preputial separation is a developmental effect, DPR considered it unlikely to result from *in utero* exposure alone. Rather, it was viewed as secondary to reduced postnatal body weight in the pups. In addition, because no effects on reproductive function were observed in the 2-generation rat reproductive toxicity study, fipronil-induced delayed preputial separation did not appear to have consequential longer-term effects. In conclusion the delayed preputial separation in pups was not considered an acute effect. This is described in detail in VII.A.4. Developmental Toxicity Endpoints, below.

In a comparative thyroid assay (CTA), decreased thyroid hormone (T4) levels were quantified in fetuses on GD 20 at a LOEL of 1 mg/kg/day, resulting in a NOEL 0.3 mg/kg/day (Coder, 2019). A related increase in liver weights was seen in pups on PND 4 and 21. Thyroid hormone

deficiency during development, even of short duration, can lead to irreversible brain damage. However, because T4 levels in the CTA were first measured after 15 consecutive *in utero* doses, the time course of the hormone decrease in fetuses was essentially unknown. Moreover, technical issues reduced the reliability of the measured thyroid hormone levels in the CTA. Consequently, DPR did not consider the CTA results adequate to establish a critical acute POD. This is described in detail in VII.A.4. Developmental Toxicity Endpoints and in VII.A.5. Thyroid Toxicity, below.

In a chronic toxicity study in rats, decreased T4 levels were observed in adult males after one week of exposure at 0.06 mg/kg/day with a corresponding NOEL of 0.02 mg/kg/day (Aughton, 1993). DPR conducted an extensive weight-of-evidence analysis of thyroid hormone perturbations, concluding that the decreased T4 levels in adult male rats at 0.06 mg/kg/day were unlikely to be deleterious in the short-term and were not an appropriate surrogate for decreased T4 levels in pregnant females (where acute T4 reduction could have developmental consequences). This is described in detail in the VII.A.5 Thyroid Toxicity, below.

Finally, a published study conducted in adult female ICR (CD-1 IGS) mice exposed to fipronil from gestation day (GD) 1 to GD 3 showed an increased number of blastocysts with dead cells in embryos extracted from dams exposed at 0.009 mg/kg/day (Sefcikova *et al.*, 2018). The toxicological significance of these data is unknown, as the animals were not followed to parturition. Furthermore, a certain level of cell death is expected during the blastocyst stage of rodent and human embryo development. The level of cell death that might affect reproductive outcomes or development is unknown (Hardy and Spanos, 2001; Sefcikova *et al.*, 2018).

VII.A.2. Subchronic Point of Departure

Support for the critical subchronic critical POD of 0.02 mg/kg/day based on decreased serum T4 levels

Although the subchronic POD was derived from a chronic rat study, the effects were noted within a subchronic duration (e.g., within 1 year of exposure) (Aughton, 1993). Confidence in this determination was based on the several factors. First, T4 decreases were sustained (from week 1 until the end of the study), they were dose-dependent, and carried pairwise statistical significance at the LOEL of 0.06 mg/kg/day dose above. Second, short-term thyroid hormone deficiencies in human adults are unlikely to be harmful. However, sustained decreases in T4 in adult rats are toxicologically relevant to humans as they can generate clinical manifestations of hypothyroidism including lethargy, hyporeflexia, poor motor coordination, and memory impairment (Bernal, 2000). Therefore, decreases in T4 alone are sufficient for establishing a critical endpoint.

Additional support for the critical subchronic POD from observations of convulsions and death in adult male rats

Convulsions and death occurred in one male at week 23 at the LOEL of 0.06 mg/kg/day. At necropsy, this animal was identified with a brain neoplasm, raising the possibility that the

convulsions and death were caused by a CNS neoplasm. DPR reexamined the study database to determine if there was a correlation between brain neoplasms and convulsions throughout the entire dose range and duration of the study. As is detailed in Table 47 below, there were 3 instances of co-occurrence, 3 instances of CNS tumors alone, and 9 instances of convulsions alone in males. In females, all CNS neoplasms occurred in controls or low-dose animals (0.03 mg/kg/day; n=3), while all instances of convulsions occurred at the mid-high dose (1.6 mg/kg/day; n=3) or high dose (17 mg/kg/day; n=12). In other words, no absolute statements are possible regarding an etiologic role for CNS tumors in the genesis of convulsions in the week 23 male (or in the other 2 males in the Aughton study where convulsions and CNS tumors co-occur). Fipronil is an overt convulsant through GABA-gated chloride channels, and therefore may have caused the convulsions in the week 23 male at 0.06 mg/kg/day. Not only was there a single male that died following subchronic treatment, two additional males exhibited convulsions at the same dose later in the study, and 12 more animals (male and female) exhibited convulsions at the next higher dose, five of which died. This is valid support for the designated subchronic POD of 0.02 mg/kg/day.

Table 47. Incidence of CNS tumors and convulsions in the 2-year chronic toxicity study of Aughton (1993)

Animal ID	Sex	Dose (mg/kg/day)	CNS tumor	Date tumor detected	Convulsions?	Time of convulsions (weeks)
121	M	0.06	oligodendroglioma	week 23	yes	23
145	M	0.06	n/a	n/a	yes	61
127	M	0.06	astrocytoma	week 71	yes	69
177	M	1.3	n/a	n/a	yes	60
171	M	1.3	astrocytoma	week 67	no	n/a
202	M	13	n/a	n/a	yes	1
224	M	13	n/a	n/a	yes	1
237	M	13	n/a	n/a	yes	1
238	M	13	n/a	n/a	yes	1
638	M	13	n/a	n/a	yes	1
207	M	13	astrocytoma	week 63	yes	1
645	M	13	n/a	n/a	yes	3
627	M	13	n/a	n/a	yes	51
248	M	13	astrocytoma	week 86	no	n/a
222	M	13	spinal chordoma	week 87	no	n/a
274	F	0	astrocytoma	week 44	no	n/a
264	F	0	astrocytoma	week 80	no	n/a
345	F	0.02	astrocytoma	week 70	no	n/a
764	F	1.6	n/a	n/a	yes	1
433	F	1.6	n/a	n/a	yes	55
446	F	1.6	n/a	n/a	yes	21, 30, 32, 37
787	F	17	n/a	n/a	yes	1

Table 47. Incidence of CNS tumors and convulsions in the 2-year chronic toxicity study of Aughton (1993)

Animal ID	Sex	Dose (mg/kg/day)	CNS tumor	Date tumor detected	Convulsions?	Time of convulsions (weeks)
478	F	17	n/a	n/a	yes	54
453	F	17	n/a	n/a	yes	57
456	F	17	n/a	n/a	yes	57
482	F	17	n/a	n/a	yes	63
487	F	17	n/a	n/a	yes	68
480	F	17	n/a	n/a	yes	83
496	F	17	n/a	n/a	yes	23, 31
470	F	17	n/a	n/a	yes	35, 38
492	F	17	n/a	n/a	yes	42, 57, 61, 62
455	F	17	n/a	n/a	yes	56, 61
499	F	17	n/a	n/a	yes	59, 72, 78

The total number of animals in each dose group was 50.
M: male; F: female: n/a: not applicable (no tumor detected).
Data from Aughton (1993).

Additional empirical support for the critical subchronic POD

As noted in the Hazard Identification section, three other studies generated subchronic PODs of similar magnitude to the critical value, increasing the strength of the critical POD determination.

- A subchronic NOEL of 0.05 mg/kg/day based on delayed preputial separation and decreased startle response in rats at 0.9 mg/kg/day was established in the developmental neurotoxicity study (Mandella, 1995). However, the significant dose gap between the NOEL and LOEL may be consistent with a higher NOEL value.

- A subchronic $BMDL_{10}$ of 0.05 mg/kg/day based on hepatic periacinar hypertrophy was set in the 13-week mouse study (Broadmeadow, 1991). Although the primary function of liver is to respond to xenobiotic load, this effect was constantly observed in repeat-dose studies with fipronil.

- A subchronic NOEL based on urine found in the FOB observation area (suggestive of autonomic dysfunction) of 0.03 mg/kg/day in rats (Driscoll and Hurley, 1993). However, this value was based on an indirect observation rather than a direct observation in the experimental animals.

VII.A.3. Chronic Point of Departure

The critical chronic oral POD of 0.02 mg/kg/day was derived from a rat chronic dietary study.

The critical chronic oral POD of 0.02 mg/kg/day was based on convulsions and mortality, sustained decreases in T4, and increased progressive senile nephropathy at 0.06 mg/kg/day (Aughton, 1993). There were, however, several effects were observed at the study NOEL. These effects included decreased serum albumin, increased absolute liver, thyroid and spleen weights, and increased incidence of progressive senile nephropathy. The data underlying these observations were insufficiently robust to support critical LOEL designation.

Because inhalation and dermal toxicity studies with chronic duration were not available, the chronic dermal and inhalation toxicity were gauged using the chronic oral POD. This decision carries uncertainties similar to those accompanying the acute and subchronic route extrapolation.

VII.A.4. Developmental Toxicity Endpoints

PODs from developmental toxicity studies were not used as critical values in the characterization of acute risks to humans.

Developmental toxicity studies typically employ a repeated dose regimen. However, they are potentially useful for identifying acute PODs, either when the findings are reported shortly after the onset of exposure or when there are developmental effects that could have resulted from a single or short-term exposures. Consistent with this proviso, DPR concluded that the PODs from the available developmental studies were not appropriate for use as critical values in the characterization of acute risks to humans (see explanation above). However, they were applicable both for characterizing subchronic exposures (DNT study) or were useful in the overall weight of the evidence analysis of fipronil-induced toxicities (CTA, developmental and reproductive studies in rats and rabbits.

Five registrant-submitted studies evaluated the potential effects of fipronil on development. These included developmental studies in rats and rabbits, a reproductive toxicity study in rats, a DNT study in rats and a CTA in rats (Mandella, 1995; Coder, 2019). The effects reported in these studies included decreased T4 levels in fetuses and increased liver weight in pups, decreased body weights and convulsions in pups, reduced pup viability, decreased litter size, dead/missing pups, decreased startle response and developmental delays.

In studies from the published literature, effects that could be considered developmental or reproductive in nature (i.e., sperm with altered motility, increased incidence of dead cells in blastocysts) had unclear toxicological meaning (de Barros *et al.*, 2016a; Sefcikova *et al.*, 2018).

The DNT and CTA studies evidenced developmental effects at doses lower than maternal effect. Delayed preputial separation was observed after 25 doses *in utero* and via lactation (LOEL of 0.9 mg/kg/day and NOEL of 0.05 mg/kg/day (Mandella, 1995). This effect was likely related to the reduced body weights of the male pups, and thus required repeated exposues. In the CTA, decreased thyroid hormone levels were measured in GD 20 fetuses after 15 days of *in utero* exposure (LOEL of 1 mg/kg/day and NOEL of 0.3 mg/kg/day (Coder, 2019). The critical subchronic NOEL of 0.02 mg/kg/day for decreases in T4, convulsions and convulsion-associated deaths in adult rats will be protective against the developmental toxicities seen after repeated

exposures in the DNT study (NOEL of 0.05 mg/kg/day) and in the CTA (NOEL of 0.3 mg/kg/day). However, fetal T4 levels were first measured in the CTA on GD 20. It is conceivable that hormone decreases could have occurred after a single or a few *in utero* doses, thus making the NOEL of 0.3 mg/kg/day also applicable to acute exposures. This value is lower than the critical acute oral POD of 0.77 mg/kg/day based on decreased hindlimb splay in rats. DPR did not select the NOEL of 0.3 mg/kg/day as the critical acute oral POD due to uncertainties regarding the reliability of the measured thyroid hormone levels in the CTA (see discussion below in the Thyroid Hormone Measurements section).

VII.A.5. Thyroid Toxicity

PODs based on short-term decreases of thyroid hormone levels in rats were not used as critical values in the characterization of acute risks to humans.

Repeated exposure to fipronil caused thyroid toxicities in rats evidenced by decreased T4 levels, increased thyroid weight and histopathology in adult and developing rats, and thyroid tumors at sufficiently high doses. Sustained T4 elimination is the required first step for all subsequent thyroid pathologies. In adult rats, decreased T4 levels were observed following 1 week of treatment. However, as described in section VII.A.1 above and detailed below, DPR did not select a critical POD based on this effect to estimate acute risks to humans.

Decreased Thyroxine in Male Rats after Short-term Exposure

Decreased thyroxine (T4) levels were observed at 1.6 and 0.06 mg/kg/day in female and male rats respectively after one week of exposure in a combined chronic and oncogenic study (Aughton, 1993). Because the exposure duration was short-term, DPR examined if this effect could be used for setting the critical acute POD.

The decreased T4 in male rats occurred at a treatment level 40x lower than the BMD_{10} (equivalent to the LOEL) for decreased hindlimb splay from the acute neurotoxicity study (i.e., 2.09/0.06 mg/kg/day). This is concerning because neurological and developmental effects can result from prenatal (maternal) or perinatal hypothyroidism during critical developmental periods (DeLong and Adams, 1991). Thyroid hormone deficiency, even of short duration, during specific timing in development can lead to irreversible brain damage (Bernal, 2000). Numerous studies have reported deficits in cognition (decreased IQ scores or similar measures) in children with decreased neonatal thyroid hormone levels, despite maternal levels that are within the traditional "normal" reference ranges (OEHHA, 2015). Therefore, short-term changes in circulating thyroid hormone levels can be used in risk assessment to serve as an upstream indicator of possible developmental effects in humans, even without histopathological findings (Woodruff *et al.*, 2008; EFSA, 2018). However, a careful weight-of-evidence analysis indicated that the decreased T4 levels seen in adult male rats at 0.06 mg/kg/day were not an appropriate surrogate for decreased T4 levels in pregnant females or developing animals. The decision was justified by the following considerations:

- Reductions in T4 levels were measured in pregnant females after 15 days of fipronil administration and after one week in non-pregnant females. This effect occurred at similar dose levels: the LOEL for pregnant dams was 3 mg/kg/day (Coder, 2019) and the LOEL for non-pregnant females was 1.6 mg/kg/day (Aughton, 1993).

- In the comparative thyroid assay (CTA), reductions in T4 levels were measured in GD 20 rat fetuses and PND 4 pups at 1 and 3 mg/kg/day, respectively, after 15 to 20 days of exposure (Coder, 2019). These doses are similar to those that caused T4 decreases in pregnant and non-pregnant females (3 and 1.6 mg/kg/day, respectively) (Aughton, 1993; Coder, 2019).

- An acute study in male Long-Evans rats showed decreased T4 following a gavage dose of 10 mg/kg fipronil, with no effect measured at 5 mg/kg (Moser *et al.*, 2015). In contrast, adult male CD rats appeared to be more sensitive to fipronil with a LOEL for T4 decreases of 0.06 mg/kg/day (Aughton, 1993). Regardless, short-term thyroid hormone deficiencies in adults are unlikely to be harmful (Bernal, 2000).

- Humans are likely to be less sensitive to changes in circulating thyroid hormone levels than rats, as they possess better thyroid hormone storage and buffering capabilities (US EPA, 1998; International Agency for Research on Cancer, 1999).

In conclusion, the critical acute oral POD of 0.77 mg/kg/day is considered protective of acute, treatment-related changes in thyroid hormone.

Thyroid Hormone Measurements

Because thyroid hormone levels play an important role in the hazard identification for fipronil, metrics used to assess the quality of thyroid hormone data are described herein. To be considered adequate for risk assessment, thyroid hormone effects must be dose-dependent and significant. In addition, the presence or absence of related effects, including changes in thyroid organ weight or histopathology, is considered. The magnitude of change in thyroid hormones should be ≥ 20% in either direction to be considered a robust effect, as this would be outside the range of normal variation (US EPA, 2011e). In addition, the coefficient of variation (the standard deviation divided by the mean) for each exposure group should be less than 25–30% for T3 and T4 (US EPA, 2011e; Li *et al.*, 2019). Finally, the mode of action by which thyroid parameters are altered and the biological significance of those alterations are also considered.

A dose-dependent and significant 24% decrease in T4 levels occurred in male CD rats following one week of daily (oral) fipronil exposure at 0.06 mg/kg/day in the combined chronic and oncogenicity study in rat (Aughton, 1993). The coefficient of variation for this dose was 33%, which was just outside the recommended range of 25–30%. Because this effect was observed after just one week of fipronil exposure, it could be considered an acute effect. However, the results were not ultimately used in determining the acute critical POD because decreased T4 levels in adult rats and humans are unlikely to be deleterious in the short term.

In the CTA, decreased T4 levels were measured in dams on GD 20 at 3 mg/kg/day and in fetuses on GD 20 at 1 mg/kg/day, generating NOELs of 1 mg/kg/day (dams) and 0.3 mg/kg/day (fetuses) (Coder, 2019). These effects were significant ($p < 0.05$) and dose-dependent. T4 levels decreased by 19% in the pups and 26% in the dams. The coefficient of variation was 24% for pup data and 26% for dam data. These results provided confirmation that neither toxicologically relevant thyroid hormone decreases nor thyroid-dependent overt developmental impacts were likely to occur in rats at or below the fetal NOEL of 0.3 mg/kg/day. Because T4 levels were first measured after 15 consecutive in utero doses, the exact timeline of the hormone decreases in fetuses is not known. Therefore, the results from the CTA study could be used for setting an acute critical POD to protect against possible developmental effects in humans.

DPR's analysis unveiled issues with the reliability of the measured thyroid hormone levels in the CTA. Importantly, many of the individual data points for hormone values were not within the ion ratio tolerance established by the investigator. The T3 or T4 ion ratio is equivalent to the primary ion peak divided by a second, confirmatory ion peak. The study-calculated ion ratios labeled as sample were not within the tolerance. In addition, a total of 286/855 T4 measurements (33%) were not within the tolerance with the rate ranging from 0 to 88.2% for T4 measurements in different exposure groups. The highest percent fail in T4 data was in negative control samples. Numerous T3 measurements also failed.

While the methods and data were certified by the study quality assurance representative, the failed ion ratio observations were not mentioned in the study report. DPR later learned that US EPA requested an additional thyroid hormone quality measure for this study which was not part of the CTA protocol. The conducting laboratory performed the ion ratio analyses in which primary and confirmatory samples were both analyzed and the resultant data were compared. The conducting laboratory used an analytical calibration curve plot to derive a curve mean and ± 20% tolerance. Sample ion ratios that fell outside that range were not within tolerance. It is possible that this method was too stringent, as the tolerable range was derived from a calibration curve instead of biological specimens that tend to be more variable than standards. Many of the failed samples were just outside of the tolerable range (e.g., 20.3% instead of 20%). There was no apparent correlation between percent fail and population (dams or fetuses) or exposure groups.

Despite the uncertainty regarding the reliability of the measured thyroid hormone levels in the CTA, effects such as increased thyroid weight and histopathology observed at higher doses suggested that the changes in blood thyroid hormone levels were representative of potential physiologic or pathologic change. For purposes of this assessment, data flagged as outside of tolerance increased the level of uncertainty of the toxicological significance of the data. Therefore, while there was qualitative value to the study results, the analytical integrity precluded the use of the acute results from the CTA to derive a quantitative acute POD.

Hepatic-Induced Thyroid Toxicity

Fipronil caused thyroid toxicity but did not act directly on the thyroid. Rather, it induced hepatic (liver) metabolism, which led to an increase in elimination of T4. This caused hyper-stimulation of the thyroid, increased thyroid weight, hypertrophy and hyperplasia, and, at sufficiently high doses, thyroid tumors (Peters *et al.*, 1991b; Peters *et al.*, 1991a; King, 1992; Aughton, 1993; Coder, 2019).

