Department of Pesticide Regulation
Environmental Monitoring Branch
Surface Water Protection Program
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Study 296: The Washoff Potential of Fipronil from Dogs Treated with Fipronil Pet-Care Products

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1.0 INTRODUCTION

Fipronil and its major degradates are toxic to aquatic invertebrates at relatively low concentrations and frequently detected in surface waters of California (Ensminger et al. 2013). Fipronil is a phenylpyrazole insecticide registered for uses including structural pest control, bait and gel products, and topical flea and tick treatment for pets. There are limited data available to identify the sources and fate of fipronil and degradates during wastewater treatment; however, fipronil has been detected in wastewater treatment plant effluent indicating sewershed sources exist and treatment is not completely effective (Heidler and Halden 2009). The application of pet products and subsequent indoor bathing of animals represents a potential source to the wastewater sewershed. There are 109 fipronil containing products registered for use in California, of which 76 products are labeled for pet application. Based on California sales data, over 70,000 pounds of products for pest management on pets were purchased during 2013. Common spot-on product labels claim to be waterproof and to remain effective after bathing. The purpose of this study is to evaluate fipronil concentrations in rinsate derived from typical pet grooming practices during the first wash post-application. This is the first study of its kind to directly measure pesticide concentrations derived from common pet grooming practices. Information gained from this study will be crucial in evaluating sources of fipronil into the sewershed.

2.0 OBJECTIVES

The goal of the project is to characterize the washoff potential from pets treated with fipronil products as a result of common bathing practices. Results will be used to evaluate the relative mass contribution from common pet products to wastewater treatment systems. Objectives of the project are as follows:

1) Determine potential of spot-on pet products used according to the product label to contribute fipronil and fipronil degradates to the waste stream

2) Quantify mass of fipronil and fipronil degradates in wash rinsate from commonly used spot-on products
3) Evaluate effect of time between application and bathing on fipronil wash off concentrations
4) Using hypothesis test procedure to evaluate differences in the washoff mass between controlled study participants and non-controlled general population.

3.0 PERSONNEL

The study will be conducted by staff from the CDPR's Surface Water Protection Program (SWPP), Environmental Monitoring Branch under the general direction of Nan Singhasemanon, Senior Environmental Scientist, Supervisory. Key personnel are listed below:

Project Leader: Jennifer Teerlink, Ph.D.
Field Coordinator: KayLynn Newhart
Reviewing Scientist: Robert Budd, Ph.D.
Statistician: Yina Xie, Ph.D.
Laboratory Liaison: Sue Peoples
Analytical Chemistry: Center for Analytical Chemistry, Department of Food and Agriculture (CDFA)

4.0 STUDY PLAN

Products intended for use on dogs have the highest reported sales in California of topically applied pet application products. This, in conjunction with the common practice of indoor bathing, make dogs the ideal study group and therefore the focus of this study. Label instructions for spot-on treatments are based on the size of animal and suggest use of the product once every 30 days. The goal of this study is to quantify the fipronil washoff potential of applied spot-on treatments as a function of pet size and timing of application throughout the 30 day period of treatment.

4.1 Product Application

Volunteers, already using fipronil-containing pet care products on their pet, will be solicited to participate in the project. Volunteers will be enlisted to represent two size groups and three time periods (Table 1). In the event that an insufficient number of dog volunteers are found, monthly consecutive washoff events of the same dog may be included for 2, 7, and 28 day time points. Timing of reapplication will be consistent with label instructions. Subject information will be recorded for each dog (breed, weight, age, product applied, relative activity level, etc.). Subjects will coordinate with SWPP staff to schedule product application date and subsequent washoff. Prior to application, participants will be instructed to thoroughly wash the pet to remove residual

concentrations. Product application will be according to label, and participants will record the date and time of application and provide label to SWPP staff. In addition, participants will be asked to note any activity during the course of the study that may influence the results (e.g., swimming, brushing).

Table 1- Proposed sampling scheme.

Controlled Washoff Experiment			
Pet Size	**2 days**	**7 days**	**28 days**
S	6	6	6
L	6	6	6
Subtotal			**36**
Standard Washoff Sampling			
Small Dogs			18
Large Dogs			18
Subtotal			**36**
QA/QC			
Equipment Blanks			12
Sample Duplicates			4
Pet Carryover			2
Subtotal			**18**
Total Samples			**90**

Note: L = large (≥45 lbs), S = small (<45 lbs)

