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Requirements for insecticide and repellent treated articles

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Requirements for insecticide and repellent treated articles

Final report

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Preface

Within the last years an increasing number of insecticide-treated articles to protect humans or animals against arthropod bites have appeared on the market. These include clothing, wrist bands, hair bands, outdoor equipment (sleeping bags, tents) to protect humans, but also different devices to protect animals. Recently, even repellent-treated articles have become available, due to new techniques like micro-encapsulation. Such articles fall under the biocides legislation and may have to be authorised before placing on the market.

For authorisation of a biocidal product sufficient efficacy must be proven, and an assessment of the health and environmental risks must be conducted. However, guidance is lacking on how to assess the efficacy of such articles, and how to estimate exposure from them.

Therefore, the Swedish Chemicals agency has commissioned a study to close this gap. The present study was carried out by Dr. Hans Dautel, IS Insect Service GmbH, Berlin. The project leader at the Swedish Chemicals Agency was Ulrike Frank. Birgitta Malmgren, Lena Konovalenko and Jörgen Magnér were members of the project group. The project was conducted during summer and autumn 2020.

Content

Glossary

AATCC – American Association of Textile Chemists and Colorists

ADI – Acceptable daily intake

AS – Active substance

BDU – Battle Dress Uniforms (military)

BfR – Bundesinstitut für Risikobewertung (German Federal Institute for Risk Assessment)

BPC – Biocidal Products Committee

BPR - Biocidal Products Regulation

BW – Body weight

CPT – Complete protection time

DEET – N, N-Diethyl-m-toluamide

EFF WG – Efficacy Working Group of the BPC

EBAAP – Ethyl-butyl-acetyl-aminopropionate

EPA - Environmental Protection Agency

EU - European Union

FAO – Food and Agriculture Organization of the United Nations

KD – Knock-down

KEMI – Kemikalieinspektionen, Swedish Chemicals Agency

LLIN - Long-Lasting Insecticidal Net

OECD - Organisation for Economic Co-operation and Development

PT – Product Type

RH – Relative humidity

SD – Standard deviation

SPC – Summary of product characteristics

TL - Technische Lieferbedingungen (Technical Specifications)

US EPA - United States Environmental Protection Agency

UV – Ultraviolett

WHO - World Health Organisation

WHOPES - World Health Organization Pesticide Evaluation Scheme

WG - Working Group

Summary

Many insecticide- or repellent-treated articles are on the market to protect humans or animals against biting arthropods. These may be considered biocidal products according to the Biocidal Products Regulation (BPR), requiring efficacy evaluation for authorisation.

In this study, available data for efficacy evaluation of such treated articles were gathered via a literature search. Relevant data about testing methods and performance standards, which could be used for a guidance, were extracted from literature. Additionally, available information was gathered with respect to risk assessment of treated articles and the most relevant exposure parameters are described.

Treated articles were grouped into five categories: 1. Human apparel, 2. Treated articles used close to the human body, or indoors, 3. Articles for outdoor use, 4. Treated articles to protect animals, and 5. Mosquito nets.

Laboratory knock-down (KD) tests provide valuable data on the baseline efficacy of tested articles. There are established setups available to evaluate KD and mortality of target organisms. Clear cut-off values for KD times however, indicating sufficient efficacy, are only available for few species.

For product authorisation, simulated-use tests or field tests are mandatory to prove sufficient efficacy. Suggestions are made for suitable simulated-use or field tests according to product type and target organisms. The latter include target organisms as diverse as mosquitos, sand flies, midges, biting flies, wasps, ticks, lice, bed bugs, house dust mites, and others.

Different tests and testing strategies are suggested for the abovementioned product categories. For human apparel (1), test systems already in use for repellent tests (e.g. arm-in-cage tests, room tests, tick repellent tests) can be utilized with only slight modifications. The same applies for many articles used close to the human body, or indoors (2). Concerning treated articles intended to reduce outdoor populations of blood-sucking arthropods (3), we suggest field tests as most adequate. This is also true for a number of target species that are parasitizing cattle and horses (e.g. biting flies) (4). For mosquito nets (5), a good evaluation scheme, proposed by the World Health Organisation (WHO), is available.

However, the present study also revealed that certain simulated-use tests, e.g. to evaluate bite protection rather than repellency against ticks, are lacking. Likewise, simulated-use tests are lacking for lice and house dust mites.

Product efficacy can be influenced by a variety of parameters. Washing probably is the most relevant one decreasing efficacy. Therefore, we suggest to adopt the concept of complete protection time (CPT) as a measure how long the article will remain efficacious during its lifetime. Furthermore, it remains uncertain, whether all treated articles need a so-called regeneration time (up to a few days) after washing to restore full efficacy, as it is known for certain bed nets. We therefore suggest determining the regeneration time and inform the consumer of any post-washing waiting periods before using the washed fabric. Interestingly also heat, e.g. ironing, can profoundly decrease efficacy.

Good guidance on assessing health risks of usage of treated articles is available from the WHO. The most relevant exposure is considered to be by dermal contact. Guidance how to assess environmental risks, in contrast, is lacking in the literature.

The results of this study have been compiled into a draft guidance document, which can be found in Appendix II.

1 Introduction

In recent years, more and more insecticide- or repellent-treated articles have appeared on the market: shirts and trousers which are supposed to protect against mosquito bites, hair-bands which claim to protect against head-lice or dog vests which promise protection against ticks. Such treated articles may - depending on the claim - be considered biocidal products according to the Biocidal Products Regulation (BPR, Regulation (EU) 528/2012) with the consequence that they must be authorised before placing on the market. However, if such products are submitted for authorisation, guidance is lacking how to assess their efficacy and what data need to be requested to estimate exposure from such articles. The present study aimed at

(i) collecting available data that might be used for efficacy evaluation of treated articles, and for their exposure assessment,
(ii) formulating adequate performance standards for such articles and
(iii) making suggestions for meaningful claims.