Direct toxicity to the liver was observed in registrant-submitted and published studies in rat, mouse, and dog and in one published case study with a human who ingested fipronil to self-harm (Bharathraj *et al.*, 2015). The effects (hepatocyte damage, lipid accumulation, increased oxidative stress, increased absolute and relative liver weights, changes in blood chemistry parameters, altered transport and metabolism, and mitochondria dysfunction) are similar to symptoms that characterize nonalcoholic steatohepatitis or fatty liver disease in humans. Fatty liver disease compromises liver function and can result in additional disease and death (McCullough, 2006; Fisher *et al.*, 2008; Hardwick *et al.*, 2010; Canet *et al.*, 2014; National Institute for Health and U.S. National Library of Medicine, 2015).

One published study showed that fipronil binds *in vitro* and *in silico* to human serum albumin and competitively inhibits the binding of a heme-Fe(III) complex (Ascenzi *et al.*, 2018). This could reduce the direct toxicity of fipronil and/or impair binding of endogenous molecules such as T4, which could increase hepatic clearance of T4.

VII.A.6. Route-Specific PODs

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

DPR uses route-specific critical endpoints whenever appropriate. However, if a POD is based on target organ or tissue toxicity monitored by one exposure route (usually oral) but not another (dermal or inhalation), that endpoint may be used to establish critical values for the other routes, as recommended by the US EPA (Rowland, 2008). For fipronil, oral PODs were used to characterize dermal and inhalation toxicity. As stated previously, both dermal and inhalation studies with acute or short-term duration endpoints were available. However, the route-specific studies did not measure the most sensitive endpoints, and, in the case of inhalation, calculation of an internal dose relied on default values that are associated with additional uncertainty. Therefore, the oral POD was used to calculate risk for all routes.

Regardless of the endpoints chosen, DPR recognizes that toxicokinetics are likely different by the different routes, which creates another source of uncertainty. For example, chemicals do not undergo hepatic first pass metabolism by the inhalation and dermal routes, which increases the possibility that inhalation or dermal exposure creates a different set of metabolites. Absorption and distribution can also be different by different routes that may result in different internal doses at the target tissue.

VII.A.7. Oncogenicity

This RCD did not include a cancer risk estimate for the thyroid follicular tumors observed in rats after chronic exposure to fipronil.

As described below, a cancer risk estimate for fipronil was not estimated because fipronil is not mutagenic and because the weight of evidence supports a mode of action for thyroid tumors in rats that is likely not relevant to humans.

Fipronil induced thyroid follicular cell adenomas and carcinomas in CD rats (Aughton, 1993). Because tumor incidence rates at the high dose (13–17 mg/kg/day) were higher than controls by significant margins in both males and females, a fipronil-based etiology is likely. Such data indicating oncogenesis usually trigger a quantitative cancer analysis using multistage linear extrapolation. Evidence supports that fipronil induces thyroid tumors in rats through a process of hypothalamic-pituitary-thyroid axis disruption. This mode of action (MOA) is well-recognized in the rat. However, the same MOA is less relevant to humans due in part to more efficient serum thyroid buffering in humans. Various chemicals including phenobarbital and propyzamide operate by this MOA in rats. The process starts with hepatic enzyme induction, followed by enhanced T4 metabolism and clearance, decreased negative feedback on the hypothalamus, and consequent increased TSH secretion by the pituitary gland. Sustained elevations in TSH in turn causes follicular cell hypertrophy, hyperplasia and, ultimately, neoplasia (Papineni *et al.*, 2015).

To determine if chemically-induced thyroid tumorigenesis operates through this pathway, fulfillment of a specific set of requirements is recommended (US EPA, 1998). The evidence in rats for each is listed below.

1. Increased cellular growth: Increased liver weights occurred in at least nine registrant-submitted studies at doses as low as 0.3 mg/kg/day in rat pups in the CTA (Coder, 2019) and at 0.06 mg/kg/day in adult rats in the chronic mouse study (Broadmeadow, 1993). Thyroid cell hypertrophy was detected in rats at as low as 1.7 mg/kg/day (Holmes, 1991a; Aughton, 1993; Peters, 1996).

2. Hormonal changes: Decreased T4 levels were the most sensitive endpoint in the two registrant-submitted studies that measured thyroid endpoints (Aughton, 1993; Coder, 2019). Decreased T4 was also observed in rats in several published studies (Leghait *et al.*, 2010; Moser *et al.*, 2015). Increased TSH levels were observed in rat (Roques *et al.*, 2013), and increased T4 clearance was observed in rats and sheep (Leghait *et al.*, 2010; Roques *et al.*, 2012).

3. Site of action: Increased hepatic metabolism leading to increased T4 clearance from blood plasma was observed in rats following fipronil administration (Peters *et al.*, 1991b; Leghait *et al.*, 2009). These observations indicated strong liver involvement. In addition, fipronil induced hepatic enzymes involved in thyroid hormone clearance, including UDP-GT, CYPs, carboxylesterases, and membrane transport proteins in rats (Roques *et al.*, 2012; Roques *et al.*, 2013) and in human cell cultures (Das *et al.*, 2006). Fipronil also induced hepatic cytochrome P450 levels and activity in rats and in human cells (Tang *et al.*, 2004; Caballero *et al.*, 2015) and upregulated CYP mRNA and long non-coding RNA

in human cell cultures (Mitchell *et al.*, 2016; Mitchell *et al.*, 2017). Both human and rat liver microsomes metabolize fipronil, although the evidence suggests that rats have a higher intrinsic clearance of fipronil than humans (Tang *et al.*, 2004). High fipronil clearance would decrease the negative feedback on the pituitary through the hypothalamus, leading to sustained TSH stimulation of the thyroid and consequent tumor formation. Thus, the weight of evidence suggests that with its enzyme induction and increased metabolism, the liver is the primary target organ. Altered thyroid function is secondary to the primary effects on the liver.

4. Dose correlations: Induction of rat thyroid tumors occurred at doses also associated with the primary liver-mediated effects noted above (i.e., 13 to 17 mg/kg/day for males and females, respectively) (Aughton, 1993). While liver effects also occurred at a lower dose, the data suggest that consequent changes in thyroid hormone concentrations were likely insufficient to support tumorigenesis.

5. Reversibility: Effects on thyroid hormone levels in the rat combined chronic/ oncogenicity study were reversible; T4 levels rose and TSH levels declined after fipronil was removed from the diet (Aughton, 1993). If a chemical works through hypothalamic-pituitary-thyroid axis disruption, levels of thyroid-pituitary hormones return to normal after cessation of chemical dosing, decreasing the possibility of tumor induction.

In conclusion, the weight of evidence suggests that fipronil-induced hepatic clearance of serum T4 is a necessary precursor to the development of thyroid tumors in rats. Such a process is unlikely to be operative in humans because humans buffer thyroid hormone changes more efficiently. Nevertheless, the critical chronic POD of 0.02 mg/kg/day, which is based partly on the precursor event for tumors at 0.06 mg/kg/day, should be protective of any possible tumor formation in humans.

VII.A.8. Estimate of Oral Absorption

This assessment assumes that the majority of orally administered fipronil is absorbed though the gut and reaches the general circulation, resulting in equivalent administered and internal doses. However, because ~50% of the dose is excreted in the feces, combined with the confirmation of enterohepatic recirculation, systemic absorption could be lower because a major fraction of the dose may not reach the general circulation. If true, the actual oral POD could be lower than the critical POD cited in this document (i.e., fipronil would be more toxic) by a factor determined by the absorption rate. In calculating human risk from oral exposure, as long as the assumed oral absorption rate assumption (100%) is the same as that used for calculating human exposure by this route, oral MOE would not be affected. Similarly, the dermal MOE would accurately represent dermal risk because the human dermal exposure reflected an experimentally verified 4.3% absorption rate. However, there is uncertainty in determining risk from the inhalation route since the percent absorption in humans (Jiang, 2022) has not been verified.

VII.A.9. Toxicity of Fipronil Metabolites and Degradates

This assessment evaluated the human risk from exposure to the parent compound fipronil only. However, the toxicological properties of fipronil depend not only on the parent, but also on its bioactive sulfone and sulfide metabolites, and the desulfinyl photoproduct. The effects of the fipronil derivatives are particularly relevant in cases when exposure to these compounds occurs independent of exposure to the parent. This is the case for exposure from drinking water where the sulfone, sulfide and amide metabolites, and the photodegradate were detected separately in the surface water in California (Jiang, 2022). Therefore, a complete picture of the potential health risks of fipronil would include not only toxicity data on fipronil and its derivatives, but also their potential for human exposure, especially as dietary tolerances include those derivatives. But because exposure estimates for the derivatives are limited, total risk from exposure to fipronil-plus-derivatives may be underestimated in this assessment. Additional information on fipronil metabolites and degradates can be found in Appendix II.

VII.B. Exposure Appraisal

VII.B.1. Handler, Home User, and Residential Post-Application Exposure Appraisal

The exposure assessment used the best information available at the time of this assessment. When possible, fipronil specific data were used to analyze identified exposure scenarios using registered fipronil products, specific application methods, and specific user groups. However, for scenarios that lacked fipronil specific information, either default values suggested by standard US EPA or DPR practices (Beauvais *et al.*, 2007; US EPA, 2012c) or surrogate data from pesticides with physiochemical properties and application methods similar to fipronil were used. Scenarios which employed surrogate data included handler scenarios for structural liquid concentrate and turf products, post-application scenarios for structural bait gel and bait strip products, and applicator scenarios for structural dust products. In so doing, uncertainties may have been introduced into the analysis including the potential for overestimation of certain handler exposures. Steps were taken to provide reasonable worst-case scenarios based on legal label. This provides a conservative, and therefore protective, approach to the exposure estimations.

Of the almost 6000 human health illness reports analyzed, approximately 80% were associated with the use of pet products. DPR only evaluates legal label uses for its exposure scenarios. It is not known how many of the illnesses may have been due to illegal use or use that did not adhere to label instructions. It is important to follow all manufacturers' instructions on the label in order to reduce potential exposure. However, because of the large number of illness reports associated with pet products, the exposure and risk from home use of these products were carefully evaluated.

Home use of flea and tick products for companion animals can potentially lead to inhalation exposure, dermal exposure, and/or incidental oral exposure. The low vapor pressure of fipronil (2.8×10^{-9} mmHg at 25°C) reduces the risk of inhalation of spot-on products for home users and reduces inhalation risk for children and adults following use of either spot-on or spray products. To assess dermal and incidental hand-to mouth exposures associated with home use, DPR used

label-recommended monthly applications to companion animals. A significant level of radiolabeled fipronil was detected in the stratum corneum and viable epidermis of the application zone (neck) of beagle dogs up to 56 days post-application (Cochet *et al.*, 1997). This persistence combined with regular 30-day applications may underestimate dermal and incidental hand-to-mouth exposures. However, since the transferable (e.g., available) amount of fipronil on companion animals rapidly decreases within 28 days after treatment, there is a lowered risk of longer term exposure (Jiang, 2022). Even so, estimates of post-application object-to-mouth exposures can be further refined to reflect more accurate multi-day or multi-episode exposure profiles to reflect product-specific retreatment intervals (US EPA, 2012c).

Additional information on how specific data gaps were addressed and the confidence in the exposure analysis and methodologies for fipronil are found in the Exposure Assessment Document (Jiang, 2022).

VII.B.2. Dietary Exposure Assessment Appraisal

Uncertainties in the exposure to fipronil residues in food were introduced with the use of analytical limits of detection (LOD) or tolerances as surrogates for pesticide monitoring residue concentrations. While thousands of food commodity samples have been screened for fipronil residues, only a small number showed positive detections (0.35% of PDP samples; 2007–2017). Importantly, the input residue values for all commodities identified as major contributors to the acute dietary exposure were based on LOD values. Additional uncertainty arose from the assumption that fipronil exposure only results from the consumption of foods based on commodities with tolerances. That is, no illegal uses of fipronil in food crops were included in this analysis.

Uncertainties were also associated with the consumption database embedded in the current DEEM-FCID™ (version 4.02), which is based on the NHANES 2-day food consumption survey data for 2005 through 2010. A consumption survey from recent years would more accurately represent the population's consumption patterns.

Finally, the acute dietary exposure was estimated using a Tier 2 point-estimate analysis, which employs tolerance or upper bound measured residues. These assumptions are conservative, as it is unlikely that all of the commodities consumed in a given day will contain fipronil residues at the highest legally allowed or measured level. Since the resulting exposures produced MOE levels above the DPR screening MOE targets (500 and 1000 at the 95^{th} and 99^{th} percentiles, respectively), further refinements were not performed.

VII.C. Other Regulatory Agencies

United States Environmental Protection Agency (US EPA)

The US EPA completed a Draft Risk Assessment for Registration Review in 2020 (US EPA, 2020). It revised and updated regulatory values from a previous 2015 memorandum, 2011

scoping document, and 2007 human health risk assessment (US EPA, 2007; US EPA, 2011c; US EPA, 2015). The most significant changes include:

- Removal of an FQPA safety factor applied to short- and intermediate-term oral exposure for lack of thyroid hormone data.
- Reduction of the interspecies extrapolation factor from 10 to 3 for short- and intermediate- duration exposures that were based on thyroid hormone endpoints.
- Revised short- and intermediate-term POD.
- An increase in the POD value for the developmental neurotoxicity study in rat.

The US EPA (2020) acute reference dose (aRfD) for acute dietary exposure was 0.025 mg/kg/day, based on effects in the acute neurotoxicity study in rat with total uncertainty/safety factors of 100x (US EPA, 2020). The chronic reference dose (cRfD) for chronic dietary exposure was 0.0002 mg/kg/day, for effects in the combined/oncogenicity study in rat, with total safety factors of 100x. US EPA also developed levels of concern for short-, intermediate- and long-term incidental oral, dermal and inhalation exposure (US EPA, 2020).

European Food Safety Authority (EFSA)

In 2006 the European Food Safety Authority (EFSA) published their conclusions on a draft human health risk assessment prepared for proposed uses of fipronil (EFSA, 2006). The Acceptable Daily Intake (ADI) of 0.0002 mg/kg/day was based on the no observed adverse effect level (NOAEL) of 0.02 mg/kg/day in the rat oncogenicity study with the EFSA standard safety factor of 100. An aRfD of 0.009 mg/kg/day was derived from the developmental neurotoxicity study in rat with a developmental NOAEL of 0.9 mg/kg/day, with the EFSA standard safety factor of 100. The assessment concluded that no genotoxic or oncogenic potential was demonstrated, and that the induction of thyroid follicular cell tumors is specific to rat and not considered relevant to humans.

French Food Health Safety Agency (AFSSA)/ French Environmental Health Safety Agency (AFSSE)

In 2005 the French Food Health Safety Agency (AFSSA) and French Environmental Health Safety Agency (AFSSE) completed a human health risk assessment on fipronil (Agence Francaise de Securite Sanitaire des Aliments, 2005). The ADI of 0.0002 mg/kg/day was based on the NOAEL of 0.02 mg/kg/kg/day in the chronic rat study with 100x safety factors. An aRfD of 0.009 mg/kg/day was based on a NOAEL of 0.9 mg/kg/day for neurobehavioral effects in offspring and reduced body weights and food consumption in dams in a developmental neurotoxicity study in rat, with safety factors of 100x. This aRfD replaced a previous aRfD of 0.003 mg/kg/day. The assessment concluded that the available experimental data do not give reason to suspect oncogenic risk from fipronil in humans, as the thyroid follicular tumorigenesis observed in rats can be explained by a rat-specific biological mechanism.

Australian Pesticide and Veterinary Medicine Authority (APVMA)

In 2003, the Australian Pesticide and Veterinary Medicine Authority (APVMA) initiated a risk assessment for fipronil following receipt of 73 adverse reports in humans and animals involving fipronil veterinary products from 1997 to 2006 (APVMA, 2011). The current Australian ADI for fipronil is 0.0002 mg/kg/day (set in June 1994). It was derived from the NOEL of 0.02 mg/kg/day in the chronic/oncogenicity rat study with safety factors of 100x. The ADI includes the parent compound and metabolites. The Australian aRfD of 0.02 mg/kg is based on a NOEL of 2.5 mg/kg for decreased landing foot splay in an acute oral neurotoxicity study in rats (APVMA, 2011). The aRfD incorporates a safety factor of 100x.

VIII. CONCLUSION

The purpose of this risk characterization document was to evaluate the human health risks associated with fipronil exposure.

Data submitted by registrants, published in scientific journals, and reported by other regulatory or scientific bodies were reviewed for toxicological endpoints in mammals. Critical acute, subchronic, and chronic points of departure determined from these studies were combined with human exposure estimates to characterize risks from handler and residential exposure, as well as from food and drinking water. Oral and dermal acute and chronic reference doses were also developed as target exposure levels for adults and children.

All of the critical points of departure for fipronil were based on effects observed in oral toxicity studies in rats and mice. Non-oncogenic risks were calculated as margins of exposure, a ratio of the critical points of departure to the estimates of exposure. An analysis of the uncertainties inherent in this risk characterization resulted in designation of 100 as the target for all scenarios evaluated. The target margin of exposure is the product of an interspecies uncertainty factor (UF_A) of 10 and an intraspecies uncertainty factor (UF_H) of 10. Values falling below the target of 100 are considered to pose a potential health risk.

Risks from single and multiple, or aggregate, exposure scenarios were calculated for both handlers, home users, and adults and children exposed post-application. Although considered a contributor in certain scenarios, DPR did not include food or drinking water exposures in the aggregate risk calculations because of the inconsequential change to overall risk. Likewise, oncogenic risk was not calculated. The chronic POD is based on an upstream non-oncogenic effect (decreased T4) at a dose level that is much lower than the level where tumors occurred in rats (0.02 mg/kg/day versus 13 mg/kg/day), and oncogenesis is likely caused by an extra thyroidal mechanism that is not relevant to humans. Therefore, the chronic POD is considered protective of cancer in humans.

Handler margins of exposure considered to pose a potential health risk include:

- Short-term dermal exposure to pet spray products
- Seasonal exposure to pet spray and spot-on products as well as structural liquid concentrate and turf granules
- Annual exposure to pet products and liquid concentrate

Scenarios that pose a potential risk to home users include:

- Acute dermal exposure for users who apply pet spray at home

Post-application residential exposures for adults considered to pose a potential health risk include:

- Seasonal exposure to pet spray products

Post-application residential exposure for children considered to pose a potential health risk include:

- Short-term oral exposure to turf granules
- Seasonal exposure to spot-on products
- Seasonal dermal and oral exposure to pet spray products

Reference doses (RfDs) were calculated using total uncertainty factors (UF) of 100, which included 10x for interspecies extrapolation and 10x for intrahuman variability. The acute oral RfD was 0.008 mg/kg. The acute dermal RfD was 0.18 mg/kg (adjusted for 4.3% absorption). The subchronic and chronic oral RfDs were 0.0002 mg/kg/day. The subchronic and chronic dermal RfDs were 0.005 mg/kg/day (adjusted for absorption).

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APPENDIX I. FIPRONIL SYSTEMATIC LITERATURE REVIEW

Specific Aims

- Conduct literature searches and use systematic review methods to identify studies published until June 16, 2022, that are relevant to understanding the potential human health hazards of fipronil as outlined in the PECO (population, exposure, comparator, and outcome) criteria.
- Track potentially relevant supplemental material, including mechanistic evidence informative of mode of action (MOA), potential genotoxicity, human illness reports, epidemiology studies, and ADME information. Studies conducted in non-mammalian model systems (*i.e.*, nematodes and fish) are not included at this time.

Methods and Results

Database Searches

On June 16, 2022, a literature search was performed in PubMed (www.ncbi.nlm.nih.gov) using the common pesticide name "fipronil" as the key word (because names for fipronil derivatives all include the word "fipronil", this search also found publications on metabolites and on the photodegradate). The resultant studies (1624) were exported from PubMed as a .csv file and imported into Microsoft Excel. Twelve additional papers not referenced in PubMed were identified in the fipronil references and were added to the list. Five more articles were suggested for consideration by OEHHA and underwent rigorous review. This search superseded previous systematic literature reviews (SLRs).

