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SUBJECT: ESTIMATION OF EXPOSURE TO PERSONS IN CALIFORNIA TO AMITRAZ®
          IN SPOT-ON FORMULATION ANTI-FLEA AND TICK TREATMENTS FOR
          DOGS, DOG COLLARS FOR ANTI-TICK TREATMENT, AND PLASTIC
          STRIPS FOR THE IN-HIVE CONTROL OF VARROA DESTRUCTOR MITES

I was asked to address exposure and risk for use scenarios not specifically addressed in the original Risk Characterization Document (RCD) for Amitraz. Five scenarios were addressed, including post-application exposures to adults and children from spot-on and pet collar products, and handler exposures to those placing beehive treatment strips. Assumptions and details of calculations are given in this memo.

Based on central nervous system effects in humans reported in the RCD, the no-observed-effects-level (NOEL) is 0.125 mg/kg/day; the RCD states that a Margin of Exposure equal to or greater than 10 is considered adequate for the protection of human health when calculated with a NOEL from a human study. All but one of the examined scenarios exceeded this target; the exception was the post-application exposure (collar) to a 1- 2 year old child (Table 1).

Table 1: Exposure Estimates and Associated Margins of Exposure (MOE)

Formulation	Age	Dermal (mg/kg/day) [1]	HTM (mg/kg/day) [2]	Total (mg/kg/day) [3]	MOE
Post-application					
spot-on	1 – 2 yrs	0.0056	0.0004	0.006	21
spot-on	adult	0.00009	n/a [4]	0.00009	1357
collar	1 - 2 yrs	0.088	0.023	0.11	1
collar	adult	0.0054	n/a	0.0054	23
Handler					
beehive strip	adult	0.00003	n/a	0.00003	4870

[1] Daily dose of Amitraz absorbed percutaneously
[2] Daily dose of Amitraz absorbed via hand-to-mouth (HTM)
[3] Total (dermal + HTM) daily dose of Amitraz
[4] n/a: not applicable

Estimation of Amitraz[®] (Spot-on Formulation) Exposure to 1<2 year old Child

The acute exposure estimates calculated for the 1 to 2 year old child are **0.0056 mg/kg/day** (Table 1) for percutaneously absorbed Amitraz and **0.0004 mg/kg/day** (Table 1) for the amount absorbed orally from hand-to-mouth activity. Based on the low vapor pressure of the spot-on formulation, exposure to Amitraz is not anticipated via the inhalation route. The total amount of Amitraz absorbed daily, estimated by summing the amounts absorbed percutaneously and orally, is **0.006 mg/kg/day** (Table 1). Based on central nervous system effects in humans reported in the RCD, the NOEL is 0.125 mg/kg/day; the RCD states that an MOE equal to or greater than 10 is considered adequate for the protection of human health when calculated with a NOEL from a human study. The MOE (referred to as MOS in the document), is **20.6** (Table 1). No estimates were generated for these potential exposures in the Amitraz RCD (Frank, 1995), in EPA's Amitraz RED (U.S. EPA, 1995), or the subsequent TRED (U.S. EPA, 2006a).

<u>Estimation of Dermal Exposure to Amitraz for 1 to 2 yr old Child</u>
The formulae shown below, obtained from EPA's 2012 version of the Standard Operating Procedures for Estimating Residential Pesticide Exposure Assessment (EPA SOP) (U.S. EPA, 2012), were used to estimate exposure:

$$E = TC * TR * ET$$

E = exposure (mg/day)
TC = transfer coefficient (cm^2/hr)
TR = transferable residue (mg/cm^2)
ET = exposure time (hours/day)

$$TR = AR * F_{AR} / SA$$

TR = transferable residue (mg/cm^2)
AR = application rate or amount applied to animal (mg)
F_{AR} = fraction of the application rate available as transferable residue
SA = surface area of the pet (cm^2)

Absorbed dermal dose, normalized to body weight, is calculated as:

$$D = E * AF / BW$$

D = dose (mg/kg-day)
E = exposure (mg/day)
AF = absorption factor (dermal)
BW = body weight (kg)

Transfer Coefficient and Exposure Time

E = TC * TR * ET

The EPA SOP transfer coefficient (TC) was generated using data from a surrogate exposure study of pet groomers bathing dogs with a shampoo formulation containing carbaryl. The groomers lathered each dog in the shampoo, let the dog soak in the formulation for 5 minutes, and then rinsed the shampoo off of each dog. Samples were obtained through hand washes and internal dosimeters at the end of the study. The TC, 1400 cm^2/hr, was generated by dividing the rate of exposure (ug/hr) by the concentration of residue on the pet (ug/cm^2). The exposure time (ET) of contact between the infant and the dog from the EPA SOP is 1 hour/day. Since the ET is 1 hour/day, the aforementioned EPA SOP TC of 1400 cm^2/hr is equivalent to 1400 cm^2/day.

