M E M O R A N D U M

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DATE: January 17, 2023

SUBJECT: Response to the Fipronil Task Force Request for Reconsideration of Findings in DPR's 2021 Fipronil Draft Risk Characterization Document

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Background

The registrants, Fipronil Task Force, LLC (FTF), reviewed the draft Risk Characterization Document (RCD) for fipronil (January 2021) prepared by the Human Health Assessment Branch (HHA) of the Department of Pesticide Regulation (DPR). Their comments were submitted to HHA on May 20, 2021 in a document titled "Request for Reconsideration of DPR's Draft Fipronil Risk Characterization Document." This memorandum contains HHA's responses to comments by FTF, specific to the toxicology, hazard identification, and risk characterization sections of the RCD. Responses to comments pertaining to the Exposure Assessment Document are covered in a separate memorandum.

This memorandum is divided into three parts to correspond to comments received from FTF on the Critical Acute Points of Departure, Critical Subchronic Points of Departure, and Overall Risk Assessment Conclusions. Responses to the Executive Summary and Introduction parts of the FTF document were not prepared as these sections did not contain specific comments. Note that references cited in the FTF review are not included in the reference section of this memorandum.

DPR sincerely appreciates the efforts made by the Registrants and its representatives to review the draft RCD. When appropriate, FTF's comments were taken into account in the final fipronil RCD. Responses to selected comments are detailed below.

Request for Reconsideration: Critical Acute Point of Departure (POD)

Revised Critical Acute Oral POD

FTF Comment: In the draft RCD, an acute oral POD of 0.87 mg/kg/day was selected based on a Benchmark Dose (BMD) analysis, or Benchmark Dose Software (BMDS) modeling, to model a BMD and BMDL (lower limit of a 95% confidence interval of the BMD) for hindlimb splay in rats in an acute oral neurotoxicity study (Hughes, 1997). As two acute oral neurotoxicity studies with fipronil are available in the database, from which acute oral endpoints may be derived… It is first critical to note that BMD modeling is neither necessary nor appropriate for this endpoint. The use of the NOAEL/LOAEL approach using DPR's established no effect level of 2.5 mg/kg bw from Hughes et al (1997) is more appropriate than a BMD approach (DPR, 2021). The purpose of the BMD approach is to use dose-response modeling to refine dose levels that correspond to specific response levels near the low end of the observable range of the data (i.e., "benchmark dose levels"). This approach incorporates and conveys more information than the NOAEL/LOAEL approach (EPA, 2012a). In the case of fipronil, this refinement is not needed as a clear and acceptable NO(A)EL is available from the Hughes et al (1997) study…

> **DPR Response**: DPR uses the benchmark dose (BMD) modeling approach to derive points of departure (PODs) for all data amenable to modeling, regardless of whether an experimental dose was designated as a study no effect level. This aligns with the US Environmental Protection Agency's (US EPA) definition of a point of departure as,
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> > *"The dose-response point that marks the starting point for low-dose extrapolation. The POD may be a NOAEL/LOAEL, but ideally is established from BMD modeling of the experimental data, and generally corresponds to a selected estimated low-level of response (US EPA, 2012, p. 73)."*
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> Specific to the Hughes (1997) and Gill et al (1993) studies, DPR considered the 9–10% decrease in hindlimb splay at both study no-observed-effect levels (NOELs) to be within the observed range of variation because this effect did not increase in magnitude or reach statistical significance over a 5-fold dose increase (0.5 to 2.5 mg/kg/day). However, this did not eliminate the possibility that an effect was present at those doses. Indeed, the BMD model recognized some level of decreased hindlimb splay at the NOEL of 2.5 mg/kg/day to be treatment-related (i.e., above the normal variation). The benchmark dose approach is ideal for datasets of this type, as it only applies a biologically and statistically appropriate benchmark response level (BMR) but makes no assumption as to the presence or absence of a treatment response at a given dose. As such, DPR retained the acute critical POD derived with BMD modeling of the hindlimb splay data from Hughes (1997). DPR concluded that a 10% BMR was appropriate for modeling changes in hindlimb splay based on the data from Hughes (1997). After re-modeling the dataset with the most recent software version (BMDS v 3.2), the updated critical acute oral $BMDL_{10}$ is now 0.77 mg/kg/day with a BMD of 2.09 mg/kg/day. For detailed discussions on the BMD modeling of the critical acute POD for

fipronil please refer to the Risk Assessment and Risk Appraisal sections as well as the BMD appendix in the final RCD.

FTF Comment, continued: While the modeled data pass the "goodness of fit" test in the BMD modeling software, the output of the data is inconsistent with the results of the study as reported by DPR and do not accurately characterize the dose-response relationship with respect to adversity or biological relevance. In a study that has an established NOAEL, such as Hughes et al, the modeled BMDL should be above the established NO(A)EL to indicate an accurate description of the modeled effect. If the modeled BMDL is below the NO(A)EL, then the model is predicting the empirical NO(A)EL to be an adverse effect level, which is not only an inconsistency, but also an inaccuracy…

DPR Response: A modeled BMDL need not be above an established NOAEL (or NOEL) to be valid. Because the NOEL is entirely based on dose spacing and statistical power (i.e., number of animals per dose), it is more than possible that the BMDL can be lower than the NOEL. According to page 16 of the US EPA technical guidance on benchmark dose modeling cited by FTF (US EPA, 2012), an affirmative answer to each of the following questions enables use of the BMD approach:

1. Are there sufficient data?
2. Is there a biologically or statistically significant trend?
3. Are there enough dose groups?
4. Is the dose-response relationship amenable to modeling? (*i.e.*, Is there a clear dose-response relationship? And is the desired BMR near the range of observed responses?)
5. Are there adequate model fits and estimates of BMDs and BMDLs?

Because all criteria for valid BMD modeling are fulfilled by the fipronil database, DPR holds that the use of modeling is not only valid but provides a more accurate POD and is thus preferable to the NOEL of 2.5 mg/kg.

FTF Comment, continued: …[T]he chosen BMR (i.e., 10% decrease in hindlimb splay) is not biologically significant, nor does it have a specific quantitative threshold that is considered to be biologically significant or "adverse" …

DPR Response: There is no generally agreed upon biologically significant level for reduced hindlimb splay. DPR chose 10% as the lower limit of a biologically detectable response for designation as the acute BMDL. This value was supported by the data as the BMR of 10% remains near the range of observation. 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 acute neurotoxicity rat

studies, and (*3*) 10% is at or near the limit of sensitivity of these measurements for observing a statistically significant decrease in hindlimb spay.

