

# New Methods and Strategies for Monitoring Susceptibility of Fleas to Current Flea Control Products\*

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## CLINICAL RELEVANCE

A flea larval bioassay was developed by an international team of scientists to monitor the susceptibility of fleas (*Ctenocephalides felis*) to imidacloprid (Advantage, Bayer Animal Health). The assay was validated using laboratory and field isolates of *C. felis*. Flea eggs representing different field isolates of *C. felis* were collected by veterinarians in the United States, United Kingdom, and Germany. Of the 972 flea isolates obtained during the 5-year study, 768 contained sufficient numbers of eggs to conduct the larval bioassay. Greater than 5% survival occurred for only six of the field isolates evaluated. Further evaluation and analysis of these isolates demonstrated that they did not differ significantly in their susceptibility to imidacloprid from the reference strains used to develop the assay. Collections of field flea isolates will continue in an attempt to detect and document any change in the susceptibility of field flea populations to imidacloprid.

### ■ INTRODUCTION

Modern host-targeted flea control products, such as Advantage/K9 Advantix (imidacloprid/imidacloprid and permethrin, Bayer Animal Health), Capstar (nitenpyram, Novartis Animal Health), Frontline (fipronil, Merial), Program and Sentinel (lufenuron, Novartis Animal Health), and Revolution and Stronghold (selamectin, Pfizer Animal Health), have had dramatic impacts on our ability to control cat fleas (*Ctenocephalides felis*).<sup>1-3</sup> These agents have essentially replaced previous products that contained traditional flea insecticides (e.g., pyrethrins, pyrethroids, carbamates, organophosphates). There are increasing concerns in the veterinary community about the development of resistance to the newer flea control agents. These concerns are fueled predominantly by field reports that performance of these products has decreased since they were introduced. Concerns are also based on the documented resistance to traditional flea control products mentioned previously (Table 1).<sup>4</sup> Presumed resistance to the newer flea control agents is usually unfounded or supported by limited research. In the majority of cases, product failures were likely related to compliance

issues. However, there appear to be occasional reports of reduced susceptibility (resistance?) to newer products. Strains of *C. felis* with reported reduced susceptibility to lufenuron and fipronil have been discovered and maintained in the laboratory.<sup>5-7</sup> Molecular characterizations of at least some of these flea strains indicated that resistance genes were present.<sup>8</sup> These studies are interesting, but they provide no definitive information on the true prevalence of resistant fleas in pet populations. Such prevalence information would be invaluable to those who must respond to inquiries about resistance. A scientifically sound method of measuring the susceptibility of modern flea control products that could provide a means of measuring the continuing performance of flea insecticides would be useful. This article describes such a method for monitoring susceptibility of cat fleas to imidacloprid (Advantage) and summarizes results of an international imidacloprid susceptibility monitoring effort.

### ■ DEFINITION OF FLEA INSECTICIDE RESISTANCE

Development of resistance to previously lethal insecticides is a powerful and potentially

**TABLE 1. Examples of Insecticides for Which Cat Flea (*C. felis*) Resistance Has Been Documented<sup>a</sup>**

<i>Insecticide</i>	<i>Resistance Ratio<sup>b</sup></i>
Malathion	190
Carbaryl	20
Permethrin	12
Diazinon	11
Chlorpyrifos	10
Fenthion	10
Cyfluthrin	6.8
Cypermethrin	5.2
Propoxur	4.4

<sup>a</sup>Modified from reference 4.

<sup>b</sup>Ratio of the lethal dose of insecticide required to kill a field strain of *C. felis* to the dose required to kill a reference strain.

pervasive natural phenomenon.<sup>9</sup> The World Health Organization defines insecticide resistance as “the development of an ability of a strain of some organism [fleas in this case] to tolerate doses of a toxicant [insecticide] that would prove lethal to a majority of individuals in a normal population of the same species.”<sup>10</sup> Insecticide resistance can occur via several biologic mechanisms and can involve all or a portion of a population (Table 2).<sup>11,12</sup> It is important to remember that insects develop resistance genes through natural processes (e.g., mutation, recombination). Resistant insects then emerge as the predominant phenotype in a population because insecticides select for them. Stated differently, insecticides kill what they can and leave what they cannot. Surviving insects may then pass their resistance genes to their offspring. Eventually, this causes the population to change from one that was once fully susceptible to one with reduced susceptibility. The degree of susceptibility in a population depends on the pro-

**TABLE 2. Examples of Mechanisms of Insecticide Resistance<sup>11,12</sup>**

<i>Resistance Mechanism</i>	<i>Reason for Ectoparasite Survival</i>
Decreased penetration of the ectoparasite cuticle	Altered ectoparasite exoskeleton prevents or decreases entry of the insecticide
Increased metabolism of insecticide	Increased production of enzymes by the ectoparasite results in enhanced destruction of the insecticide
Altered insecticide target site	Change(s) in the binding site (target site) of the insecticide results in a decreased insecticidal effect

portion of resistant and susceptible insects that compose that population. It is important that veterinarians understand that insecticides do not “cause” resistance, they simply contribute to the process of resistance development by “allowing the survival” of resistant insects.

