Abstract # 194

Non-animal Efficacy Testing Approaches for Ectoparasiticides

Katherine Groff, MS, PETA Science Consortium International e.V., Germany

Background

Prior to the registration and sale of new flea and tick control products for animal companions, regulatory guidelines require that these ectoparasiticides are evaluated for toxicity and efficacy, which involves testing on dogs or cats in laboratories. For example, to meet regulatory requirements for demonstration of product efficacy, dogs are artificially infested with upwards of 100 fleas or 50 ticks and experience adverse effects, which can include severe anemia from the infestations and toxicity from the test chemicals.[1] Additional animals are used to rear insects and arachnids for later use in testing. Here, we show how *in silico* models, *in vitro* assays, and weight of evidence approaches can replace the use of animals for the development of these products.

In Silico, Ex Vivo, and *In Vitro* Models

Computational approaches: *In silico* models for absorption, distribution, metabolism, and excretion are used to inform *in vitro* efficacy assays, and they have also been used to elucidate mechanism of action.[2,3]

Hair clipping *ex vivo* assay: Hair from dogs and cats wearing treated collars is harvested on numerous days and used in ectoparasite contact tests.[4]

Mortality contact tests: Numerous tests expose eggs, larvae, nymphs, or adults to the test substance directly or via filter paper to evaluate mortality.[5-12]

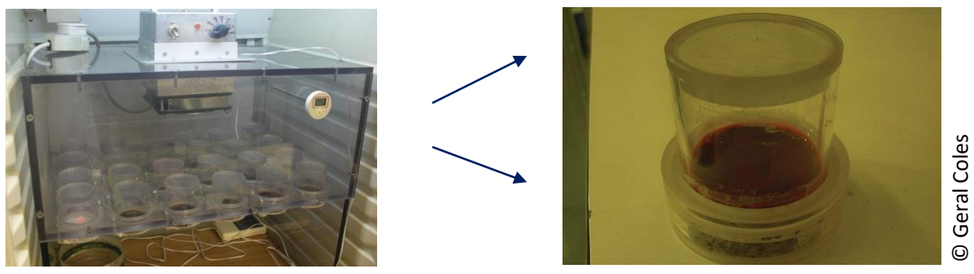
Deterrent and repellant assays: Ectoparasites are exposed to the test substance or its vapors in the presence of attraction pheromeones and effect is studied.[13]

Cell voltage clamp assay : Electrophysiological recordings from isolated insect nerve cells are used to assess the effects of the test substance.[14]

Up Close on Artificial Membrane Tests

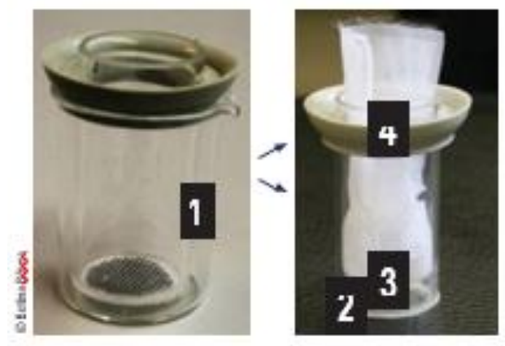
For efficacy testing, artificial membrane systems have been developed to evaluate both oral and topical ectoparasiticides that are added to the blood in the *in vitro* system or applied to the membranes. Artificial membranes essentially act as skin, allowing fleas or ticks to naturally attach and feed on blood or media through the membrane. Silicone membranes have been used to feed ticks and a parafilm membrane system to feed fleas. While artificial membrane systems may be used by companies for in-house screening, at this time, regulatory agencies still require efficacy testing in animals.

Fleas



Heated flea artificial membrane system with tray of feeding units (L) and an insert (R). The insert shows a single feeding unit with the blood meal, below which are the parafilm membrane and the fleas.

Ticks



Tick artificial membrane system (L) with an insert that is placed inside (R). A glass beaker (1) contains the blood meal or feeding media above which the insert is placed. The insert consists of a glass tube with a silicone membrane at the bottom (2). Attachment stimuli (mechanical, olfactory, and host-contact chemo-stimuli conditions that encourage ticks to attach to the membrane and feed) and ticks are placed on the membrane. To confine the ticks, a cap with netting (3) is placed above them. A rubber stopper (4) is placed on the insert to ensure that the blood meal is below the silicone membrane with the ticks when the insert is placed in the beaker.

In Vitro Advantages

- Allow greater control and standardization, including the ability to control components of the blood meal and quantify dose effects of products
- Permit direct observation of flea or tick attachment to the membrane, feeding, reproductive output, and mortality
- Eliminate variability caused by using different animals when conducting *in vivo* studies
- Eliminate costs associated with animal use

In Vitro Considerations

- Mechanical, olfactory, and host-contact chemo-stimuli conditions affect the ability to feed fleas and ticks using *in vitro* methods
- The blood source (e.g. different animal hosts or human blood) and quality affect success. For ticks, the blood meal (and membrane) must be long-lasting
- The use of an anticoagulant and an antibiotic or antifungal component to avoid contamination of the *in vitro* feeding systems may affect the success of feeding

Moving Forward

A weight-of-evidence approach would combine approaches described here and expert judgement to determine efficacy without the use of dogs and cats in laboratories. Industry, government, academia, and other organizations are collaborating to identify the scientific and logistical gaps that prevent the implementation of *in vitro* and *in silico* methods to evaluate the efficacy of flea and tick control products and to outline the steps required to further develop, gain confidence in, and gain regulatory acceptance of identified testing strategies.

Case Studies

- Krull and colleagues studied the optimization of an *in vitro* feeding system for ticks, such as the effects of different blood meal treatments and blood preservation. They found that carbon dioxide levels and the addition of antibiotics to blood are important factors affecting feeding success.[15]
- Li and colleagues, Krober and Guerin, and Kuhnert and colleagues used silicone membrane systems to confirm the efficacy of commercial products that are intended to control different types of ticks.[16-18]
- Kernif and colleagues examined modifications to improve methods of rearing fleas and found that human blood can be used successfully as a blood meal.[19]
- Williams and colleagues used a parafilm membrane system to demonstrate that a commercial flea product controlled flea reproduction.[20]
- Banks and colleagues tested a variety of compounds by adding them to the blood meal in a parafilm membrane system, which allowed for the determination of their potency levels and the optimum systemically active flea control product.[21]
- The European Medicines Agency scientific discussion on Comfortis® evaluates the product's active ingredient in three *in vitro* assays using an artificial membrane feeding system for fleas. The resulting signs of toxicity were similar to those found in other insect species.[22]

For references, see www.thepsci.eu/ectoparasiticides