Level 1. Title and abstract screen

Article titles and abstracts were screened for relevance to the fipronil PECO (Populations of interest, Exposures, Comparators, and Outcomes) criteria (Table I.1).

Table 48. PECO Criteria Used for the Fipronil Systematic Literature Review

PECO Element	Evidence
P (population)	**Human:** Any population and life stage (occupational or general population, including children and other sensitive populations). **Animal:** Nonhuman mammalian animal species (whole organism) of any life stage (including preconception, in utero, lactation, peripubertal, and adult stages).
E (exposure)	**Relevant forms and synonyms:** Fipronil-sulfone Fipronil-sulfide Fipronil-desulfinyl **Routes:** I. Oral (gavage or dietary) A. Acute or short-term (up to one week): LOEL under 7.5 mg/kg-

Table 48. PECO Criteria Used for the Fipronil Systematic Literature Review

PECO Element	Evidence
	day or NOEL under 3 mg/kg-day B. Subchronic or chronic: LOEL under 0.5 mg/kg-day or NOEL under 0.1 mg/kg-day II. Inhalation: Any III. Dermal: Any
C (comparator)	**Human:** A comparison or referent population exposed to lower levels (or no exposure/exposure below detection limits), or exposure for shorter periods of time. *Case reports and case series will be tracked as "potentially relevant supplemental information."*
	Animal and Other: A concurrent control group exposed to vehicle-only treatment or untreated control.
O (outcome)	All health outcomes.

Three members of HHA staff decided whether each article was relevant, not relevant, potentially relevant supplemental material, or unclear based on the information provided in the publication title and abstract. Only references marked "relevant", "potentially relevant" or "unclear" proceeded to the next screening phase. At least two people independently reviewed each abstract. During the level 1 screening, publications categorized as "relevant" or "potentially relevant" and forwarded for higher levels of review; publications categorized as "not relevant" were determined not to contain data relevant to the effect of fipronil on human health and were excluded from the further analysis, and the reasons for exclusion are summarized in Table I.2. The reasons for exclusion are useful in case publication's relevance is reconsidered in the future for other purposes.

Table 49. Reasons for Exclusion Used at the Level 1 of the Fipronil Systematic Literature Review

Reason for Exclusion	Description of reason
Environmental Fate/Risk	Studies focused on environmental fate or environmental risk of fipronil
No Exposure	Exposure data were not provided/available
Foreign Language	Study was not available in English*
Application/Efficacy	Study was focused on efficiency of fipronil for anti-flea and tick treatment, or for application methods
Derivatives	Compound studied was not fipronil or relevant derivatives
Removal	The study focused on methods of fipronil removal from waste/surface water
Non-Mammalian	Model organism was not mammalian (*i.e.*, birds, fish, amphibians, crustaceans, reptiles, worms)
Review/Editorial/Commentary	Publication without original data or analysis
Target Organism	Study of effects on target organisms (*i.e.*, insects, arachnids, or fungi)

Table 49. Reasons for Exclusion Used at the Level 1 of the Fipronil Systematic Literature Review

Reason for Exclusion	Description of reason
Detection	The study focused on methods of fipronil detection
Other	Other categories do not apply

*When possible, a google translator was used to translate papers in English

Results: Of the initial 1641 studies, 192 were identified as potentially relevant for human health risk assessment and underwent a full text review. Of these 192 papers, 73 were later found not to meet stringent criteria for research quality or RCD relevance (see details below) during the full text screening. Information reported in these 73 studies was not included into the RCD or the supplementary materials.

Level 2: Full text screening for PECO criteria and supplemental material

Full texts of articles were reviewed in-depth for methodologies and findings. This included the creation of study summaries that described the study design (i.e. animal model, sex, exposure route, doses tested, key endpoints, results, and NOEL/LOEL values (where applicable). For this final SLR, DPR additionally screened potentially relevant articles for experimental design quality, present of necessary controls and appropriate vehicles, percent of active ingredient and purity of compound, and whether the journal that published the article was "predatory". DPR follows established criteria for identifying predatory journals and publishers (Laine and Winker, 2017; Elmore and Weston, 2020). Most reputable biomedical journals are indexed in MEDLINE, the premier bibliographic database for journals on life sciences and biomedicine (https://www.nlm.nih.gov/medline/index.html); DPR scrutinizes and/or flags journals with articles in PubMed that are not also indexed in MEDLINE. Studies that did not meet study quality criteria were tagged with a reason for exclusion as indicated in Table I.3. Articles were screened for the fulfillment of PECO criteria listed in Table I.1, and their potential to inform the selection of critical points of departure. Dates of screening decisions and results were recorded. Because DPR had already reviewed registrant-submitted data, the final PECO criteria were focused to find articles with effects below a certain exposure level (LOEL under 7.5 mg/kg-day or NOEL under 3 mg/kg-day for acute exposure and LOEL under 7.5 mg/kg-day or NOEL under 3 mg/kg-day for subchronic or chronic exposure). Articles not meeting the PECO criteria but containing data that were potentially relevant as supplemental material were tagged with appropriate supplemental information tags (Table I.4) and included in the weight-of-evidence analysis.

Table 50. Reasons for Exclusion Used at the Level 2 of the Fipronil Systematic Literature Review

Reason for Exclusion	Description of reason
Experimental Design	Improper controls or vehicles
Fipronil Purity	Purity of active ingredient 80% or less

Table 50. Reasons for Exclusion Used at the Level 2 of the Fipronil Systematic Literature Review

Reason for Exclusion	Description of reason
Journal Quality	Reporting journal does not meet minimum quality standards, including not indexed in PUBMED or MEDLINE and not in the Directory of Open Access Journals (DOAJ) list
Other	Other categories do not apply

Results: Of the initial 192 studies, 6 were identified as meeting all PECO criteria. An additional 111 studies were identified as containing potentially relevant information and/or contributing to the weight of evidence for the effect of fipronil on human health. Out of these 111 studies, 14 studies were identified as potentially relevant to ADME/PBPK, 3 as potentially relevant to genotoxicity, 11 to epidemiology, 7 to illness reports, and 82 were identified as potentially relevant supplemental mechanistic studies (Table I.4). All 6 PECO studies, one ADME/PBPK and one genotoxicity studies were also included in the list of potentially relevant supplemental mechanistic studies. Relevant information from articles deemed important for weight-of-evidence was recorded and included into the RCD, when appropriate. Results of this review are summarized in Table I.4.

Table 51. Supplemental Information Categories for Fipronil Publications.

Supplemental Material Tag	Number of Publications	Studies Reported
Mechanistic	82	Studies were performed in human or nonhuman mammalian animal species of any life stage (including preconception, *in utero*, lactation, peripubertal, and adult stages), or in a relevant *in vitro* model such as cell lines, primary cultures or protein extracts derived from those species
ADME/PBPK	14	Studies describing absorption, distribution, metabolism or excretion (ADME) of fipronil, as well as physiologically-based pharmacokinetic (PBPK) models
Genotoxicity	3	Studies investigating genotoxicity or mutagenicity of fipronil
Epidemiology	11	Studies describing health-related sequelae in exposed populations
Illness reports	7	Studies describing illnesses or fatalities in humans following fipronil exposure

Level 3: In-depth review and analysis for Point of Departure (POD) designation

Studies identified though PECO screening were reviewed in-depth for methodologies and findings. Data analyses were performed when appropriate, and data suitable for Benchmark Dose (BMD) modeling were identified.
Results: None of the articles identified through PECO screening contributed a POD for the fipronil RCD. However, several were useful for weight-of-evidence analysis and subchronic POD support.

Quality Control

Following the completion of screening review phases, the data and conclusions expressed in prepared review documents were subjected to quality control review to ensure fidelity to the original publications. Every stage of systematic literature review

Summary:

A summary of the systematic review process used for fipronil and the corresponding results is provided in Table I.5. Database search, conducted on June 16, 2022 identified 1624 articles potentially relevant to the fipronil RCD. Seventeen (17) articles were added to the screening list from other sources. Title and abstract screening identified 192 articles potentially meeting fipronil PECO criteria and/or as containing valuable supplementary information. The rigorous screening of article full texts identified 6 articles fully meeting PECO criteria, and additional 111 articles that contained relevant supplementary information. These supplementary information was categorized as fipronil mechanism (82 publications), ADME-PBPK (14 publications), illness reports (7 publications), genotoxicity (3 publications) and epidemiology (7 publications). None of the articles identified through PECO screening contributed a POD for the fipronil RCD.

Table 52. Overview of Systematic Literature Review Results for Fipronil

Level review	Criteria	Objective(s)	Number of Publications
Initial search	Key word “fipronil”	NCBI database search and references	1641
Level 1	Title and abstract reviewed to identify relevance to critical endpoint determinations, mechanism, ADME-PBPK, illnesses, genotoxicity or epidemiology	Review of full texts	192

Table 52. Overview of Systematic Literature Review Results for Fipronil

Level review	Criteria	Objective(s)	Number of Publications
Level 2	Full texts reviewed for PECO criteria relevance	Review of full text, relevant selected	6 potential POD, 117 relevant supplemental information
Level 3	In-depth review and data analysis	Develop POD	0

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Elmore, S. A., and Weston, E. H. 2020. Predatory Journals: What They Are and How to Avoid Them. *Toxicol Pathol* 48:607-610.

Laine, C., and Winker, M. A. 2017. Identifying predatory or pseudo-journals. *Biochem Med (Zagreb)* 27:285-291.

APPENDIX II. FIPRONIL METABOLITE AND PHOTODEGRADATE TOXICOLOGY PROFILE

The overall toxicological properties of fipronil depend on the parent compound and on its derivatives (metabolites and photoproduct, see Figure II.1.) (Hainzl *et al.*, 1998). The effects of the fipronil derivatives are particularly relevant in cases where exposure to a derivative occurs independently of exposure to parental fipronil, such as occurs with the environmental photodegradate. A complete picture of the potential health risks resulting from fipronil exposure would include toxicity data on its derivatives and their potential for human exposure, especially considering that the dietary fipronil tolerances are established for the combined residues of fipronil and three derivatives (two metabolites and a photodegradate). Because exposure estimates for the derivatives are limited, total risks from exposure to fipronil may be underestimated in this assessment.

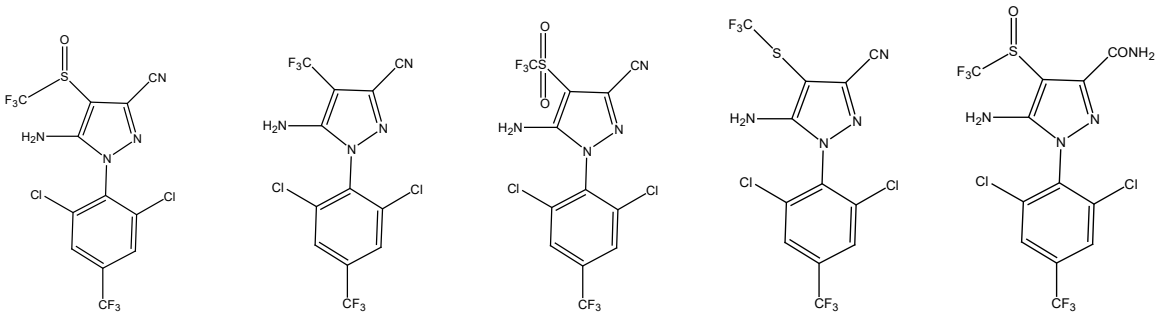

Figure II.4. Chemical Structure of Fipronil, Metabolites and Photoproduct

Fipronil-desulfinyl is the principal photoproduct in plants and soils (Hamon *et al.*, 1996). The database for fipronil-desulfinyl is extensive, consisting of toxicokinetic studies in rats, goats and laying hens, acute studies in rats and mice, subchronic studies in rats, mice and dogs, a combined chronic and oncogenic study in rats, developmental toxicity and acute neurotoxicity studies in rats, a gene mutation study in *Salmonella typhimurium*, an *in vitro* chromosomal aberration study in human lymphocytes, an *in vivo* chromosome aberration study in mice, and a oxidative stress study on mitochondria isolated from rat livers.

Limited toxicity data were available for two of the major Phase I metabolites (fipronil-sulfone and fipronil-sulfide). These metabolites are formed through oxidation. Fipronil-sulfone is the major metabolite in both vertebrates and insects (Hamon *et al.*, 1996). It has been detected in humans as well as rats, goats, hens, rabbits and cows (Powles, 1992; Byrd, 1994; Stewart, 1994a; Stewart, 1994b; Mohamed *et al.*, 2004). Studies available on fipronil-sulfone included an acute study in mice, a gene mutation study in *Salmonella typhimurium*, an *in vitro* chromosomal aberration study in human lymphocytes, and an oxidative stress study on mitochondria isolated from rat livers. For fipronil-sulfide, the database included an acute study in rat and a gene mutation study in *Salmonella typhimurium*.

In addition, a report was submitted by the registrants that compared the toxicity of fipronil to its derivatives (Blacker, 1997).

The toxicology profiles for fipronil-desulfinyl, fipronil-sulfide and fipronil-sulfone are detailed below.

I. Fipronil-Desulfinyl

I.A. Toxicokinetics

I.A.1. Oral – Rat

A study on the kinetics of fipronil-desulfinyl was performed on six different groups of Sprague Dawley rats (Totis and Fisher, 1994). In Groups 1, 2 and 4, 5 rats/sex/group received a single oral gavage dose of [phenyl-U-^{14}C]-fipronil-desulfinyl at 1 or 10 mg/kg. In Group 3, 8 rats/sex received 14 consecutive non-radiolabeled daily doses followed by administration of a single ^{14}C labelled oral dose of 1 mg/kg to 5 rats/sex/group. In Group 5, 7 rats/sex/group received a single oral dose of radiolabeled fipronil-desulfinyl of 1 mg/kg. In Group 6, 3 male rats received a single radiolabeled oral dose at 1 mg/kg.

Recovery of fipronil-desulfinyl was 96.86 ± 3.73%. Elimination was greater in the feces than in the urine. The highest mean fecal excretion rate was in the high-dose (10 mg/kg) animals (70 and 56% in males and females, respectively). In the 1 mg/kg groups, 61 and 46% of elimination occurred via the feces in males and females treated with a single dose, respectively, and 61 and 53% was recovered in feces from males and females, respectively, treated with multiple doses. Mean recovery in urine was 8.8 and 10.7% in single high-dose males and females, 6 and 4% in single low-dose males and females, and 10 and 11% in repeated low-dose males and females.

Mean recovery of radioactivity in tissues after 168 hours post dosing was 20% and 30% in the high-dose males and females, 27 and 41% for the single low-dose males and females and 22 and 32% for the repeated low-dose males and females, respectively. The highest concentrations of radioactivity were present in fat. At the high dose, all tissues had detectable radioactivity. Radioactivity levels were dose-dependent and appeared in relatively high levels in liver, adrenals, skin, fur, intestine and ovaries.

In the blood, the mean C_{max} in the high-dose group was 2.03 ± 0.47 and 2.31 ± 0.90 µg radioactivity equivalents/g in males and females at a T_{max} of 72.53 ± 9.08 and 70.52 ± 8.30 hours, respectively. The elimination half-life was 170.10 ± 21.20 hours and 220.60 ± 55.71 hours in males and females, respectively, with estimated areas under the curve (AUC) of 503.40 and 539.86 µg equivalents/g, indicating comparable bioavailability in both sexes. The C_{max} mean values at the low-dose were 0.14 ± 0.02 and 0.15 ± 0.03 µg equivalents/g, with T_{max} mean values of 45.93 ± 13.63 and 60.65 ± 17.14 hours in males and females, respectively. Elimination half-life mean values were 156.26 ± 17.89 and 209.90 ± 13.75 hours for males and females, respectively, with an AUC of 33.18 ± 5.13 and 49.45 ± 7.33 µg equivalent/g, indicating a slightly lower bioavailability in males than in females.

Up to 17 different radiolabeled entities were detected in urine and up to 13 different compounds in feces. Trace levels of intact compound were detected in urine, whereas parental fipronil-desulfinyl was the main compound in feces. Fipronil-desulfinyl subject to intensive metabolism by Phase I enzymes *in vivo*. Elimination occurred mostly via the fecal route, indicating either poor oral bioavailability, enterohepatic recirculation, or both. Small differences were seen between the single- and repeated-dose experiments. Presence of radioactivity in the carcass and the relatively long half-life indicate partitioning into a deep compartment, *e.g.,* fat.

I.A.2. Oral – Goat

Three lactating goats, one per dose level, received oral doses of [^{14}C]-phenyl-labeled fipronil-desulfinyl twice daily by gelatin capsule for 7 days at dietary concentrations of 0.05, 2, or 10 ppm (Johnson *et al.*, 1996). Radiolabel was followed in urine, feces, whole blood, plasma, and milk during exposure, and fat (peri-renal and omental) liver, kidneys, and skeletal muscle at termination.

Fecal excretion accounted for 20 – 50% of the administered label, constituting the major route of excretion at each concentration. Urinary excretion ranged from 3% to 7% of the total exposure. Tissue accumulation was significant: 7 – 26% was found in total body fat. No difference between specific activities in renal fat and omental fat was detected. Liver had the second highest specific activity, comprising 2.2 – 4.4% of the administered dose. Skeletal muscle had lower specific activity than liver, but represented a much larger proportion of body mass, accounting for a larger proportion of total recovered radioactivity (4 – 9% of administered dose). Milk accounted for 1 – 5% of total administered dose. Exhaled CO_2 was not measured in this study, but a rat disposition study (Totis and Fisher, 1994) determined that CO_2 residue was trivial.

About 50% of the administered 0.05 ppm radiolabel was absorbed. Accumulation of residues in milk reached steady state at ~100 hours. This is consistent with the relatively long retention time previously observed in rat, both for the parent fipronil and for the photodegradate. Quantitative distribution in organs and excreta was evaluated only in the goat exposed to 10 ppm. In urine samples, a sulfate conjugation product of the pyrazole amine group was the most significant metabolite [21 – 31% of the total recovered residues (TRR)], with a glucuronide of that same amino group comprising 5 – 6% of the TRR. The only other identified metabolite was MB 46400, where the trifluoromethyl carbon was replaced by a carboxylic acid (6 – 8% of the TRR). Fipronil-desulfinyl comprised 1 – 2% of the urinary TRR and 72 – 77% of the TRR in feces. Liver radiolabel was dominated by fipronil-desulfinyl (58% of TRR), with no other single residue exceeding 3.4% of the TRR. Fipronil-desulfinyl comprised 49%, 70%, 86%, 82% and 94% of the kidney muscle, renal fat, omental fate and milk TRRs, respectively.

I.A.3. Oral – Laying Hen

^{14}C-phenyl-labeled fipronil-desulfinyl was given orally by capsule to groups of 5 laying hens at dietary concentrations of 0.05, 2, or 10 ppm for 14 days (McCorquodale *et al.*, 1996). Eggs were collected throughout the study days and were separated into whites and yolks. Excreta was

collected prior to the first dose administration and at 24 hours intervals thereafter. Animals were killed approximately 23 hours following the final dose. A total of 68.0 – 81.4% of the total administered radiolabel was recovered: 53 – 71% was recovered in excreta, with recovery percentage declining with increased dose; 4.2 – 6.7% was recovered in eggs, with 2 – 3 fold higher levels detected in yolks than in whites; and 3.99 – 6.34% was recovered in tissues. The highest tissue residue levels were in omental fat, partially formed eggs, liver and skin fat.