FTF Comment, continued: If it were appropriate or scientifically justified to evaluate hindlimb splay by BMD modeling, one would consider a response level of one SD from the control based on the BMD Technical Guidance (2012a). Based on this information, a BMD analysis was conducted by the FTF using one SD of the control mean value as the BMR (BMDS Software Version 3.2) and resulted in a BMD_{1SD} of 4.4 mg/kg bw and a $BMDL_{1SD}$ of 1.6 mg/kg bw (Appendix A) … The BMDU (upper limit of the 95% confidence interval of the BMD) for this model was defined as infinity. As the ratio of BMDU/BMDL reflects the uncertainty in the BMD model (EFSA, 2017a), this reflects a potential "infinite" amount of uncertainty and further erodes the confidence in the BMD modeling to adequately describe the dose-response for this study. Thus, there is no adequate or appropriate BMD model, and a NOAEL/LOAEL approach is the most appropriate and accurate for human health risk assessment…

DPR Response: As per US EPA (2012), DPR uses 1 standard deviation (SD) as a default when other BMRs are not identifiable and as a standardized basis for comparison. DPR calculated a BMD_{1SD} of 4.4 mg/kg/day and a $BMDL_{1SD}$ of 1.6 mg/kg/day for the hindlimb splay data using a BMR of 1 SD and the BMDS v3.2 model. However, the BMR of 10% versus the default of 1 SD was chosen because the 10% effect level is supported by the data and the $BMDL_{10}$ value of 0.77 mg/kg/day is supported by the overall acute toxicity database (for example, the inhalation study in rats established an acute NOEL of 0.8 mg/kg/day; refer to the Hazard Identification and Appraisal Sections in the final RCD). The $BMDU_{10}$ for the $BMDL_{10}$ of 0.77 mg/kg/day was 6.88 mg/kg/day, derived with the Exponential 4 model. The $BMDU_{1SD}$ for the $BMDL_{1SD}$ of 1.6 mg/kg/day was undefined or infinite (also derived with the Exponential 4 model). The $BMDL_{1SD}$ (1.6 mg/kg/day) derived with a BMR of 1 SD was not used to establish the POD. Nevertheless, per BMDS v3.2, the 1 SD model with an undefined BMDU was considered "viable and recommended." Meaning, out of the 9 models included in the 1 SD analysis, the BMDS v3.2 program recommended using the Exponent 4 model with an undefined BMDU. Per the US EPA (2012) guidance, BMDU is reported for multistage cancer models (page 58) and for applications where a one-sided upper confidence interval is useful – for example, a 10% increase in extra risk (page 68) (US EPA, 2012). With a one-sided lower confidence interval (BMDL), "the interval extends to the mathematical (infinity) or natural upper limit" (page 68). Because DPR is interested in characterizing the data at the lower end of the model (i.e., the BMD/BMDL), this 1 SD model is valid and is appropriate to use for comparison to the 10% effect model because the Exponent 4 model passed all goodness-of-fit tests, has the lowest AIC, and passed the visual inspection.

FTF Comment, continued: The FTF also attempted to conduct BMD modeling on the hindlimb splay from the Gill et al. (1993) study using a BMR of 1SD, but the data did not fit any of the available models. However, the low magnitude of the effects at the 5 mg/kg dose level (12%) is supportive of 2.5 mg/kg bw from the Hughes (1997) study being a clear no effect level and an

appropriate NOAEL for POD selection. As discussed above, the Hughes et al study was conducted at doses of 0, 2.5, 7.5, or 25 mg/kg bw to further refine the NOAEL of 0.5 mg/kg bw from the Gill et al study dosed at 0, 0.5, 5, or 50 mg/kg bw…

> **DPR Response**: DPR concurs that the hindlimb splay data from Gill et al. (1993) were not amenable to modeling. However, the magnitude of effects at the low end of the response curve in one study (Gill *et al.*, 1993) do not negate the results of validated BMD modeling of data from another study (Hughes, 1997).

Acute Dermal POD: Revised Acute Oral POD is Protective

FTF Comment: In the draft RCD, the Department concluded that the acute NOAEL from a subchronic dermal study was based on decreased body weight and food consumption… [I]t is critical to note that no appropriate endpoints to assess acute exposures are available in the dermal toxicity database, including the studies mentioned by DPR. No adverse effects were observed in the repeated dose dermal toxicity study at any dose level and that the acute dermal LD50 studies are not appropriate for risk assessment consideration. Rather, the proposed oral NOAEL of 2.5 mg/kg bw is protective of all findings in repeated and acute dermal studies and the most appropriate for assessing dermal risks because an acute neurotoxicity study with dermal application is not available…

> **DPR Response**: DPR agrees that the available dermal toxicity studies were inadequate to provide a defensible critical POD and that the critical acute dermal value should therefore come from the critical acute oral study. However, for the reasons articulated in the preceding paragraphs, the BMDL value was appropriately established as the acute POD instead of the study NOEL.

FTF Comment, continued: In the subchronic dermal study in rats (Henwood, 1997), no treatment-related effects were reported that are attributable to a single-dose or acute exposure. Furthermore, no adverse effects were observed in this study and the NOAEL is the highest dose tested of 1,000 mg/kg bw/day. DPRs conclusion that acute dermal NOEL for the study was 500 mg/kg bw/day based on decreased bodyweight and food consumption does not reflect an adverse response which is appropriate for risk assessment POD selection… Therefore, there is no evidence to support that these changes, which were observed only during week 1, were treatment-related or adverse…

> **DPR Response**: Between study days 1 and 8, control male rats gained 45 ± 75 grams while the animals exposed to 1000 mg/kg/day gained 29 ± 15.3 grams ($p < 0.05$), a 24% deficit (Henwood, 1997). The acute dermal LOEL and NOEL values were therefore appropriately set at 1000 and 500 mg/kg/day based on a reduction in body weight gain during the first week. Food consumption was also reduced at 1000 mg/kg/day (180 ± 23.6 grams vs. 198 ± 15.9 grams in controls, not statistically significant). While the precise reason for the body

weight gain deficit was not clear, DPR recognizes it as evidence of a failure to thrive and thus a valid endpoint for LOEL designation in this study.

FTF Comment, continued: Additionally, changes in bodyweight and bodyweight gain are not generally accepted as acute effects or the result of a single dose and require additional investigation into palatability and food consumption as described above…

DPR Response: As noted in the prior response, DPR regards a 24% deficit in body weight gain over the first week of a study to be a toxicological effect. While such a response cannot be ascribed to a single dose, a week of daily exposures is indicative of a short-term response, which is similar to an acute response in a regulatory context. While the acute designation in this study had no effect on the ultimate risk assessment because oral exposure was used to represent the acute dermal situation, it is worth noting DPR has used body weight gain deficits over several days to drive acute risk assessment PODs in the past, most notably for 1,3-dichloropropene (https://www.cdpr.ca.gov/docs/whs/active_ingredient/1_3-d.htm).