The study used to generate the TC is not an ideal surrogate for dermal exposure to Amitraz in spot-on formulation. The carbaryl was in a detergent, making it more miscible in water. Following the shampoo step, each dog was rinsed. During this step, any AI present on the hands would potentially be rinsed off along with the shampoo. Moreover, internal dosimeters were used by the investigators. The dosimeters were worn underneath the groomer's clothing which consisted of a short-sleeved shirt and a pair of pants. In addition, the groomer wore a smock as an outer garment. However, a 1<2 year old child would likely not be wearing a smock and might also not be wearing a shirt. On page 40 of a previously written memorandum by the EPA (U.S. EPA, 2003), the authors state that the shampoo study "should not be used for residential exposure assessments because protective clothing (i.e., smock and long pants) were worn over the whole-body dosimeters and adjusting the data using negative protection factors which [_sic_] is generally not considered appropriate." Because of these reasons, the approach in the EPA SOP was not used.

Another potential approach to estimating a TC for contact with the treated dog is through use of the estimated child's hug surface area (SA), EPA's estimated exposure time (ET) between the child and treated dog, and EPA's replenishment number (N_Replen). To be health protective, the 1<2 year old child is assumed to hug versus pet the treated dog. The estimated hug surface area for the 1<2 year old was derived from data obtained from EPA. According to EPA, the total body SA of the 1<2 year old is 5300 cm^2 (U.S. EPA, 2012). The U.S. EPA Exposure Factors Handbook lists the percentages of the total body SA for the head, trunk, arms, and hands as being 16.5, 35.5, 13.0, and 5.7, respectively (U.S. EPA, 2011). Since the hug SA would involve the anterior head, trunk, arms and hands, the percentages were halved and summed to get a total of 35.35% of the total body SA or 1874 cm^2. The exposure time (ET) of contact between the infant and the dog was obtained from the EPA SOP and is estimated to be 1 hour/day. The number of hugs per day was estimated using the replenishment number (N_Replen), which was also presented in the EPA SOP but only for estimating hand-to-mouth exposure. The N_Replen was

estimated to be 4 events/hr. This point estimate was used by EPA to estimate the number of times a child pets the dog per hour. However, for estimating dermal exposure for the child, petting the dog was made equivalent to hugging. Multiplying the duration of contact (1 hr/day) with the replenishment number (4 hugs/hr), gives 4 hugs/day. Hence, the daily TC for the child hugging the dog is equal to 4 x 1874 cm^2/day or 7496 cm^2/day.
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Since the ET was factored into estimating the TC, it was removed from the exposure formula:

E = TC * TR

Transferable Residue

The next component of the exposure formula is the transferable residue (TR). As stated earlier, the formula for this variable is:

E = TC * TR

TR = AR * F$_{AR}$ / SA

TR = transferable residue (mg/cm^2)
AR = application rate or amount applied to animal (mg)
F$_{AR}$ = fraction of the application rate available as transferable residue
SA = surface area of the pet (cm^2)

The application rate (AR) was obtained from the Amitraz product labels for the spot-on formulation. The rate is expressed in units of mg of AI applied/cm^2 of pet surface area. Each product label is designated for a range of pet weights. After converting these weight-ranges to surface areas using a formula from the EPA Wildlife Exposures Factors Handbook (U.S. EPA, 1993), the peak maximum application rate of 265.9 mg of Amitraz applied to a dog with a surface area (SA) of 1868 cm^2 was used for estimating exposure. This amount of AI and surface area combination provided the highest exposure estimate, barely, of the weights listed on the product labels.

The other component of the formula, the fraction of the applied amount of Amitraz available as transferable residue (F$_{AR}$), is from a surrogate study on another pesticide called Fipronyl (Brickel, 1997). Five transferable residue studies for the spot-on formulation were found. In two of the studies, the investigators used a gloved mannequin hand to pet the treated dogs and measure AI transfer (Wrzesinsk, 2009 and 2010). Three of the studies were carried out using the human hand covered with a cotton glove (Brickel, 1997; Bach, 2002; Jennings et al., 2000). The human hand studies are more representative since the hand, much like a child's face, chest, arms, and hands during a hug, conforms to the petting surface more effectively than the mannequin

hand which is relatively rigid. Hence, the F_{AR} used for estimating exposure was obtained from a study utilizing the human hand. Of the three human hand studies available, only one study was chosen for estimating exposure. This study titled, "Dislodgeable Residues of Fipronil Following Topical Application of Frontline® Spot-on Treatment to Dogs" (Brickel, 1997), was selected because the sampling timepoints nearly spanned the length of the 30-day efficacy period. This span of residue data is important since it provides information on the transferable residue trend from the day of application to nearly the end of the efficacy period. In addition, the petting event consisted of 5 strokes along the treated dog with a cotton gloved hand, covering the bulk of its body. This type of petting more closely approximates a 1<2 year old's hug than the petting protocol of the Jennings et al. study. In that study the sample was collected by, "vigorously petting the dog's hair forward and back along his back and down both sides for a 5 minute period" (Jennings et al, 2000). Another advantage of the selected study is that the investigators measured the transfer of both the parent AI and its major metabolites, summing the amounts to get the total amount of transfer. The sample recoveries obtained in this study for gloves spiked with 30 µg of Fipronyl/glove were greater than 90%. The sample residues measured in the study ranged from 4.5 to 4302 µg of Fipronyl/glove.