The purpose is to formulate a draft guidance for efficacy testing including possible claims, and to describe the most relevant exposure parameters. This work can in future be integrated into the framework of EU guidance and can help with the assessment and authorisation of insecticide- and repellent-treated articles.

Both, the diversity of products intended to protect humans and animals from arthropod attack, and the very different biology of target organisms make a meaningful structuring of the topic challenging. To take both efficacy and exposure into account, the treated articles were divided into five product categories (see chapter 4.1). Within each category, target species or species groups like ticks or mosquitos are separately treated, because their biology dictates different testing approaches (see chapter 4.2).

As the lifespan of treated articles is considerably longer as compared to repellents directly applied to the skin or the fur, factors decreasing efficacy during use have to be taken into account. These are described in chapter 4.3 and suggestions how to integrate them into efficacy assessment are made.

To be able to carry out a risk assessment, the exposure from articles, often worn close to the body and over a longer period of time, has to be assessed. Additional parameters as compared to repellents directly applied to the skin are relevant for exposure assessment. Such parameters are described in chapter 4.4.

The draft guidance can be found in Appendix II. Appendix I lists the results of an internet research for treated articles on the market (table A1) and gives an overview over the retrieved literature together with a short description of the content for every reference (table A2).

2 Previous research

The current report used existing guidelines or guidance documents (e.g. from the WHO, the BPR, or the United States Environmental Protection Agency (EPA)) as a starting point for the draft guidance (see Appendix II). Also, the literature cited in the tender for this report, and an internal unpublished report by KEMI (Konovalenko & Magnér 2020) served as a source for further searches. Published literature is available on efficacy of different active substances, application on textiles, wear and tear, testing methods and different target organisms. The findings of a number of these articles were also taken into account for the present report.

In the past 2-3 years a number of revisions of the BPR guidance (European Chemicals Agency, 2018) on efficacy for Product Type 19 (PT19) have been written and were distributed to interested parties in industry and to test institutes for comment. These draft guidance documents are unpublished yet, but provide highly improved test descriptions compared to the existing BPR guidance, with regard to PT19. Some also included suggestions for efficacy testing of treated articles, and are thus referred to in this report, too.

3 Methods

3.1 Internet research: Diversity of products

A limited Internet search was performed to get an overview of the diversity of products that are available on the market and that are likely treated articles according to the BPR. Thereby the focus was on including different categories of articles with only one or few representatives each rather than collecting all producers or distributors e.g. of outdoor clothing protecting against arthropods. The research was performed in German and in English to cover most of the product types available within Europe. The results were transferred into an Excel sheet showing data with

a) the key word, under which the product was found
b) the name of the product
c) the description of the product including an efficacy claim
d) the internet addresses

for each item. Please find the results in table A1 in the Appendix.

The list is far from comprehensive but representatively shows the diversity of products on the market. Virtually all types of human clothing including hats, buffs, and shoes equipped with chemical protection against insects can be purchased. The same applies for pet animals and even horses. In addition, there are numerous other treated fabrics (e.g. blankets, scarfs, mattress liners, etc.) and other items (e.g. wristbands, collars, clip-ons, etc.) available including outdoor equipment like sleeping bags, tents, mosquito nets and even products to locally reduce outdoor species like ticks (tick rolls). Concerning the target species against which the treated articles are intended to be used, there is likewise a great diversity. In fact, virtually all arthropod species affecting human or animal health are included (e.g. mosquitos, stinging and biting flies, ticks, mites, bed bugs, fleas, lice).

3.2 Literature research: Testing methods

A literature research was conducted with the aim to get information on test systems that may be relevant to test treated articles, and information on parameters relevant for product efficacy and risk assessment. The literature cited in the tender for this report, and an internal unpublished report by KEMI (Konovalenko & Magnér 2020) served as starting point for further searches. Further literature was searched using Google scholar and ISI Web of Science. In a first step, recent literature (2017 up to July 2020) was searched using the following key words (all key words must appear per bullet point):

- Impregnated fabric permethrin
- Treated fabric permethrin
- Fabric tick
- Fabric mosquito
- Fabric insect

This served to find the most recent articles in the area.

Follow-up searches were performed with literature up to August 2020 to cover specific topics. The following key words (all key words must appear) gave relevant results:

- musca* insectic* net*
- taban* insectic* fabric*

- taban* treat* fabric*
- taban* treat* net*
- acaric* fabric*
- mite treat* fabric*
- mite permethrin
- mite barrier
- lice treat* fabric*
- permethrin *neotrombicula*

The potential value of a given publication was assessed from the abstract. If the publication was relevant, the information concerning test systems and parameters influencing risk assessment were extracted from the publication and transferred into a spreadsheet, whereby text was reduced to its absolute minimum.

The results were transferred into spreadsheet with the following columns:

a) Reference (the reference is cited)
b) Use type (use category as described in chapter 4.1)
c) Article category (description of the respective article)
d) Intended use/claim
e) Target species (species or species group)
f) AS
g) Mode of action (active upon contact, or in the gas phase/active as repellent or insecticide/acaricide)
h) Test system/purpose of article (the relevant test system is described or mentioned, or, if the publication deals with other topics, the topic is described)
i) Efficacy level (performance standards are described, or most relevant test result)
j) Efficacy parameters (parameters influencing product efficacy, and thus, also efficacy in the course of a test, are mentioned)
k) Exposure parameters (parameters influencing exposure are mentioned)
l) Non-target effects (possible non-target effects are mentioned)
m) Miscellaneous (other information, e.g. washing procedures, possibly relevant for this study)

Please find the spreadsheet in table A2 in appendix I.

This list is likewise far from being comprehensive as the main goal of the present work was to identify relevant test systems rather than to give a complete literature overview. It is apparent from this list, that there are only few test systems regularly used by researchers. These include the WHO cone test and the arm-in-cage test, both mainly used with mosquitos. Targeting crawling arthropods, KD tests, as described in chapter 8.3, were used quite frequently. Most other test systems are designed for certain arthropod species and often can only be used for other species with species-specific modifications.