FTF Comment, continued: DPR also concluded from the acute LD50 study in rabbits that the lowest acute dermal LOEL was 100 mg/kg/day for hyperactivity, diarrhea, and bloody kidney in rabbits. In this study, rabbits were treated with a single dermal dose of fipronil at 100, 250, 500, 1000, or 2000 mg/kg bw for 24 hours (Myers and Christopher, 1992). However, LD50 studies are not appropriate for use in quantitative risk assessment or for point of departure selection. Acute toxicity studies designed to identify the median lethal dose (LD50) and not establish a NO(A)EL or LO(A)EL value. The exposure duration of the LD50 study is 24 hours and uses a semi-occlusive wrap which does not reflect expected human exposure scenarios. Additionally, acute toxicity studies do not have a control group…

DPR Response: The acute LD50 study in rabbits (Myers and Christopher, 1992) was not used for quantitative risk assessment or as the basis of a POD because a functional observational battery (FOB) was not performed, negating the possibility of determining an endpoint of risk assessment significance. Furthermore, the dose range was higher than that relevant to critical POD determination. Identification of a dermal LOEL from Myers and Christopher was to emphasize that a NOEL could not be determined, and that use of the critical acute oral BMDL as the acute/short term POD was strongly indicated.

Acute Inhalation POD: Revised Acute Oral is Protective

FTF Comment: For acute inhalation toxicity, the draft RCD proposed an acute inhalation LOEL based on effects that were not observed after acute exposures or were only observed at the dose above the LOEL… However, …the no observable adverse effect concentration (NOAEC) for acute inhalation toxicity effects is 0.005 mg/L, equivalent to a NOAEL of 4.8 mg/kg bw/day. The proposed oral endpoint of 2.5 mg/kg bw is therefore the most appropriate for assessing

inhalation risks because it is protective of all acute effects observed in the inhalation study and an acute neurotoxicity study with inhalation exposure is not available…

DPR Response: DPR set the same acute NOEL as FTF. However, 0.005 mg/L converts to an internal dose of 0.8 mg/kg/day, not 4.8 mg/kg/day as stated in the Request for Reconsideration. As such, the proposed acute oral endpoint of 2.5 mg/kg/day would not protect from effects noted at the 0.005 mg/L range. In the RCD, air concentrations were converted to equivalent 1-day doses using the default rat breathing rate of 0.96 m^3/kg/day and normalized for exposure duration with the following equation:

$$\text{Dose (mg/kg/day)} = \text{Concentration (mg/m}^3\text{)} \times \frac{0.96\ \text{m}^3}{\text{kg.day}} \times \frac{4\ \text{hours}}{24\ \text{hours}}$$

FTF Comment, continued: Therefore, selection of the NOAEC and LOAEC of 0.0001 mg/L and 0.005 mg/L, respectively, is not appropriate for an acute POD because no relevant effects were reported within the initial days of exposures (i.e when acute exposures are relevant) at 0.005 mg/L. The appropriate NOAEC is 0.005 mg/L and the appropriate LOAEC is 0.03 mg/L based on the effects reported on Day 2 of exposure.

DPR Response: DPR set the acute POD at 0.005 mg/L (0.8 mg/kg/day) based on Adamo-Trigiani (1999) in agreement with FTF's proposed value. However, the subchronic POD was set at 0.001 mg/L (0.16 mg/kg/day) 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 (0.8 mg/kg/day). Note: the NOAEC was 0.001 mg/L not 0.0001 mg/L as stated in the Request for Reconsideration.

FTF Comment, continued: Decreased bodyweights were also not observed following acute exposure to fipronil at the reported NOAEC and LOAEC of 0.0001 and 0.005 mg/L, respectively (Table 3). In male rats, a statistically significant decrease was only observed at the high-dose and only following 8-days of exposure. In female rats, decreased bodyweights were not observed until Day 22 of the study. Additionally, while a statistically significant decrease in bodyweights was observed, they are not considered to be biologically relevant as they are less than 10% and within the normal biological variation as discussed in the previous section. As no biologically significant changes in absolute bodyweight occurred at the DPR reported LOEL of 0.005 mg/L, changes in food consumption and bodyweight gain are also not considered to be toxicologically relevant. Furthermore, as previously discussed, effects on bodyweight over the course of a week are not generally accepted for regulatory purposes or as scientific precedent as being the result of a single-dose or as being relevant to acute exposure. Additionally, changes in liver weights and clinical chemistry changes were not considered to be adverse in the absence of liver histopathology or 2-fold changes in at least 2 liver enzymes (EPA, 2002).

DPR Response: FTF correctly pointed out that decreased bodyweights were not observed following acute inhalation exposure to fipronil at the LOEL of 0.005 mg/L. However, this concentration resulted in a significant decrease in body weights in females after repeated exposures for 22–28 days. DPR established a subchronic inhalation NOEL of 0.001 mg/L based on this effect. Moreover, the multiplicity of parallel organ weight and clinical chemistry changes at the LOEL of 0.005 mg/L (i.e., increased relative and absolute liver weights changes in cholesterol, bilirubin, globulin, prothrombin time, and activated thromboplastin time) were suggestive of liver toxicity even in the absence of overt liver histopathology. The point takes on greater significance when the observations of liver pathology in mice are taken into account. For this reason, DPR considers these effects to be toxicologically significant.

DPR also identified effects at the highest tested concentration (LOEL of 0.03 mg/L) that occurred after a short-term exposure, including significant decreases in body weights in males first measured at treatment day 8 and clinical signs on day 2. These observations were the basis for setting an acute/short-term inhalation NOEL at 0.005 mg/L. DPR maintains that body weight decreases, particularly when sustained over a short time period or in the context of other effects, are toxicologically significant and appropriate for acute NOEL designation.

Review of DPR-Cited Literature Studies: Revised Critical Oral POD is Protective

FTF Comment: Based on several literature studies, the draft RCD concluded that the literature supports an acute NOEL ranging from 1 to 5 mg/kg bw… DPR proposes that the Caballero (2015) study is appropriate for POD derivation. While this study is well-conducted and provides mechanistic information into the fipronil liver and thyroid toxicity, the effects at the LOEL of 5 mg/kg/day are not considered adverse and are not considered appropriate for acute POD selection. In Caballero (2015), rats were orally (gavage) treated with fipronil at doses of 1, 5, 10, or 15 mg/kg bw/day for 6 days. Determinations of cytochrome P450 (CYP) enzyme activities were carried out in hepatic microsomes isolated from treated rats… As shown in Table 5 above, minimal effects (less than 2-fold) on hepatic enzymes were reported at 5 or 10 mg/kg/day. Elevations of hepatic enzymes are an expected adaptive response to a xenobiotic. It is generally accepted that changes in hepatic enzymes of less than 2-fold in the absence of liver weight changes and liver histopathological effects are not considered to be adverse for human health risk assessment (EPA 2002; Pandiri et al, 2017; Maronpot et al, 2010; Hall et al, 2012). Additionally, while CYP induction may be a biomarker for liver effects, it is a biomarker for repeated dose liver effects, and not typically associated with single-dose or acute exposures. Furthermore, it is generally considered an adaptive response and investigated to determine the mode of action of a chemical and not establish an adverse effect level (Maronpot et al, 2010). Therefore, the effects at the reported LOEL of 5 mg/kg/day are not considered adverse and are not considered appropriate for acute POD selection…