Sampling for residue transfer for the study titled, "Dislodgeable Residues of Fipronil Topical Application of Frontline® Spot-on Treatment to Dogs", was conducted 1, 4, and 8 hours after treatment. In addition, samples were collected 1, 2, 4, 7, 14, 21, and 28 days after treatment. The amount of transferred residue decreased over the course of the study. The residues transferred on the day of the application were the highest measured in the study. Six glove samples were generated at each of the three (i.e., 1-, 4-, and 8-hour) time points. The total mean percentage of the applied dose transferred to the cotton glove on the day of the application is 2.389%. The highest mean percentage transferred to the glove on the day of the application was 1.08% taken at the 4 hour time point. Only one time point was taken on each of the subsequent days where transferred residues were collected. The mean percentages of the amount applied on days 1, 2, 4, 7, 14, 21, and 28 are 0.506, 0.470, 0.233, 0.176, 0.0433, 0.0214, and 0.00668%, respectively. The highest mean percentage (i.e., 1.08%) of the three timepoints sampled on the application day was used for estimating acute Amitraz exposure that the 1<2 year old child gets from hugging the treated dog. Since the F_{AR} is a ratio, the percentage of the applied amount (i.e., 1.08%) was converted to the decimal form of 0.0108.

To be health-protective, the 1<2 year old child is assumed to be shirtless, maximizing the contact between bare skin and the fur of the treated dog. However, the F_{AR} study used for estimating exposure, utilized a hand fitted with a cotton glove and the cotton glove can load much more AI than the bare hand. To adjust for this increased loading, a correction factor, generated in another study, was incorporated into the exposure estimation. In the correction factor investigation the treated surface was carpet which was treated via broadcast or aerosol application methods. In the study, the amount of AI loaded onto a gauze pad used to wipe a 100 cm^2 section of chlorpyrifos treated carpet was compared with that loaded onto the bare hand pressed on and dragged across the carpet. The data showed that the transfer of chlorpyrifos from the carpet to the bare hand was

23 to 24 times less than that transferred to the gauze wipe (Lu, 1999). Hence, the middle value of 23.5 was used to adjust for the potential difference in loading between the cotton glove in the petting studies and the bare skin of the 1<2 year old's hug. To adjust for the anticipated difference in loading, the F_{AR} value of 0.0108 derived from the petting study was divided by 23.5 to get 0.00046. As a result, the new TR is equal to 6.55 x 10^{-5} mg/cm^2.

Exposure

E = TC * TR

Based on the TC and TR generated for Amitraz, the exposure (E) for the 1 to 2 year old hugging a dog treated with the spot-on formulation is 0.49 mg/day. This exposure estimate can be expressed as a concentration by dividing the 0.49 mg by the aforementioned estimated surface area of the face, chest, arms, and hands (i.e., 1874 cm^2). The result is 0.262 µg/cm^2.

Dose
Only a small fraction of the Amitraz transferred to the epidermis of the infant is likely to be absorbed. In order to estimate the amount of Amitraz absorbed from this concentration, a dermal absorption study using rats dosed with the applied dermal concentrations of 1, 10, 100, or 1000 µg/cm^2 was used (AgrEvo USA Company [1994] Registration Package 287-0104). Although 1 µg/cm^2 is the dosage closest to the estimated skin concentration (i.e., 0.262 µg/cm^2), the recoveries at this dosage were poor. Hence, the absorption data could not be used. Therefore, the absorption result generated for the next closest level, 10 µg/cm^2, was used to estimate dermal absorption. The percent of the applied dose recovered at this level was 94.15%. The percent of the applied Amitraz absorbed at this level is 12.64% at 120 hours after the application. The percent of the applied amount of Amitraz remaining on the skin 120 hours after application is 0.476%. Since this remaining AI may eventually be absorbed, it was combined with the percent of Amitraz absorbed to get 13.1%. The decimal value of this percentage or 0.131 is the absorption factor (AF) and was used to calculate the dose (D) for estimating exposure.

Three other dermal absorption studies were discovered. However, they were not appropriate for estimating exposure. Two of the studies focused on dermal absorption of emulsified Amitraz in the dog and pig (NOR-AM Chemical Company and, the UPJOHN Company [1984] Registration Package 287-041, and NOR-AM Chemical Co. [1992] Registration Package 287-084). Four animals were used in each study. The treated areas were relatively large and left uncovered. The mean of the reported absorption values of the dog and pig studies were 2.6% and 6.7%, respectively. However, the percent absorptions only covered excreted amounts and, unlike the rat study, not the amount left in the tissues. No Amitraz recovery was listed for the dog study, and the pig study listed a wide range of recoveries (57 to 81%). This was attributed to the overly gentle skin washing procedure used by the investigators of the study. The third study was a well-conducted dermal absorption study utilizing rats. This study was not used, however, because the dosage (i.e., 100 µg/cm^2) is substantially greater than the estimated skin concentration in

comparison with the 10 µg/cm^2 dose concentration used in the selected study (NOR-AM Chemical Co. [1992] Registration Package 287-084).