Furthermore, existing guidelines (WHO, BPR) and relevant standards (DIN, NEN, AATCC, Technical specifications of different Armed Forces) were examined for their applicability for testing insecticide/repellent treated materials.

From the literature search and from existing guidance documents or guidelines, suitable test systems were selected. The considerations for selecting certain tests are documented in chapter 4. The aim was to use existing guidelines whenever possible, or to adapt these for the evaluation of treated articles. The aim was also to standardise test systems or test principles across different products, whenever possible.

3.3 Parameters influencing efficacy

Parameters that might influence product performance were extracted from the literature. We discuss those relevant parameters in chapter 4.3 and suggest different consequences for such parameters. Some parameters, like washing, can be included in the testing procedures, others can lead to information on the product label. Both testing regimes and information might be useful for the consumer to avoid possible product failure. These considerations have also been included in the “claims” section of the draft guidance.

3.4 Parameters relevant for risk assessment

Parameters that might be relevant for risk assessment (both health risk and environmental risk) were extracted from the literature. We discuss those relevant parameters in chapter 4.4 and suggest different consequences.

4 Results

4.1 Grouping of articles

Both, the variety of treated articles on the market, and the variety of target species make it very difficult to structure the whole topic of efficacy testing of treated articles. It was proposed in the tender for this study, to place treated articles into one of five categories. Based on the internet and literature research we follow this with a slight modification (see below definition of groups 2 and 3) and group the articles as follows:

1. Human apparel
2. Treated articles used close to the human body or indoors
3. Articles for outdoor use
4. Treated articles to protect animals
5. Mosquito nets

Human apparel is a quite well-defined category, including all types of clothing (trousers, shirts, jackets, etc.) as well as hats and shoes.

More difficult was the distinction between treated articles for outdoor use and articles to protect humans other than apparel. We found it appropriate to group all articles together that act close to the human body, regardless if they are used outdoors or indoors. Therefore, also sleeping bags and tents are included in this second group, as well as all devices used indoors. Overall, this second category includes quite different products like wristbands (kept on the arm), stickers (affixed on clothing) clip-ons (personal dispenser clipped to the belt), mattress liners (against house dust mites), lice hairbands, sleeping bags, tents, blankets, curtains, treated chairs or banks, or insect barrier fabric strips (e.g. wrapped around furniture, against bedbugs). All are intended to be used close to the human body. If used indoors (e.g. insect barriers) humans also likely come into close contact to them.

The third category is restricted to those articles for outdoor use which do not come into close contact with humans and includes e.g. mobile insecticidal walls, eave ribbons, tick rolls, bee- or wasp repellents. This grouping may facilitate both efficacy and exposure assessment.

The fourth, quite well-defined category includes all articles to protect animals (e.g. horse blankets, dog vests, dog sleeping mats, etc.).

The fifth category is restricted to mosquito nets.

4.2 Selection of suitable test systems

In the following, the reasonings for choosing specific tests systems for the draft guidance are described.

We first discuss the suitability of knock- down (KD) and mortality tests. These are often necessary as basic laboratory tests to show sufficient efficacy of treated articles during product development. KD tests may be used for efficacy evaluation of products from different categories e.g. human apparel, articles of outdoor use and articles to protect animals. Thereafter, we discuss the suitability of further tests, particularly simulated-use tests, that may be used to evaluate marketable products. These types of test have to take relevant use conditions and relevant target organisms into account. Therefore, we have selected them for every article category as described above and for every target organism within the article category.

4.2.1 Basic laboratory tests to evaluate knock-down and mortality of target species

Measuring KD or mortality in target species is straightforward: specimens are continuously exposed to test fabric and (i) time to KD is measured individually until 100% KD (yielding a mean ± SD KD time) or, (ii) the percentage of knocked-down individuals or their mortality after a fixed exposure time is evaluated.

4.2.1.1 Flying insects

For flying insects, particularly mosquitos, basically five test systems are in use:

- The cone test according to WHO (2013b) or deviations thereof (Gopalakrishnan et al. 2019; Faulde et al. 2016)
- The tube test (WHO 1998)
- The ball test (WHO 1998)
- The petri dish test (Richards et al. 2018)
- The tunnel test (WHO 2013b)

The cone test is the most frequently used test during the past decades, regardless of the drawback that mosquitos may rest on the stopper closing the opening of the cone or on the cone`s glass surface. This reduces the “real” contact time of mosquitos with the fabric. As mentioned in the WHO (2013b) guideline, this is particularly relevant when the fabric to be tested has a so-called “excito-repellent effect” inducing mosquitos to leave the test surface.

To overcome this, petri dish assays were performed (Sullivan et al. 2019; Connally et al. 2019) to increase forced contact time of mosquitos with the fabric by test-volume reduction. However, Richards et al. (2018) did not find a statistically significant increase of mortality in petri dish tests as compared to cone tests. The petri dish assay also involves intermittent cooling of mosquitos (-20°C for 45 s) before transfer (to and from the petri dish) with possible negative effects on their fitness. Therefore, we recommend the well-established cone test for KD or mortality evaluation of mosquitos (and other flying insects). Additionally, the cone test may be used with any type of fabric, be it soft or stiff.

The tube test (also called WHO susceptibility test) is also frequently used and the test equipment can be purchased, so that different laboratories can work with the same equipment, facilitating comparisons between studies. It is, however, not suitable for testing soft fabrics like mosquito nets, as it is not possible to line the inner wall of the tubes with this material properly. Nevertheless, the tube assay was specifically designed for an easy transfer of mosquitos to the tube and may be used as an alternative to the cone test.

Another alternative is the ball test (WHO 1998), in which mosquitos have no opportunity to rest on untreated surface (in contrast to the cone or tube test). Additionally, individual KD-times of mosquitos may be measured. The test could be used for other flying insects as well. However, the equipment must be custom-made, which prevents standardisation between laboratories.

