Regulatory Toxicology and Pharmacology 88 (2017) 349–355

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Commentary

Itching for change: Embracing modern flea and tick product development

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ARTICLE INFO

Article history:
Received 17 May 2017
Accepted 5 July 2017
Available online 6 July 2017

Keywords:
Fleas
Ticks
Dogs
Cats
Product testing
Ectoparasiticides
In vitro feeding

ABSTRACT

The development and regulatory approval of ectoparasiticides, including flea and tick control products, involves decades-old methods and the use of large numbers of animals to evaluate toxicity and efficacy. Animals also are used to rear (breed and feed) fleas and ticks for later use in testing. Non-animal methods for regulatory-required testing and rearing currently exist and, with further development, others could soon become available. Here we provide an overview of the state-of-the-science of non-animal methods for rearing and regulatory-required efficacy testing of flea and tick control products. Several remaining challenges as well as recommendations on the steps needed to replace animals in the evaluation of these products are discussed.

1. Introduction

The burgeoning companion animal industry, which sells parasite control products (ectoparasiticides) such as flea and tick treatments for dogs and cats, grosses billions of dollars annually. In 2015, expenditures on companion animal products in the United States (U.S.) alone amounted to more than $60 billion (American Pet Products Association, 2016). Government agencies in the U.S., European Union (E.U.), and other countries regulate ectoparasiticides, which are treated as drugs, pesticides, or biocides depending on product specifications such as the route of exposure. Regulatory agencies require testing of the products, most of which is currently conducted on animals, prior to registration and sale to the public. To bring a new product to market, animals are used in three areas: 1) toxicity testing; 2) rearing fleas and ticks for later use in efficacy testing; and 3) efficacy testing.

2. Toxicity testing

Thousands of mice, rats, guinea pigs, and rabbits are used to assess a product's potential toxicity. For example, U.S. and E.U. regulatory guidelines state that a variety of human health effects tests, such as acute, subchronic, and chronic toxicity tests as well as mutagenicity, carcinogenicity, neurotoxicity, and reproductive toxicity tests, must be conducted for new products intended to be applied to companion animals (European Union, 2012; EPA, 1998b).

Additional toxicity studies are conducted on "target" animals (in this case, dogs or cats), including margin of safety determinations using both the recommended dose and overdoses, studies for the proposed treatment periods and longer treatment durations, reproductive toxicity tests, dermal adverse reaction studies for topically-applied products, and oral toxicity testing (EPA, 1998a; European Medicines Agency, 2008; Intervet Inc, 2014; Novartis Animal Health US Inc.). Both young and adult animals are included in these tests, depending upon label claims (EPA, 1998a). In a representative set of toxicity studies for one product that is indicated solely for flea control, more than 400 dogs, cats, puppies, and kittens were used in toxicity tests, often dosed daily with the test chemical, for up to nine months before being killed (Novartis Animal Health US Inc., 2003a; Novartis Animal Health US Inc.). These social animals often are housed alone, with housing regulations varying by country. For example, in toxicity studies for another product, 200 dogs, cats, puppies, and kittens were kept for up to 180 days in solitary cages less than 1.4 m^2 in size with solid walls (Bayer Healthcare LLC, 2012). Not only do animals suffer the psychological effects of these conditions, they may experience adverse effects from the chemicals, such as inappetence, vomiting, diarrhea, ataxia, and seizures and other neurological symptoms

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http://dx.doi.org/10.1016/j.yrtph.2017.07.002
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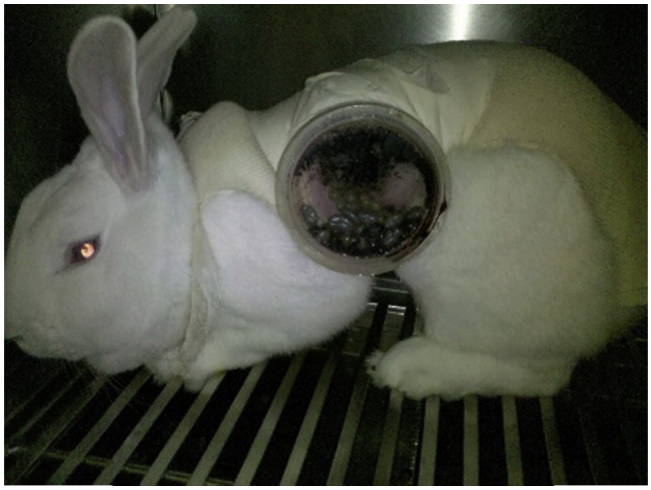

Fig. 1. Ticks being fed on a rabbit in the U.S. ©PETA.

(EPA, 1998a; CVMP, 2006, 2011, 2013; Bayer Healthcare LLC, 2011; Novartis Animal Health US Inc.; Zoetis Inc, 2016; Intervet Inc, 2014).