Using this absorption factor (AF), the exposure value in mg/day, and the estimated body weight of the infant of 11.4 kg, provided in the EPA SOP, the following EPA SOP formula was used to calculate the dose:

D = E * AF / BW
D = dose (mg/kg-day)
E = exposure (mg/day)
AF = absorption factor (dermal)
BW = body weight (kg)

Based on the exposure (E), absorption factor (AF), and body weight (BW), the 1 < 2 year old hugging a dog treated with the spot-on formulation is estimated to absorb Amitraz percutaneously at a rate of **0.0056 mg/kg/day**.

Appraisal of Estimate for Dermal Exposure to Amitraz for 1 to 2 yr old Child
Three assumptions made in order to generate the exposure estimate may lead to under- or overestimation of exposure. The assumption that the entire hug surface area of the child contacts the dog during a hug may overestimate exposure. Moreover, the assumption that the F_{AR} is representative of the AI transfer occurring during a hug may under- or overestimate exposure. Finally, the assumption that the dermal absorption factor (AF) is representative of the dermal absorption experienced by the 1<2 year old hugging the treated dog may lead to underestimation. The dose concentration used in the dermal absorption study used to derive the AF is 10 µg/cm^2. However, the estimated dose concentration experienced by the 1<2 year old is 0.262 µg/cm^2. The AF may underestimate exposure since evidence suggests that the rate of dermal absorption of pesticides increases with decreasing skin concentration (Thongsinthusak et al, 1999). Moreover, in addition to the absorbed Amitraz, the AF was made to include the amount left on the treated section of skin. This amount left on the skin was assumed to eventually be absorbed. This assumption may have led to overestimation of exposure.

Estimation of Hand-to-Mouth Exposure to Amitraz for 1 to 2 yr old Child
The formulae, obtained from EPA's 2012 version of the Standard Operating Procedures for Estimating Residential Pesticide Exposure Assessment, were used to estimate exposure:
E = [HR * (F$_M$ * S$_{AH}$) * (ET * N_Replen) * (1 - [1 - SE] $^{Freq\_HTM/N\_Replen}$)]

E = exposure (mg/day)
HR = hand residue (mg/cm^2)
F$_M$ = fraction of hand mouthed
S$_{AH}$ = surface area of hand
ET = exposure time (hours/day)

N_Replen = # of replenishment intervals/hr
SE = salivary extraction
Freq_HTM = frequency of hand-to-mouth events

The HR was calculated using the TR, calculated previously for dermal exposure, and the surface area of the 1 to 2 year-old child's hand (S_{AH}). The S_{AH} (150 cm^2) was obtained from the EPA SOP. The F_M value, 13% or 0.13, was also obtained from the EPA SOP. The ET and N_Replen, as stated earlier, were estimated by EPA to be 1 hr/day and 4 events/hr. The event in this case consists of the child petting or hugging the dog and replenishing the amount of Amitraz on the hand. The SE and Freq_HTM were obtained from the EPA SOP and are 48% or 0.48, and 20 hand-to-mouth events per hour, respectively. The hand-to-mouth exposure (E) value generated for the 1 < 2 year old child is **0.005 mg/day**.

The daily dose of Amitraz for the 1 to 2 year old child was calculated using the previously stated estimated body weight of 11.4 kg. The daily dose is **0.0004 mg/kg/day**.

Appraisal of Estimate for Hand-to-Mouth Exposure to Amitraz for 1 to 2 yr old Child
Some of the point estimates used in the EPA SOP formula may lead to overestimation of exposure. The SE was derived from a study which measured the amount of pesticide removed from the hand with gauze pads wetted with saliva (Camann, D.E. et al., 1989). This methodology may have overestimated the SE from hand-to-mouth events due to the scrubbing action of the gauze pad (Driver et al., 2013). In addition, it is possible that the amount of time needed to remove the pesticide from the hand is substantially longer than the estimated amount of time the child mouths the hand (Driver et al., 2013). The Freq_HTM events are assumed to represent the hand going into the mouth. However, the data used by EPA to get this rate does not specify whether the hand goes into the mouth or just to the outside of the mouth (Xue et al., 2007, and Xue et al., 2010). Driver et al (2013) cite data from a study using 1 to 2 year old children (Leckie et al., 2000) and suggest that hand-in-mouth events constitute only 46% of the total hand-to-mouth events. In addition to the point estimates, the formula used in the EPA SOP does not incorporate potential losses of Amitraz from the hand due to the child touching surfaces not containing pesticide, or from hand washing.

Removal of pesticide from the hand by mouthing is assumed by EPA to be a non-linear process. Removal of Amitraz from the hand via hand-to-mouth events is represented by the following portion of the formula: $(1 - [1 - SE]^{Freq\_HTM / N\_Replen})$. Using this portion of the formula creates a loss of Amitraz from the hand which is non-linear over time. Hence, relative to a linear relationship, more hand-to-mouth events are required to remove the Amitraz. This may underestimate exposure.