**■ SIGNS OF RESISTANCE TO FLEA CONTROL PRODUCTS**

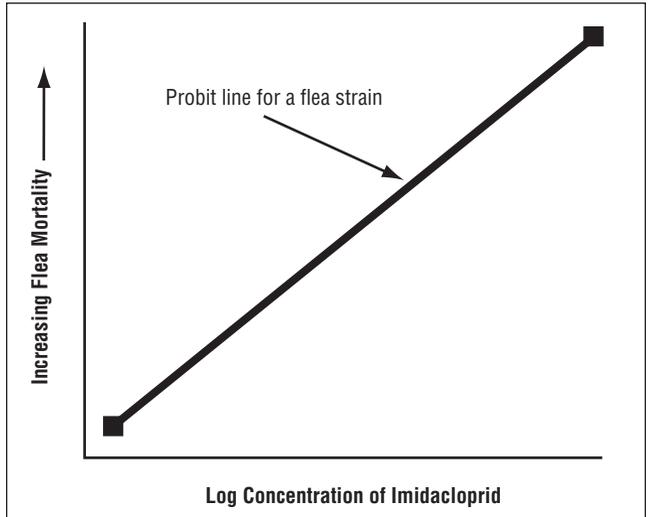
Obviously, resistance to flea control products will become apparent to veterinarians and pet owners because of the presence of fleas when none were seen previously or because greater numbers of fleas are observed than in the past. However, it may be difficult to differentiate emerging resistance from variations in flea populations caused by seasonal or annual fluctuations in rates of flea challenge, inconsistencies in client compliance habits, or changes in the environments to which pets are exposed. True resistance is likely to develop more slowly than flea population changes caused by the factors just mentioned. Pet owners are more likely to complain about seeing fleas sooner after

treatment or to report seeing a small increase in the number of fleas on their pets over time. They might also report the development or recrudescence of flea allergy dermatitis when it either did not exist or was previously controlled. These complaints would likely give way to complaints of flea numbers continuing to increase, even when products are administered as directed by veterinarians and product package inserts. Another hint of resistance might be an improvement in flea control when a different product with a different mechanism of action is used. However, success in controlling fleas by switching products may not always confirm that resistance is the real problem. Pet owner compliance may be better with one product than another, or differences in dose banding between

products may mean that a larger dose of the new product is being administered. Only after resistance has been confirmed by appropriate laboratory assays can we truly attribute product failures to resistance. These problems were responsible in part for the monitoring initiative described in this article.

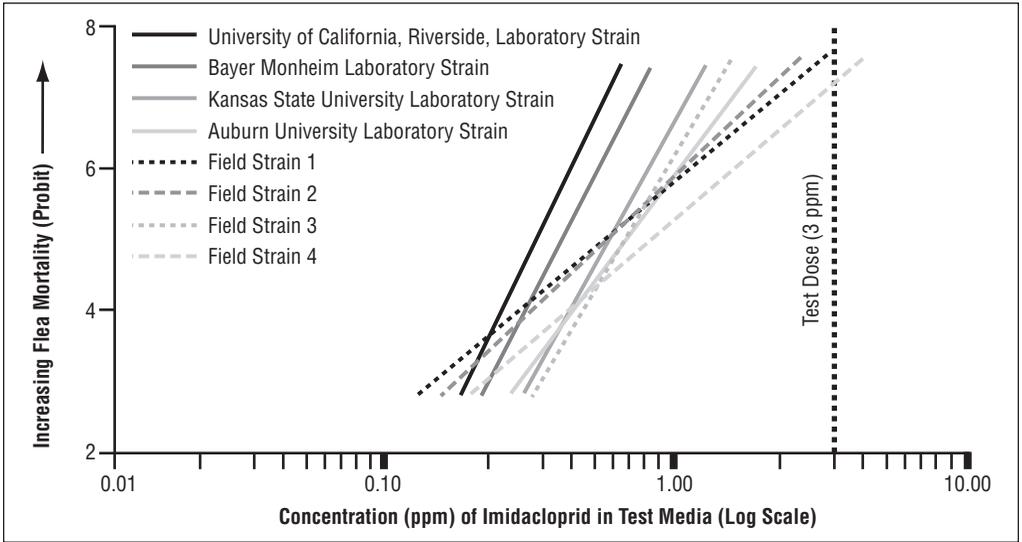
#### ■ DEVELOPMENT OF A LABORATORY BIOASSAY TO MONITOR SUSCEPTIBILITY OF FLEAS TO IMIDACLOPRID