DPR Response: DPR agrees that Caballero's observation is consistent with an adaptive hepatic response. To clarify, the RCD does not contend that those observations are either adverse or appropriate for POD derivation. However, global induction of CYP activity is relevant to the ultimate designation of the acute POD because the suggestion of a concerted hepatic response at a LOEL dose (5 mg/kg/day; NOEL = 1 mg/kg/day) (Caballero *et al.*, 2015) was similar to the relevant dose range used in the critical Hughes (1997) neurotoxicity study. As mentioned previously, stimulation of liver metabolic capacity is critical to the damaging effects of fipronil on thyroxin levels in this dose range.

FTF Comment, continued: In Moser (2015), the selected NOEL of 5 mg/kg bw based on decreased T3 and T4 and altered metabolic profiles does not impact acute POD selection and the NOAEL of 2.5 mg/kg bw is protective of all effects observed in this study…

DPR Response: As with Caballero (2015), Moser (2015) was cited as support for the acute LOEL/POD of 7.5 mg/kg / 0.87 mg/kg mainly because it provided evidence of responsivity in a dose range relevant to the critical designation (Moser *et al.*, 2015). The POD has been remodeled to a value of 0.77 mg/kg.

FTF Comment, continued: In Martins (2009), male Wistar rats were administered a single oral gavage dose of fipronil at 0, 1, 10, 30, or 100 mg/kg bw in distilled water. Open field and elevated plus maze parameters were evaluated. A single dose of fipronil showed behavioral changes, characterized by a reduction of motor activity in the open field and elevated plus maze. A review of the data tables in Martins (2009), specifically for number of rearing, movement time in the open maze, and decreased time in the closed arm of the elevated cross maze did not reveal any treatment-related changes at 10 mg/kg bw. In the open field after one hour of exposure, rearings were only decreased at the 30 and 100 mg/kg bw dose levels and movement time was also only decreased at 30 and 100 mg/kg bw dose levels and did not show a clear dose-response relationship. There was no clear indication of an effect at 10 mg/kg bw at any time point in the elevated cross maze tables. Further, even if effects at 10 mg/kg bw were identified, the critical acute oral POD of 2.5 mg/kg bw is protective. Additionally, as this study was non-guideline, the available guideline neurotoxicity studies are considered to be more reliable.

DPR Response: Upon reexamination, DPR found methodological problems with Martins (2009). For example, the investigators used an 80% fipronil formulation without the proper controls. In addition, the study was a Master's thesis published in Portuguese. DPR therefore removed Martins (2009) as support for the critical acute POD.

Request for Reconsideration: Critical Subchronic Oral Point of Departure

Critical Subchronic Oral POD

FTF Comment: The draft RCD proposed that the critical POD for assessing subchronic oral, dermal, and inhalation routes of exposure and risk is derived from a chronic oral study in rats. Because the oral POD is the most appropriate for fipronil subchronic POD selection for all routes of exposure for human health risk assessment, it is critical that it is derived in accordance with sound scientific practices as well as regulatory guidance and precedent… In the draft RCD, an oral POD of 0.02 mg/kg/day was selected as the critical POD based on decreases in serum thyroxine (T4) levels, convulsions, and death in rats from a chronic oral study (Aughton, 1993). Additionally, DPR concluded that several subchronic studies supported the use of the chronic toxicity study for all subchronic durations… For fipronil, it is first critical to note that toxicity increases significantly over-time, especially beyond one year of exposure. The conclusion of increased toxicity over time is consistent with both the review herein by the FTF and the conclusions of EPA's registration review risk assessment (EPA, 2020). Therefore, chronic exposure studies are not appropriate for assessment of risk from subchronic (short- or intermediate-term) exposure scenarios. Further, several studies have been improperly assigned NOEL/LOEL values by DPR...

> **DPR Response**: DPR defines a subchronic period of exposure in a toxicology study as greater than seven days to one year. PODs based on impacts occurring over subchronic timeframe are relevant to assessing risk from intermediate/seasonal exposures (DPR, 2017). Therefore, regardless of the total exposure time covered by a study, a subchronic designation is appropriate if the effects occurred within the indicated exposure duration.
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> The critical subchronic POD was based on toxicological endpoints that occurred within the 7 days – 1 year exposure period. These included decreased circulating thyroxin levels detected in male rats at every tested time point between weeks 1 and 50, and convulsions and death in one male at week 23 (Aughton, 1993). Direct support for this designation came from three subchronic guideline compliant studies summarized below. Additional details follow.
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> - 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). Effects were detected following 25 days of fetal exposure (15 days *in utero*, 10 days during lactation).
> - Autonomic dysregulation in rats evidenced by presence of urine in the FOB observation noted at weeks 4 and 9 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).
> - Significant dose-dependent increase in hepatocyte periacinar hypertrophy in male CD-1 mice at the LOEL and the lowest dose tested (0.13 mg/kg/day) in a 13-week subchronic oral toxicity study (Broadmeadow, 1991). Modeling resulted in a $BMDL_{10}$ of 0.05 mg/kg/day (10% effect, Dichotomous-Hill model).

Combined Chronic/Carcinogenicity Study in Rats: Not Appropriate for Subchronic POD

FTF Comment: As stated, the combined chronic/carcinogenicity study in rats is not appropriate for subchronic POD selection. In the draft RCD, DPR established a NOEL of 0.02 mg/kg bw/day

based on convulsions, death, and decreased thyroid hormones in the combined chronic/ carcinogenicity toxicity study in rats (Aughton, 1993)… [O]nly one animal (male #121) was identified with convulsions and/or death in the 0.06 mg/kg bw/day group prior to 6 months (week 23), although a dose response at this week was not identified for mortality (0, 0, 1, 0, and 2 at 0, 0.02, 0.06, 1.3, and 13 mg/kg bw/day, respectively). Further, this animal was identified with a brain neoplasm (report page 1690-1691), which directly correlates with the appearance of convulsions and death. The occurrence of brain neoplasms is not an effect of fipronil and did not occur in a treatment-related manner among any species in any study in the fipronil database. Therefore, the effects in this animal are considered to be incidental to fipronil treatment and related to the brain neoplasm, which is not an effect of fipronil toxicity. Further, selecting a POD for an effect in 1 out of 80 animals, without a dose-response, is not scientifically sound or consistent with current regulatory practice.