I.A.4. Dermal – Rat

The extent of dermal absorption of ^{14}C-fipronil-desulfinyl was examined in male rats (Cheng, 1996). Twenty four rats per dose (4 rats per time point) were exposed to 0.08, 0.8 or 8 mg fipronil-desulfinyl in 1.0% carboxymethylcellulose. Two animals received vehicle only. The skin was washed and animals sacrificed at 0.5, 1, 2, 4 10 or 24 hours post dose, when blood, urine and feces were also collected. Mean recovery of total radioactivity ranged from 93 to 103%, with the majority (90 to 102%) occurring in the skin wash. Radioactivity in blood, excreta and carcass accounted for less than 3% of the total applied radioactivity. DPR estimated that the dermal absorption of fipronil-desulfinyl (3.2%) is similar to that of fipronil (4.3%) (DPR, 1999b).

I.B. Acute Toxicity

I.B.1. Oral – Rat

Fipronil-desulfinyl (98.6%) was administered by gavage to 5 Sprague Dawley rats/sex/group at 3, 10, 20, or 30 mg/kg (Dange, 1993b). Rats were monitored for 14 days thereafter. Mortality occurred in 0/5, 0/5, 3/5, and 5/5 males and 0/5, 0/5, 4/5, and 5/5 females at ascending doses. Animals displayed dyspnea, bradypnea, hunched posture, and tonic and clonic convulsions at 30 mg/kg. Reduced motor activity and nasal discharge was observed at 10 to 30 mg/kg. An apparent hyper-reaction to noise appeared at all dose levels without a clear dose-response. Treatment-related pathological findings occurred at 30 mg/kg and included enlarged livers with marked lobular pattern (2/5 in males), pale livers (2/5 in females), and hyper-salivation (1/5 males and 5/5 females). The investigators calculated LD_{50} values of 18 mg/kg for males and 15 mg/kg for females. Fipronil-desulfinyl is a Toxicity Category I oral toxicant.

I.B.2. Dermal – Rat

A single dose of 2000 mg/kg fipronil-desulfinyl (98.6%) was applied to the clipped dorsal skin of 5 Sprague Dawley rats/sex for 24 hours to investigate acute toxicity following dermal exposure (Dange, 1993a). Two females died; one death was attributed to the test substance. Clinical observations in males included subdued behavior, piloerection, chromodachryorrhea in nose and eyes, and polypnea (rapid or panting respiration). In females, lacrymation, reduced motor activity, piloerection, bradypnea, palpebral ptosis, tremors, soiled fur, curled up at handling and increased motor activity were noted. Slight body weight loss occurred during the first week, but body weight gain occurred during the subsequent week in all animals. The terminal weights of surviving animals exceeded their initial weights. Autolysis was observed in the two rats that died during the study. White areas in the liver were observed in 1 of the

decedents, and red areas in the liver were observed in 1 surviving female rat at terminal necropsy. The LD_{50} for males and females was > 2000 mg/kg, making it a Toxicity Category III toxicant.

I.B.3. Intraperitoneal – Mouse

A published study in mice was available from literature with administration via intraperitoneal injection (Hainzl and Casida, 1996). Male Swiss-Webster mice were treated via intraperitoneal injection with fipronil or fipronil-desulfinyl. All compounds were dissolved in DMSO. Eight to 17 mice were used for each LD_{50} determination. Toxicity was evaluated 24 hours after treatment. Further experimental details such as doses and the treatment protocol were not provided. The LD_{50} for desulfinyl-fipronil was 23 mg/kg (Hainzl and Casida, 1996), which was approximately 2-fold more toxic than the parent (41 mg/kg). The study also measured the mouse GABA receptor IC_{50} for fipronil and fipronil-desulfinyl. The IC_{50} for fipronil-desulfinyl was less than one-tenth that of the parent, indicating its greater inhibitory potential at the mouse receptor (IC_{50} = 1010 nM for fipronil and 97 nM for fipronil-desulfinyl).

I.C. Subchronic Toxicity

I.C.1. Oral 14-day – Rat

An exploratory 14-day toxicity study was conducted in the rat by oral gavage (Dange, 1994c). Fipronil-desulfinyl (98.6%) was administered to 5 Sprague Dawley rats/sex/dose at 0.3, 1, 3 or 10 mg/kg/day for 14 days. Between days 5 and 8, 1 female died at 3 mg/kg/day and all animals died at 10 mg/kg/day. Clinical signs including piloerection, chromodachryorrhea, prostration, excessive reaction to noise, curled up at handling, hunched posture, nasal discharge, and few feces were observed at 3 and 10 mg/kg/day. Convulsions preceding death occurred in 3 males and 2 females at 10 mg/kg/day. Decreased body weights were observed at 10 mg/kg/day in males and females at day 5. Decreased body weight gain was observed at 3 mg/kg/day and 10 mg/kg/day in males and females. Average feed consumption rates decreased at 3 mg/kg/day in females and at 10 mg/kg/day in males and females. Neutrophils increased in a dose-dependent manner up to 240%, achieving statistical significance at 3 mg/kg/day ($p < 0.01$). A dose-dependent decrease in total bilirubin occurred in males and females, achieving statistical significance at 3 mg/kg/day dose ($p < 0.05$). Increased serum total protein was observed in females at 3 mg/kg/day ($p < 0.05$). Brain congestion was noted in all decedents. There was an increase in severity and occurrence of thyroid cell atrophy in males but not in females. The investigators considered the effect treatment-related at 3 mg/kg/day.

The NOEL was 1 mg/kg/day, based on blood chemistry effects and mortality seen at 3 mg/kg/day.

I.C.2. Oral 28-day – Rat

Fipronil-desulfinyl (97.5%) was administered to 10 Sprague-Dawley rats/sex/group in the diet at 0, 0.5, 3, 30, or 100 ppm for 28 consecutive days (Dange, 1995b). All rats at 100 ppm died

between days 5 and 15. One male at 30 ppm died on day 6. Mean dose levels were 0, 0.04, 0.23, and 2.2 mg/kg/day in males and 0, 0.04, 0.24, and 2.32 mg/kg/day in females (mean doses at 100 ppm were not included due to total mortality). Clinical signs included curling up on handling, thin appearance, crusty skin, and piloerection. There were dose-related body weight and food consumption decrements. A sharp reduction in thyroid hormones was detected at 30 and 100 ppm: On day 7, Triiodothyronine (T3) was 46% lower in surviving females ($p < 0.001$) and Thyroxine (T4) was lower by 63% in males ($p < 0.05$) and by 50% in females ($p< 0.01$) at 100 ppm. In animals dosed at 30 ppm, T4 was lower by 33% in males ($p < 0.001$); on day 23, both sexes had reduced T4 (49% in males and 61% in females, $p < 0.01$) and T3 was 33% lower in males ($p < 0.01$).

The NOEL was 3 ppm (0.23 mg/kg/day) based on mortality and reductions in thyroid hormones at 30 ppm.

I.C.3. Oral 90-day – Rat

Fipronil-desulfinyl (97.5%) was administered to 10 Sprague Dawley rats/sex/group via diet at 0, 0.5, 3, 10, or 30 ppm, equivalent to 0, 0.029, 0.177, 0.594, and 1.772 mg/kg/day for males and 0, 0.035, 0.210, 0.709, and 2.101 mg/kg/day for females (Dange, 1994b). In addition to standard subchronic study parameters, measurements of T3, T4, and thyroid stimulating hormone (TSH) were taken at study weeks 2 and 10.

Clinical signs including irritability to touch in both sexes at 10 ppm and 30 ppm and in 1 male at 3 ppm. Increased motor activity was seen in 1 female at 10 ppm and in 9/10 females at 30 ppm. Five out of 10 males at 30 ppm curled up on handling. Mortality occurred at 30 ppm in 1 male and in 3 females on days 11 and 13. Treatment at 30 ppm significantly reduced body weight in females from day 8 to 36 and in males by 8 – 15% from days 8 to 71 ($p < 0.05$). At 10 ppm, body weight was significantly decreased in males by 7 – 5% from day 57 to 78 ($p < 0.05$). Food consumption was transiently reduced at 30 ppm in males and females; decrement was significant through day 15 in males ($p < 0.01$) and through day 8 in females ($p < 0.05$).

Significant changes in clinical chemistry parameters occurred in females treated with 30 ppm fipronil-desulfinyl. These include reduced cholesterol ($p < 0.05$), reduced triglycerides ($p < 0.05$), and reduced bilirubin ($p < 0.01$). Treatment-related histopathology was limited to decedents, and showed lymphoid depletion in the spleen, pulmonary edema in lungs, and hypertrophy of the zona fasciculata of the adrenal cortex. Increased relative brain weight was seen in males at 10 and 30 ppm ($p < 0.05$). Increased liver weight relative to body weight was observed in females at 10 ppm only ($p < 0.05$).

There was a 29% reduction of T3 at week 10 ($p < 0.05$) and a 48% reduction of T4 at week 2 ($p < 0.01$) in 30 ppm males.

The 90-day NOEL for fipronil-desulfinyl in rats is 0.5 ppm (~0.03 mg/kg/day) based on clinical signs in males at 3 ppm (~0.18 mg/kg/day).

I.C.4. Oral 28-day – Mouse

Ten OF-1 mice/sex/group were dosed with fipronil-desulfinyl (97.5%) via the diet at 0, 0.5, 3, 30, or 60 ppm, equating to mean dose levels of 0.08, 0.49, and 5.0 mg/kg/day for the lowest 3 dose levels in males, and 0.10, 0.61, 5.6, and 12.1 mg/kg/day in females (Dange, 1994d). All males at 60 ppm died by the end of week 2. Six females at 60 ppm died and 7 males and 2 females at 30 ppm died. Clinical signs at 30 and 60 ppm included increased motor activity, excessive jumping, and compulsive biting. Body weights of males treated at 60 ppm were significantly decreased by 27% after 1 week of exposure ($p < 0.05$); no males survived to the next weight measurement. Males treated at 30 ppm had significantly decreased body weight of up to 17% for the duration of the treatment period ($p < 0.05$). Females at 60 ppm also saw a 10% decrease in body weight after 1 week ($p < 0.05$). Body weight gain was significantly reduced in males at 30 ppm for all time points and at 60 ppm for the one time point measured ($p < 0.05$). Food consumption was decreased in males at 30 ppm for the first two weeks and in males and females at 60 ppm for the first week. Relative liver weights were elevated by 19% in males at 30 ppm and by 22% in females at 60 ppm ($p < 0.05$). Centrilobular hypertrophy was remarkably increased in males at 30 ppm.

The NOEL for fipronil-desulfinyl in mouse is 3 ppm or 0.49 mg/kg/day based on clinical signs, mortality and liver changes at 30 ppm.

I.C.5. Oral 90-day – Mouse

Ten OF1 mice/sex/group received 0, 0.5, 2 or 10 ppm fipronil-desulfinyl (96%) in the diet for 13 weeks (Bigot, 1996). The mean achieved doses were 0, 0.08, 0.32 and 1.74 mg/kg/day in males and 0, 0.11, 0.43 and 2.15 mg/kg/day in females. All 10 males and 1 female from the 10 ppm group died or were euthanized in moribund condition before study termination. Death occurred in males on days 20, 28, 39, 48, 52, 62 and 84 and in the females on day 5. The only clinical signs noted for these animals were excessive jumpiness and/or irritability in 3 males, indicating possible neurological effects. There were no treatment effects on mean body weight or food consumption.

The mean serum alkaline phosphatase level in 10 ppm females was 61% higher than that of the control ($p < 0.05$) and was increased in a dose-dependent manner. Conversely, the alkaline phosphatase level in males decreased with increasing dose, and differences were not significantly different from control. There were no treatment related effects on the absolute or relative organ weights, although liver enlargement was seen in 3/10 and small thymus was seen in 4/10 male decedents at 10 ppm. The incidence of centrilobular hypertrophy in the liver increased in 10 ppm male decedents (0/10 at 0 ppm vs. 6/10 at 10 ppm).

The subchronic dietary NOEL for mice is 2 ppm or 0.32 mg/kg/day, based on mortality in both sexes and increased alkaline phosphatase levels in females at 10 ppm.

I.C.6. Oral 28-day – Dog

Two Beagle dogs/sex/dose received fipronil-desulfinyl (97.5%) in the diet at 0, 27, 80 or 270 ppm for 28 days (Dange, 1995a). This study was a preliminary, range-finding study. The mean intake levels were 0, 1, 1.9 and 2.3 mg/kg/day in males and 0, 1, 1.7 and 2.3 mg/kg/day in females. Mortality (2/2) occurred at both 80 and 270 ppm.

At 80 ppm, 1 dog/sex was sacrificed moribund on day 10 and the remaining dogs were sacrificed on day 15. Animals displayed reduced motor activity, staggering steps, irritability, increased salivation, absent or few feces and emaciation. All animals at 270 ppm were sacrificed on day 10 due to moribundity caused by a lack of food consumption beginning on day 5. Few or no feces and emaciation were noted at 270 ppm. Soft feces were occasionally observed in males at 27 ppm. One male in the 27 ppm group vomited on day 20, and the other male displayed fear and convulsions on day 29.

Body weights and food consumption at 27 ppm were comparable to controls. All animals lost weight at 80 ppm (0.3 to 1.2 kg) and at 270 ppm (0.3 to 1.8 kg). At 80 ppm, food consumption was decreased starting on day 4. At 270 ppm, all animals had a marked decrease in food consumption beginning on day 2 and were generally not eating from day 6 to 10. There was a dose-dependent decrease in weight gain in both males and females.

At terminal necropsy, one female at 27 ppm had a pale liver and both males had lower relative thymus weights compared to controls. The 80 ppm animals sacrificed on day 10 revealed pale abnormal liver color in one male and multifocal whitish areas on the liver, small thymus, and multifocal red areas on the lung in one female. Sacrifice of the remaining 2 animals at 80 ppm on day 15 revealed small thymus in both male and female with mottled appearance of the liver in the male and pinpoint black spots on the gastric mucosa in the female. At 80 ppm, there was marked thymus atrophy, diffuse sinusoidal leukocytosis in liver, and centrilobular hepatocytic enlargement in all dogs. Additionally, mild multifocal hydropic degeneration of the hepatocytes and chronic hepatitis with periportal fibrosis were noted in one male and one female.

The NOEL was < 27 ppm (< 1 mg/kg/day) based on convulsions and incidence of pale liver at 27 ppm.

I.C.7. Oral 90-day – Dog

Five Beagle dogs/sex/dose received fipronil-desulfinyl (96%) in the diet at 0, 3.5, 9.5, or 35 ppm for 90 days (Dange, 1996). The mean fipronil-desulfinyl intake was 0, 0.10, 0.27, and 0.95 mg/kg/day for males and 0, 0.10, 0.29, and 1.05 mg/kg/day for females.

One 35-ppm female was sacrificed on day 28 with increased salivation, prostration, writhing and tremors, noisy breathing, and dyspnea. Microscopy revealed marked coronary arteritis and myocardial necrosis. The investigators considered these effects common to dogs of this age and did not attribute the death to treatment.

Body weight, food consumption, and hematology were comparable to controls at all dose levels. Excessive barking and aggressive behavior were noted on day 84 and increased salivation, irritability, and tremors were noted on day 86 in one female at 35 ppm. At week 13, a male in the 9.5 ppm dose group displayed increased alanine aminotransferase and alkaline phosphatase activities relative to controls. A significant increase in group mean urine pH was noted for males at 35 ppm ($p < 0.01$). A low incidence of mild to moderate coronary arteritis and slight to moderate thymus involution was observed across all groups (including controls) and both sexes. Gross necropsy, organ weights, and histopathology were unremarkable.

The NOEL of 9.5 ppm (0.29 mg/kg/day) was based on aggressive behavior and increased salivation, irritability, and tremors in one high dose female.

I.D. Chronic Toxicity and Oncogenicity – Rat

Fipronil-desulfinyl (96%) was administered via the diet at 0, 0.5, 2 or 10 ppm to 10 Sprague-Dawley rats/sex/group for sacrifice after 53 weeks (interim group) and to 60 rats/sex/group for sacrifice after 104 weeks (terminal sacrifice group) (Bigot, 1998). Animals were observed daily for clinical signs. Body weights and food consumption were measured weekly for the first 13 weeks and once every 4 weeks thereafter. Hematology was performed on the interim group at 26 and 52 weeks and clinical chemistry was performed at weeks 25 and 51. Hematology was performed in the terminal sacrifice group at weeks 26, 52, 78 and 104, and clinical chemistry was performed at weeks 25, 51, 77 and 103. The mean fipronil-desulfinyl intake during the treatment period from week 1-53 was 0, 0.028, 0.113 and 0.563 mg/kg/day in males and 0, 0.039, 0.143 and 0.733 mg/kg/day in females. The mean fipronil-desulfinyl intake during the treatment period from week 1-101 was 0, 0.025, 0.098 and 0.497 mg/kg/day in males and 0, 0.032, 0.127 and 0.546 in females.

By 26 weeks of treatment at 10 ppm there was an increase in the mortality rate in females. Investigators subsequently reduced the dietary level from 10 to 6 ppm for females only. The authors state that the survival rates of all control and treated groups were in the same range as the survival rates from historical controls in the conducting laboratory. Survival rates were provided for 4 historical control studies, but no details were given on the strain or number of animals or the date range of these studies.

Chronic exposure to fipronil-desulfinyl resulted in chromodachryorrhea (bloody tears due to excessive secretion of porphyrins) that was dose-responsive and significantly increased in the high-dose females ($p < 0.05$). Aggressive behavior and irritability to the touch were increased in high dose males and females ($p < 0.05$ for males, $p < 0.001$ for females). Convulsions were observed in all exposed groups and in control males and were statistically significantly increased relative to controls in females at 2 and 10 ppm ($p < 0.05$ and $p < 0.01$, respectively). Males exposed to 2 ppm experienced convulsions before any other group (day 85). In females, the onset of convulsions occurred earlier with increasing dose, in a dose-dependent manner. No changes were observed in body weight, food consumption, urinalysis, organ weights, or histopathological findings at necropsy.

The high dose group males had statistically significant increases of sub-epithelial mixed cell infiltration in the stomach ($p < 0.05$). A dose-dependent increase in incidences of lung congestion/hemorrhage occurred in females, which was statistically significant at the high dose ($p < 0.05$). There was a statistically significant decrease in bilirubin and in triglycerides and an increase in glucose in females at high dose during week 26 only.

The chronic NOEL for fipronil-desulfinyl in the rat was 0.5 ppm or 0.025 mg/kg/day due to mortality in males and increased convulsions in females at the LOEL of 2 ppm.

I.E. Genotoxicity

The below table (Table II.1) describes genotoxicity studies for fipronil-desulfinyl and for fipronil metabolites fipronil-sulfide and fipronil-sulfone.