In the tunnel test, finally, mosquitos are tested in a 60 cm long (25 x 25 cm wide) tunnel. They are released into the tunnel from one side and are attracted by a live, immobilised host (e.g. a guinea pig or a rabbit) placed on the other side of the tunnel. A treated test net equipped with holes is placed in the middle of the tunnel and the mosquitos have to find the holes and pass the net in order to reach the host. They thereby have to contact the net and may thereafter be unable or unwilling to find the host. The WHO (2013b) guideline suggests to use this test if the cone test revealed an insufficient efficacy of the test net. In order to reduce

animal testing, however, we suggest not to use the WHO tunnel test whenever possible (for further information see chapter 4.2.5).

Performance standards

For tests with mosquitos, performance standards are available for the WHO cone test (WHO 2013b), tube test and ball test (WHO 1998) that we recommend taking up into the guidance. For example, the WHO (2013b) recommends 100% KD of mosquitos within ≤ 71.5 min when they are continuously exposed to treated fabric. In the cone test, mosquitos are exposed to treated fabric for 3 min, and KD must either be ≥ 95% 1 h after end of exposure, or mortality must be ≥ 80% 24 h after end of exposure.

Performance standards for KD tests with stable flies and other insects are described in Clark & Pearce (2019) in similar assays but with different exposure periods (e.g. 24 h). Britch et al. (2018) also used a continuous 24 h exposure in tests with other flying insects and suggested an arbitrary benchmark of 90% mortality at the end of the exposure period. This benchmark was met in tests with *Culex quinquefasciatus*, *Stomoxys calcitrans*, and *Phlebotomus papatasi*, but not with *Musca domestica* that showed only a mortality of > 80 and < 90%.

Literature may provide more data on KD times and mortality in flying insects (for an overview see Banks et al. 2014).

4.2.1.2 Crawling arthropods

Crawling arthropods are usually placed on test fabric and kept in place either by an inverted petri dish or an uncovered glass ring placed on that fabric. We recommend a glass ring, due to less chance of air saturation with active substance molecules in the test system. In addition, optimal humidity conditions can easily be maintained in this open test system. It can be used for virtually all crawling insects and even mites with some adaptations in scale. In the following, this test is referred to as “KD test” for crawling arthropods.

The Technical Specification (TL 8305-0331, 2020) is a set of requirements for permethrin-treated fabric issued and applied by the German Armed Forces. It describes standards with respect to technical and chemical aspects as well as to the biological efficacy, treated fabrics must meet. The WHO tube test is recommended by this Technical Specification for KD evaluation in ticks. However, ticks are more difficult to observe inside the tube lined with fabric and individual tick specimens leaving the fabric surface (at the end of the tubes) during a test must be placed back. In contrast, there is no need for tick handling when keeping the ticks in place with a glass ring (its inner wall coated with fluon). We therefore favour this KD test method for the BPR.

Performance standards

The TL 8305-0331 licensing conditions recommend that fabric shall provide mean KD times of ≤ 71.5 ± 12 min for *Ae. aegypti*, ≤ 27.1 min for *I. ricinus*, and, in an older version (TL 8305-0331, 2009) ≤ 60.0 ± 21.0 min for silverfish (*L. saccharina*). Battle Dress Uniforms (BDUs) providing such an efficacy level were extensively tested in the field and proved highly effective (> 98%) in preventing tick and mosquito bites (Most et al. 2017; Faulde et al. 2015). This suggests that the KD times measured in a laboratory test may translate to a real protection (against ticks and mosquitos) in the field and can be used as standards in the BPR, too. The Dutch standard (NEN 8333, 2017) for fabric testing against *I. ricinus* nymphs is basically the same as the TL 8305-0331.

However, data for other tick species tested on fabric with the same proven efficacy level are, to our knowledge, currently not available. This is particularly true for adult ticks that may need higher doses to be knocked-down than nymphs (Prose et al. 2018). The data of Prose et al. suggest that ≥ 90% KD 1 h after a 3 min exposure on treated fabric may be feasible for adults of different tick genera and thus be used as a standard to prove sufficient KD. In general, however, we favour to measure individual KD times until 100% KD and report the results as mean ± SD KD times, as well as the time to 100% KD. This provides the most informative data and allows comparison of results between test institutes without the need to agree on an (arbitrary) fixed exposure time.

In conclusion, there are well established setups available to evaluate KD and mortality of target organisms on treated fabric. Clear cut-off values for KD times indicating sufficient efficacy of the fabric, however, are only available for few species, i.e. mosquitos (WHO 2013b; TL 8305-0331) and *I. ricinus* nymphs (TL 8305-0331).

For testing which takes the conditions of use into account, however, the selection of test systems has to be more specific, both concerning the articles category and the target organisms. Thus, suitable test system are listed in the following, distinguished for every group of target organisms within every article category.

4.2.2 Human apparel

There are many products on the market claiming to protect against arthropods. The main target species, however, belong to mosquitos and ticks. As for repellents, also for treated clothes, simulated-use tests should be mandatory for product evaluation. Field tests are of ethical concern if target species, e.g. mosquitos or ticks, can act as vectors for human pathogens. If field tests were conducted by applicants, they could nevertheless provide additional information useful for product authorisation.

4.2.2.1 Mosquitos

4.2.2.1.1 Laboratory tests

As described above, the WHO cone test (and WHO tube test or ball test) should be suitable to show the baseline efficacy of test fabric in a laboratory setting.

4.2.2.1.2 Simulated-use tests

For tests with mosquitos, guidelines are available, particularly from the WHO, the EPA and also the BPR guidance, dealing with insecticides/acaricides and/or repellents. For efficacy testing of clothing, these can be used with only minor modifications.