DPR Response: FTF raised the possibility that the convulsions and death sustained by a singleton male at week 23 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 R.1 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. This is valid support for the designated subchronic POD of 0.02 mg/kg/day. The analysis of data on CNS tumors and convulsions are now added to the Risk Appraisal section of the final RCD.

In response to the FTF contention that DPR selected a critical endpoint that is not scientifically sound or consistent with current regulatory practice, DPR would like to restate that the subchronic POD was derived from a chronic rat study, however the effects were noted after a subchronic duration (e.g., within 1 year). The effects included decreases in T4 (discussed below) and convulsions and mortality in a single male rat at week 23. All effects were treatment related. Decreases in T4, alone, are sufficient for establishing a critical endpoint. T4 was consistently and significantly decreased from week 1 until the end of the chronic study. Convulsions are a serious effect of fipronil exposure, as noted above. 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.

TABLE R.1. 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
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.

FTF Comment, continued: The changes in thyroid hormones in this study are also not considered to be relevant for subchronic human health risk assessment. The toxicity database for fipronil evaluated thyroid histopathology in multiple subchronic and chronic duration studies in rats, mice, and dogs. Fipronil's toxicological database clearly demonstrates that thyroid histopathology does not occur prior to one-year of exposure at doses relevant to subchronic risk assessment. Additionally, the comparative thyroid assay (CTA), which is a more sensitive study and better designed to detect adverse outcomes relating to thyroid hormone fluctuations, identified that 1 mg/kg bw/day was the critical POD for thyroid toxicity following subchronic exposure to adult animals. The 1 mg/kg bw/day POD for thyroid toxicity is consistent with DPR's NOEL value for the CTA.

DPR Response: Reduction in circulating thyroid hormones, independent of thyroid histopathology, may have deleterious effects on tissues all over the body (EFSA, 2018). For this reason, DPR holds that fluctuations in thyroid hormone levels are toxicologically significant, particularly if sustained over longer periods and associated with thyroid tumor formation, as is the case for fipronil.

Aughton (1993) showed that one week of daily dietary exposure of male rats to fipronil resulted in a dose-dependent reduction in serum T4 levels (Aughton, 1993). At 0, 0.02, 0.06, 1.3 and 13 mg/kg/day, T4 levels were 2.93 ± 0.5, 3.02 ± 0.6, 2.23 ± 0.74*, 1.16 ± 0.7*** and 0.00 ± 0*** µg/dL, respectively. A similar response profile was observed at 50 weeks: 5.95 ± 1.1, 5.51 ± 1.0, 4.83 ± 0.6**, 3.90 ± 0.7*** and 2.07 ± 0.4*** µg/dL, respectively. An increase in thyroid tumors (adenomas plus carcinomas) in males at 0.06 mg/kg/day detected at the end of the study paralleled the drop in T4 concentration: 0/49, 1/48, 5/50*, 3/50 and 17/50*** (note: p <0.05*, 0.01**, 0.001***).

The Coder (2018) study was designed to detect outcomes relating to thyroid hormone fluctuations during pregnancy and in the developing organisms. However, the real significance of Coder study to the fipronil risk assessment was that it removed the T4 effect measured by one week of exposure in males by Aughton from consideration as an acute / short term endpoint. This was because the threshold dose in the Aughton study, 0.06 mg/kg/day, was unlikely to induce fetal effects if pregnant females were exposed.

Subchronic Neurotoxicity (rats): Revised Critical Oral POD

FTF Comment: The subchronic neurotoxicity study is the critical study for POD selection for subchronic (short- and intermediate-term) exposures. As discussed below, the NOAEL of 0.3 mg/kg bw/day based on exaggerated startle response and exaggerated tail-pinch response at the high-dose of 9 mg/kg bw/day is consistent with central nervous system effects reported in other studies for fipronil and is considered the LOAEL and critical POD for fipronil subchronic (short- and intermediate-term) subchronic risk assessment… However, as explained in greater detail in the acute oral section of this Request for Reconsideration (page 4), the BMD evaluation does not accurately describe the data as the reported BMD of 0.02 mg/kg bw/day and BMDL of 0.01

mg/kg bw/day are both below the DPR-established NOAEL of 0.03 mg/kg bw/day for the study. When the BMDL is below the NOAEL, then the model is predicting the empirical NOAEL to actually be an adverse effect level, which is not only an inconsistency, but also an inaccuracy.

> **DPR Response**: The methodology behind the designation of BMD and BMDL values that are lower than study NO[A]ELs is explained earlier in this document. However, after remodeling the data with BMDS v3.2, DPR determined that the model was not an appropriate fit due to saturation at the high dose. Therefore, DPR updated the RCD with a NOEL of 0.5 ppm (0.03 mg/kg/day) based on presence of urine in the observation area and exaggerated tail pinch response at the LOEL of 5 ppm (0.3 mg/kg/day).

FTF Comment, continued: Further, the effects described in the draft RCD at the NOAEL were neither treatment-related nor biologically significant. Exaggerated tail pinch response was only observed in 1/10 male animals at 0.3 mg/kg bw/day on week 9 (Table 8). No effects were reported at this dose in females at any time, or during week 4 or week 13 evaluations. Additionally, no statistically significant changes were observed on the presence of urine in the observation area at this dose level (Table 9). While a non-statistically significant increase in urine was observed in the 0.3 mg/kg bw/day dose, this effect alone is not considered to be biologically significant or treatment-related due to the lack of other corresponding indicators of an effect on the autonomic nervous system (ANS) (e.g., heart ate, pupillary response or respiratory rate) and high incidence in the controls (5/10). This is further supported by the DNT and acute neurotoxicity studies, which do not demonstrate an impact of fipronil on the ANS. However, the increased number of animals with exaggerated startle response and exaggerated tail-pinch response at the high-dose of 9 mg/kg bw/day is consistent with central nervous system effects reported in other studies for fipronil and is considered the LOAEL for the study, with a NOAEL of 0.3 mg/kg bw/day. The NOAEL of 0.3 mg/kg bw/day is consistent with EPA (2020), EFSA (2006), and APVMA (2003) conclusions.

> **DPR Response**: FTF's assignment of a NOAEL / LOAEL at 0.3 / 9 mg/kg/day for the Driscoll and Hurley (1993) study based on exaggerated startle and tail pinch responses is rational based on the incidences of these parameters in males at weeks 4 (0/10, 0/10, 0/10 and 4/10 at 0, 0.03, 0.3 and 9 mg/kg/day, respectively, for both parameters) and 9 (0/10, 0/10, 1/10 and 2/10, also for both parameters). However, the response in a single male at 0.3 mg/kg/day for both tail pinch and startle at week 9 encouraged a deeper look at other parameters. There is a possible dose-response for the presence of urine in the observation area at weeks 4 and 9 (week 4: 5/10, 6/10, 9/10, 10/10; week 9: 5/10, 4/10, 7/10 and 9/10), which is likely an autonomic effect. In addition, males appeared to be more sensitive that females in all three parameters (females showed no response for any of these measures), lending weight to the analysis. DPR did not consider the apparent lack of other ANS parameters to be crucial, as the three neurological effects discussed here were mutually supporting.