Table 53. Genotoxicity Studies Conducted with Fipronil Photodegradate or Metabolites

Chemical	Test Type	System	Species, Strain or Culture	Dose or Concentration	S9	Result	Reference/Study Status
Fipronil-Desulfinyl	Gene Mutation	Ames Reverse Mutation Assay	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA138	10, 25, 50, 100, 250 µg/plate, 60 hr	±	Negative	(Percy, 1993)
Fipronil-Desulfinyl	Gene Mutation	HGPRT Forward Mutation	Chinese hamster lung V79 cell line	15, 30, 60, 80 or 100 µg/ml	±	Negative	(Adams, 1996a)
Fipronil-Desulfinyl	Chromosomal Aberration	*In vitro* Chromosome Aberration	Human lymphocytes (♂)	5, 15, 30, 60, 125, 250, 500 or 625 µg/ml for 18 or 32 hr	±	Negative	(Adams, 1996b)
Fipronil-Desulfinyl	Chromosomal Aberration	*In vivo* Chromosome Abberation	CD-1 Swiss mouse bone marrow erythrocytes (♀ + ♂)	2, 4, 8 or 16 mg/kg for 24, 48 or 72 hr	n/a	Negative	(Proudlock, 1996)
Fipronil-Sulfide	Gene Mutation	Ames Reverse Mutation Assay	*S. typhimurium* TA98, TA100, TA1535, TA1537	10, 25, 50, 100 and 250 µg/plate, 72 hr	±	Negative	(Percy, 1994)
Fipronil-Sulfone	Gene Mutation	Ames Reverse Mutation Assay	*S. typhimurium* TA98, TA100, TA1535, TA1537	0.32, to 500 µg/plate, 48 hr	±	Negative	(Clare, 1988b)
Fipronil-Sulfone	Chromosomal Aberration	*In vitro* Chromosome Aberration	Human lymphocytes (♀ + ♂)	75, 100 or 300 µg/ml for 3 hr	±	Negative	(Marshall, 1988b)

Table 53. Genotoxicity Studies Conducted with Fipronil Photodegradate or Metabolites

Chemical	Test Type	System	Species, Strain or Culture	Dose or Concentration	S9	Result	Reference/Study Status
Fipronil-Desulfinyl and Fipronil-Sulfone	Oxidative Stress In Vitro	Mitochondrial respiration, ATP synthesis, mitochondrial membrane potential, Ca^{2+} efflux	Wistar rat liver cells	0.5- 25 µM	n/a	Positive	(Tavares *et al.*, 2015)

I.G. Developmental Toxicity – Rat

Fipronil-desulfinyl (99.2%) was administered to sperm-positive female CD rats (25 per group) by oral gavage from gestation days (GD) 6 to 15 at doses of 0.2, 1.0 or 2.5 mg/kg/day (Foulon, 1997). Dams were examined daily for signs of illness. Body weights were recorded on GD 0, GD 6-16 and on GD 20. Dams were sacrificed on GD 20 and the reproductive tract and fetuses were examined.

No mortality occurred in the dams. A significant decrease in weight gain occurred in dams at 1.0 and 2.5 mg/kg/day from GD 6-12 and at 2.5 mg/kg/day from GD 6-26 ($p < 0.01$). Reduced food consumption was seen at 2.5 mg/kg/day from GD 9-12 and GD 12-16 ($p < 0.01$). A significant increase in hair loss and scabbing in dams occurred at 2.5 mg/kg/day ($p < 0.05$).

At 2.5 mg/kg/day, there was a dose-dependent, significant increase in non-ossification of the 5th and 6th sternebrae in fetuses ($p < 0.01$). In addition, there was an increase in ossification delay of pubic bones and the caudal vertebrae at 2.5 mg/kg/day. Fetal body weights were statistically significantly decreased by 2.4% in females ($p < 0.01$) and 2.5% in males ($p < 0.05$).

The maternal NOEL is 0.2 mg/kg/day due to statistically significant decreases in body weight during the dosing period at 1.0 mg/kg/day. The developmental NOEL is 1.0 mg/kg/day due to fetal body weight change and ossification delays at 2.5 mg/kg/day.

I.H. Acute Neurotoxicity – Rat

Fipronil-desulfinyl (99.5%) was administered as a single oral gavage dose to 12 Crl: CD BR rats/sex/dose at 0.5, 2 or 12 mg/kg in corn oil (Hughes, 1996). Animals were observed for 14 days following treatment. FOBs were performed before treatment, at time of peak effect (TOPE, determined to be 6 hours in a range-finding experiment conducted in the same laboratory) and at days 7 and 14 post-dosing. All animals from the control and high dose groups were subjected to pathological evaluation. Brain, spinal cord, peripheral nerves (sciatic, sural and tibial), dorsal root and gasserian ganglia were examined for pathology.

No mortality was observed. Significant decreases in body weight gains were observed at 12 mg/kg in males and females during week 1 (22 – 25%, $p < 0.01$) and a 30% increase in weight gain was

seen in males during week 2 ($p < 0.05$). Food consumption decreased in 12 mg/kg males and females during week 1 ($p < 0.01$) with no effect on food utilization/conversion. Clinical signs (soft stool and change in appearance of the fur) were also seen in the controls and were attributed to the corn oil vehicle.

Significant changes were observed in the FOBs conducted in animals dosed at 12 mg/kg group, compared with the control group, in both sexes. At the 6-hour TOPE, there was an increase in forelimb strength in females, a decrease in hindlimb splay in males and females, and a decrease in rectal temperature in males and females ($p < 0.01$). On day 7, a significantly decreased forelimb grip strength in males and an increase in mean activity counts in females at 12 mg/kg were observed ($p < 0.05$). There was also a significant increase in mean activity counts in females at 2 mg/kg on day 7 ($p < 0.05$). However, the mean activity counts were the same for all three fipronil treatment doses; a lack of dose-response over the 24-fold range lacks plausibility as a treatment effect. At day 14, there was a significant increase in mean rearing events in females and a decrease in immediate righting reflex in males at 12 mg/kg (both $p < 0.05$), which were highly variable and may not have been due to treatment.

A total of 4/12 control males had axonal degeneration in nerve tissues compared to 6/10 males in the high-dose group; the increase was not statistically significant. One of 5 female controls and 2/5 high-dose females displayed axonal degeneration. The report authors suggest that axon degeneration is not treatment related. However, the modest increase combined with the fact that authors did not evaluate tissues from the low- or mid-doses made it impossible to determine if this was due to treatment.

The acute neurotoxicity study NOEL for rats was 2 mg/kg due to decreased body weight gain and food consumption and observations in the functional observational battery in the 12 mg/kg group.

II. Fipronil-Sulfone

II.A. Acute Toxicity – Mouse

A published study in mice was available from open literature with administration via intraperitoneal injection (Cole *et al.*, 1993). Male Swiss-Webster mice were treated via intraperitoneal injection with fipronil and its metabolite fipronil-sulfone. All compounds were dissolved in DMSO. Eight to seventeen mice were used for each LD_{50} determination. Toxicity was evaluated 24 hr after treatment. Further experimental details such as doses and the treatment protocol were not provided. The LD_{50} for fipronil was reported as 32 mg/kg and the LD_{50} was reported as 80 mg/kg for fipronil-sulfone.

II.B. Genotoxicity

A gene mutation study, a chromosome aberration study, and an oxidative stress study with fipronil-sulfone are described in the genotoxicity Table II.1 in the fipronil-desulfinyl section, above.

III. Fipronil-Sulfide

III.A. Acute Toxicity – Rat

Fipronil-sulfide was orally administered to 5 Sprague Dawley rats/sex/dose at 50, 65, 90 or 120 mg/kg (Dange, 1994a). Mortality was observed in both sexes at doses higher than 50 mg/kg within the first week following treatment. Body weight gains were observed in all surviving rats except 1 female at 50 mg/kg during the two-week post-exposure period. Common clinical signs include excessive jumps at 50 mg/kg in males and females. Subdued behavior, fear, mucoid feces, clonic convulsions, slight tremor, curls up at handling, piloerection, hunched posture, reduced motor activity, soiled fur and excessive vocalization were observed at the higher doses. Stomach distended with gas and moderately enlarged liver were observed in rats at terminal autopsies and at autopsy of decedents. The LD_{50} for males and females is 83 mg/kg. Fipronil-sulfide is a Toxicity Category II acute oral toxicant. The LOEL of 50 mg/kg is based on excessive jumping in both sexes and failure to gain weight in one female.

III.B. Genotoxicity

A gene mutation study with fipronil-sulfide is described in the genotoxicity Table II.1 in the fipronil-desulfinyl section, above.

IV. Comparative Toxicity of Fipronil, its Metabolites and the Environmental Photodegradate

Fipronil and fipronil-desulfinyl share a similar pharmacokinetic profile and mode of action (interaction with the GABA receptor). The pharmacokinetic profiles for absorption, distribution, elimination, and blood kinetics in rats were similar for fipronil and desulfinyl, with feces being the main route of elimination. Acute studies for fipronil-desulfinyl showed the photodegradate to be more toxic than fipronil by the oral route but similarly toxic by dermal and IP administration. The toxicity profile for fipronil-desulfinyl was characterized by mortality and clinical signs of neurotoxicity (increased/decreased motor activity in rats, convulsions) while fipronil showed selective organ toxicity (liver and thyroid) in addition to neurotoxic effects. Unlike the parent fipronil, chronic exposure of rats to fipronil-desulfinyl did not result in thyroid toxicity or tumors. Fipronil and fipronil-desulfinyl may differ in their ability to affect the thyroid gland. It has been suggested that the sulfoxide group of fipronil, which the photodegradate lacks, may be responsible for the thyroid effects (US EPA, 2000).

The limited acute toxicity studies in rats and mice indicate that the sulfide and the sulfone have mammalian toxicity similar to the parent compound, but the sulfone may be a stronger inhibitor of the GABA receptor. Chronic toxicity studies were not available for the sulfone or the sulfide metabolites to evaluate their effects on the liver or thyroid function. The toxicity of other identified fipronil metabolites (detrifluoromethylsulfonyl-fipronil and the amide RPA200766) have not been evaluated in mammals.

Neither the metabolites nor the photodegradate caused mutations or chromosome aberrations. However, fipronil-desulfinyl and fipronil-sulfone caused increased oxidative stress *in vitro*.

V. Conclusions

Based on the limited data available for the metabolites fipronil-sulfone and fipronil-sulfide, toxicity is comparable to the parent. However, the lack of subchronic, chronic, developmental and neurotoxic data preclude further comparison. Based on the acute LD_{50} values in rat and mice, oral and dermal desulfinyl is more toxic than the parent. However, the points of departure (POD) established in the available studies are comparable, and the critical POD for fipronil will be protective of desulfinyl exposure.

An important consideration is a lack of exposure data for the metabolites and for fipronil-desulfinyl. As such, characterizing risk from total exposure to fipronil and its derivatives cannot be conducted at this time.

In conclusion, the critical POD established for fipronil in this risk assessment are assumed to be protective against the toxicity of fipronil-desulfinyl. Table II.2 compares the critical points of departure (POD) for fipronil to the POD established in the respective studies with fipronil-desulfinyl.

Table 54. Fipronil Critical POD compared to Toxicity of Fipronil-Desulfinyl

Duration	Fipronil	Fipronil POD	Fipronil-desulfinyl	Fipronil-desulfinyl POD
	Critical Endpoint	(mg/kg/day)	Endpoints	(mg/kg/day)
Acute	Decreased hindlimb splay in an acute oral neurotoxicity study in rats	0.77[a]	Altered forelimb grip strength, decreased hindlimb splay, decreased rectal temperature and decreased body weight gain and food consumption in an acute oral neurotoxicity study in rats	2.0[d]
Subchronic	Convulsions and mortality and sustained decreases in T4 after subchronic oral exposure in a chronic study in rats	0.02[b]	Irritability to touch and aggressive behavior in a 90-day subchronic oral study in rats	0.029[e]
Chronic	Convulsions and mortality, sustained decreases in T4, and increased progressive senile nephropathy in a chronic oral study in rats	0.02[c]	Mortality in males and increased convulsions in females in a chronic oral study in rats	0.025[f]

POD: point of departure; T4: thyroxine thyroid hormone.
[a]Benchmark dose modeling (BMDS, version 3.2) of hindlimb splay data from acute neurotoxicity study in rat (Hughes, 1997). NOEL of 2.5 mg/kg/day.
[b]Combined chronic and oncogenic study in rat (Aughton, 1993).
[c]Combined chronic and oncogenic study in rat (Aughton, 1993).
[d]Acute neurotoxicity study in rat (Hughes, 1996).
[e]Subchronic 90-day oral study in rat (Dange, 1994b).
[f]Combined chronic and oncogenic study in rat (Bigot, 1998).

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APPENDIX III. STATISTICS USED IN FIPRONIL ONCOGENESIS ANALYSIS

The Department of Pesticide Regulation (DPR) used the Poly-3 test (Bieler and Williams, 1993) to assess the prevalence of hepatocellular carcinomas in male mice from the combined chronic and oncogenic study conducted with fipronil (Broadmeadow, 1993). The Poly-3 test provides survival-adjusted quantal-response that modifies the Cochran-Armitage linear trend test. More specifically, this method modifies the denominator in the quantal estimate of carcinoma incidence to approximate more closely the total time at which the animals were at risk. In this appendix, DPR presents the results of the Poly-3 test adjusted denominators on the hepatocellular carcinoma mouse data, and the results of the sample size at risk of carcinoma starting on day 409 when the first carcinoma was detected and the sample size at risk of adenoma on day 317 when the first adenoma was detected. The results of the Cochran-Armitage trend test and the Fisher's exact test for pairwise comparisons between the control and every other dose group are also presented. These tests were performed with R version 4.2.1 (R Core Team, 2020). Annotated R code is included at the end of this appendix. The purpose of this appendix is to provide all of the tables and results of these analyses performed on the mouse data from Broadmeadow (1993) for full transparency.

Poly-3 Test – Incidence of Carcinoma and Adenoma in Male Mice

A Poly-3 test was performed on the original sample size to adjust the denominator of the rate of hepatocellular carcinomas in male mice. Table III.1 displays the original sample size for each dose, the sample size after the first adenoma was detected on day 317, the number of adenomas, the denominator after the first carcinoma was detected on day 409, the number of carcinomas, and the adjusted denominator after the Poly-3 test adjustment. The R package MCPAN was used to perform the Poly-3 adjustment (Schaarschmidt, 2018).

Table 55. Denominators for mouse data

Dose	0 (control) mg/kg-day	0.01 mg/kg-day	0.06 mg/kg-day	1.18 mg/kg-day	3.43 mg/kg-day
Original sample size	52	52	52	52	52
At-risk animals on day 317	47	50	44	40	47
Adenomas	10	3	2	6	6[a]
At-risk animals on day 409	41	39	34	32	42
Carcinomas	1	1	2	1	5
Adenoma + Carcinoma	11	4	4	7	10[a]
Poly-3 adjusted sample size	38.5	39.4	34.5	32.3	39.2

[a]One male in the 3.43 mg/kg-day group developed both an adenoma and a carcinoma. Both are reported here.

Cochran-Armitage Trend Test – Male Mice

DPR performed a Cochran-Armitage trend test (Cochran, 1954) to assess the dose-response of adenomas and hepatocellular carcinomas in male mice. To perform the test with the Poly-3 adjusted denominators, the number of carcinomas occurring at each dose was subtracted from the Poly-3 test adjusted denominators (Table III.2). Both one- and two-sided p-values were calculated, with statistical significance assessed at the α=0.05 level. The trends of the carcinoma data were statistically significant with both the Poly-3 adjustment, and without. The R package DescTools was used to perform the Cochrane-Armitage trend tests (Signorell, 2022).

Table 56. Carcinoma data for Cochran-Armitage trend test based on Poly-3 adjusted denominators. Results of Cochran-Armitage trend test based on Poly-3 adjusted denominators: Z: 2.1396; two-sided p-value = 0.032; one-sided p-value = 0.016

Dose	Carcinomas	No Carcinomas
0 (control)	1	37.5
0.01 mg/kg-day	1	38.4
0.06	2	32.5
1.18	1	31.3
3.43	5	34.2

Table 57. Carcinoma data for Cochran-Armitage trend test based on day 409 denominators. Results of Cochran-Armitage trend test based on day 409 denominators: Z: 2.0387; two-sided p-value = 0.041; one-sided p-value = 0.021

Dose	Carcinomas	No Carcinomas
0 (control)	1	40
0.01 mg/kg-day	1	38
0.06	2	32
1.18	1	31
3.43	5	37

Table 58. Adenoma data for Cochran-Armitage trend test based on Poly-3 adjusted denominators. Results of Cochran-Armitage trend test based on Poly-3 adjusted denominators: Z: 0.324; two-sided p-value = 0.746; one-sided p-value = 0.373

Dose	Adenomas	No Adenomas
0 (control)	10	28.5
0.01 mg/kg-day	3	36.4
0.06	2	32.5
1.18	6	26.3
3.43	6[a]	33.2

[a]One male in the 3.43 mg/kg-day group developed both an adenoma and a carcinoma.

Table 59. Adenoma data for Cochran-Armitage trend test based on day 317 denominators. Results of Cochran-Armitage trend test based on day 317 denominators: Z: 0.420; two-sided p-value = 0.674; one-sided p-value = 0.337

Dose	Adenomas	No Adenomas
0 (control)	10	37
0.01 mg/kg-day	3	47
0.06	2	42
1.18	6	34
3.43	6[a]	41

[a]One male in the 3.43 mg/kg-day group developed both an adenoma and a carcinoma.

Table 60. Adenoma and Carcinoma data for Cochran-Armitage trend test based on Poly-3 adjusted denominators. Results of Cochran-Armitage trend test based on day 317 denominators: Z: 1.173; two-sided p-value = 0.241; one-sided p-value = 0.121

Dose	Adenomas and Carcinomas	No Adenomas or Carcinomas
0 (control)	11	27.5
0.01 mg/kg-day	4	35.4
0.06	4	30.5
1.18	7	25.3
3.43	10	29.2

Table 61. Adenoma and Carcinoma data for Cochran-Armitage trend test based on day 317 denominators. Results of Cochran-Armitage trend test based on day 317 denominators:
Z: 1.274; two-sided p-value = 0.203; one-sided p-value = 0.101

Dose	Adenomas and Carcinomas	No Adenomas or Carcinomas
0 (control)	11	36
0.01 mg/kg-day	4	46
0.06	4	40
1.18	7	33
3.43	10	37

Fisher's Exact Test

Fisher's exact tests (Fisher, 1922) were also completed to test the results of each dose value compared to the control. None of the comparisons showed a statistically significantly higher rate in any doses compared to controls. Both one- and two-sided p-values were calculated, with statistical significance assessed at the α=0.05 level. For four of the comparisons including the adenoma data, the control mice had a statistically significantly higher rate than mice in the treated groups. The one-sided tests only evaluated whether mice in the treated groups had higher rates of adenomas or carcinomas than the control mice or not. None of the one-sided tests were statistically significant.

Table 62. Results of pairwise Fisher's exact tests on incidence of carcinomas in mouse data, comparing control to treated groups, using Poly-3 adjusted denominators

Dose (mg/kg/day)	0	0.01	0.06	1.18	3.43
Poly-3 adjusted sample size[a]	39	39	35	32	39
Carcinomas observed	1	1	2	1	5
Two-sided p-value	ref	1.0	0.59	1.0	0.20
One-sided p-value[b]	ref	0.75	0.45	0.70	0.10

[a]Rounded to the nearest integer to perform the Fisher's exact test.
[b]Testing whether the incidence of carcinomas in the treated groups is larger than control

Table 63. Results of pairwise Fisher's exact tests on incidence of carcinomas in mouse data, comparing control to treated groups, using animals at risk after day 409 denominators

Dose (mg/kg/day)	0	0.01	0.06	1.18	3.43
At-risk animals on day 409	41	39	34	32	42
Carcinomas observed	1	1	2	1	5
Two-sided p-value	ref	1.0	0.59	1.0	0.20
One-sided p-value[a]	ref	0.74	0.43	0.81	0.11

[a]Testing whether the incidence of carcinomas in the treated groups is larger than control

Table 64. Results of pairwise Fisher's exact tests on incidence of adenomas in mouse data, comparing control to treated groups, using Poly-3 adjusted denominators

Dose (mg/kg/day)	0	0.01	0.06	1.18	3.43
Poly-3 adjusted sample size[a]	39	39	35	32	39
Adenomas observed	10	3	2	6	6[c]
Two-sided p-value	ref	0.07	0.03	0.58	0.40
One-sided p-value[b]	ref	0.99	0.99	0.84	0.92

[a]Rounded to the nearest integer to perform the Fisher's exact test.
[b]Testing whether the incidence of adenomas in the treated groups is larger than control
[c]One male in the 3.43 mg/kg-day group developed both an adenoma and a carcinoma.