In response to the needs described above, an international team of veterinarians, parasitologists, and entomologists was convened to establish a laboratory assay capable of monitoring the susceptibility of *C. felis* to imidacloprid.<sup>13-16</sup> After considering prior models, target flea life



**Figure 1.** A dose response is displayed as a probit line, which is obtained by converting the response (mortality) at each concentration of imidacloprid to its corresponding probit. Each probit is graphed against the log of each concentration of imidacloprid that was tested. Probit lines are “fingerprints” of a flea strain’s response to an insecticide. A steep line (greater slope) indicates that the mixture of fleas in the population responds to a narrow dose range (more genetically homogeneous population). A shallower line indicates the opposite.

cycle stages, and the logistics of recovery and shipment of isolates of *C. felis* from veterinary clinics to the different research laboratories, a larval bioassay was identified as the most likely to fulfill the research objective. Initial research focused on use of laboratory flea strains to establish baseline susceptibility to imidacloprid. A dose response (probit line; Figure 1) was generated for flea strains from Auburn University; Kansas State University; University of California, Riverside; and Bayer Laboratories, Monheim, Germany. The imidacloprid concentrations used in the assay ranged from 0.005 ppm to 3 ppm (mg/L). Because laboratory strains are likely to differ in their responses to imidacloprid compared with pet strains of fleas, the assay was further calibrated using 17 field flea strains collected from pet animals.<sup>17</sup> As suspect-



**Figure 2.** Examples of imidacloprid dose–response (probit) lines for four laboratory and four field strains of *C. felis*. The selected target dose of 3 ppm was based on dose responses of both laboratory and field strains. The steeper slope for the laboratory strains suggests a more homogeneous response than was observed for the field strains.

ed, the range of responses of laboratory fleas differed from that of pet fleas. The dose of imidacloprid required to kill 50% of the laboratory fleas ( $LD_{50}$ ) ranged from 0.07 to 0.77 ppm, whereas the range of  $LD_{50}$  values for the field strains was 0.06 to 1.51 ppm. These data confirmed our suspicions that flea strains collected from pet animals consisted of fleas with a wider range of responses than the laboratory strains (Figure 2).

Because the objective of this research was to monitor large numbers of pet isolates of fleas, the larval bioassay had to be modified. Instead of using a range of dosages to produce a dose–response line (probit), a single dose (diagnostic dose) of 3 ppm was selected for evaluation.<sup>17</sup> The selection of this dose was based on the previous laboratory and field data and was done with care. Selecting a diagnostic dose that was too low would result in the identification of an excessive number of flea isolates with surviving fleas—an erroneous result suggestive of

reduced susceptibility to imidacloprid. Selecting a diagnostic dose that was too high might fail to identify flea strains whose susceptibility to imidacloprid might be truly reduced compared with reference laboratory strains and field isolates.

### ■ SAMPLING OF CAT AND DOGS FOR FLEA EGGS<sup>17</sup>

Veterinary clinics throughout the United States, Germany, and the United Kingdom were asked to participate in the field collections. To obtain flea eggs, cats or dogs were placed in cages for a period of 1 to 24 hours. Paper was placed beneath the floor walks in each cage. At the end of the collection period, the animals were brushed or combed gently to remove haircoat debris, including any remaining flea eggs. (Most eggs dropped to the paper during normal animal movement and grooming.) The debris from the paper was examined with a hand magnifier to ensure that flea eggs

were present and that there were sufficient numbers of eggs to conduct the assay. Eggs and haircoat debris were then passed through a sieve and funnel and collected into a glass tube. The glass tube was packaged in a Styrofoam cooler containing several layers of insulation, moistened gauze pads, and ice packs to promote safe shipment to one of the participating laboratories. A questionnaire was provided to all participating clinics; information requested included household and pet information, such as signalment, other pets on the premises, use of environmental and on-animal flea control products, level of flea infestation, whether fleas were a recurring or first-time problem, and the like. Flea eggs received from US veterinary clinics were evaluated at Auburn University,

able flea eggs is necessary. Twenty flea eggs are added to each of the imidacloprid and control dishes. If more than 40 eggs are present in the sample, the eggs are distributed to treated and control dishes as follows:

- 60 available eggs—two treatments and one control
- 80 available eggs—three treatments and one control
- 100 available eggs—three treatments and two controls
- 120 available eggs—four treatments and two controls
- 140 available eggs—five treatments and two controls

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***The veterinary community should be very cautious about presuming that flea product failures are caused by resistance.***

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Kansas State University, or University of California, Riverside; flea eggs from the United Kingdom at the Royal Veterinary College; and flea eggs from Germany at the Bayer Laboratories in Monheim, Germany.