Developmental Neurotoxicity Study Revisions: Revised Subchronic Oral POD is Protective

FTF Comment: When reviewed in the context of the fipronil database, the developmental and neurotoxic effects observed in the developmental neurotoxicity (DNT) support the review critical oral POD of 0.3 mg/kg bw/day from the subchronic neurotoxicity study. In the fipronil DNT study (Mandella, 1995), 30 mated female Sprague Dawley rats were administered fipronil in the diet from gestation day (GD) 6 through lactation day (LD) 10 at doses of 0, 0.5, 10 or 200 ppm fipronil. The average daily intake of fipronil calculated from food consumption was 0, 0.05, 0.9 or 15 mg/kg/day. The pups were indirectly exposed to fipronil for a total of 25 days (15 days in utero and 10 days via lactation) … As described below, the effects observed at 0.05 and 0.9 mg/kg bw/day are neither biologically significant nor toxicologically relevant. Therefore, the appropriate NOAEL for the DNT study is 0.9 mg/kg bw/day with a LOAEL of 15 mg/kg bw/day…

> **DPR Response**: The developmental neurotoxicity (DNT) study by Mandella (1995) showed lasting and significant dose-dependent reductions in pup bodyweights in females starting at postnatal day 0 and extending through postnatal day 21 at 0.9 (5-9%) and 15 (8-34%) mg/kg/day. A similar response was observed in males at 0.9 (3-7%) and 15 (9-34%) mg/kg/day. These bodyweight effects were toxicologically relevant because they were likely the cause of the developmental effects manifesting as delayed preputial separation and decreased maximum startle responses in male pups. The absence of an effect on pup bodyweight at similar doses in the reproductive toxicity study by King (1992) and in the comparative thyroid assay (Coder, 2019) does not negate the importance of the Mandella observation. DPR views the pup bodyweight decrements observed by Mandella as toxicologically and developmentally significant and is retaining the NOEL and LOEL at 0.05 and 0.9 mg/kg/day for this study.

FTF Comment, continued: It is worth noting that the draft RCD reports doses of 0.16, 1.68, and 16.97 mg/kg bw/day to males and 0.20, 2.00, and 20.76 mg/kg bw/day to females based on the most conservative weekly consumption during the two-generation reproductive toxicity study. However, a time-weighted average is the more appropriate value for risk assessment and selecting the most conservative week is not an accurate representation of the overall dosage and not consistent with common risk assessment practices. The mg/kg bw/day dose levels in this study should be 0.25, 2.5, and 26 mg/kg bw/day to males and 0.27, 2.7, and 28 mg/kg bw/day to females based on the average compound intake of P0 animals…

> **DPR Response**: Normalized dose reporting for reproductive toxicity studies is often problematic because maternal bodyweights change radically as the fetuses grow. Because the precise period of the dosing regimen most relevant to toxic effects is usually unclear, DPR chose to list the most health conservative (i.e., lowest) dose to identify PODs. In any case, the differences between the lowest values used by DPR and the time-weighted averages recommended by FTF are relatively small and inconsequential to DPR's final assessment.

FTF Comment, continued: In the DNT study, the effect on foreskin separation, although statistically significant based on DPR statistical analysis, was not statistically significant as calculated by the study authors from the control group, and the mean of the 0.9 mg/kg bw group (45.4 ± 2.9 days) is within half a standard deviation of the control group (44.0 ± 2.5 days) (Table 13). Further, in the DNT study report (Table L-2; page 215) lists the mean day as the criteria for preputial separation for each animal. However, the day that preputial separation is attained *[emphasis original]* is used for the evaluation of this endpoint, not the mean day (in accordance with OECD 443). Table L-8 (page 221) of the DNT study reports lists all of the days associated with the preputial separation process (i.e. beginning to completion). The day of attainment for each animal was identified as the last day reported in this table. Based on the day of attainment for preputial separation presented in Table 14 below, there is no effect on preputial separation at 0.9 mg/kg bw/day. The mean day of attainment was 45.7 days in the control, 46.9 days at 0.05 mg/kg bw/day, 47.1 days at 0.9 mg/kg bw/day, and 51.0 days at 15 mg/kg bw/day. Additionally, the available historical control data for mean preputial separation time for studies conducted within the laboratory report the mean range from 42.8 - 48.5 days for six studies, which further supports that the effects reported in the DNT study at 0.09 mg/kg bw/day are within the historical control values for the laboratory (Mandella and Rodwell, 1998) and, thus, are neither treatment-related nor adverse…

DPR Response: The OECD 443 guideline regarding the appropriate time to gauge preputial separation does not state in any clear manner that the mean day for this process is invalid, remarking only that:

> *"All selected F1 animals are evaluated daily for balano-preputial separation or vaginal patency for male/female respectively commencing before the expected day for achievement of these endpoints to detect if sexual maturation occurs early." (p. 11, paragraph 46, https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd-tg443-508.pdf).*

In addition, USEPA's guidance with respect to this endpoint makes no specification at all concerning the time of achievement of preputial separation:

> *"Included as adverse effects for males should be delay or failure of testis descent, as well as delays in age at preputial separation or appearance of sperm in expressed urine or ejaculates." (https://www.epa.gov/sites/default/files/2014-11/documents/guidelines_repro_toxicity.pdf; page 49).*

While FTF's analysis of the day of attainment of separation data does not indicate statistical significance at 0.9 mg/kg/day, a biologically significant trend is readily apparent. The mean-day-of-separation measure clarifies what is evident using the day-of-attainment measure. In addition, the mean values for the 10 ppm and 200 ppm groups exceeded historical control

means in the same rat strain from four studies conducted in the same laboratory between 1989 and 1995, with means of 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) (Mandella and Rodwell, 2005). Because the reported mean measurements are close to the one-day requirement of the OECD guideline, DPR considers it to be an appropriate endpoint and that the data are consistent, with an effect at 0.9 mg/kg/day.

FTF Comment, continued: Furthermore, effects on preputial separation must be evaluated with body weight effects (Carney et al, 2004) to determine if the effect is secondary to pup body weight or a true treatment related effect. Effects on preputial separation should be reported as the day preputial separation is attained expressed as a covariate with pup body weight on Day 21. Minimally, the age and body weight on the day of attainment must be reported to determine if the effect was only secondary to pup body weight or a true treatment related effect indicating a reproductive developmental effect (Foster and McIntyre, 2002). Evaluating preputial separation within the context of bodyweight is further affirmed in regulatory guidance…

DPR Response: DPR recognized the plausibility that the delay in preputial separation observed in male rat pups in the Mandella DNT study was secondary to reduced pup body weight. A relationship between pup body weight gain and developmental delay is well recognized but does not decrease the importance of the latter observation.