As standard simulated-use test for clothing we recommend the arm-in-cage test, probably one of the best evaluated simulated-use tests available. As even this test system is continuously improved, we recommend adopting the following modifications:

- A minimum of 20 landings/minute should be provided to conduct an arm-in-cage test, and
- a test area of defined size on the forearm of test subjects should be used instead of the whole arm.

These modifications result from an inter-calibration study, carried out in three European laboratories, as presented at the meeting of the BPC WG for efficacy (V/2019) and discussed among the efficacy WG members. The study revealed that the landing pressure rather than mosquito density inside the test cage improves test reproducibility. Also, an exposure area of defined size, being the same among all test subjects (cited according to Konovalenko & Magnér 2020) is advantageous in this respect.

If mosquito bites are to be recorded, a particular problem becomes evident: treated fabrics might either be thin, enabling mosquitos to bite through, or may be of a thickness that mechanically prevents mosquito bites. If protection from mosquito bites is claimed for a treated jacket thick enough to prevent bites mechanically, the added value of a chemical "mosquito proof" is questionable, unless it also provides protection of uncovered skin (i.e. claiming a "halo" effect). In this case, arm-in-cage testing only makes sense, if also uncovered body parts are exposed to the test species. Otherwise, the untreated control fabric would already provide 100% protection. If, however, such a jacket is claimed to protect against arthropods in general, or against mosquitos and e.g. ticks, it may have an added value even without "halo" effect. This highlights the importance of precise claims.

Many (but not all) of the treated clothing currently on the market are impregnated with insecticide (permethrin) showing very low vapour pressure and thus little or no "halo" effect (Tangena et al. 2018). In these cases, primarily those body parts covered by treated cloth may be protected. Studies with ticks (Eisen et al. 2017; Prose et al. 2018), bed bugs (Jones et al. 2015), and lice (Sholdt et al. 1989), however, show that already very short exposure times on treated fabric, insufficient to cause immediate KD, may render the parasite unwilling to further engage in host-seeking and/or biting. The results of Orsborne et al. (2016) and Mulatier et al. (2019) suggest that this may also be true for mosquitos. If so, mosquitos in the field that first land on treated clothing (and not on uncovered skin) may take off and not further try to bite, thus reducing the number of host-seeking individuals in the immediate vicinity of the person wearing the treated apparel. It is unlikely that such an effect can be demonstrated in an arm-in-cage test. If, in such a test, half of the test area on the arm is covered by fabric and the other half is not, significant numbers of mosquitos will just by chance land on uncovered skin and be scored as not repelled.

Therefore, a room test as additional simulated-use test for treated fabric may be useful. It more closely simulates the natural situation, when mosquitos approach from a larger distance and could land anywhere on the whole body rather than on a small piece of forearm. The test system we describe in the draft guidance (Appendix II) is based on Orsborne (2016). The test room should have a minimum size of 1.80 x 1.80 x 1.80 m (larger setups are possible, for instance tests in greenhouses as described in Revay et al. (2013), based on the EPA (1999) guideline). Such a room test can prove a significant reduction in mosquito bites, represented by the number of landings, compared to a control. The number of landings on treated clothes is to be recorded as well as KD and mortality of mosquitos after the test. If the number of landings decrease in the course of the test and the number of knocked-down individuals

and/or mortality increases compared to the control, this may be indicative for product efficacy (because a high proportion of mosquitos first landed on treated clothes (as opposed to untreated body parts)). KD and mortality values, however, are only supplemental information to estimate performance of the products. The key outcome is reduction of mosquito bites.

4.2.2.2 Other flying blood feeding insects

If claims include prevention of bites from other flying parasites like midges, stable flies, sandflies, or blackflies, virtually the same test systems can be used: arm-in-cage tests and room tests. Weeks et al. (2019) for instance, successfully tested sandflies (*Phlebotomus papatasi*) in an arm-in-cage setup. Test conditions should be adapted to the needs of the target species, to provide optimal conditions for their host-seeking.

4.2.2.3 Ticks

4.2.2.3.1 Laboratory tests

The KD effect on ticks can be tested as described above for crawling arthropods.

In addition, there are more elaborate or sophisticated test methods available. The Moving-Object Bioassay, a highly standardized laboratory repellent test that yields results very close to simulated-use tests with human volunteers (Dautel et al. 2013), is inherently suited to test treated fabrics and is also listed in the draft guidance (and in the BPR). Also, the tick irritancy test described by Eisen et al. (2017) may be useful to answer specific questions.

Eisen et al. (2017) showed that ticks being only shortly exposed to treated fabric may achieve a dose that is insufficient to induce KD (ticks still show normal walking behaviour), but nevertheless renders the ticks unwilling to ascend a vertically held finger (finger ascension assay). This behaviour holds on for the next minutes or hours and may indicate that such ticks are unable or unwilling to bite for that time period. After longer time periods post exposure, the ticks restore their normal behaviour and readily ascend a finger again (> 90% of ticks). This may in part explain the very low rates of tick-bites found in a field study, where BDUs were tested (Faulde et al. 2015). A finger ascension assay could in principle be used as supplemental test after a tick KD test on clothes, to further evaluate ticks that were not repelled in the test for their ability to bite. However, to unequivocally show that an unwillingness to climb a finger is equivalent to an inability to bite, further tests are necessary. Therefore, we do not mention the fingertip assay in the draft guidance.

4.2.2.3.2 Simulated-use tests

For tick repellents, a simulated-use test that has been in use for at least two decades is available. In detail it is described in the EPA (2010) guideline as well as in the BPR guidance (European Chemicals Agency, 2018). The latest revision of the corresponding chapter in the latter guidance (TNsG_PT19_Ticks_Draft-DE_180815.pdf) suggests an adaptation of the described test method for treated clothes. Therefore, we suggest using the test described in that document.