**■ THE IMIDACLOPRID SUSCEPTIBILITY FLEA LARVAL BIOASSAY**

To conduct the assay, imidacloprid is dissolved in acetone and mixed with a larval flea-rearing media at the diagnostic rate of 3 ppm.<sup>14</sup> After the imidacloprid-acetone mixture is added to rearing media, the acetone is allowed to evaporate. For control media (see below), only acetone is added to the rearing media. The imidacloprid-treated and control media are then transferred to glass dishes. To determine the susceptibility of field isolates, a minimum of 40 vi-

- 160 available eggs—six treatments and two controls
- 180 available eggs—six treatments and three controls

Any remaining eggs are placed “en masse” into rearing media and allowed to mature to adult fleas. This provides adult fleas for propagation and maintenance on cats if test results suggest that additional analysis is required. All glass dishes are covered and incubated at 26°C to 28°C and 75% to 80% relative humidity for 28 days. Each dish is examined after 11 to 14 days for the presence of larvae and/or pupae. Determination of the number of hatched eggs and/or pupae allows for accurate calculation of the number of viable eggs that were present in the samples. After 28 days, live adult fleas present in treated and control dishes are enumerat-

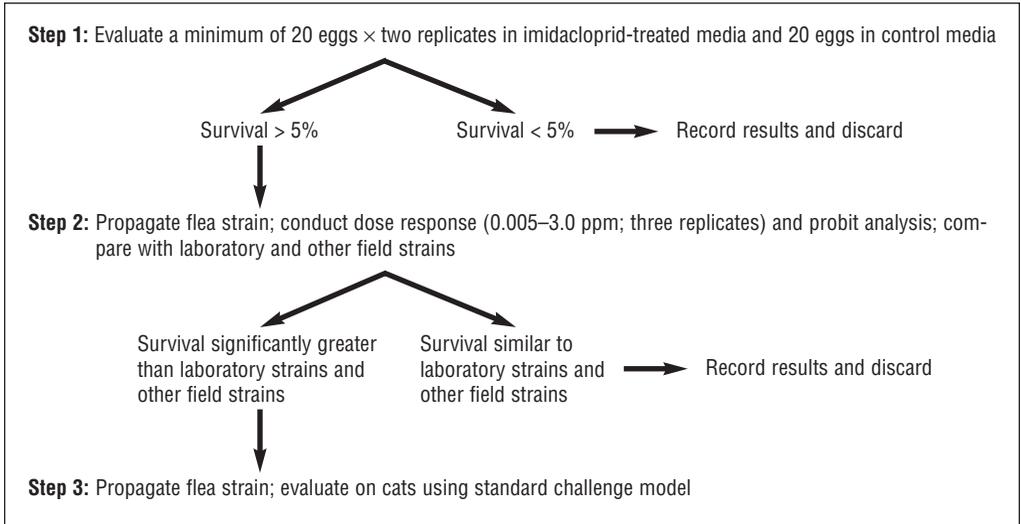


Figure 3. Algorithm for evaluation of flea eggs in the imidacloprid larval susceptibility bioassay.

ed. If the number of adult fleas in the treated dishes does not exceed 5% of the number of eggs placed, the results are recorded and the fleas are discarded. If the number of adult fleas in the treated dishes exceeds 5% of the number of eggs placed, the flea isolate is placed on laboratory cats for propagation, and a repeat of the diagnostic dose assay or a complete dose response (0.005–3.0 ppm) is performed. Additional strategies dictated that if survival remained significantly higher than laboratory strains or field isolates, the candidate flea strain would be propagated further and evaluated on cats using an *in vivo* challenge model. A summary algorithm for evaluation of flea eggs is presented in Figure 3.

### ■ STATISTICAL ANALYSIS

Flea strains for which adult emergence exceeded 5% and for which complete dose–response studies were conducted were analyzed by probit analysis using a POLO software program (now available as Polo Plus, LeOra Software, Menlo Park, CA).<sup>18,19</sup>