FTF Comment, continued: Evidence of neurotoxicity observed in the DNT is also consistent with the fipronil database. The effect on startle response (as calculated by DPR) for pups is shown in Table 15, below. While a statistically significant change as calculated by DPR on maximum startle response at 0.9 mg/kg bw/day in males is reported, the percent decrease observed was slight (13%) and occurred in the absence of any other effects. Additionally, the cumulative mean maximum startle response time at 0.9 mg/kg bw/day (109.02) was within the standard deviation of the control group (124.94 ± 48.26), and % change was less than the % SD (~40%), supporting that the statistically significant change is incidental and not a treatment-related or adverse event.

DPR Response: The definition of a "slight response" may vary. However, both DPR and FTF agree as to the evidence for neurotoxicity based on the maximum startle response in males, even at the 0.9 mg/kg/day dose. Moreover, reduced pup weights and delayed preputial separation were also present at that dose. All three observations were sufficient to support designation of 0.9 and 0.05 mg/kg/day as the LOEL and NOEL doses, respectively.

FTF Comment, continued: Furthermore, the other neurotoxic effects observed (unable to stay afloat PND 6, unable to swim in a straight line PND 6, unable to keep head out of water PNDs 6 and 14) in this study had a more robust response at the high-dose and were not observed at 0.9 mg/kg bw/day. Based on the weight-of-evidence, 0.9 mg/kg bw/day is the NOAEL for neurotoxicity and the LOAEL is 15 mg/kg bw/day under the conditions of the DNT study. The

NOAEL for neurotoxicity is consistent with EPA (2020), EFSA (2006), and APVMA (2003) conclusions…

> **DPR Response:** The presence of more robust signs of neurotoxicity at 15 mg/kg/day does not invalidate the observations at 0.9 mg/kg/day. Rather, the latter are appropriately viewed as threshold changes indicative of dose responsiveness. As such, they are useful endpoints for risk assessment.

Subchronic Mouse Oral Toxicity Revisions: Revised Subchronic Oral POD is Protective

FTF Comment: When reviewed in the context of current regulatory practices and policies, observed liver effects in the mouse subchronic oral toxicity study are not considered to be adverse at doses below 3.2 mg/kg bw/day. Therefore, the proposed critical oral POD of 0.3 mg/kg bw/day from the subchronic neurotoxicity study is protective of all effects observed. In the subchronic feeding study in mice, (Broadmeadow, 1991), CD-1 mice (12/sex/dose) were administered fipronil for 13 weeks at dietary levels of 0, 1, 3, 10, or 25 ppm (0, 0.13, 0.38, 1.27, or 3.2 mg/kg bw/day for males and 0.17, 0.57, 1.72, or 4.53 mg/kg bw/day for females) … However, periacinar (centrilobular) hypertrophy is not adverse on its own. Hepatocellular hypertrophy is a common adaptive response to xenobiotics. Based on current regulatory guidance and precedent, hepatocellular hypertrophy is only considered adverse in the presence of corroborative indicators of liver toxicity such as a greater than 2-fold increase in liver enzymes or clinical chemistry parameters related to liver toxicity, increased liver weights, and/or permanent cellular damage such as hepatocellular necrosis (EPA, 2002). There is no corroborating evidence of liver damage at 0.13, 0.38, or 1.27 mg/kg bw/day in this study or within the toxicological database. No statistically significant changes in liver weights were observed at these doses and adverse histopathological effects (focal necrosis) were only observed at the high-dose of 3.2 mg/kg bw/day…

> **DPR Response**: The parameter upon which the Broadmeadow subchronic LOEL was based is more accurately described as periacinar hypertrophy with cytoplasmic vacuolation. DPR regards the presence of vacuolation as part of the evidence for tissue damage. Moreover, the dose responsiveness of this endpoint in males was significant at the two higher doses (incidence at week 53 at 0, 0.01, 0.06, 1.18 and 3.43 mg/kg/day was 0/14, 2/15, 2/19, 7/17** and 12/18***; p <0.01**, 0.001***). This was accompanied by an increase in severity at the high dose. Finally, liver weights relative to body weight was significantly elevated at 0.06 (12% over controls), 1.18 (20%) and 3.43 (51%) mg/kg/day. The combination of liver histopathology and organ weight change is strong evidence for establishment of the NOEL and LOEL at 0.06 and 1.18 mg/kg/day, respectively.

Comparative Thyroid Assay (rats) Revisions: Revised Subchronic Oral POD is Protective

FTF Comment: In the comparative thyroid assay (CTA) (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 … However, the effects at 0.3 mg/kg bw/day in both fetuses and offspring at GD 20, PND 4, and PND 21 are not biologically relevant. There were no statistically significant changes in T3 or TSH levels and, while statistically significant, the decreases in T4 levels were less than 20% and not considered to be biologically relevant (EPA, 2018). Additionally, there was no apparent dose response between the groups 3, 4, and 5, and the group 5 mean was within the range of control standard deviation. Furthermore, during late gestation, T4 level is supplied by both the dams and by fetal production, and no corresponding decreases were observed in GD 20 dams or PND 4 pups (Table 16) to corroborate the observed change (Moog et al, 2017). Therefore, the weight-of-evidence supports that the statistically significant decrease in T4 at this dose was incidental and not biologically relevant…

> **DPR Response**: It is difficult to discern what FTF means by its statement "…the weight-of-evidence supports that the statistically significant decrease in T4 at this dose (i.e., 0.3 mg/kg/day) was incidental and not biologically relevant" since DPR set the developmental NOEL at that dose (0.3 mg/kg/day).