Table 65. Results of pairwise Fisher's exact tests on incidence of adenomas in mouse data, comparing control to treated groups, using animals at risk after day 317 denominators

Dose (mg/kg/day)	0	0.01	0.06	1.18	3.43
At-risk animals on day 317	47	50	44	40	47
Adenomas observed	10	3	2	6	6[b]
Two-sided p-value	ref	0.04	0.03	0.58	0.41
One-sided p-value[a]	ref	0.99	0.99	0.85	0.92

[a]Testing whether the incidence of adenomas in the treated groups is larger than control

[b]One male in the 3.43 mg/kg-day group developed both an adenoma and a carcinoma.

Table 66. Results of pairwise Fisher's exact tests on incidence of carcinomas and adenomas in mouse data, comparing control to treated groups, using Poly-3 adjusted denominators

Dose (mg/kg/day)	0	0.01	0.06	1.18	3.43
Poly-3 adjusted sample size[a]	39	39	35	32	39
Carcinomas and adenomas observed	11	4	4	7	10
Two-sided p-value	ref	0.08	0.09	0.59	1.0
One-sided p-value[b]	ref	0.99	0.98	0.81	0.69

[a]Rounded to the nearest integer to perform the Fisher's exact test.
[b]Testing whether the incidence of carcinomas and adenomas in the treated groups is larger than control

Table 67. Results of pairwise Fisher's exact tests on incidence of carcinomas and adenomas in mouse data, comparing control to treated groups, using animals at risk after day 317 denominators

Dose (mg/kg/day)	0	0.01	0.06	1.18	3.43
At-risk animals on day 317	47	50	44	40	47
Carcinomas and adenomas observed	11	4	4	7	10
Two-sided p-value	ref	0.049	0.09	0.60	1.0
One-sided p-value[a]	ref	0.99	0.99	0.83	0.69

[a]Testing whether the incidence of carcinomas and adenomas in the treated groups is larger than control

Day of Carcinoma Occurrence

Table 68. Day of carcinoma occurrence in Broadmeadow (1993) male mouse data

Animal Number	Dose (mg/kg-day)	Day of Carcinoma Occurrence
16	0	409
73	0.01	547
107	0.06	547
112	0.06	547
186	1.18	548
212	3.43	532
224	3.43	547
237	3.43	548
243	3.43	429
254	3.43	549

R Code – Male Mice

The code below is what was used to calculate the results of the Cochran-Armitage tests and the Fisher's exact tests above. The code starts with performing the Poly-3 test using the Bieler and Williams delta method of adjustment along with the Williams contrast type. The results of this test provide the adjusted denominators which are input in the code and used for the Cochran-Armitage tests and the Fisher's exact tests.

```
## Poly-k analysis   ##
require(MCPAN)
dat <- read.csv("animaldata2.csv",head=T)
tst2 <- poly3test(dat$day,dat$tumor,f=dat$doseg, method="BW",type="Williams")
tst2
### Cochrane-Armitage ###

require(DescTools)
dos <- c(0,0.01,0.06,1.18,3.43)
carcin <- c(1,1,2,1,5)
noncarcinpoly3 <- c(37.5,38.4,32.5,31.3,34.2)

xx2table <- matrix(c(carcin,noncarcinpoly3),ncol=2)
colnames(xx2table) <- c(1,0)
rownames(xx2table) <- dos
CochranArmitageTest(xx2table)
CochranArmitageTest(xx2table,alternative="one.sided")

noncarcin <- c(40,38,32,31,37)

xx3table <- matrix(c(carcin,noncarcin),ncol=2)
```

```
colnames(xx3table) <- c(1,0)
rownames(xx3table) <- dos
CochranArmitageTest(xx3table)
CochranArmitageTest(xx3table,alternative="one.sided")

# adenomas and poly3 #
adenomas <- c(10,3,2,6,6)
nonadspoly3 <- c(28.5,36.4,32.5,26.3,33.2)

xx4table <- matrix(c(adenomas,nonadspoly3),ncol=2)
colnames(xx4table) <- c(1,0)
rownames(xx4table) <- dos
CochranArmitageTest(xx4table)
CochranArmitageTest(xx4table,alternative="one.sided")

nonadenomas <- c(37,47,42,34,41)

xx5table <- matrix(c(adenomas,nonadenomas),ncol=2)
colnames(xx5table) <- c(1,0)
rownames(xx5table) <- dos
CochranArmitageTest(xx5table)
CochranArmitageTest(xx5table,alternative="one.sided")

# adenomas and carcinomas and poly3 #

adcars <- c(11,4,4,7,10)
nonadcarspoly3 <- c(27.5,35.4,30.5,25.3,29.2)

xx6table <- matrix(c(adcars,nonadcarspoly3),ncol=2)
colnames(xx6table) <- c(1,0)
rownames(xx6table) <- dos
CochranArmitageTest(xx6table)
CochranArmitageTest(xx6table,alternative="one.sided")

nonadcars <- c(36,46,40,33,37)

xx7table <- matrix(c(adcars,nonadcars),ncol=2)
colnames(xx7table) <- c(1,0)
rownames(xx7table) <- dos
CochranArmitageTest(xx7table)
CochranArmitageTest(xx7table,alternative="one.sided")

## Fisher's exact test comparing control to each dose level ##
```

```
# carcinomas poly3 adjusted denom #
xx8table <- matrix(c(1, 1, 37.5, 38.4), ncol=2)
colnames(xx8table) <- c(1,0)
rownames(xx8table) <- c(0,0.01)
xx9table <- matrix(c(1, 2, 37.5, 32.5), ncol=2)
colnames(xx9table) <- c(1,0)
rownames(xx9table) <- c(0,0.06)
xx10table <- matrix(c(1, 1, 37.5, 31.3), ncol=2)
colnames(xx10table) <- c(1,0)
rownames(xx10table) <- c(0,1.18)
xx11table <- matrix(c(1, 5, 37.5, 34.2), ncol=2)
colnames(xx11table) <- c(1,0)
rownames(xx11table) <- c(0,3.43)
fisher.test(xx8table)
fisher.test(xx8table, alternative="less")
fisher.test(xx9table)
fisher.test(xx9table, alternative="less")
fisher.test(xx10table)
fisher.test(xx10table, alternative="less")
fisher.test(xx11table)
fisher.test(xx11table, alternative="less")

#carcinomas day 409 denominators #
xx12table <- matrix(c(1, 1, 40, 38), ncol=2)
colnames(xx12table) <- c(1,0)
rownames(xx12table) <- c(0,0.01)
xx13table <- matrix(c(1, 2, 40, 32), ncol=2)
colnames(xx13table) <- c(1,0)
rownames(xx13table) <- c(0,0.06)
xx14table <- matrix(c(1, 1, 40, 31), ncol=2)
colnames(xx14table) <- c(1,0)
rownames(xx14table) <- c(0,1.18)
xx15table <- matrix(c(1, 5, 40, 37), ncol=2)
colnames(xx15table) <- c(1,0)
rownames(xx15table) <- c(0,3.43)
fisher.test(xx12table)
fisher.test(xx12table, alternative="less")
fisher.test(xx13table)
fisher.test(xx13table, alternative="less")
fisher.test(xx14table)
fisher.test(xx14table, alternative="less")
fisher.test(xx15table)
```

```
fisher.test(xx15table, alternative="less")

#adenomas day poly3 denominators #
xx16table <- matrix(c(10, 3, 29, 36), ncol=2)
colnames(xx16table) <- c(1,0)
rownames(xx16table) <- c(0,0.01)
xx17table <- matrix(c(10, 2, 29, 33), ncol=2)
colnames(xx17table) <- c(1,0)
rownames(xx17table) <- c(0,0.06)
xx18table <- matrix(c(10, 6, 29, 26), ncol=2)
colnames(xx18table) <- c(1,0)
rownames(xx18table) <- c(0,1.18)
xx19table <- matrix(c(10, 6, 29, 33), ncol=2)
colnames(xx19table) <- c(1,0)
rownames(xx19table) <- c(0,3.43)
fisher.test(xx16table)
fisher.test(xx16table, alternative="less")
fisher.test(xx17table)
fisher.test(xx17table, alternative="less")
fisher.test(xx18table)
fisher.test(xx18table, alternative="less")
fisher.test(xx19table)
fisher.test(xx19table, alternative="less")

#adenomas day 317 denominators #
xx20table <- matrix(c(10, 3, 37, 47), ncol=2)
colnames(xx20table) <- c(1,0)
rownames(xx20table) <- c(0,0.01)
xx21table <- matrix(c(10, 2, 37, 42), ncol=2)
colnames(xx21table) <- c(1,0)
rownames(xx21table) <- c(0,0.06)
xx22table <- matrix(c(10, 6, 37, 34), ncol=2)
colnames(xx22table) <- c(1,0)
rownames(xx22table) <- c(0,1.18)
xx23table <- matrix(c(10, 6, 37, 41), ncol=2)
colnames(xx23table) <- c(1,0)
rownames(xx23table) <- c(0,3.43)
fisher.test(xx20table)
fisher.test(xx20table, alternative="less")
fisher.test(xx21table)
fisher.test(xx21table, alternative="less")
fisher.test(xx22table)
fisher.test(xx22table, alternative="less")
```

```
fisher.test(xx23table)
fisher.test(xx23table, alternative="less")

#adenomas and carcinomas poly3 denominators #
xx24table <- matrix(c(11, 4, 28, 35), ncol=2)
colnames(xx24table) <- c(1,0)
rownames(xx24table) <- c(0,0.01)
xx25table <- matrix(c(11, 4, 28, 31), ncol=2)
colnames(xx25table) <- c(1,0)
rownames(xx25table) <- c(0,0.06)
xx26table <- matrix(c(11, 7, 28, 25), ncol=2)
colnames(xx26table) <- c(1,0)
rownames(xx26table) <- c(0,1.18)
xx27table <- matrix(c(11, 10, 28, 29), ncol=2)
colnames(xx27table) <- c(1,0)
rownames(xx27table) <- c(0,3.43)
fisher.test(xx24table)
fisher.test(xx24table, alternative="less")
fisher.test(xx25table)
fisher.test(xx25table, alternative="less")
fisher.test(xx26table)
fisher.test(xx26table, alternative="less")
fisher.test(xx27table)
fisher.test(xx27table, alternative="less")

#adenomas and carcinomas day 317 denominators #
xx28table <- matrix(c(11, 4, 36, 46), ncol=2)
colnames(xx28table) <- c(1,0)
rownames(xx28table) <- c(0,0.01)
xx29table <- matrix(c(11, 4, 36, 40), ncol=2)
colnames(xx29table) <- c(1,0)
rownames(xx29table) <- c(0,0.06)
xx30table <- matrix(c(11, 7, 36, 33), ncol=2)
colnames(xx30table) <- c(1,0)
rownames(xx30table) <- c(0,1.18)
xx31table <- matrix(c(11, 10, 36, 37), ncol=2)
colnames(xx31table) <- c(1,0)
rownames(xx31table) <- c(0,3.43)
fisher.test(xx28table)
fisher.test(xx28table, alternative="less")
fisher.test(xx29table)
fisher.test(xx29table, alternative="less")
fisher.test(xx30table)
```

```
fisher.test(xx30table, alternative="less")
fisher.test(xx31table)
fisher.test(xx31table, alternative="less")
```

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APPENDIX IV. BENCHMARK DOSE MODELING OF ENDPOINT DATA FOR FIPRONIL

Modeling with Benchmark Dose Software (BMDS; version 3.2)

The Department of Pesticide Regulation (DPR) used a benchmark dose (BMD) approach to derive points of departure (PODs) for all data amenable to modeling. The US EPA's Benchmark Dose Software (BMDS; version 3.2) was used to estimate the threshold of toxicity for each endpoint. For this risk assessment, modeled data included quantal data (dichotomous incidence response) and continuous data (exact values and standard deviations from reported measurements). The threshold or Benchmark Response (BMR) level for a given effect was 10% for quantal or continuous data, and 5% for developmental effects (US EPA, 2012a). BMDS converted the data for each endpoint into a family of related mathematical models. Each model provided a benchmark dose (BMD) value as well as a value representing the 95% lower bound of the BMD (BMDL), which was considered the POD for the observed effect.

In the BMD approach, the data for each endpoint were used to generate a family of models. Each model was evaluated over the full dose range to select a "best" model for each data set. The evaluation process was based on a hierarchical examination of:

- the results from statistical tests for goodness-of-fit,
- the lowest Akaike Information Criteria (AIC) score for relative goodness-of-fit,
- closeness of BMD and BMDL to each other and to the nearest dose levels for goodness-of-fit and model dependence,
- visual inspection of lines over data points for goodness-of-fit and toxicological plausibility,
- the magnitude of residuals for goodness-of-fit, and
- considerations of sample size, variability, and whether there is a maximum response at the high dose.

The most appropriate models for each endpoint were next evaluated as part of the hazard identification process for their fitness to provide PODs for risk assessment. This evaluation reconsidered factors that included the toxicological plausibility of the effect and the quality of the data, as well as the relative magnitude of the threshold of toxicity represented by the BMDL. Models and PODs that were considered in the fipronil risk assessment are described in this appendix.

I. Acute Neurotoxicity Study in Rats: Modeling of Hindlimb Splay

Continuous hindlimb splay data from male rats in an acute neurotoxicity study (Hughes, 1997) were modeled with a 10% effect level (BMR_{10}) and constant variance.

Exponential4 was the best-fit model, with a BMD_{10} of 2.09 mg/kg and a $BMDL_{10}$ of 0.77 mg/kg. Raw data are shown in Table IV.1. BMDS analysis results are shown in Table IV.2, and the best-fit model plot is shown in Figure IV.1.

Table 69. Hindlimb splay data in male rats, 7 hours post-treatment with fipronil, in acute neurotoxicity study

Dose (mg/kg)	N	Mean (cm)	SD
0	10	8.7	1.58
2.5	10	7.8	1.38
7.5	10	6.7**	1.07
25	10	6.3**	2.08

** Indicates p-value < 0.01; SD: standard deviation.
Data from Hughes (1997).

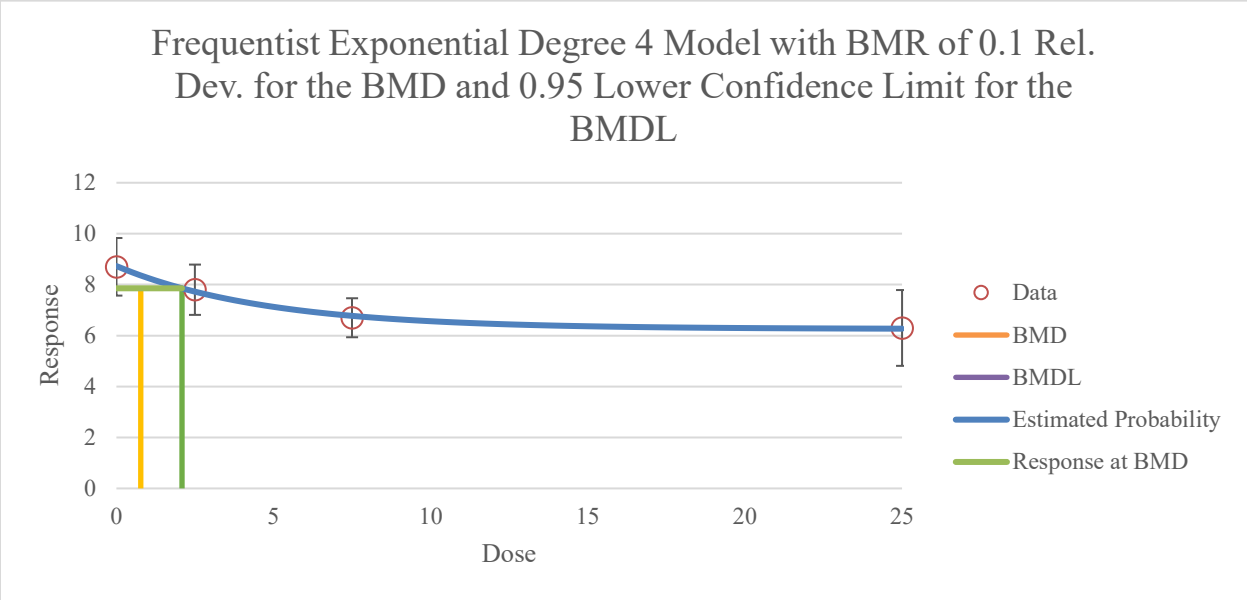

Figure IV.5. Plot of best-fit (Exponential 4) model of hind limb splay in male rats following exposure to fipronil in acute neurotoxicity study (Hughes, 1997)

Table 70. BMDS analysis of hindlimb splay data in male rats after fipronil exposure in acute neurotoxicity study

Model	BMD	BMDL	BMDU	Test 4 P-Value	AIC	Scaled Residual for Dose Group near BMD	Scaled Residual for Control Dose Group	BMDS Recommend
Expo2	8.69	5.52	18.48	0.13	155.47	-1.48	1.12	Viable - Alternate
Expo3	8.69	5.52	18.48	0.13	155.47	-1.48	1.12	Viable - Alternate
Expo4	**2.09**	**0.77**	**6.88**	**0.81**	**153.50**	**0.16**	**-0.06**	**Viable - Recommended**
Expo5	2.41	0.78	7.38	NA	155.44	0.00	0.00	Questionable
Hill	2.42	0.48	6.80	NA	155.44	0.00	0.00	Questionable
Poly3	9.98	6.81	20.37	0.11	155.89	-1.56	1.24	Viable - Alternate
Poly2	9.98	6.81	20.17	0.11	155.89	-1.56	1.24	Viable - Alternate
Power	9.98	6.81	20.15	0.11	155.89	-1.56	1.24	Viable - Alternate
Linear	9.98	6.81	20.16	0.11	155.89	-1.56	1.24	Viable - Alternate

Expo: Exponential model; Ploy: polynomial degree model; BMD: benchmark dose; BMDL: lower 95th percentile limit of BMD; AIC: Akaike Information Criteria score for relative goodness-of-fit. Blue highlighting and bold text: Final BMD and BMDL. All analyses were frequentist, restricted with relative deviation of 10%.
Data from Hughes (1997).

II. Developmental Neurotoxicity Study: Modeling of Rat Pup Body Weight

BMD analysis was performed on male and female pup body weight data from all time points in the developmental neurotoxicity (DNT) study (Mandella, 1995). Data were modeled using a 5% effect level (BMR_{05}).

The Exponential5 model provided the best-fit, with a BMD_{05} of 0.68 mg/kg and a $BMDL_{05}$ of 0.38 mg/kg/day for male rat pups on post-natal day (PND) 17. Raw data are shown in Table IV.3. Analysis results are shown in Table IV.4, and the best-fit model plot is shown in Figure IV.2. The BMDL 0.38 mg/kg/day was appropriate for use as an endpoint for decreased pup body weight, however, it was not the most sensitive endpoint in the DNT study.

The Fipronil Task Force (FTF) also analyzed pup body weight data from the DNT study as well as from the two-generation reproductive study in rat (King, 1992), (Fipronil Task Force, 2015). Four continuous BMD models were tested to fit pup body weight data at 1 standard deviation (SD), and at 5% and 10% extra risk levels. FTF determined that the most conservative BMDL was derived from the DNT study, with 5% extra risk, on female pup body weight at PND 21. The resultant $BMDL_{05}$ of 0.39 mg/kg/day was the same as DPR's $BMDL_{05}$ for the PND 17 male pups.

Table 71. Body weight data for PND 17 male rat pups following fipronil exposure *in utero* and during lactation in the developmental neurotoxicity study

Dose (mg/kg/day)	N	Mean (grams)	SD
0	26	41.7	3.7
0.05	29	41.2	4.5
0.9	29	38.9	5.1
15	21	31.3	3.6

SD: standard deviation. Data from Mandella (1995).