4.2.2.4 Lice

There may be clothes on the market, claimed to protect against body lice. These should be tested in simulated-use tests or field tests. We are not aware of any simulated-use test with lice using treated clothing. A possible idea might be to test lice on treated fabric placed on the forearm of volunteers similar as with ticks (a tick may not crawl >3 cm upwards or remain on treated fabric for >3 min; see chapter 8.3.1.3.3). When placed on the human body, head lice walk upwards to reach the scalp (Galassi et al. 2019), rendering it a possible test method.

However, it is unclear whether this strictly applies also to body lice, an ecotype of the species showing a different distribution on the body than head lice. Therefore, field tests are suggested to evaluate treated clothes against body lice. Benkouiten et al. (2014) tested permethrin-treated clothes against human body lice in the field. This publication may serve as a basis to design such field tests.

For further information regarding lice and test methods see chapters 8.3.1.4 and 8.3.2.5.

4.2.2.5 Conclusion

In conclusion, human apparel can in general be evaluated in test systems that are already in use for repellent tests. There, the principle of Complete Protection Time (CPT) is used. The EPA (2010) defines CPT as “the time from application of a repellent until efficacy failure as it is defined in each study—for example, the time from application until the first efficacy failure event confirmed within 30 minutes by a second similar event”. Common repellents like DEET, Icaridin, or EBAAP show an efficacy time in the range of hours after application on skin. In the case of treated clothes, this time may extend to months or years of usage, even up to the expected lifetime of these clothes. To determine any CPT, clothes have to be tested after certain use times, or number of washings according to the claim. Suggestions for claims and tests are described in chapter 8.2.

4.2.3 Treated articles to be used close to the human body or indoors

Treated articles within this category include very different products like wristbands (kept on the arm), stickers (affixed on clothing) clip-ons (personal dispenser clipped to the belt), lice hairbands (kept on the head), mattress liners (against house dust mites), sleeping bags, tents, blankets, curtains, treated chairs or banks, and insect barrier fabric strips (e.g. wrapped around furniture, against bedbugs). All are intended to be used close to the human body. If used indoors (e.g. insect barriers) humans may also come into close contact to treated surfaces.

However, most of these items can be tested in similar approaches, provided similar behavioural characteristics of the target species. Most target species belong to mosquitos, but products against ticks, bedbugs, lice, or house dust mites are also available on the consumer market.

Laboratory tests like the WHO cone test and the WHO tube test (flying insects), or knock-down tests (crawling insects) as described in chapter 4.2.1 may be suitable tests for determining baseline product efficacy in a laboratory setting. This may fully apply to products like mattress liners, sleeping bags, tents, blankets, curtains, and insect barriers, but less so for products which essentially claim a spatial effect like wristbands, hairbands, stickers, and clip-ons.

In general, simulated-use tests should be mandatory for product authorisation. In the following, simulated-use tests according to target species and product are suggested.

4.2.3.1 Mosquitos

4.2.3.1.1 Simulated-use tests

Products to be kept on or at an arm (e.g. wristlets) can be tested with an arm-in-cage test as described for repellents in the latest version of the BPR.

Products for which a protective effect for the whole human body is claimed can be tested in a room test (e.g. sleeping bags, tents, blankets, clip-ons, wristband, and stickers) using the same

or a similar set-up as described in chapter 8.3.1.1.3. In case of volatile active substances (ASs), care must be taken that the room air does not saturate but is sufficiently ventilated.

Among the articles listed, tents may be a special case because the fabric is thin, but usually exhibits a mechanical barrier against mosquitos if the skin is not directly contacting it from the inside. Impregnation with pyrethroids of low vapour pressure usually does not prevent mosquito bites through the fabric (Faulde et al. 2012), particularly if it is thin. Thus, the benefit of using a treated tent as opposed to an untreated one should be described by the applicant. A possible benefit could be that the tent reduces the number of bites even if mosquitos accidentally enter the tent, for example, when the door is left open. This could be tested in a room test, similar as described in Orsborne et al. (2016). Mosquitos could be released into the test room with a test subject inside the open tent for 1 h. Mosquitos should come into contact with the tent and subsequently be rendered unable or unwilling to bite thus reducing the number of bites compared to a control (untreated open tent).

Possible test scenarios like this are not described separately for each product (e.g. sleeping-bags, blankets) but could be adapted from e.g. Orsborne et al. (2016).

A difficult case is treated curtains intended to reduce mosquito numbers inside rooms. Toledo et al (2015) describe a randomized controlled trial in a large number of households. The baseline activity of such curtains may be evaluated by standard laboratory KD tests (e.g. cone test) at the beginning and after certain time periods (months). However, when used indoors, the efficacy of such curtains likely depends on the frequency they are used as resting sites for indoor mosquitos. As there are usually many potential hiding or resting places inside living rooms for mosquitos, the curtain has to compete with such places. Evaluating the efficacy of such a curtain in a room test seems unrealistic if tests are performed inside a bare room, where the curtain would be the only attractive resting place for mosquitos. If a curtain was tested in a room test, we therefore suggest equipping this room with furniture similar as would be present in private living rooms to provide alternative resting or hiding places for mosquitos.

4.2.3.2 Other flying blood-feeding insects

These may be tested in an arm-in-cage tests or room tests, whereby test conditions like temperature, RH, daytime, etc. must be adapted to the target species.

4.2.3.3 Ticks

As laboratory tests, the KD tests described in chapter 4.2.1.2 for crawling insects should be performed. This could be done with all devices consisting of fabric like blankets, sleeping-bags, mattress liners, or tents.

Because of ethical reasons it is not possible to let test persons intentionally be bitten by ticks. A device dispensing repellents or acaricides should be able to discourage ticks from normal host-seeking behaviour on the host. If such a spatial effect is claimed (e.g. for devices like stickers, clip-ons, wristbands), we suggest using the standard repellent test as described in the BPR revision document (TNsG_PT19_Ticks_Draft-DE_180815.pdf). Specifically, a tick should be prevented from walking upwards on a host for more than 3 cm or walk appr. 1 cm upwards into a test area and then stay there for ≥ 1 min as in repellent tests. The rationale for this is, that ticks, when picked up by a person in the field, should not be able to crawl under clothes, where the effect of the test device most likely would be diminished or even be lost. Therefore, it should not walk on clothes for distances larger than a few centimetres.

