



## IN-VITRO EFFICACY ASSESSMENT OF EARTH ANIMAL® REPELLENT SPRAY AGAINST DIFFERENT ARTHROPODS

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### Efficacy assessment of EARTH ANIMAL repellent spray

In the conditions of these trials, with the samples of the spray tested, the arthropod strains and methodologies used, the EARTH ANIMAL repellent spray has proved a relevant repellent effect towards fleas, ticks, flies and mosquitoes.

Indeed, the EARTH ANIMAL repellent spray was efficient enough to deter the ticks *Ixodes ricinus* and *Rhipicephalus sanguineus* from staying on the attractive stimulus and therefore to potentially avoid ticks' feeding attachment to the host.

Similarly, the odour of the EARTH ANIMAL repellent spray significantly repels the cat fleas *Ctenocephalides felis*, the mosquitoes *Aedes aegypti* and flies *Musca domestica* as these insects spent significantly less time in the zones baited with the odour of the EARTH ANIMAL repellent spray.

## I. INTRODUCTION

The goal of this laboratory study is to determine the efficacy of an EARTH ANIMAL repellent spray against different arthropods gravitating around pets. Due to the lack of official standard methodology, the testing procedures used are in concordance and inspired by scientific literature as required by the technical notes for guidance on biocide product evaluation PT19 (EC 2012).

As recommended by the European directive 63/2010/UE, *in-vitro* testing were used to assess this product efficacy, allowing the avoidance of animal testing which is against AB7-industries' ethic policy.

All the arthropod species tested are the one targeted by the product and mentioned in the European directive to evaluate biocide products for PT18 and PT19 (EU 2012).

Two different ticks' species were used to assess this product. The ticks, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae), is one of the main target of the product. It has a worldwide distribution and is commonly find in USA. It is mentioned as a test species by the European biocide directive (EC 2012). The second specie tested, *Ixodes ricinus* (Linnaeus, 1758) (Acari: Ixodidae), belongs to another genus that is also present in USA. Again, this species is mentioned as a test species by the European biocide directive (EC 2012).

The most common flea; the cat flea *Ctenocephalides felis* (Bouché, 1835) (Siphonaptera: Pulicidae), is one of the main target as it parasites all pets (cats as well as dogs) worldwide and belongs to the genus *Ctenocephalides* spp. requested by the European biocide directive (EC 2012).

The mosquito's species tested, *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae) is known to have a highly aggressive behaviour. It belongs to the genus *Aedes* spp. requested by the European biocide directive (EC 2012) and mosquitoes of this genus are present in USA. These efficacy results on mosquito repellence give an idea of repellence power of the tested product on other flying haematophagous insects such as sand flies that are considered as pets' parasites.

The fly's species tested was the common fly *Musca domestica* (Linnaeus, 1758) (Diptera, Muscidae) requested by the European biocide directive (EC 2012). Even if this insect rarely causes a direct health problem on pets, its presence may strongly disturb and annoy them.

The EARTH ANIMAL repellent spray tested contains a total of 2% of active biocide ingredients: Virginia Cedarwood oil [CAS: 8000-27-9] (1%) & Peppermint oil [CAS: 8006-90-4] (1%).

The repellent spray qualifies for registration as a 40 C.F.R. §152.25(f) exemption under EPA rules (EPA 2015a, 2015b).

## I. RESULTS

### 1. TICKS' TRIALS

		behaviour acts (%)				n
		start	walk	cling	stay	
<i>Rhipicephalus sanguineus</i>	Spray	100	75.0	4.2	4.2	24
	control	100	100	100	83.3	24
<i>Ixodes ricinus</i>	Spray	100	66.7	0	0	12
	control	100	100	83.3	83.3	12

(numbers represent the % of ticks carrying the behavioural act)  
(n = number of replicates)

The EARTH ANIMAL repellent spray demonstrated a good repellent activity against the ticks *R. sanguineus*; walking, clinging and staying on the stimulus being significantly reduced in presence of the EARTH ANIMAL repellent spray compared to control (Chi<sup>2</sup> test,  $p < 0.01$ ).