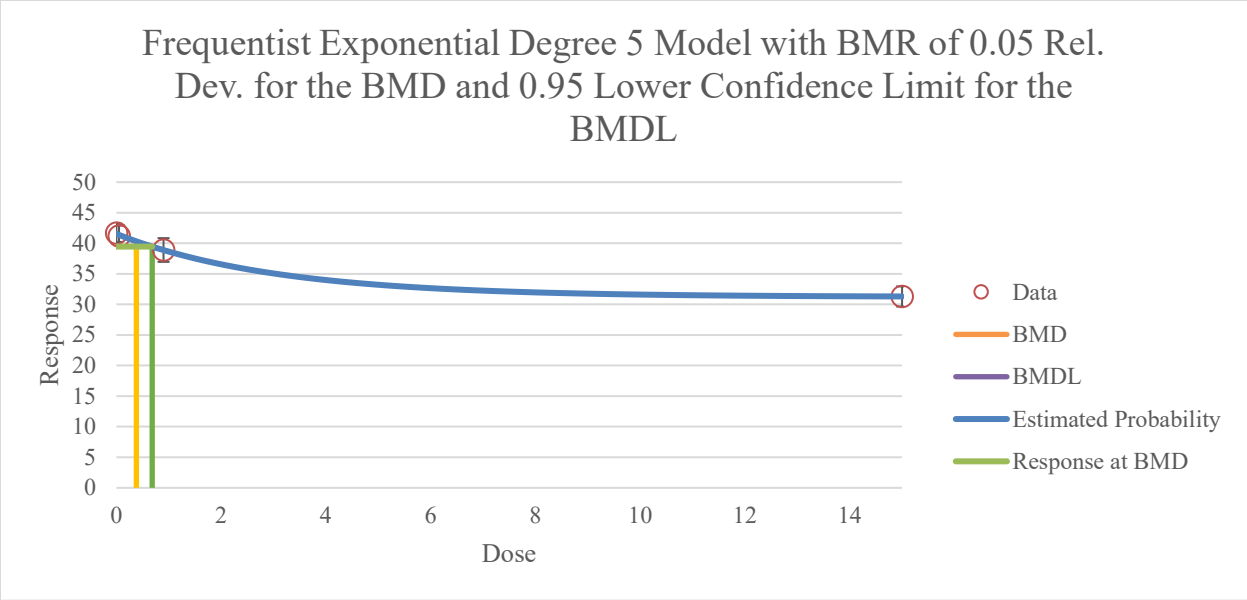

Figure IV.6. Plot of best-fit (Exponential Degree 5) model of pup body weight in male Sprague Dawley rats following fipronil exposure *in utero* and during lactation from Mandella (1995) developmental neurotoxicity study

Table 72. BMDS analysis of PND 17 body weight data in rat pups following fipronil exposure in utero and during lactation in developmental neurotoxicity study

Model	BMRF	BMD	BMDL	BMDU	Test 4 P-Value	AIC	Scaled Residual for Dose Group near BMD	Scaled Residual for Control Dose Group	BMDS Recommendation
Expo2	0.05	2.85	2.36	3.56	0.14	611.75	-1.59	1.02	Viable - Alternate
Expo3	0.05	2.85	2.36	3.56	0.14	611.75	-1.59	1.02	Viable - Alternate
Expo4	0.05	0.68	0.38	1.98	0.77	609.92	0.02	0.20	Viable - Alternate
Expo5	**0.05**	**0.68**	**0.38**	**1.98**	**0.77**	**609.92**	**0.02**	**0.20**	**Viable - Recommended**
Hill	0.05	0.85	0.73	1.10	NA	612.03	0.00	0.32	Questionable
Poly3	0.05	3.19	2.71	9.20	0.12	612.05	-1.64	1.06	Viable - Alternate
Poly2	0.05	3.19	2.71	7.25	0.12	612.05	-1.64	1.06	Viable - Alternate
Power	0.05	3.19	2.71	12.16	0.12	612.05	-1.64	1.06	Viable - Alternate
Linear	0.05	3.19	2.71	3.88	0.12	612.05	-1.64	1.06	Viable - Alternate

PND: postnatal day; Expo: Exponential model; Poly: polynomial model. BMD: benchmark dose; BMDL: lower 95th percentile limit of BMD; AIC: Akaike Information Criteria score for relative goodness-of-fit. Blue highlighting and bold text: Final BMD and BMDL. All analyses were frequentist, restricted with relative deviation of 5%.
Data from Mandella (1995).

III. Subchronic 13-week Oral Mouse Study: Modeling of Periacinar Hypertrophy

Dichotomous periacinar hypertrophy data in male mice exposed to fipronil via the diet for 13 weeks (Broadmeadow, 1991) were modeled with a 10% effect level (BMR_{10}).

DPR used the Dichotomous Hill model, with a BMD_{10} of 0.11 mg/kg/day and a $BMDL_{10}$ of 0.05 mg/kg/day. Raw data are shown in Table IV.5. Analysis results are shown in Table IV.6, and the plot of the best-fit model is shown in Figure IV.3.

Table 73. Incidence of periacinar hypertrophy in male mice after 13-weeks of daily fipronil exposure

Dose (mg/kg/day)	N	Effect
0	12	0
0.13	12	2
0.38	12	3
1.27	12	6
3.2	12	10

Data from Broadmeadow (1991).

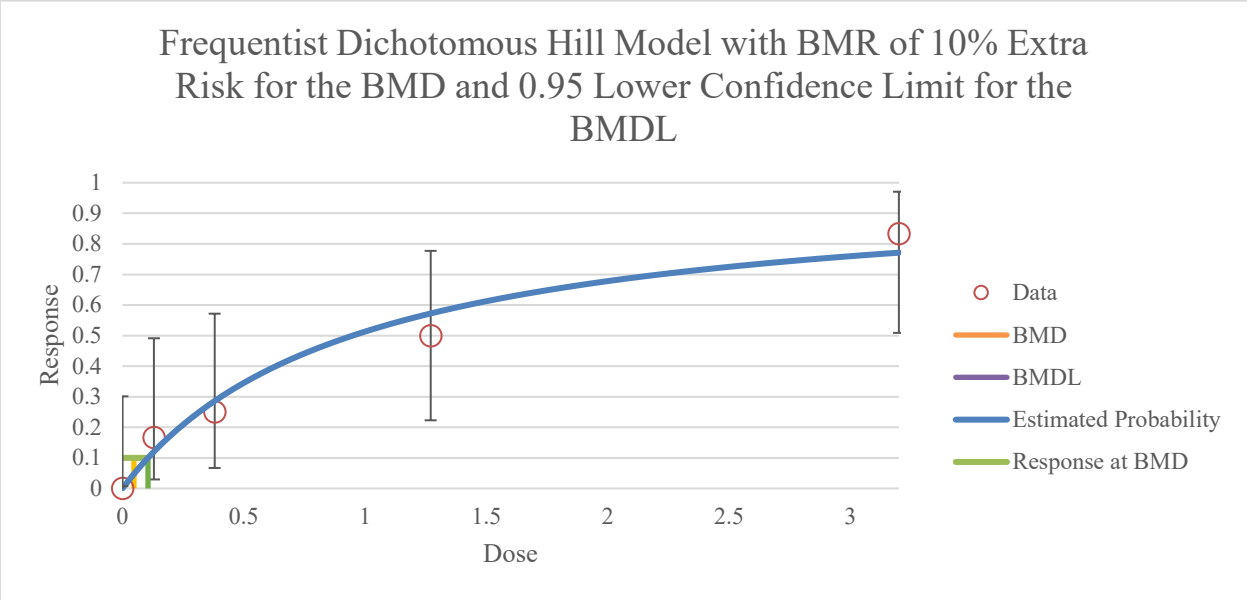

Figure IV.7. Plot of best-fit (Hill) model of periacinar hypertrophy incidence in male mice following 13-weeks of daily fipronil exposure (Broadmeadow, 1991)

Table 74. BMDS analysis of periacinar hypertrophy data in male mice following 13 weeks of daily fipronil exposure

Model	BMD	BMDL	BMDU	P Value	AIC	Scaled Residual for Dose Group near BMD	Scaled Residual for Control Dose Group	BMDS Recommendation
Dichotomous Hill*	**0.105**	**0.047**	**0.257**	**0.841**	**56.591**	**0.492**	**0.000**	**Viable - Alternate**
Gamma	0.174	0.117	0.546	0.716	57.052	0.996	-0.371	Viable - Alternate
Log-Logistic	0.105	0.061	0.257	0.841	56.591	0.492	0.000	Viable - Alternate
Multistage Degree 4	0.174	0.117	0.516	0.716	57.052	0.996	-0.371	Viable - Alternate
Multistage Degree 3	0.174	0.117	0.516	0.716	57.052	0.996	-0.371	Viable - Alternate
Multistage Degree 2	0.174	0.117	0.516	0.716	57.052	0.996	-0.371	Viable - Alternate
Multistage Degree 1	0.174	0.117	0.302	0.716	57.052	0.996	-0.371	Viable - Alternate
Weibull	0.174	0.117	0.533	0.716	57.052	0.996	-0.371	Viable - Alternate
Logistic	0.513	0.354	0.743	0.434	59.974	0.538	-1.328	Viable - Alternate
Log-Probit	0.108	0.017	0.241	0.634	58.671	0.485	0.000	Viable - Recommended
Probit	0.494	0.355	0.694	0.442	59.868	0.552	-1.298	Viable - Alternate

BMD: benchmark dose; BMDL: lower 95th percentile limit of BMD; AIC: Akaike Information Criteria score for relative goodness-of-fit. Blue highlighting and bold text: Final BMD and BMDL. All analyses were frequentist, restricted with extra risk of 10%. *Dichotomous Hill was used instead of log-probit because it had the lowest AIC and best visual fit.

Modeling with Bayesian Statistics-Based BMD (BBMD)

Comparative analysis of BMD modeling using frequentist and Bayesian approaches

The US EPA BMD Software (BMDS, versions 3.2) used in the fipronil RCD employs a frequentist-based statistical approach (maximum likelihood estimation) for dose–response model fitting to estimate BMDs. More recently, a Bayesian statistics-based BMD (BBMD) methodology with a Markov chain Monte Carlo algorithm was developed to generate distributional BMD estimates [https://benchmarkdose.org, (Shao and Shapiro, 2018)]. DPR currently is in the process of evaluating PODs derived using the Bayesian BMD method. Our comparative analysis revealed a similar range of BMDLs derived from BMD modeling using either the frequentist or Bayesian approach (Table IV.7).

Table 75. BMDL/BMD derived from the frequentist (BMDS) and Bayesian (BBMD) approaches

Study	Endpoint	NOEL (mg/kg/day)	BMR	BMDL/BMD (mg/kg/day)		
				BMDS 3.2	BBMD-HW	BBMD-MA
Hughes, 1997	Decreased hindlimb splay in male rats	2.5	10% Rel Dev	0.77 / 2.09 (Expo 4)	0.17 / 1.02 (Expo 4)	0.28 / 7.23
Hughes, 1997	Decreased hindlimb splay in male rats	2.5	1 SD	1.56 / 4.43 (Expo 4)	0.38 / 2.59 (Expo 4)	0.59 / 16.41
Mandella, 1995	Decreased body weight in male rat pups at PND17	0.05	5% Rel Dev	0.38 / 0.68 (Expo 5)	0.37 / 0.69 (Expo 4)	0.46 / 2.15
Broadmeadow, 1991	Increased incidence of periacinar hypertrophy in male mice	n/a (LOEL = 0.13)	10% Extra risk	0.05 / 0.11 (Hill)	0.12 / 0.19 (Quantal linear)	0.13 / 0.27

BMR: Benchmark response; BBMD: Bayesian BMD; BBMD-HW: Models with highest posterior model weight in Bayesian BMD modeling; BBMD-MA: model average in Bayesian BMD modeling; Expo: exponential model; Bayesian BMD modeling was performed using the web-based application (benchmarkdose.com).

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APPENDIX V. PUBLISHED STUDIES EXCLUDED FROM THE FIPRONIL GENOTOXICITY ASSESSMENT

Through systematic literature review the Department of Pesticide Regulation (DPR) identified 20 published studies in the open literature relating to genotoxicity of fipronil (latest systematic literature review conducted on June 16, 2022). DPR uses a screening process to identify journal articles that could be useful in human health risk assessment. To be eligible for consideration in selecting critical PODs, in the weight of the evidence or in genotoxicity analyses, published papers must meet a set of minimum study acceptance criteria (US EPA, 2012b). Seventeen of the 20 studies were excluded from use in assessment of genotoxicity because they did not meet one or more of the study acceptance criteria, specifically:

1. The toxic effects are in an appropriate test animal species.
2. Treatment(s) are compared to acceptable controls (studies which use a solvent vehicle should also include solvent vehicle controls).
3. Adequate data are provided on the chemical tested (i.e., test article characterization, exact nature and source of the pesticide; the percent active ingredient and/or the purity of the test compound).
4. The study results (findings) are adequately reported.
5. The study findings are relevant to assessing human health risks.

Table V.1 outlines the published studies excluded from the fipronil genotoxicity assessment, and the reasons for exclusion.

Table 76. Published studies excluded from the fipronil genotoxicity assessment

	Study Reference	Study Design	Reason for Exclusion
1	(Ghisi Nde *et al.*, 2011)	**Test article:** 2.5 % Fipronil formulation Termidor (BASF) **Test system:** Caspian white fish (Rhamdia quelen) **N:** 15/dose **Exposure route:** test compound added to fish tanks **Dosing schedule:** Daily for 60 days **Doses:** 0, 0.05, 0.10 and 0.23 µg/L **Vehicle:** not specified **Endpoints:** Nuclear morphological alterations at 0.10 and 0.23 mg/ml	This study used a commercially formulated product with unknown formulation ingredients, the control vehicle was not described, and the test species is not relevant for human health risk assessment.
2	(de Oliveira *et al.*, 2012)	**Test article:** 80% Fipronil formulation - Reagent 800 WG, (BASF) **Test system:** 5-6 weeks old female Swiss mice **N:** 5/dose **Exposure route:** intraperitoneal **Dosing schedule:** single injection **Doses:** 0, 15, 25, 50 mg/kg **Vehicle:** distilled water **Endpoints:** DNA damage (Comet assay of nucleated cells) and micronucleus induction in reticulocytes in peripheral blood 24 h after exposure at 50 mg/kg/day	This study used a commercially formulated product with unknown formulation ingredients and unacceptable vehicle controls (animals received distilled water).
3	(Girgis and Yassa, 2013)	**Test article:** Fipronil product with unknown purity (Agrovetzaschita, S.P. Company) **Test system:** albino rats **N:** 10/dose **Exposure route:** oral **Dosing schedule:** single exposure **Doses:** 0, 25 and 50 mg/kg **Vehicle:** not specified **Endpoints:** Increases in chromosomal aberrations and micronuclei in bone marrow cells 24, 48 and 96 hours post dosing	This study used a fipronil product with unknown percent purity and the control vehicle was not described. The storage and preparation of dosing solutions were not detailed. The description of the test animals, study conduct including exposure (gavage or diet) and the methods used for the cytogenetic analyses and micronuclei assay were inadequate.
4	(Celik *et al.*, 2014)	**Test article:** 7.5% Fipronil formulation FIBREX 75 (described by authors as having 412.5 mg fipronil in 5.5 ml) **Test system:** Human peripheral blood lymphocytes **N:** 3 male donors **Dosing schedule:** fipronil added to cell cultures **Concentration:** 0, 0.1, 0.3, 0.7 µg/mL for 72 hours **Vehicle:** Not described **Endpoints:** Significant increase in sister-chromatid exchanges and micronucleus formation, , and DNA damage in a Comet assay	This study used commercially formulated product with unknown formulation ingredients, the source of the fipronil product and the control vehicle was not described. Additional concerns with this study included the unknown exposure temperature, duration, and the test concentration in the Comet assay, as well as the use of X-ray contrast agent that may not be entirely inert to the cell.

Table 76. Published studies excluded from the fipronil genotoxicity assessment

	Study Reference	Study Design	Reason for Exclusion
5	(de Morais *et al.*, 2016)	**Test article:** 80% Fipronil formulation Reagent 800 WG (BASF) **Test system:** Drosophila melanogaster **N:** 40 flies/sex/dose **Exposure route:** larval immersion **Dosing schedule:** 48 hours **Concentrations:** 0, 0.3; 0.7; 1.5 or 3.0 x 10^{-5} mM **Vehicle:** not specified **Endpoints**: mutagenicity, recombinogenicity and carcinogenicity in somatic cells of D. melanogaster at all doses with cytotoxicity at 0.7x10^{-5} mM and higher concentrations	This study used a commercially formulated product with unknown formulation ingredients and unacceptable vehicle controls (water).
6	(Lovinskaya *et al.*, 2016)	**Test article:** Fipronil product not specified **Test system:** 2–3 month-old male BALB/cYwal mice **N:** 5/dose **Exposure route:** intraperitoneal **Dosing schedule:** single and repeated (10 days) exposures **Doses:** 0, 4.75, 9.50, 19.00, and 31.70 mg/kg/day **Vehicle:** water **Endpoints:** Significant DNA damage in cells of liver and spleen at ≥ 9.50 mg/kg and in lung cells at all doses, chromosomal aberrations in bone marrow cells and structural abnormalities of spermatocytes at 19 and 31.7 mg/kg/day.	This study used fipronil of unknown source and purity, and unacceptable controls (water).
7	(Mohammed *et al.*, 2016)	**Test article:** Fipronil formulation 20% (Yong-nong Bioscience Co, Ltd) **Test system:** Japanese quaill **N:** 10 birds/dose **Exposure route:** Oral gavage **Dosing schedule:** Single dose **Doses:** 0, 1.13, 2.26, 5.65. 11.3 mg/kg **Vehicle:** distilled water **Endpoints:** Dose-dependent increases of DNA strand breaks in liver cells of quails, liver histopathology, reduced body weight and food intake, and mortality	This study used a commercially formulated product with unknown formulation ingredients, unacceptable controls (animals were exposed to water) and the test species is not relevant for human health risk assessment.
8	(Yildirim and Agar, 2016)	**Test article:** Fipronil purity not described but was purchased from Sigma and likely was of high purity **Test system:** roots of Vicia faba seedlings **N:** 15 seeds/dose **Exposure route:** seeds soaked in unspecified fipronil solution **Dosing schedule:** 7 days **Concentrations:** 0, 0.5, 1, 2, 3, and 4 ppm **Vehicle:** Not specified **Endpoints:** Dose dependent changes in genomic DNA template stability, decreased amount of root length and increased level of protein	This study did not detail the storage, preparation of dosing solutions, the vehicle and the controls. In addition, the test organism is not appropriate for human health risk assessment.