4.2.3.4 Bed bugs

For bed bugs, treated fabric or other material acting as a barrier to prevent access of bed bugs to furniture or beds may be on the market. Such fabric may be wrapped e.g. around beds, sofas, or other furniture, acting as a classical repellent barrier. Thus, repellent tests evaluating this effect can be used. The test methods we propose are simulated-use tests based on Van der Pan et al. (2019), Wang et al. (2013) and Todd (2011). All use CO_2 and heat as attractants to motivate bed bugs to cross the repellent barrier. Van der Pan et al. (2013) use a ventilated three-chamber-system having the advantage, that there is no saturation of test chambers with any potentially volatile test compound, and CO_2. Wang et al. (2013) and Todd (2011) used perhaps more realistic "open tests" setups in the sense that the situation in a living-room is simulated. However, saturation of the room air, particularly with CO_2, must be prevented and the motivation of the bed bugs to walk over the treated fabric might be somewhat lower than in the three-chamber-system due to (i) a larger distance between attractant source and bed bugs than in the three-chamber-system, and (ii) a lack of direct air current from attractant source to bed bugs. Particularly the heat source placed quite distant from the repellent barrier seems to be ineffective in the "open test" as bed bugs are attracted to heat sources only at very small distances of a few centimetres (DeVries et al. 2016). We therefore suggest to use the three-chamber-system as it seems to be a worst-case test and is highly standardised. The other test system could be used if specific claims have to be tested.

If a barrier is tested against bedbugs, the width of the test barrier must be no larger than the smallest one to be marketed. The label should state that cutting the barrier to smaller widths will decrease efficacy.

4.2.3.5 House dust mites

House dust mites produce allergens that may be highly problematic for sensitized persons. Measures to reduce mite numbers indoors include reducing the relative humidity (RH) inside rooms, removing all carpets as potential mite breeding sites, and/or perform pertinent cleaning to remove any debris as potential food for mites. An additional option to reduce mite numbers may be mattress liners, as beds can be prominent breeding sites for house dust mites. Such liners may be finely woven to physically prevent migration of mites from the mattress through it, but there are also liners treated with acaricide on the market.

There is a guideline available from the American Association of Textile Chemists and Colorists (AATCC; e.g. AATCC 194-2013 test method). This describes precisely the conduction of a long-term test (6 weeks) and the aimed effect of treated fabric on a mite colony. We suggest this test as a simulated-use test.

There are further publications available that may be used to test fabric intended to protect humans from house dust mites. Wongkamchai et al. (2005) describe a mortality test where mites are exposed to test surfaces for 24 h and mortality determined thereafter. This may be adapted for treated fabric. Mahakittikun et al. (2009) describe a heat escape method to test whether mites can migrate through treated or untreated fabric. This test may be useful if treated mattress liners are claimed to prevent mite migration through the fabric. There is also a field test described where mattress liners are tested in private households with sufficient mite abundance over many months (Cameron & Hill, 2002). From a medical point of view, the number of mites should be below 100 individuals/gram of dust and allergen levels (Der p1, the main allergen of the mite) should be < 2 µg/g dust (as cited in Cameron & Hill, 2002).

4.2.3.6 Lice

There are products on the market like wristbands, hairbands, or scarfs claimed to protect against infestation with human lice, focussing on head lice. Human head lice (*Pediculus humanus capitis*) and body lice (*Pediculus humanus humanus*) are regarded to be the same species (Light et al. 2008) and can both be used as surrogate test organisms for each other. If a person acquires head lice from another one, this most likely happens by direct contact from head to head (Galassi et al. 2019).

To determine the KD times of lice, we suggest standard KD tests as described in chapter 4.2.1.2. Sholdt et al. (1989) performed KD tests with body lice on treated fabric. The results show an exposure time of 75 min to induce 100% KD in lice. This could be used as a tentative cut-off time for continuous exposure on treated fabric. Even after short exposure times (15, 30, 60 s), lice mortality was 100% 12 h later (but not at 6 h after exposure). To be in line with KD tests with other arthropods, we tentatively suggest 100% mortality (determined 24 h after exposure) after a 2 min exposure of lice on treated fabric as a cut-off criterion.

Tests should be conducted with adult lice, preferably within 1 d after their last blood meal. If claimed separately, also juvenile stages should be tested. If efficacy against eggs is claimed, eggs of an age of 0-1 d and 4-5 d may be used to test efficacy on eggs with and without developed nerve cells. Typical conditions to keep all louse stages are 32°C and 76% RH.

If a treated article is claimed as a barrier for lice preventing access of lice across this treated article, efficacy should be proven in a simulated-use test. The same applies if the treated article is claimed to prevent infestation of humans by lice.

However, we are not aware of any simulated-use test with lice using treated articles. To overcome this, we developed an in-house test to evaluate a possible repellent effect of fabric or other devices against lice. It is a choice test on a vertical surface, where lice could easily walk on. Heat is used as an attractive stimulus to increase the louse`s motivation to cross the repellent barrier (positive thermotaxis) and the set-up is based on the natural behaviour of lice entering a host and searching for a warm skin surface.

With this method, the efficacy of test products like bracelets, hair tils or hairbands against human lice can be evaluated. We share this method and describe it in the draft guidance.

4.2.4 Articles for outdoor use

4.2.4.1 Devices to reduce the local abundance of outdoor flying insects

Devices to reduce outdoor numbers of flying arthropods (mosquitos, sandflies, etc.) were tested by Britch et al. (2010, 2018). They consist of “mobile walls” covered by treated fabric placed in the surrounding of military camps. Target species attracted by humans may rest or hide on such surfaces receiving a dose of insecticide to be knocked-down or killed thus reducing the local abundance of host-seeking specimens.