### ■ RESULTS AND DISCUSSION

A total of 972 flea isolates were obtained from veterinarians in the United States, Germany, and the United Kingdom during the 5-year study period (Table 3). Of the 972 isolates received, 768 contained a sufficient number of eggs to conduct a larval bioassay. The number of flea strains obtained from and evaluated in the United States (355) was slightly less than the number obtained from and evaluated in Germany and the United Kingdom (413). All flea isolates evaluated at all laboratories were identified as *C. felis*. As demonstrated in Table 3, the number of isolates varied during each of the submission years. The greatest number of flea isolates was received and assayed in 2004 and the fewest in 2002. This variation likely reflects the differences in annual rates of flea challenge and, thus, the number of infested animals and/or the willingness of collaborating clinics to submit flea eggs to the participating laboratories. Table 4 summarizes hosts from which flea eggs were collected. Information on host origin of fleas was available for 962 of the 972

**TABLE 3. Numbers of Flea Egg Collections Received and Tested<sup>a</sup> in the Participating Laboratories (2001–2005)**

<i>Participating Laboratory</i>	<i>No. of Eggs Received/Tested</i>				
	<i>2001</i>	<i>2002</i>	<i>2003</i>	<i>2004</i>	<i>2005</i>
Auburn University College of Veterinary Medicine	45/36	18/16	20/16	45/40	25/21
Kansas State University College of Veterinary Medicine	39/25	16/11	26/16	40/34	27/22
Bayer Animal Health, Agricultural Center, Monheim, Germany	37/23	27/26	25/25	51/41	12/12
University of California, Riverside, Department of Entomology	46/26	17/15	22/17	44/40	26/20
The Royal Veterinary College, Department of Pathology and Infectious Diseases	73/53	59/50	57/40	67/52	108/91
<b>Total</b>	<b>240/163</b>	<b>137/118</b>	<b>150/114</b>	<b>247/207</b>	<b>198/166</b>
<b>Grand Total Received/Tested: 972/768</b>					

<sup>a</sup>A minimum of 40 eggs is required to conduct the laboratory test.

**TABLE 4. Hosts from Which Flea Eggs Were Obtained<sup>a</sup>**

<i>Host</i>	<i>Study Year</i>					<i>Total</i>
	<i>2001</i>	<i>2002</i>	<i>2003</i>	<i>2004</i>	<i>2005</i>	
Cat	166	112	110	194	163	745
Dog	70	25	38	51	33	217
<b>Total</b>	<b>236</b>	<b>137</b>	<b>148</b>	<b>245</b>	<b>196</b>	<b>962</b>

<sup>a</sup>Only specimens with complete host information are included.

flea isolates. The majority (745 of 962 [77%]) of flea isolates were obtained from cats; the remainder (217 of 962 [23%]) were obtained from dogs. We attribute the difference in the number of source host species to the ease with which cats are maintained in cages compared with dogs. Differences in host origin of fleas would not likely affect the results obtained in

the bioassay because *C. felis* is not rigidly host specific and dogs and cats in the same household are likely to share the flea isolate.<sup>20,21</sup>

A sample of 535 of the questionnaires returned during the 2002 to 2004 sampling period provided interesting background information on habits and prior treatments of some of the source animals. Many pet owners (55%)

**TABLE 5. Locations of Participating US Veterinary Clinics (2001–2005)**

State	Number of Clinics Submitting Isolates
Missouri	12
California	7
Florida	7
Texas	4
Georgia	3
Tennessee	3
Virginia	3
Alabama	2
Kansas	2
Louisiana	2
Massachusetts	2
New Jersey	2
Oklahoma	2
South Carolina	2
Wisconsin	2
Illinois	1
Indiana	1
Kentucky	1
Maryland	1
Minnesota	1
Mississippi	1
Ohio	1

were not sure when their pet was last treated with a flea control product. Approximately 31% indicated that their pet had never been treated. Five percent stated that their pet had been treated 1 to 2 months previously; 4%, 2 to 6 months previously; and 4%, 6 to 12 months previously. Regarding use of specific products, 22% of respondents used imidacloprid (Advantage), 14% used fipronil (Front-

line), and 2% used lufenuron (Program). The remaining respondents either used another product or were not sure of the product used. Information provided by veterinarians on the level of infestation on dogs or cats (2001–2005,  $n = 741$ ) indicated that 367 of the dogs or cats had high levels of infestations (>50 fleas), 276 had medium infestations (20–50 fleas), and 98 had low (<20) infestations. However, these data must be interpreted with caution because visual assessments of flea burdens are not as accurate as actual counts.

For purposes of discussion, we divided the United States into four geographic regions.<sup>22</sup> Forty-eight percent (30 of 62) of the participating veterinary clinics were located in the southeast (Table 5). Among southeastern states, Florida ( $n = 7$ ), Texas ( $n = 4$ ), and Georgia, Tennessee, and Virginia ( $n = 3$  each) had the most participating clinics; Alabama, Louisiana, Oklahoma, and South Carolina had two participating clinics each. Although attempts were made to sample clinics from as many geographic regions as possible, the milder climatic conditions in the southeastern United States were more likely to support the flea life cycle and consequently would likely yield a greater number of pets with demonstrable flea burdens. The fewer number of participating clinics in the western (7), midwestern (20), and northeastern (5) United States again reflects the climatic influences on flea prevalence.