The EARTH ANIMAL repellent spray also demonstrated a good repellent activity against the ticks *I. ricinus*; walking, clinging and staying on the stimulus being significantly reduced in presence of the EARTH ANIMAL repellent spray compared to control (Chi<sup>2</sup> test,  $p < 0.05$ ).

Therefore, we can conclude that the EARTH ANIMAL repellent spray gave relevant repellent results against both species of ticks *I. ricinus* and *R. sanguineus*.

## 2. FLEAS' TRIAL

		Behavioural Index		Wilcoxon test
		mean	SD	
Spray	<i>Ctenocephalides felis</i>	-0.663	± 0.050	$p < 0.01$

(n = 6 replicates per tested spray)

Each replicates had a behavioural index below -0.25 with therefore the mean behavioural index significantly lower than 0 (Wilcoxon test,  $V=0$ ,  $p < 0.01$ ), demonstrating that the fleas avoid the arms baited with the odour of the EARTH ANIMAL repellent spray.

Therefore, we can conclude that the EARTH ANIMAL repellent spray gave relevant repellent results against the fleas *C. felis*.

## 3. MOSQUITOES' TRIAL

		Time spent in		n
		attractive zone	escape zone	
<i>Aedes aegypti</i>	Spray [	00:59,7 ± 00:15,4	01:56,0 ± 00:15,6	10
	t-test (**= $p < 0.01$ )		**	**
	control	01:53,4 ± 00:04,3	01:05,7 ± 00:04,3	10

(mean time ± SD in min:sec,msec)  
(n = number of group of 5 mosquitoes)

Mosquitoes spent significantly less time in the attractive zone placed upwind next to the attractive stimulus if it is combined with the EARTH ANIMAL repellent spray (t-test,  $t=3.20$ ,  $p < 0.01$ ). Similarly, mosquitoes spent significantly more time in the escape zone placed downwind from the attractive stimulus if it is combined with the EARTH ANIMAL repellent spray (t-test,  $t=2.99$ ,  $p < 0.01$ ).

Therefore, we can conclude that the EARTH ANIMAL repellent spray gave relevant repellent results against the mosquitoes *A. aegypti*.

## 4. FLIES' TRIAL

		% of time spent in the test arm		Wilcoxon test	n
		mean %	SD		
<i>Musca domestica</i>	Spray	16.2%	± 6.1%	***	10
	Mann-Whitney test		***		
	control	49.2%	± 6.5%	n.s.	47

(statistical tests with n.s. =  $p > 0.05$  & \*\*\* =  $p < 0.001$ )  
(n = number of group of 3 flies)

In the olfactometer, flies spent significantly less time in the arm baited with the odour of the repellent spray (Wilcoxon test,  $W=100$ ,  $p>0.001$ ), whereas they spent a similar amount of time in both unbaited arms of the control trial (Wilcoxon test,  $W=1173$ ,  $p>0.05$ ). Consequently, flies spent significantly less time in the baited arm of the test trial with the repellent spray than on the unbaited arms of the control trial (Mann-Whitney test,  $W=457.5$ ,  $p<0.001$ ).

Therefore, we can conclude that the repellent spray gave relevant repellent results against the flies *M. domestica*.

## II. CONCLUSION

In the conditions of this trial, with the samples tested, arthropod strains and methodologies used, the EARTH ANIMAL repellent spray has proved a relevant repellent effect.

Indeed, the EARTH ANIMAL repellent spray managed to avoid the two ticks' species *Ixodes ricinus* and *Rhipicephalus sanguineus* to walk, attach and stay on the attractive stimulus. Moreover, the odour of the EARTH ANIMAL repellent spray managed to significantly repel cat fleas *Ctenocephalides felis*, flies *Musca domestica* and mosquitoes *Aedes aegypti*.

### **Disclaimer**

*There were no circumstances which can have affected the reliability of the data presented in this report. The results described in this report were generated in-vitro and on the provided samples not damaged by actual conditions of use or storage. The trials have been conducted on laboratory strains of the model arthropods requested by European biocide regulation (EC 2012), and the local arthropods' strains can be different in other labs or in real conditions of use.*