FTF Comment, continued: As DPR concluded for organ weights in PND 21 pups, the organ weight changes at PND 4 and PND 21 were also relatively small and not associated with liver histopathology. This is further supported by the lack of liver effects (organ weight and gross pathology) in pups in the comparative thyroid assay and by the lack of liver effects (gross pathology) in the 2-generation reproduction study in pups at similar doses. Following sub-chronic exposure, liver effects were only observed in parental animals at dose levels at and above 2 mg/kg bw/day in both 90-day study in rats and 2-generation rat reproductive study, supporting that 1 mg/kg bw/day is a NOAEL for subchronic exposure. Following EPA (2002) guidance on liver toxicity, these changes in liver weight are not considered to be adverse or an appropriate POD for risk assessment as they are common adaptive response to xenobiotics. Therefore, based on the weight of evidence, the offspring/fetal study NOAEL is 1 mg/kg bw/day with a LOAEL of 3 mg/kg bw/day based on thyroid hormone fluctuations in fetuses on GD 20 and pups on PND 4 and PND 21, as well as thyroid histopathology in PND 21 pups…

> **DPR Response:** DPR based its developmental NOEL of 0.3 mg/kg/day on observations of reduced T4 in fetuses on gestation day 20 (19% compared to controls; $p < 0.05$) and increased absolute liver weights in males on postnatal day 4 (19%; $p < 0.01$) and in both sexes on postnatal day 21 (14% in males, $p < 0.05$; 15% in females, $p < 0.05$) at the LOEL of 1 mg/kg/day. A reduction of T4 in fetuses can have serious developmental consequences. Unfortunately, Coder (2019) did not follow the pups after weaning and did not subject the pups to tests designed to detect behavioral anomalies. While the increased liver weights were not associated with histopathology, they are likely reflective of the enhanced cytochrome

P450 activity that caused the T4 reduction, as explained earlier in this document. When taken together, DPR considers both observations to be appropriate for developmental LOEL designation.

Rabbit Developmental Toxicity Study Revisions: Revised Subchronic Oral POD is Protective

FTF Comment: For the rabbit developmental study (King, 1990), DPR concluded that the NOEL for maternal toxicity was 0.1 mg/kg bw/day based on decreased BW gain. The NOEL for developmental effects was the highest dose tested, 1 mg/kg bw/day, as no effects were observed on the developing fetuses. However, a review of the absolute mean body weights does not reveal a biologically relevant effect. As shown in Table 17 below, exposure to 1 mg/kg bw/day only resulted in a mean body weight loss up to 5%. As this is below 10%, which is the generally accepted decrease for adversity by regulatory authorities (EPA and PMRA) and, in the absence of any other effects, the decrease in mean body weight is not considered to be biologically relevant. Decreases in maternal body weight gain, in the absence of adverse effects on mean body weight, are not considered to be adverse. Further, there is no clear dose-response for decreased bodyweights and the effect is not clearly related to treatment. Based on this weight of evidence, the maternal NOAEL for this study is 1 mg/kg bw/day…

DPR Response: To clarify, the observed effect was reduced body weight gain, not body weight loss. DPR's approach was to analyze the percent (%) body weight gain compared to controls. During the first 3 dosing days (gestation days 6–8), maternal weight gains at 0, 0.1, 0.2, 0.5 and 1.0 mg/kg/day were 100%, 67%, 50%*, 50%* and 50%*, respectively. For gestation days 6-10 those figures were 100%, 45%***, 64%*, 55%*** and 55%**. [Note: p <0.05*, 0.01**, 0.001***] This pattern was maintained throughout gestation, although significance at 0.2 mg/kg/day was not always achieved. The apparent effect at 0.1 mg/kg/day for gestation days 6–10 lacked a clear dose response. In conclusion, the effects when expressed in terms of absolute body weights may appear minimal, however they attain significance when expressed as a percent of control body weight gains. DPR considered this study as support for the critical subchronic POD of 0.02 mg/kg/day.

Review of DPR Cited Literature Studies: Revised Subchronic Oral POD is Protective

FTF Comment: Two literature studies cited by in the draft RCD were reported with LOEL/NOEL values that may impact critical POD selection. In the Bano and Mohanty (2020) study, the effects of fipronil on immune system parameters was investigated and DPR reported a NOEL of <0.475 mg/kg bw/day based on reduced relative thymus weight, reduced number of splenocytes and thymocytes, and reduced proliferative indices in splenocytes and thymocytes in mice administered fipronil via oral gavage from PND 31 to 60. However, these effects are not considered to be adverse effects or relevant to risk assessment. As this is a mechanistic study exploring precursor effects which may lead to immunotoxicity, it is not appropriate for assigning

a risk assessment LOAEL. Furthermore, immunotoxicity is not expected for fipronil, no effects on immune system organs or hematology were observed in the extensive repeated dose toxicological database in any species. In addition, the immunotoxicity study was waived by EPA (2020) and further investigation of immunotoxicity endpoints is not expected to impact risk assessment. Therefore, the DPR-assigned NOEL/LOEL is not considered relevant for critical POD selection and no relevant effects for risk assessment were observed…

> **DPR Response**: The focus of DPR's analysis of Bano and Mohanty (2020) was on effects to the spleen, and their potential relationship to overall fipronil toxicity. However, upon re-examination DPR found methodological problems with this paper and removed it as support for the subchronic point of departure.

FTF Comment, continued: In the Sefcikova (2019) study, the developmental effects of fipronil were investigated and DPR reported a NOEL of <0.009 mg/kg bw/day based on increased percentage of dead cells in blastocysts, more blastocysts with dead cells, and altered development of embryos in mice administered fipronil from gestation day 1 to gestation day 3. As with the previous study, these effects are precursors to potential developmental toxicity and are not considered to be adverse or relevant to risk assessment. There is no clear link between the reported effects and a functional impairment on reproductive or developmental toxicity in mammals. The potential reproductive and developmental toxicity of fipronil has been thoroughly investigated in guideline studies, and clear NOAELs have been established, which protect for all potential developmental effects observed. Therefore, this NOEL/LOEL is not considered relevant for critical POD selection and no relevant effects for risk assessment were observed…

> **DPR Response:** The toxicological significance of the observed effects on mouse embryo count and blastocysts was unclear, particularly in relation to implantation, pregnancy, and potential developmental outcomes. The lack of a mouse 2-generation study precluded the ability to detect such effects in that species. No similar sequalae were described in the rat 2-generation reproductive toxicity study. The presence of an *in vivo* effect on mouse embryos at this very low maternal dose was indication that the guideline studies may not adequately detect effects on preimplantation parameters. However, embryonic impacts were noted at 0.9 mg/kg/day, including decreased numbers of isolated cells, slower transition from oviduct to uterus, increased degraded embryos, and a decrease in the number of blastocysts. Because of the duration of the study, DPR removed it from consideration in the subchronic POD development. However, the study is retained in the developmental toxicity section and discussed further in the Risk Appraisal section in the final RCD.

Request for Reconsideration: Overall Risk Assessment Conclusions

FTF Comment: The following tables present the revised risk estimates for fipronil based on the specific Requests for Reconsideration substantiated herein. Please note that the revised PODs and dermal absorption values utilized in the MOE calculations are included below each table…

> **DPR Response:** The differences between the MOEs calculated by FTF in Tables 20-23 and DPR's MOEs reflect the use of different critical PODs and dermal absorption values. DPR maintains that its toxicity endpoints and exposure estimates, and thus its MOEs, are preferable to those recommended by FTF due to their stronger scientific support, as detailed in previous sections of this document. Responses to exposure estimates are found in a separate memo.

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