The majority of field flea egg strains (US, UK, and German) were submitted to the assay laboratories from May through December (Figure 4); peak submission months were August, September, and October. These results are not surprising and again reflect climatic influences on the flea life cycle and development. Clearly, as mean diurnal temperatures increase during the summer months (a climatic trend seen in all geographic regions sampled), a shorter *C. felis* life cycle combined with the accumulation of environmental

flea stages leads to greater adult emergence. Similar seasonal abundances of *C. felis* were observed in other studies conducted in the United States.<sup>23</sup> However, in tropical environments, *C. felis* is less likely to demonstrate significant seasonality because of the absence of significant seasonal differences in temperature and humidity.<sup>23</sup>

Of the 768 flea isolates that were suitable for the bioassay, only six had an adult survival rate of more than 5% of eggs evaluated (Table 6). These isolates were obtained from California (four isolates), Florida (one isolate), and Louisiana (one isolate). Adult emergence rates ranged from 5% to 13.33%. As discussed previously, these isolates were retested either at the diagnostic dose or using the dose–response assay to determine if they were significantly different from laboratory strains in their responses to imidacloprid. Results of a repeat of the 3 ppm challenge or probit analysis of the full dose response for these isolates indicated that either no survival was observed when isolates were retested or that the susceptibility of these isolates was not significantly different from susceptible laboratory strains maintained at the participating laboratories. Therefore, further testing of these flea strains on animals was deemed unnecessary. Adult emergence exceeding 5% was not observed for any of the flea isolates evaluated in the United Kingdom or Germany. These results do not provide assurance that resistance to imidacloprid does not exist in pet flea populations. Results of this study do suggest that the frequency of resistance, if present in pet

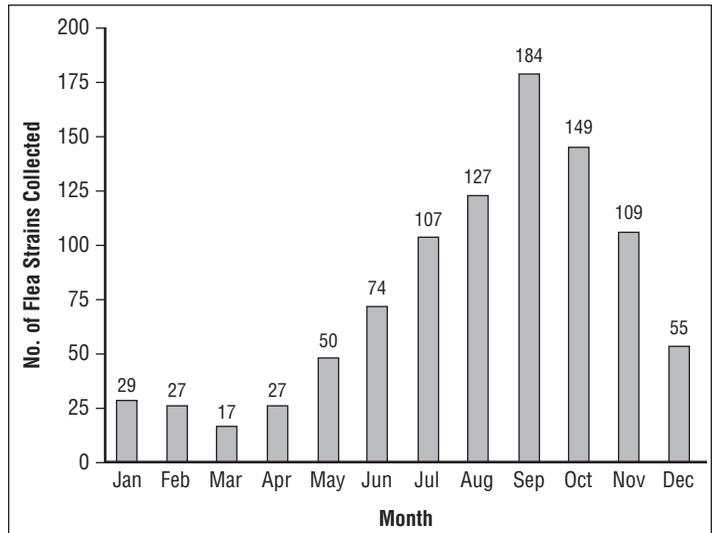


Figure 4. Flea strains collected by month (all geographic locations, 2001–2005).

populations, appears low at the present time. Because responses of fleas from the United States, Germany, and the United Kingdom were similar in the larval assay, it also appears that there are currently no demonstrable geographic susceptibility differences of *C. felis* to imidacloprid.

It is interesting that *C. felis* was the only flea recovered from dogs and cats in the United States in this survey. Several additional flea species, including *Ctenocephalides canis*, *Pulex irritans*, *Pulex simulans*, *Echidnophaga gallinacea*, *Xenopsylla cheopis*, and certain rabbit (e.g. *Cediopsylla* spp, *Odontopsyllus* spp) and additional rodent fleas (e.g. *Orchopeas* spp, *Polygenis* spp), have been recovered from dogs in different regions of the world.<sup>20,24</sup> In many situations, these fleas were recovered from rural or feral dogs or from wild canids. Because our samples were obtained from “cared-for” animals, the preponderance of *C. felis* probably reflects the habits of these animals and the habitats they visit. *C. canis* has been recorded with some frequency in some regions of the world,