Estimation of Amitraz (Spot-on Formulation) Exposure to Adult

Estimation of Dermal Exposure to Amitraz for Adult
The exposure estimate calculated for the adult is **0.00009 mg/kg/day** (Table 1) for percutaneously absorbed Amitraz. Based on the low vapor pressure of the spot-on formulation, exposure to Amitraz is not anticipated via the inhalation route. Except for the TC and BW, the estimate was generated using the same formulae and point estimates as those used for estimating exposure to the 1 to 2 year old child. The TC was generated in the same manner but instead of the surface area of the child's hug, the estimated combined surface area of the adult's palms and fingers (i.e., 139.46 cm^2/hand or 278.92 cm^2 for both hands), was incorporated into the exposure formula (Agarwal and Sahu, 2010). The BW for the adult used in the formula is 80 kg. The estimated skin concentration of Amitraz on the adult hand is 0.39 µg/cm^2. Based on this concentration, the AF used to estimate exposure was the same as that used for the 1<2 year old exposed via hugging the treated dog (i.e., 13.1%). Based on central nervous system effects in humans reported in the RCD, the NOEL is 0.125 mg/kg/day; the RCD states that an MOE equal to or greater than 10 is considered adequate for the protection of human health when calculated with a NOEL from a human study. Based on the estimated dosage and the aforementioned NOEL, the MOE (referred to as MOS in the document), is **1357** (Table 1).

Estimation of Amitraz (Dog Collar) Exposure to 1- 2 year old Child

The acute exposure estimates calculated for the 1 to 2 year old child are **0.088 mg/kg/day** (Table 1) for percutaneously absorbed Amitraz and **0.023 mg/kg/day** (Table 1) for the amount absorbed orally from hand-to-mouth activity. Based on the low vapor pressure of the dog collar formulation, exposure to Amitraz is not anticipated via the inhalation route. The total amount of Amitraz absorbed daily, estimated by summing the amounts absorbed percutaneously and orally, is **0.11 mg/kg/day** (Table 1). Based on the critical NOEL of the RCD, the MOE for this dose is **1** (Table 1).

Estimation of Dermal Exposure to Amitraz for 1 to 2 yr old Child
The exposure estimate for dermal exposure of the 1 to 2 yr old child to Amitraz was generated using hand exposure data obtained from a journal article (Davis, 2008). The article described a study conducted to measure exposure to tetrachlorvinphos (TCVP) from treated collars on a variety of dogs. The experiment consisted of petting experiments conducted on 55 pet dogs of differing breeds and sizes. The study was conducted by volunteers wearing cotton gloves, who petted the dogs constantly for 5 minutes. The gloves were then removed and extracted for residue analysis. To test for sample recovery, gloves and t-shirts were spiked with a range of TCVP levels (i.e., 0.5 to 2000 µg). The spike recoveries ranged from 85 to 102% with a mean recovery of 95%. The petting events and subsequent glove samples were generated before the collar was

fitted to the dog, and then 4 hours, 3, 7, 14, 28, 56, 84, and 112 days after collar placement. The dog was petted in specific areas: on the back near the base of the tail, the neck area with the collar in place, and the neck area with the collar removed. All sample residue levels peaked 7 days after the dogs were fitted with the collar. The highest value obtained was from the neck area with the collar in place and generated a mean concentration of 24 mg/glove. The lowest residue level was consistently obtained from the back near the base of the tail (mean = 130 µg/glove). The specific value was not given for day 7. However, based on the graph (Figure 1 in the article), the level was approximately the same throughout the entire study. To be health-protective the 1<2 year old child was assumed to hug the neck area with the collar in place. Hence, the 24 mg/glove value was used to estimate exposure. The amount of TCVP in each collar was 4.8 grams (14.55% AI). Hence, the collar weighed 33 grams. The largest Amitraz collar available is 27.5 grams. Therefore, this collar was used to estimate exposure since it is closest in size to the TCVP collar of the study. Since the Amitraz collar is 9% AI, then the amount of Amitraz in the product collar is 2.475 g. Dividing 2.475 g with 4.8 g gives 0.52; multiplying 24 mg with 0.52 gives 12.48 mg. As with the spot-formulation residue transfer study, the investigators in the dog collar residue transfer study wore cotton gloves. To be health-protective, the 1<2 year old is assumed to not be wearing a shirt. Therefore, as with the spot-formulation exposure estimates, the correction factor generated in the Lu and Fenske (1999) study (i.e., 23.5) was used to reduce the glove residue of 12.48 mg to 0.53 mg.

Another correction is needed for the duration of the sampling for the collar study versus the anticipated duration of the 1<2 year old's hug. As mentioned earlier, each sample in the residue transfer study was collected by petting the dog constantly for 5 minutes. This type of interaction is not representative of the child's hug since the hug would likely be shorter and less vigorous. This duration and vigor was not corrected for in the Lu and Fenske (1999) study, since the gauze pad was merely wiped three times across the 100 cm^2 treated carpet in one direction and then three times in the perpendicular direction. This would likely take a matter of seconds. In contrast the petting sampling events in the collared dog residue transfer study took 5 minutes of constant petting with a cotton glove. The percentage of applied AI transferred to the glove is likely much higher than that transferred to the gauze pad in the Lu and Fenske (1999) study.