Table 76. Published studies excluded from the fipronil genotoxicity assessment

	Study Reference	Study Design	Reason for Exclusion
9	(Karaismailoglu, 2017)	**Test article:** Fipronil purity and source not specified **Test system:** roots of plant Allium cepa (onion) **N:** 5 onion bulbs/dose **Exposure:** roots soaked in unspecified fipronil solution **Dosing schedule:** 6, 12 and 24 h **Doses:** 0, 1, 2.5, 5, and 10 ppm **Vehicle:** Not specified **Endpoints:** Statistically significant increases in chromosome aberrations and micronuclei in somatic cells of the plant	This study did not specify the purity of the test compound, the vehicle and the controls, and the test organism is not appropriate for human health risk assessment.
10	(Ziliotto *et al.*, 2017)	**Test article:** 9.8% Fipronil formulation Frontline plus® **Test system:** adult crossbred dogs **N:** 5/sex **Exposure route:** dermal **Dosing schedule:** single application **Doses:** 0, 6.7 mg/kg **Vehicle:** not described **Endpoints**: Negative for genotoxicity based on lack of increased DNA damage in peripheral blood nucleated cells at 3, 8 and 24 h, measured in a Comet assay	This study used a commercially formulated product with unknown formulation ingredients and unacceptable controls (untreated control dogs assayed just before they received a single dermal dose). The formulation also contained methoprene, a growth regulator used as an insecticide.
11	(Ardeshir *et al.*, 2019)	**Test article:** Fipronil 95% purity (Moshkfam Fars Chemical Co) **Test system:** Caspian white fish **N:** 12 fish/dose **Exposure route:** fipronil solutions added to fish tank **Dosing schedule:** 14 days **Doses:** 0, 0.1, 1, 5 and 10 µg/L) **Vehicle:** not specified **Endpoints:** Increased DNA strand breaks in livers measured by the Comet assay at all concentrations.	This study used unacceptable controls (ground water instead of solvent or vehicle) and the test species is not relevant for human health risk assessment.
12	(de Morais *et al.*, 2019)	**Test article:** 80% Fipronil formulation Reagent 800 WG (BASF) **Test system:** plant Tradescantia pallida **N:** 25 stems /dose **Exposure:** fipronil added to water **Dosing schedule:** 8 h **Concentrations:** 0.025; 0.05; 0.1; 0.2; 0.4; 0.8 and 1.6 g/L **Vehicle:** distilled water **Endpoints**: Increased micronuclei in tetrads of T. pallida at 0.2 g/L and higher concentrations.	This study used a commercially formulated product with unknown formulation ingredients, unacceptable control (distilled water) and the test species is not a relevant for human health risk assessment.
13	(Amaeze *et al.*, 2020)	**Test article:** 2.5% Fipronil-EC formulation **Test system:** Catfish Clarias gariepinus **N:** 25 stems /dose **Exposure:** fipronil added to water **Dosing schedule:** 96 hours **Concentrations:** 0, 6.5, 7.5, 8.5, 9.5, 10.5 µg/L **Vehicle:** dechlorinated water **Endpoints**: Increased nuclear abnormalities in red blood cell in catfishes, no micronuclei formation observed.	This study used a commercially formulated product with unknown formulation ingredients, unacceptable controls (dechlorinated municipal water) and the test species is not relevant for human health risk assessment.

Table 76. Published studies excluded from the fipronil genotoxicity assessment

	Study Reference	Study Design	Reason for Exclusion
14	(de Oliveira *et al.*, 2020)	**Test article:** 80% Fipronil formulation - Reagent 800 WG, (BASF) **Test system:** Amazonian turtles (Podocnemis expansa) **N:** 5 eggs /dose **Exposure:** eggs incubated in fipronil solution **Dosing schedule:** 59 days (from beginning of egg incubation to hatching) **Doses:** 0, 4, 400 ppb **Vehicle:** distilled water mixed with sand **Endpoints:** Changes suggestive morphotoxicity and aneuploidogenicity in erythrocytes of pups from treated eggs at 4 but bot at 400 ppb, , no micronuclei formation or DNA damage observed.	This study used a commercially formulated product with unknown formulation ingredients, unacceptable controls (distilled water) and the test species is not relevant for human health risk assessment.
15	(Santos *et al.*, 2021)	**Test article:** 80% Fipronil formulation - Reagent 800 WG, (form BASF) **Test system:** tadpoles **N:** 4 /dose **Exposure:** fipronil added to tank water **Dosing schedule:** 4, 8, 12, and 16 days **Doses:** 0.00, 0.04, 0.08, 0.4 mg/L, **Vehicle:** not specified **Endpoints:** Increases of anucleated erythrocyte cells.	This study used a commercially formulated product with unknown formulation ingredients, unacceptable controls (tank water only), and the test species is not relevant for human health risk assessment.
16	(Uçar *et al.*, 2021)	**Test article:** Fipronil purity not specified (obtained from Akdeniz Chemistry**)** **Test system:** Rainbow trout **N:** not specified, 160 fish were purchased **Exposure:** fipronil added to aquarium water **Dosing schedule:** 4 days **Doses:** 0, 0.05, 0.1 and 0.2 mg/L **Vehicle:** not specified **Endpoints:** Statistically significant increase of micronucleus formation in erythrocytes, DNA damage	This study did not describe the purity of fipronil, the preparation of dosing solutions, the vehicle and the controls. In addition, the test organism is not appropriate for human health risk assessment.
17	(Tisch *et al.*, 2007)[a]	**Test article:** Fipronil 95.5% (obtained from BASF AG**)** **Test system:** tonsil mucosal epithelial cells isolated from 85 tonsillitis patients over unspecified time span **Exposure:** fipronil added to culture medium **Dosing schedule:** 1 h at 37°C **Doses:** 0 (DMSO), 0.05, 0.1, 0.5, 0.75 and 1.00 mM **Vehicle: DMSO (**concentration not specified) **Endpoints:** DNA damage measured by the Comet assay	Study quality is a concern. This study did not include methods section and provided only a cursory explanation of the experimental design. Methods for determining cytotoxicity and the software used to score comets were not described. The DMSO concentration was not indicated and fipronil concentrations may have exceeded its solubility in cell suspension medium. DPR could not determine if the test system was capable of detecting negative results. Finally, the study did not demonstrate that DNA breakage stemmed from genotoxicity as opposed to cell death (necrosis and/or apoptosis).

[a]Article originally published in German. HHA translated the paper into English with Google translator

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APPENDIX VI. FIPRONIL DIETARY EXPOSURE ASSESSMENT

DEEM OUTPUT FILES

Acute Tier 2 Dietary Exposure

DEEM Output Files available upon Request.

Chronic Dietary Exposure

DEEM Output Files available upon Request.

APPENDIX VII. EXPOSURE TABLES FOR FIPRONIL

Summary of Exposure Tables from the Fipronil Exposure Assessment Document

Table 8. Inhalation and dermal fipronil exposure for handlers applying structural LC products

Exposure pathway	STADD[a] (µg/kg/d)	SADD[b] (µg/kg/d)	AADD[c] (µg/kg/d)	LADD[d] (µg/kg/d)
Dermal contact	1.4	0.49	0.28	0.15
Inhalation	0.53	0.19	0.11	0.059

a: short-term absorbed daily dose (STADD) = exposure (88.9 and 5271 µg/lb for inhalation and dermal respectively) × fipronil handled (0.42 lb/d) × absorption rate (0.043 for dermal and 1 for inhalation) ÷ body weight (70kg)
b: seasonal average daily dose (SADD) = exposure (31.9 and 1895 µg/lb for inhalation and dermal respectively) × fipronil handled (0.42 lb/d) × absorption rate (0.043 for dermal and 1 for inhalation) ÷ body weight (70kg)
c: annual average daily dose (AADD) = SADD × annual use months (7, April-October) ÷ 12
d: lifetime average daily dose (LADD) = AADD × 40 years of work in a lifetime ÷ 75

Table 10. Inhalation and dermal fipronil exposure for handlers applying structural LC products to overhead areas

Exposure pathway	STADD[a] (µg/kg/d)	SADD[b] (µg/kg/d)	AADD[c] (µg/kg/d)	LADD[d] (µg/kg/d)
Dermal contact	6.3	1.3	0.74	0.40
Inhalation	0.17	0.064	0.037	0.020

a: short-term absorbed daily dose (STADD) = exposure (28 and 24642 µg/lb for inhalation and dermal respectively) × fipronil handled (0.42 lb/d) × absorption rate (0.043 for dermal and 1 for inhalation) ÷ body weight (70kg);
b: seasonal absorbed SADD = exposure (11 and 5092 µg/lb for inhalation and dermal respectively) × fipronil handled (0.42 lb/d) × absorption rate (0.043 for dermal and 1 for inhalation) ÷ body weight (70kg);
c: annual average daily dose (AADD) = SADD × annual use months (7, April-October) ÷ 12;
d: lifetime average daily dose (LADD) = AADD × 40 years of work in a lifetime ÷ 75.

Table 13. Fipronil inhalation exposure in a post-application indoor environment.

Human receptor	Exposure pathway	STADD[a] (µg/kg/d)	SADD[b] (µg/kg/d)
Adult	Inhalation	0.023	0.0056
Child	Inhalation	0.048	0.012

a: short-term absorbed daily dose (STADD) = maximum 24-h TWA concentration (0.081 ng/L) × inhalation rate (0.59 and 0.28 m^3/kg/d for child and adult respectively);
b: seasonal average daily dose (SADD) = average 24-h TWA concentration (0.020 ng/L) × inhalation rate (0.59 and 0.28 m^3/kg/d for child and adult respectively).

Table 15. Post-application dermal and oral exposure from fipronil in indoor space

Human receptor	Exposure pathway	STADD[a] (µg/kg/d)	SADD[b] (µg/kg/d)
Adult	Dermal contact[c]	0.026	0.0022
Child	Dermal contact	0.018	0.0016
	Oral, hand-to-mouth[d]	0.064	0.0055
	Oral, object-to-mouth	0.0086	0.00073
	Incidental Oral, total	0.073	0.0062

a: short-term absorbed daily dose (STADD) was calculated using the maximum value of fipronil on indoor space in Table 14;
b: seasonal average daily dose (SADD) was calculated using the mean value of fipronil on indoor space in Table 14;
c: STADD and SADD (dermal) = fipronil concentration (0.013 and 0.0011 µg/cm^2 for STADD and SADD respectively) × transferable fraction (0.06) × transfer coefficient (6800 and 1800 cm^2/hr for adult and child respectively) × exposure time (8 and 4 hr for adult and child respectively) × dermal absorption rate (0.043) ÷ body weight (70 and 13 kg for adult and child respectively);
d: STADD and SADD (oral, hand-to-mouth and object-to-mouth) calculation equations can be found on USEPA SOP page 7-39 and 7-44 (USEPA, 2012).

Table 18. Dermal and inhalation exposure for pet groomers using a spray product

Exposure pathway	STADD[a] (µg/kg/d)	SADD[b] (µg/kg/d)	AADD[c] (µg/kg/d)	LADD[d] (µg/kg/d)
Dermal contact	52	13	13	6.9
Inhalation	1.3	0.39	0.39	0.21

a: short-term absorbed daily dose (STADD) = exposure (33 and 31788 µg/g for inhalation and dermal respectively) × fipronil handled (2.6 g/d) × absorption rate (0.043 for dermal and 1 for inhalation) ÷ body weight (70kg);
b: seasonal average daily dose (SADD) = exposure (10 and 8024 µg/g for inhalation and dermal respectively) × fipronil handled (2.6 g/d) × absorption rate (0.043 for dermal and 1 for inhalation) ÷ body weight (70kg);
c: annual average daily dose (AADD) is the same as SADD. There is no data to analyze use pattern of pet spray products over a year, so this assessment assumes year round use;
d: lifetime average daily dose (LADD) = AADD × 40 years of work in a lifetime ÷ 75.

Table 19. Dermal and inhalation exposure for a home user using a spray product

Exposure pathway	STADD (µg/kg/d)
Dermal contact	19
Inhalation	0.47

95th percentile values in Table 17 are used to calculate STADDs (Frank, 2009);
Short-term absorbed daily dose (STADD) = exposure (33 and 31788 µg/g for inhalation and dermal respectively) × fipronil handled (0.99 g/d) × absorption rate (0.043 for dermal and 1 for inhalation) ÷ body weight (70kg).

Table 21. Post-application dermal and oral exposure for pet spray products

Human receptor	Exposure pathway	STADD[a] (µg/kg/d)	SADD[b] (µg/kg/d)
Adult	Dermal contact[c]	2.4	0.51
Child	Dermal contact	4.6	0.96
	Oral, hand-to-mouth	1.1	0.22

a: short-term absorbed daily dose (STADD) was calculated using the maximum dislodgeable fipronil percentile (2.2%) in Table 20;
b: seasonal average daily dose (SADD)s were calculated using 7-day average dislodgeable fipronil percentile (0.46%) from de Fontenay et al., 1997a;
c: short-term absorbed daily dose (STADD) and SADD (dermal) = applied fipronil amount (495 mg) × dislodgeable fraction (0.022 and 0.0046 for STADD and SADD respectively) ÷ pet surface area (11000 cm^2) × transfer coefficient (5200 and 1400 cm^2/h for adult and child respectively) × exposure time (0.77 and 1 h for adult and child respectively) ÷ body weight (70 and 13 kg for adult and child respectively) × dermal absorption rate (0.043);
b: short-term absorbed daily dose (STADD) and SADD (oral, hand-to-mouth) calculation equations can be found on USEPA SOP page 8-12 (USEPA, 2012).

Table 25. Dermal exposure for groomers using pet spot-on products.

Human receptor	Exposure pathway	STADD[a] (µg/kg/d)	SADD[b] (µg/kg/d)	AADD[c] (µg/kg/d)	LADD[d] (µg/kg/d)
Adult	Dermal contact	4.2	0.71	0.71	0.38

a: short-term absorbed daily dose (STADD) = exposure (4591 µg/g) × fipronil handled (1.5 g/d) × absorption rate (0.043) ÷ body weight (70kg);
b: seasonal average daily dose (SADD) = exposure (787 µg/g) × fipronil handled (1.5 g/d) × absorption rate (0.043) ÷ body weight (70kg);
c: annual average daily dose (AADD) is the same as SADD. There is no data to analyze use pattern of pet spot-on products over a year;
d: lifetime average daily dose (LADD) = AADD × 40 years of work in a lifetime ÷ 75.

Table 26. Dermal exposure for a home user using pet spot-on products

Human receptor	Exposure pathway	STADD[a] (µg/kg/d)
Adult	Dermal contact	1.5

a: short-term absorbed daily dose (STADD) = exposure (4591 µg/g) × fipronil handled (0.54 g/d) × absorption rate (0.043) ÷ body weight (70kg).

Table 28. Post-application dermal and oral exposure for pet spot-on products

Human receptor	Exposure pathway	STADD[a] (µg/kg/d)	SADD (µg/kg/d)
Adult	Dermal contact[c]	1.4	0.090
Child	Dermal contact	2.6	0.17
	Oral, hand-to-mouth[d]	0.60	0.040

a: short-term absorbed daily dose (STADD) was calculated using the maximum dislodgeable fipronil percentile (16.77%) in Table 27;
b: seasonal average daily dose (SADD) was calculated using the 7-day average dislodgeable fipronil percentile (1.1%) from de Fontenay et al., 1997c;
c: STADD and SADD (dermal) = Applied fipronil amount (5 mg) × Dislodgeable fraction (16.77% and 1.1% for STADD and SADD respectively) ÷ Pet surface area (1500 cm^2) × Transfer coefficient (5200 and 1400 cm^2/h for adult and child respectively) × Exposure time (0.77 and 1 h for adult and child respectively) ÷ Body weight (70 and 13 kg for adult and child respectively) × Dermal absorption rate (0.043);
d: STADD (oral, hand-to-mouth) calculation equation can be found on US EPA SOP page 8-12 (USEPA, 2012).

Table 29. Handler dermal and inhalation exposure for turf granule products

Human receptor	Exposure pathway	STADD[a] (µg/kg/d)	SADD[b] (µg/kg/d)	AADD[c] (µg/kg/d)	LADD[d] (µg/kg/d)
Adult	Dermal contact	0.66	0.24	0.12	0.063
	Inhalation	0.048	0.017	0.0085	0.0045

a: short-term absorbed daily dose (STADD) = Exposure (86100 and 266 µg/lb for dermal and inhalation respectively) × fipronil handled (0.0125 lb/d) × absorption rate (0.043 for dermal and 1 for inhalation) ÷ body weight (70kg);
b: seasonal average daily dose (SADD) = Exposure (30992 and 95.5 µg/lb for dermal and inhalation respectively) × fipronil handled (0.0125 lb/d) × absorption rate (0.043 for dermal and 1 for inhalation) ÷ body weight (70kg);
c: annual average daily dose (AADD) = SADD × annual use months (6, April-September) ÷ 12
d: lifetime average daily dose (LADD) = AADD × 40 years of work in a lifetime ÷ 75

Table 30. Post-application dermal and oral exposure for turf granule product

Human receptor	Exposure pathway	STADD[a] (µg/kg/d)
Adult	Dermal, normal physical activity[b]	0.0068
	Dermal, Mowing[c]	0.00012
	Total dermal	0.0069
Child	Dermal, normal physical activity	0.0099
	Oral, hand-to-mouth	0.0021
	Oral, object-to-mouth	0.00013
	Oral, incidental soil ingestion	0.00036
	Oral, episodic granular ingestion	23
	Total oral	23

a: short-term absorbed daily dose (STADD) (dermal, normal physical activity) = TTR (0.0368 ng/cm^2) × transfer coefficient (200000 and 54000 cm^2/h for adult and child respectively) × exposure time (1.5 h) ÷ body weight (70 and 13 kg for adult and child respectively) × dermal absorption rate (0.043);
b: short-term absorbed daily dose (STADD) (dermal, mowing) = TTR (0.0368 ng/cm^2) × transfer coefficient (5500 cm^2/h) × exposure time (1 h) ÷ body weight (70 kg) × dermal absorption rate (0.043);
c: short-term absorbed daily dose (STADD) (oral, hand-to-mouth, object-to-mouth and incidental soil ingestion and episodic granular ingestion) calculation equations can be found on USEPA SOP page 3-14, 3-18, 3-22 and 3-24 (USEPA, 2012).

Table 32. Post-application dermal and oral exposure for structural bait gel product

Human receptor	Exposure pathway	STADD (µg/kg/d)	SADD (µg/kg/d)
Adult	Dermal	0.00043	0.00012
Child	Dermal	0.00031	0.000088
	Oral, hand-to-mouth	0.0011	0.00031
	Oral, object-to-mouth	0.00014	0.000041
	Oral, Total	0.0012	0.00035

a: short-term absorbed daily dose (STADD) and seasonal average daily dose (SADD) of dermal exposure = deposition residue (2145 and 619 ng/m^2 for STADD and SADD respectively) × dislodgeable fraction (0.06) × transfer coefficient (6800 and 1800 cm^2/h for adult and child respectively) × exposure time (8 and 4 h for adult and child respectively) ÷ body weight (70 and 13 kg for adult and child respectively) × dermal absorption rate (0.043);
b: STADD and SADD (oral, hand-to-mouth) calculation equations can be found on USEPA SOP page 7-39 and 7-44 (USEPA, 2012).

Table 34. Applicator dermal and inhalation exposure for structural dust product

Human receptor	Exposure pathway	STADD[b] (µg/kg/d)	SADD[b] (µg/kg/d)	AADD (µg/kg/d)	LADD (µg/kg/d)
Adult	Dermal	0.085	0.014	0.0091	0.0048
	Inhalation	0.022	0.0031	0.0021	0.0011

a: short-term absorbed daily dose (STADD) = exposure (2765 and 31.2 µg/g for dermal and inhalation respectively) × fipronil handled (0.00011 lb/d) × absorption rate (0.043 for dermal and 1 for inhalation) ÷ body weight (70kg);
b: seasonal average daily dose (SADD) = exposure (443 and 4.4 µg/g for dermal and inhalation respectively) × fipronil handled (0.00011 lb/d) × absorption rate (0.043 for dermal and 1 for inhalation) ÷ body weight (70kg);
c: annual average daily dose (AADD) = SADD × annual use months (8) ÷ 12. The number of annual use months is based on 2012-2014 PUR;
d: lifetime average daily dose (LADD) = AADD × 40 years of work in a lifetime ÷ 75

Drinking water

The recommended concentrations (point estimates) for use in drinking water exposure assessment are 0.275 ppb for the acute dietary and 0.033 ppb for the chronic dietary.

REFERENCES

Frank, J. 2009. Policy memorandum-Method for calculating short-term exposure estimates. HSM 09004. Sacramento, CA: Worker Health and Safety Branch, Department of Pesticide Regulation, California Environmental Protection Agency. Available by request at: https://apps.cdpr.ca.gov/whsrpts/hsmemo/hsmem_hsmno_action.cfm.

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