**TABLE 6. Flea Strains with Greater Than 5% Adult Emergence**

<i>Flea Strain</i>	<i>Pet's Name</i>	<i>Source/ Year Acquired</i>	<i>% Adult Emergence</i>	<i>Comments</i>
R-003-FL-02	Pinky	Florida/ 2002	13.33%	Pets in the household had been treated with Advantage. Last treatment was applied 6–12 months before collection. Further and extended analysis conducted with this isolate at the University of California, Riverside, demonstrated that the LD <sub>50</sub> and LD <sub>95</sub> were no different than those from isolates collected and sampled from the field (baseline) in 2000. Results of the assay were interpreted as normal variations in susceptibility of individuals in this strain.
B-006-CA-03	Troi	California/ 2003	6.67%	This animal was never treated for flea infestation. The assay was repeated several times in different laboratories with no survival at the discriminatory dose of 3 ppm. Results of the initial assay were interpreted as normal variations in susceptibility of individuals in this strain.
D-015-LA-03	Onyx	Louisiana/ 2003	5.00%	No available information on prior use of flea control products was available. A follow-up assay conducted at Kansas State University showed no survival at the discriminatory dose of 3 ppm. Results of the initial assay were interpreted as normal variations in susceptibility of individuals in this strain.
R-002-CA-03	Zoe	California/ 2003	7.50%	Flea eggs were acquired from cats in a multiple-cat household. Cats had been treated with Advantage. The last treatment was applied 1–2 months before collection. The laboratory was unsuccessful in rearing the next generation for additional testing. Results of the initial assay were interpreted as normal variations in susceptibility of individuals in this strain.
R-003-CA-03	Baja	California/ 2003	8.33%	Pets had been treated with Program. Last treatment for flea infestation was 2 to 6 months before collection. The laboratory was unsuccessful in rearing the next generation for additional testing. Results of the initial assay were interpreted as normal variations in susceptibility of individuals in this strain.
R-002-CA-04	Goron	California/ 2004	5.00%	Flea eggs were collected from a multiple-cat household. Cats reportedly had been exposed to flea collars (unknown active ingredient) but no other flea control products. This assay was repeated at the diagnostic dose of 3 ppm with no survivors. Results of the initial assay were interpreted as normal variations in susceptibility of individuals in this strain.

including the United States and Europe.<sup>24</sup> However its distribution tends to be spotty or restricted to dog-only kennels (e.g., greyhounds), and this species is being replaced with increasing frequency by *C. felis* on domestic dogs in many of these regions.

We believe that the larval bioassay employed in this research is a convenient and effective tool for monitoring susceptibility of field flea strains to imidacloprid. Larvae are easily obtained from eggs that can be easily collected and couriered to participating laboratories; in contrast, collection and use of adult fleas present a formidable challenge for participating veterinarians. Additionally, adult fleas that have fed often do not survive long after being removed from their hosts.<sup>21</sup> A number of bioassay methods exist for resistance or susceptibility monitoring. Such methods include immersion (insects are dipped or washed in the insecticide), residue or surface contact (insects are exposed to a dry residue on a natural or artificial substrate), topical administration (known dose of insecticide is applied directly on individual insects), and ingestion or feeding techniques (insecticide is applied to a food source that is ingested by the insect).<sup>25</sup> Our ability to detect some strains of *C. felis* with imidacloprid susceptibility characteristics that appeared to differ from reference laboratory and field strains (>5% survival) further supports the validity of the larval bioassay. Although further analysis of these strains failed to confirm that demonstrable susceptibility differences to imidacloprid did exist, the assay was capable of “flagging” those specimens in need of retesting or further evaluation. Follow-up testing revealed that the initial survival at 3 ppm represented normal variation in the population; stated differently, if we were to repeat the 3 ppm assay on this isolate many times we would see identical results in some assays and a different result in others, indicating expected

variation in response—not resistance. As stated previously, we in no way imply that our results to date confirm that resistance to imidacloprid does not exist. We will continue to monitor field populations of fleas on dogs and cats. In addition, a portion of our focus will be on pets and households with apparent flea product failures. The veterinary community should be very cautious about presuming that flea product failures are caused by resistance. We continue to believe that the vast majority of putative product failures are due to compliance issues or to a lack of understanding of the developmental biology of *C. felis*.<sup>2</sup>

In conclusion, the development of an imidacloprid susceptibility monitoring initiative is important to veterinarians, pet owners, and product manufacturers. Our capability to identify and document resistance to flea control products will allow us to better understand the molecular and biochemical basis of resistance. This in turn will allow us to employ effective prevention and control strategies. We believe that the development of an imidacloprid susceptibility assay is an excellent first step in the implementation of effective monitoring of insecticides and acaricides in the animal health arena. Perhaps the future will see the development and implementation of similar susceptibility monitoring for other flea control products, such as fipronil, selamectin, lufenuron, and methoprene.