To correct for this discrepancy, the percentage of the dose removed by the 5 minute vigorous petting technique as used in a Fipronyl spot-formulation study (Jennings et al., 2000) was compared to that removed in the 5-stroke petting method used in another Fipronyl spot-formulation study (Brickel, 1997). The 5 minute vigorous petting technique consisted of, "vigorously petting the dog's hair forward and back along his back and down both sides for a 5 minute period" (Jennings et al, 2000). This method was similar to that used in the previously described dog collar residue transfer study conducted by Davis et al. (2008), which also sampled via constant petting with cotton gloves for 5 minutes. In contrast, the 5-stroke petting method consisted of simply stroking the dog a total of 5 times from head to tail along the back and sides. This technique, with a shorter duration of contact and less vigor, more closely resembles the anticipated hug activity of the 1<2 year old child. Each Fipronyl spot-formulation residue

transfer study had numerous sampling time points. However, the only sampling timepoint to match between the two studies is the 1 day after application timepoint. In comparing the data of the two residue transfer studies at 1 day after the application, the percent of applied Fipronyl removed via the 5 minute vigorous petting method was 3.728 times greater than that removed via the 5-stroke petting method. Hence, the previous glove residue value of 0.53 mg is reduced to 0.14 mg. This adjusted residue level was utilized as a surrogate residue amount to estimate dermal Amitraz exposure to the 1<2 yr old child hugging a dog wearing a dog collar containing Amitraz.

To estimate exposure the following formulae was used:

$$E = HR * SA_{hug} * ET * N\_Replen$$

E = exposure (mg/day)
HR = hand residue (mg/cm^2)
SA_{hug} = surface area of $1 < 2$ year old child's hug (cm^2)
ET = exposure time (hr/day)
N_Replen = # of replenishment intervals/hr

$$HR = GR/SA_{hand}$$

GR = glove residue (mg)
SA_{hand} = surface area of hand (cm^2)

Absorbed dermal dose, normalized to body weight, is calculated as:

$$D = E * AF / BW$$

D = dose (mg/kg-day)
E = exposure (mg/day)
AF = absorption factor (dermal)
BW = body weight (kg)

To calculate the HR, the surface area of the glove in contact with the dog while petting was assumed to be equal to that of the palm and the palm side of the fingers for the adult hand. Hence, the surrogate value of 0.14 mg/glove became 0.14 mg/139.46 cm^2 or 0.001 mg/cm^2. As mentioned earlier, the estimated surface area of the 1 to 2 year old's hug is 1874 cm^2. Hence, the amount of Amitraz transferred to the skin via a hug is 1.91 mg. In order to estimate the amount of Amitraz absorbed from this concentration, the aforementioned dermal absorption study using rats dosed with the applied dermal concentrations of 1, 10, 100, or 1000 µg/cm^2 was used (AgrEvo USA Company [1994] Registration Package 287-0104). Although 1 ug/cm^2 is the dosage closest to the estimated skin concentration, the recoveries at this dosage were poor.

Hence, the absorption data could not be used. Therefore, the absorption result generated for the next closest level, 10 μg/cm^2, was used to estimate dermal absorption. The percent of the applied dose recovered at this level was 94.15%. The percent of the applied Amitraz absorbed at this level is 12.64% at 120 hours after the application. The percent of the applied amount of Amitraz remaining on the skin 120 hours after application is 0.476%. Since this remaining AI may eventually be absorbed, it was combined with the percent of Amitraz absorbed to get 13.1%. As previously stated, the ET is 1 hr/day, and the N_Replen is 4 intervals/hr. Calculating the exposure (mg/day) using these values generates a value of 7.66 mg/day. Multiplying this value with the decimal form of the percent absorbed (i.e., 0.131), gives the absorbed amount of 1 mg/day. Dividing this value by the estimated weight of the 1 to 2 year old child (i.e., 11.4 kg) generates a dose of **0.088 mg/kg/day**.

Another study nearly identical to the TCVP collar investigation was conducted using chlorpyrifos (CP) (Chambers et al, 2007). However, the overall and peak residue levels were substantially lower than those of the TCVP study. The authors attribute this to several potential causes: "The grand means ± SE's (from both studies) for samples taken from the collar were 16,600±1300 μg/glove for TCVP and 370±60 μg/glove for CP. This very large difference in magnitude, about 45-fold, could be attributed in part to the concentration of the insecticides in the collars (14.55% [4.8 g] active ingredient in the TCVP collar and 8% [2.54 g] active ingredient in the CP collar). The differences among the TCVP and CP studies may also be attributed to different samplers, differences in dog fur composition from one breed to another, and in all likelihood, different formulation matrices between the two types of collars leading to greater release of TCVP (for a 4-month collar) than CP (for a 6 month collar)" (Davis et al, 2008). Since the estimates are for acute exposure, the collar study with the highest measured residue levels was used for estimation of exposure.

Appraisal of Estimate for Dermal Exposure to Amitraz for 1 to 2 yr old Child
Assumptions made in order to generate the exposure estimate may lead to overestimation of exposure. The assumption that the entire hug surface area of the child contacts the dog during a hug may overestimate exposure. Another assumption which may lead to overestimation of exposure is that the child was assumed to be unprotected by clothing. Hence, no protection factors were incorporated into the formula to reduce the exposure estimate. In addition, the TCVP collar of the study used to estimate the amount of AI transferred may not be representative of the Amitraz collar. Due to the potential difference in collar formulation, the amount of residue transferred during a hug may be substantially more or less than that from the TCVP collar. Moreover, the AI residue transfer profile for the TCVP collar (i.e., transfer peaked at 7 days after fitting the collar to the dog), may also differ from that of the Amitraz collar.