Several approaches for reducing animal use in toxicity testing are currently under evaluation or have already been approved for use to satisfy many toxicity testing endpoints. These include use of existing data to obtain waivers; an additive equation for predicting the acute systemic toxicity of formulated products based on the toxicity of its ingredients; *in vitro* methods for eye irritation, skin irritation, and skin sensitization; and computational methods (EPA, 2015, 2016a, 2017; OECD, 2012, 2013a, b, 2015a-g, 2016a-c; European Chemicals Agency, 2014; CVMP, 2016).[1] While toxicity testing is not the focus of this article, approaches that reduce and replace animal use should be used whenever possible.

3. Animal use in efficacy testing

3.1. Rearing (breeding and feeding) fleas and ticks for efficacy testing

Rearing consists of breeding and feeding generations of fleas and ticks and requires raising all life stages of the parasites, i.e., egg, larva, nymph, and adult for ticks and egg, larva, pupa, and adult for fleas. Eggs of both parasites, as well as flea larva and pupa, are not maintained on live animals (Kernif et al., 2015; Greene et al., 2015; Wade and Georgi, 1988); however, animals are often used to maintain the remaining life stages (Kernif et al., 2015; Kuhnert, 1996).

To rear large numbers of parasites for efficacy testing, "host" animals, including cats, mice, and rabbits, are artificially infested in laboratories (Kuhnert, 1996; Musyoki et al., 2004; Socolovschi et al., 2009; Bonnet and Liu, 2012; Lew-Tabor et al., 2014; Liu et al., 2014; Halos et al., 2016). Rearing methods may include shaving the skin of the animals on which parasites will be contained and restricting the animals' movements so that they cannot disturb the feeding fleas and ticks (Fig. 1) (Maramorosch and Mahood, 2014).

3.2. Efficacy testing

Regulatory requirements state that active ingredients and product formulations must be assessed for efficacy using target animals. U.S. and E.U. regulatory agencies require that efficacy testing be conducted using fleas and several species of ticks, depending on the geographical region where the product will be registered and used (Table 1) and label claims (Intervet Inc, 2014; Zoetis Inc, 2016). Hundreds of dogs and cats may be artificially infested with fleas or ticks in these assessments. Label claims, such as duration of parasite control, anatomical coverage of the product, degree of parasite control, efficacy when combined with other products, and species of parasite controlled, also must be supported with data, which are usually obtained from tests on animals (EPA, 1998b; CVMP, 2015; Novartis Animal Health US Inc., 2003a, b).

Table 1
The major species of ticks and fleas used for testing ectoparasiticides for dogs and cats in the U.S. and E.U. (EPA, 2016b; CVMP, 2015).

United States	European Union
Ticks	**Ticks**
American dog tick *(Dermacentor variabilis)*	Brown dog tick *(Rhipicephalus sanguineus)*
Blacklegged tick *(Ixodes scapularis)*	Castor bean tick or sheep tick *(Ixodes ricinus)*
Brown dog tick *(Rhipicephalus sanguineus)*	Ornate dog tick *(Dermacentor reticulatus)*
Lone star tick *(Amblyomma americanum)*	Hedgehog tick *(Ixodes hexagonus)*
Fleas	**Fleas**
Cat flea *(Ctenocephalides felis)*	Cat flea *(Ctenocephalides felis)*
	Dog flea *(Ctenocephalides canis)*

In dozens of efficacy tests for one product, more than 300 dogs were each infested with as many as 110 ticks or fleas for up to 73 days. This excludes dogs used in several disease transmission studies for this product that were not regulatory requirements, one of which resulted in the deaths of nine dogs used in a control group (Merial Limited, 2010, 2011). Some dogs were held in plastic crates infested with ticks for 6 h (Merial Limited, 2010). In other studies, cats or dogs were sedated or restrained for several hours while they were artificially infested with fleas or ticks (Stanneck et al., 2012; Tielemans et al., 2014). Animals experience obvious adverse effects from being infested with tens to hundreds of fleas or ticks (Dryden et al., 2015). In one study, a cat died from anemia due to severe flea infestation, and it was noted that the experiment resulted in "profound, early flea infestations" (Novartis Animal Health US Inc., 2003b). As in toxicity testing, animals are often housed alone, depending on regulations in the country where the experiments are performed, and they suffer potential adverse effects from the test chemicals (EPA, 1998b; CVMP, 2007, 2015).

4. Alternative feeding systems for efficacy testing and rearing

4.1. Rearing

Because there are no regulatory requirements specifying that animals be used in rearing fleas and ticks, artificial membrane systems are a viable alternative. Artificial membranes essentially act as skin, allowing fleas or ticks to naturally attach and feed on blood or media through the membrane. The membranes can be modified to accommodate different species and life stages, and they permit researchers to directly observe attachment to the membrane, feeding, reproductive output, and mortality (Krober and Guerin, 2007a, b, c; Kuhnert et al., 1995; Andrade et al., 2014; Kuhnert, 1996; de Moura et al., 1997; Tajeri and Razmi, 2011;

[1] CVMP = European Medicines Agency Committee for Medicinal Products for Veterinary Use, EPA = U.S. Environmental Protection Agency, OECD = Organisation for Economic Co-operation and Development.