## REFERENCES

1. Marsella R: Advances in flea control. *Vet Clin North Am Small Anim Pract* 29:1407–1424, 1999.
2. Blagburn BL: Changing trends in ectoparasite control, in Thoday K, Foil C, Bond R (eds): *Advances in Veterinary Dermatology*. Oxford, Blackwell Publishing, 2002, pp 59–68.
3. Rust MK: Advances in the control of *Ctenocephalides felis* (cat flea) on cats and dogs. *Trends Parasitol* 21:232–236, 2005.
4. Bossard RL, Hinkle NC, Rust MK: Review of insecticide resistance in cat fleas. *J Med Entomol* 35:415–422, 1998.

5. Bardt D, Schein E: Zur problematik von therapieresistenten flohpopulationen am beispiel des stammes "Cottontail". *Kleintierpraxis* 41:561–566, 1996.
6. Payne PA, Dryden MW, Smith V, et al: Effect of 0.29% w/w fipronil spray on adult flea mortality and egg production of three different cat flea, *Ctenocephalides felis* (Bouché), strains infesting cats. *Vet Parasitol* 102:331–340, 2001.
7. Schenker R, Humbert-Droz E, Moyses EW, et al: Efficacy of nitenpyram against a flea strain with resistance to fipronil. *Compend Contin Educ Pract Vet* 23: 16–19, 2001.
8. Bass C, Schroeder I, Turberg L, et al: Identification of the *Rdl* mutation in laboratory and field strains of the cat flea, *Ctenocephalides felis* (Siphonaptera: Pulicidae). *Pest Manage Sci* 60:1157–1162, 2004.
9. Brown AW, Pal R: Insecticide resistance in arthropods. *WHO Monograph Series No. 38*. Geneva, World Health Organization, 1971.
10. World Health Organization: Present status of pesticide resistance. *Tech Rep Ser No. 818*. Geneva, World Health Organization, 1992, pp 2–17.
11. Georghiou GP: Management of resistance in arthropods, in Georghiou GP, Saito T (eds): *Pest Resistance to Pesticides*. New York, Plenum Press, 1983.
12. Roush RT: Occurrence, genetics and management of insecticide resistance. *Parasitol Today* 9(5):174–179, 1993.
13. Blagburn BL, Bach T, Bledsoe DL, et al: Advantage international flea susceptibility monitoring initiative, a 2001 update. *Compend Contin Educ Pract Vet* 23(Suppl):4–7, 2001.
14. Rust MK, Waggoner M, Hinkle N, et al: Development of a larval bioassay for susceptibility of cat fleas (Siphonaptera: Pulicidae) to imidacloprid. *J Med Entomol* 39:671–674, 2002.
15. Dryden MW, Payne PA, Blagburn BL, et al: Establishment of susceptibility profiles of cat fleas to imidacloprid and development of a program to monitor for imidacloprid susceptibility among cat flea populations—A 2002 update. *Compend Contin Educ Pract Vet* 24(Suppl):14–16, 2002.
16. Schroeder I, Blagburn BL, Bledsoe DL, et al: Progress of the international work of the "imidacloprid flea susceptibility monitoring team". *Parasitol Res* 90: S127–S128, 2003.
17. Rust MK, Denholm I, Dryden MW, et al: Determining a diagnostic dose for imidacloprid susceptibility testing of field-collected isolates of cat fleas (Siphonaptera: Pulicidae). *J Med Entomol* 42:631–636, 2005.
18. Finney DJ: *Probit Analysis*. Cambridge, Cambridge University Press, 1971.
19. Robertson JL, Preisler HK: *Pesticide Bioassays with Arthropods*. Boca Raton, FL, CRC Press, 1992.
20. Dryden MW: Biology of fleas of dogs and cats. *Compend Contin Educ Pract Vet* 15:569–579, 1993.
21. Rust MK, Dryden MW: The biology, ecology, and management of the cat flea. *Annu Rev Entomol* 42:451–473, 1997.
22. Blagburn BL, Lindsay DS, Vaughan JL, et al: Prevalence of canine parasites based on fecal flotation. *Compend Contin Educ Pract Vet* 18:483–509, 1996.
23. Durden LA, Judy TN, Martin JE, et al: Fleas parasitizing domestic dogs in Georgia, USA: Species composition and seasonal abundance. *Vet Parasitol* 130:157–162, 2005.
24. Durden LA, Traub R: Fleas (Siphonaptera), in Mullen G, Durden L (eds): *Medical and Veterinary Entomology*. San Diego, Academic Press/Elsevier, 2002, pp 103–125.
25. French-Constant RH, Roush RT: Resistance detection and documentation; the relative roles of pesticidal and biochemical assays, in Roush RT, Tabashnic BE (eds): *Pesticide Resistance in Arthropods*. New York, Chapman and Hall, 1990, pp 4–38.