4.2 Sample Collection

Each participant will be assigned a period of time (2, 7, or 28 days) in which pets will not be washed, and advised to prevent from outdoor activities such as swimming, which may affect sample results. Participants will then bring their pets to DPR's West Sacramento facility on the assigned post-application day for washing. If participants find bathing necessary before the end of the assigned period of time they will be instructed to contact SWPP staff to conduct washing and sampling collection at that time. SWPP staff will wash pets and all rinsate from a single wash event will be collected into a large basin. Rinsate volume will be measured and a 1-L sample will be taken from this basin as a representative composite of the bathing event. Rinsate volume and sample concentration will be used to calculate the mass of fipronil and degradates coming off dogs in each event. Wash basins will be washed with soap and water and rinsed with methanol between pet washing events. Equipment blank samples will be collected for each wash basin by delivering a volume of water comparable to a pet washing event. Equipment blanks will be taken before, during and after the final washing event to ensure no carryover of active ingredient from discrete washing events. To evaluate residual insecticide from previous pet care applications, a subset of participants will be asked to come to the West Sacramento facility to

allow for SWPP staff to collect a sample of pre-application rinsate as a blank. All relevant label instructions will be followed including timing between product applications.

4.3 Commercial Washoff Sampling

Label instructions exist to ensure the proper and efficacious application of pesticide products. However, there is no guarantee owners consistently follow instructions. In addition to the controlled experiment described above, we will work with regional pet grooming businesses to collect rinsate samples from pets during standard operating practices. Samples will target dogs regularly treated with fipronil products (<30 days from time of grooming). Where possible, owner surveys will be collected from general population to identify timing of application and product type. The survey information will not be as complete as pets in the controlled experiment. Scientists will work with groomer operations to devise suitable sample collection technique. Equipment blanks will be taken by rinsing bathing setup with volume of water comparable to bathing event. The mass of fipronil and fipronil degradates present in equipment blanks can be used to quantify carry over between commercial bathing events.

5.0 CHEMICAL ANALYSIS

The Center for Analytical Chemistry, California Department of Food and Agriculture, Sacramento, CA (CDFA) will conduct the analysis of fipronil and degradates for the study (Table 2). Laboratory QA/QC will follow CDPR guidelines and will consist of laboratory blanks, matrix spikes, matrix spike duplicates, surrogate spikes, and blind spikes (Segawa 1995). Laboratory blanks and matrix spikes will be included in each extraction set.

Table 2- Chemical analysis of fipronil and degradates conducted by California Department of Food and Agriculture. All samples analyzed by GC-MSD (SIM)

Analyte	Method Detection Limit (µg L^{-1})	Reporting Limit (µg L^{-1})
Desulfinyl fipronil	0.003	0.05
Desulfinyl fipronil amide	0.005	0.05
Fipronil	0.004	0.05
Fipronil amide	0.005	0.05
Fipronil sulfide	0.003	0.05
Fipronil sulfone	0.005	0.05

6.0 DATA ANALYSIS

Based on the study objectives, we propose the following statistical procedures for data analysis.

1) Exploratory data analysis will be performed to summarize the characteristics of the sample data. Plots, such as boxplots, histograms, and probability plots, will be produced to explore any potential patterns implied by the data;

2) Depending upon the data structure (e.g., normality and censoring), appropriate parametric or nonparametric estimators will be employed to give point estimates and confidence interval estimates of the mass of fipronil and fipronil degradates or mass proportion of AI being removed in a single wash.
3) Comparison procedures (e.g., ANOVA and nonparametric tests) will be conducted to evaluate whether or not there is significant difference in washoff concentration or mass proportion of AI being removed between the time of application and first wash off, and size of pet.
4) We will also utilize the hypothesis test procedures to test observed differences between the populations in the study group and those monitored at the grooming centers.

7.0 TIMELINE

Solicit Participants: Oct 2014

Product Application and Sample Collection: Nov 2014

Chemical Analysis: Nov 2014–Feb 2015

Data Report: Aug 2015

8.0 LABORATORY BUDGET

The cost for the CDFA analysis of fipronil and fipronil degradates is $600 per sample. Sampling protocol calls for 90 samples for a total analytical cost of $54,000. Supplies including purchase of pet products, wash basins, and sampling equipment will require $3,000 for a total study cost of $57,000. Costs include QA/QC sample analysis but do not include laboratory QC.

Table 2- Summary of proposed analytical and supply costs.

Item	# of samples	Total Cost
Controlled Washoff	36	21,600
Commercial Washoff	36	21,600
QA/QC Samples	18	10,800
Supplies	na	3,000
Total Cost		$57,000

9.0 LITERATURE CITED

Ensminger, M., Budd, R., Kelley, K. and Goh, K. (2013) Pesticide occurrence and aquatic benchmark exceedances in urban surface waters and sediments in three urban areas of California, USA, 2008–2011. Environmental Monitoring and Assessment, 1-14.

Heidler, J. and Halden, R.U. (2009) Fate of organohalogens in US wastewater treatment plants and estimated chemical releases to soils nationwide from biosolids recycling. Journal of Environmental Monitoring 11(12), 2207-2215.

Segawa, R. (1995) California Department of Pesticide Regulation. SOP QAQC001.00: Chemistry Laboratory Quality Control.