



**IN-VITRO EFFICACY ASSESSMENT OF
EARTH ANIMAL® REPELLENT SPOT-ON
AGAINST DIFFERENT ARTHROPODS**

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Efficacy assessment of EARTH ANIMAL repellent Spot-on

In the conditions of these trials, with the samples of the repellent Spot-on tested, the arthropod strains and methodologies used, the repellent Spot-on has proved a relevant repellent effect towards fleas and ticks.

Indeed, the repellent Spot-on 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 repellent Spot-on significantly repels the cat fleas *Ctenocephalides felis*, as this insect spent significantly less time in the zones baited with the odour of the repellent Spot-on.

I. INTRODUCTION

The goal of this laboratory study is to determine the efficacy of a repellent Spot-on 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 tick, *Ixodes ricinus* (Linnaeus, 1758) (Acari: Ixodidae), is one of the main target and the species requested by the European biocide directive (EC 2012). The second specie belonging to another genus, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae), is distributed worldwide and also mentioned as a test species by the European biocide directive (EC 2012). Both genus of these ticks are present on American soil and are known to transmit disease to pets as well as human.

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

The repellent Spot-on tested contains a total of 9% of active biocide ingredients: Cedarwood oil (Virginia) [CAS: 8000-27-9] (5%) & Peppermint oil [CAS: 8006-90-4] (4%).

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

II. RESULTS

1. TICKS' TRIALS

| | | behaviour acts (%) | | | | n |
|---------------------------------|---------|---|------|-------|------|----|
| | | start | walk | cling | stay | |
| <i>Ixodes ricinus</i> | Spot-on | 100 | 33.3 | 16.7 | 0 | 12 |
| | control | 100 | 100 | 100 | 100 | 12 |
| | | Chi ² test (n.s.=p>0.05 & ***=p<0.001) | | | | |
| <i>Rhipicephalus sanguineus</i> | Spot-on | 100 | 66.7 | 16.7 | 16.7 | 12 |
| | control | 100 | 100 | 91.7 | 91.7 | 12 |
| | | Chi ² test (n.s.=p>0.05, *=p<0.05 & ***=p<0.001) | | | | |

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

The repellent Spot-on demonstrated a good repellent activity against the ticks *I. ricinus*; walking, clinging and staying on the stimulus being significantly reduced in presence of the repellent Spot-on compared to control (Chi² test, p<0.01).

The repellent Spot-on also demonstrated a good repellent activity against the ticks *R. sanguineus*; walking, clinging and staying on the stimulus being significantly reduced in presence of the repellent Spot-on compared to control (Chi² test, p<0.05).

Therefore, we can conclude that the repellent Spot-on gave relevant repellent results against both species of ticks *I. ricinus* and *R. sanguineus*.

2. FLEAS' TRIAL

| | | Behavioural Index | | Wilcoxon test |
|---------|------------------------------|-------------------|---------|---------------|
| | | mean | SD | |
| Spot-on | <i>Ctenocephalides felis</i> | -0.418 | ± 0.093 | $p < 0.01$ |

(n = 6 replicates per tested collier)

Each replicates of the repellent Spot-on gave a behavioural index below 0 with therefore the mean behavioural index significantly lower than 0 (Wilcoxon test, $p < 0.01$), demonstrating that the fleas avoid the arms baited with the odour of the repellent Spot-on.

Therefore, we can conclude that the repellent Spot-on gave relevant repellent results against the fleas *C. felis*.

III. CONCLUSION

In the conditions of this trial, with the samples tested, arthropod strains and methodologies used, the repellent Spot-on has proved a relevant repellent effect.

Indeed, the repellent Spot-on managed to avoid the two ticks' species *Ixodes ricinus* and *Rhipicephalus sanguineus* to attach and stay on the attractive stimulus. Moreover, the odour of the repellent Spot-on managed to significantly repel cat fleas *Ctenocephalides felis*.

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.