Estimation of Hand-to-Mouth Exposure to Amitraz® for 1 to 2 yr old Child
As with the dermal exposure estimate, the residue concentration estimate for the child was calculated using the aforementioned TCVP surrogate data. In this case, instead of the hug surface area, the estimated hand surface area of the 1 to 2 year old child (i.e., 150 cm^2), was used to

estimate exposure. It's possible that the child could pet the dog just around the neck and collar area. Hence, the peak measured TCVP residue level of 24 mg/glove from Davis et al., was used to assess exposure. As shown earlier, adjusting for the difference in percent AI between the collars containing TCVP and Amitraz, generated a value of 12.5 mg/glove. Adjusting for the difference in loading between the cotton glove used in the study and the anticipated bare hand of the child, reduces the 12.5 mg/glove to 0.53 mg/hand. Dividing this value by the estimated surface area of the 1<2 year old's hand, generates an estimated Amitraz skin concentration of 0.0035 mg/cm^2. Using the same formulae and point estimates used previously for the spot-on formulation, the estimated daily dose for the 1 to 2 year old child is **0.023 mg/kg/day**.

<u>Appraisal of Estimate for Hand-to-Mouth Exposure to Amitraz for 1 to 2 yr old Child</u>
The uncertainties generated by the assumptions and approaches described in the hand-to-mouth exposure section of the spot-formulation portion of the document also apply for hand-to-mouth exposure concerning the dog collar. In addition, using the residue transfer data generated in the TCVP collar study may have led to under- or overestimation of exposure. To be health protective, the TCVP collar study was used to estimate the amount of AI transferred by Amitraz collar. However, the CP collar may be more representative of the Amitraz collar. Due to the potential difference in collar formulation, the amount of residue transferred during a hug may be substantially less than that from the TCVP collar. Alternatively, residue transfer may be greater than that generated by the TCVP collar. Moreover, the AI residue transfer profile for the TCVP collar (i.e., transfer peaked at 7 days after fitting the collar to the dog), may also differ from that of the Amitraz collar. In addition, the child was assumed to pet only the neck area of the collared dog. However, it's quite possible that the child would pet other areas of the pet which would contain lower AI residues.

Estimation of Amitraz (Dog Collar) Exposure to Adult

<u>Estimation of Dermal Exposure to Amitraz for Adult</u>

E = 2 * HRA * ET * N_Replen
E = exposure (mg/day)
HRA = hand residue amount (mg)
ET = exposure time (hr/day)
N_Replen = # of replenishment intervals/hr

D = E * AF / BW

D = dose (mg/kg-day)
E = exposure (mg/day)
AF = absorption factor (dermal)
BW = body weight (kg)

Dermal exposure was estimated for the adult using the same surrogate data as that used for the 1 to 2 year old child. The adjusted surrogate TCVP glove residue data (12.48 mg/glove) was assumed to equal the amount transferred from the collared dog to the adult's hand. This level is referred to as the "hand residue amount" or "HRA" which is in mg. This estimate was then multiplied by "2" since the owner may pet the dog with both hands. Hence, the HRA value calculated to be 24.96 mg. Since the adult is assumed to pet the dog with the bare hand, the amount 24.96 mg was divided by the previously described adjustment factor of 23.5 to get 1.06 mg. As noted earlier, the ET for adults was estimated by EPA to be 0.77 hr/day. This value and the N_Replen of 4 were then factored into the equation to generate an exposure value of 3.27 mg/day. The daily dermal concentration estimated to be transferred to the hand through petting the collared dog is 23.5 $\mu g/cm^2$. The closest dermal concentration applied in the previously described rat dermal absorption study is 10 $\mu g/cm^2$. As stated earlier, the percent of the applied amount anticipated to be absorbed at this concentration after 120 hours is 13.1%. Hence, of the 3.27 mg transferred per day, 0.43 mg is estimated to be absorbed. Dividing this value by the estimated weight of the adult (i.e., 80 kg), generates a daily dose of **0.0054 mg/kg/day** (Table 1). For comparison, the corresponding value using the CP residue data is 0.004 mg/kg/day. Based upon the critical NOEL in the RCD, the MOE for this estimated dose is **23.3** (Table 1).

Appraisal of Amitraz Dermal Exposure Estimate for Adult
The assumptions and uncertainties pertaining to this scenario are the same as those described for the hand-to-mouth exposure scenario for the 1<2 year old child petting the collared dog.

Other Sources of Estimates of Amitraz Exposure to 1 to 2 year Old Children, and Adults via the Collared Dog
No post-application residential exposure estimates were generated in the Amitraz RCD (Frank, 1995), or in EPA's Amitraz RED (U.S. EPA, 1995). The authors of the RED assumed insignificant exposure: "The Agency has assumed that the potential for contact with amitraz to children exposed to pets wearing animal collars is negligible because of the type of formulation (impregnated plastic), the low duration and frequency of exposure. In a previous Agency assessment addressing potential exposure to children resulting from impregnated pet collars, these exposures were also considered negligible."(U.S. EPA, 1995). However, post-application residential exposure estimates were generated for the TRED document (U.S. EPA, 2006a). The calculations used to generate the estimates are explained in the associated memorandum (U.S. EPA, 2006b).

The EPA exposure estimation method in the TRED document was not used to estimate exposure for the current document. The TRED document authors used a carbaryl study as a source of surrogate data and assumed that the entire amount of AI within the collar distributed to equilibrium throughout the fur of the dog. This concentration was then divided by the lifetime (i.e., 90 days) of the collar. In contrast, the aforementioned journal article used to generate estimates for the current document contained empirical petting residue data from a variety of

dogs (Davis et al, 2008). In addition, the residue data show that the amount of AI available for transfer within the fur increased rapidly, peaking at 7 days after placement of the collar on the dog. It then decreased steadily for the next 112 days of the study. The residue data obtained at the peak was used to estimate exposure in this document.

Estimation of Amitraz Exposure to Handler of Apivar® Strips

Due to a lack of AI-specific data, the acute exposure estimate for the handler applying Apivar beehive treatment strips was generated using endosulfan residue data obtained from a study on cattle ear tag applicators. This surrogate study was used because the ear tag, being a plastic impregnated with pesticide, resembles the pesticide-impregnated plastic beehive strip. Moreover, the associated handler activity, removing the ear tag from the wrapper and attaching it to the simulated ear (an approximately 4-inch x 8-inch rectangular card cut from a sheet of 0.25-inch thick ethylene vinyl acetate foam), is roughly similar to that of the handler placing the Apivar strip into the brood chamber. The ear tag study contained 15 volunteers wearing cotton gloves, underneath protective nitrile gloves, who each placed a total of 200 ear tags on simulated cow ears. Following the application, the cotton gloves of the handlers were extracted and the extracts analyzed for endosulfan residues. The spike recovery test for this study consisted of dosing gloves with a range of endosulfan concentrations. The limit of quantitation (LOQ) determined in the study is 0.005 µg/cm^2. The spike levels were at 2X, 50X, and 150X the LOQ. The mean sample recoveries at these levels were 83.3, 86.6, and 88.3 percent, respectively. The experimental samples ranged in concentration from 0.005 to 0.0695 µg/cm^2. The mean value for these sample residue data is 0.0168 µg/cm^2 or 0.0000168 mg/cm^2 while the 95th percentile is 0.0562 µg/cm^2 or 0.0000562 mg/cm^2 (KMG-Bernuth, Inc. [2010] Registration Package 182-0130). To be health-protective, the 95th percentile value was used as a surrogate AI concentration for estimating daily exposure to the Apivar beehive strip handler wearing protective gloves.

The Apivar product label instructs the handler to use "chemical-resistant" gloves. 40CFR170.240(c)(1) states, "When 'chemical resistant' personal protective equipment is specified by the product labeling, it shall be made of material that allows no measureable movement of the pesticide being used through the material during use." However, the label lacks information about which glove materials are resistant to penetration by Amitraz. Moreover, information online suggests that while rubber gloves provide protection, vinyl gloves do not (TOXNET, 2012). Since the endosulfan study incorporated the use of chemical resistant gloves, the estimate for the Apivar strip handler was not adjusted downward using a protection factor for protective gloves. Hence, the daily dermal exposure for the Apivar strip handler is 0.0562 µg/cm^2 on each hand. Assuming that the Amitraz is on the palm-side of the fingers and palm, the SA of this part of the adult hand, as mentioned earlier, is estimated to be 139.46 cm^2. Therefore, a total of 0.0157 mg of Amitraz is deposited on both hands. The dermal absorption factor used for this scenario was 13.1% since, as mentioned earlier, it was generated using a dose of 10

$\mu g/cm^2$. Of the dermal absorption studies discussed, this is the closest dose concentration to the estimated skin concentration. Hence, the estimated daily absorbed amount of Amitraz for a handler weighing 80 kg is 0.00003 mg/kg/day. The associated MOE is 4870.

Due to the low vapor pressure of Amitraz (i.e., 2.6 x 10^{-6} mmHg at 25 ° C), and the assumption that the handler of the exposure scenario is working outdoors, significant inhalation exposure to Amitraz is not anticipated (Frank, 1995).

Apivar is a Section 18 product which hasn't been used long enough to generate PUR data. However, two other anti-varroa mite beehive strip products which have been in use are Apistan® and Checkmite®. In reviewing the database for seasonal use of these products, little or no use was discovered. In the last 5 years of data (2007-2011), no use was reported except in April (0.31 lbs), October (0.63 lbs), and November (0.62 lbs) of 2007, and in August (5.25 lbs) of 2011. This amount of use is not enough to constitute seasonal use. Hence, only acute exposure was estimated (DPR, 2012).

Appraisal of Estimate for Handler of Apivar Strips
The primary source of uncertainty for this exposure estimate is the use of the surrogate data. If the endosulfan ear tag handler exposure data is not representative of the Apivar strip handler, then the estimate may under- or overestimate exposure.

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