Such devices compete with natural hiding or resting places of the target species (vegetation, natural ground, etc.) which is difficult to simulate in the laboratory. Additionally, such devices are likely intended to protect against a variety of target species that are locally abundant. We therefore suggest field tests for authorisation. The field site should provide sufficient numbers of target species during the test period and be situated in geographic and/or climate regions according to the claim. The abundance of target species is estimated by suitable traps set out before and after placement of the devices. This can be done for up to several months, or longer, depending on the claim. In parallel, samples of fabric material are

taken at regular intervals of outdoor use to monitor any decrease of KD efficacy caused e.g. by rain, sunlight, wind, etc.

A difficult point is the claim, particularly the target species, because of two reasons. Firstly, target species can vary considerably depending on both, the climate zone (e.g. northern, central, or southern Europe) and the eco-zone (e.g. wetland, forest, agricultural land, etc.). Secondly, it seems unlikely, that such a device could locally reduce the abundance of all flying insects. Potential target organisms like midges, mosquitos, horse flies, or sand flies may have very different preferences for hiding- or resting places. If the device is not attractive for any one species group, it will not be effective. We therefore suggest that such devices are tested in different eco-zones within Europe (or other parts of the world according to the claim) and the efficacy against target species groups of interest is measured. We think it is not strictly necessary that all specimens are determined down to the species level in this case. The most common species in a test area should be determined, but otherwise recording the effect against species groups (e.g. midges) could be sufficient. The label should then state, based on test results, the efficacy e.g. against mosquitos, midges, horse flies, etc. and the geographic region in Europe (e.g. temperate climate and Mediterranean climate).

One cannot expect that such devices could reduce numbers of target species by 90 % or more. Britch et al. suggested a reduction of arthropod abundance of 50 % compared to the pre-treatment number as cut-off value. We feel, however, that the efficacy should be higher than that, being e.g. 70%, to provide a significant benefit.

Such devices set up in the field may have profound effects also on non-target organisms at least on a local level. Therefore, non-target species should be monitored in parallel to the tests to account for any such effects.

4.2.4.2 Devices to reduce mosquito entry into houses

Mmbando et al. (2018) tested eave ribbons, consisting of treated sisal bands (15 cm wide, up to several m long) that are placed in the gap between the roof and wall of houses. Houses with such a gap are often used in (rural) tropical areas of Africa (and likely also in other tropical areas). The gap may be 30-40 cm wide and has been proven to be the main entry route for *Anopheles* mosquitos. The eave ribbons release transfluthrin, a relatively volatile pyrethroid and proved quite effective. Tests were performed using the experimental hut design according to WHO (2013b) and under field conditions. It seems unlikely that such a device would be marketed in Europe, because of the different construction of houses, and because malaria is not a main issue here. Also, health aspects may be an issue because inhabitants would permanently inhale the pyrethroid. Therefore, we do not include this treated article into the draft guidance.

4.2.4.3 Tick rolls

Tick rolls (German: “Zeckenrollen”, also known as “tick tubes” in the USA) are currently marketed in France, Austria, and Germany. These are cardboard rolls filled with permethrin-impregnated cotton. These are laid out in the garden, whereby 6 rolls should protect an area of 250 m². The intended effect is that mice collect the cotton from the rolls and use it as nesting material. Permethrin from the cotton will transfer to the fur of the mouse, and ticks feeding on such mice should be killed before they drop off. This type of product had already been marketed in the USA about 30 years ago with very inconsistent results with regard to efficacy (Mejon et al. 1995; Stafford, 1992; Deblinger & Rimmer, 1991).

Mice in central Europe are predominantly parasitized by the larval stage of the tick (*I. ricinus*). Only few nymphs usually feed on mice and virtually no adult ticks do so. Alternative

hosts for larvae may be any other vertebrate present in gardens, including birds or hedgehogs. Nymphs tend to prefer birds or mammals larger than mice as hosts, and adults prefer larger mammals. The life cycle of the main target species (*I. ricinus*) dictates that a possible effect cannot be seen before appr. 1 year (then, the number of questing nymphs could be reduced at the earliest) or 2 years (then, the number of questing adults could be reduced at the earliest) after laying out the rolls.

Provided the cotton balls are used as nesting material by mice and indeed work as intended, the overall efficacy in terms of a reduced number of ticks in a garden most likely depends on the composition and availability of (alternative) host species for the tick. This renders efficacy studies very difficult. Because of the complexity of the (tick host) species occurring in different gardens, we recommend performing field studies for efficacy evaluation. A high number of field sites (e.g. ≥10 test sites and control sites, each; see Drehmann et al. 2018) will be necessary, randomly allocated to either test or control site to account for differences in species composition between gardens. In addition, efficacy should be proven in at least 2 consecutive years.

Tick density should be measured by flagging or dragging a defined area in the garden for ticks (Sonenshine 1993; Vogelsang et al. 2020). A white molleton or flannel fabric, also called tick flag (e.g. 1 x 1.5 m wide), is slowly swept over the vegetation, whereby ticks cling to its underside as to a passing by host. When the flag is turned around, the ticks can be collected from it, and the number of ticks collected per garden area determined. By this way, pairs of control/test gardens should be sampled preferentially at the same day (tick activity can vary considerably according to weather conditions). Such flagging may be performed at days when weather conditions are good e.g. three times a year (April; May/June; September) covering an area of appr. 100 m² (e.g. 10 x 10 m²) or more at each time, if the garden is large enough. More frequent flagging could influence density of questing ticks by itself. Also, the transects to be flagged should be chosen by chance (i.e. should not always be exactly the same).

Concerning efficacy, it seems unrealistic to assume a reduction of host seeking ticks of ≥ 90%. This will, as a mean, probably be distinctly lower.

4.2.4.4 Wasp (or bee) repellent devices

Treated fabrics to be used as an outdoor wasp repellent (e.g. protecting people sitting at a table or in a picnic area from foraging wasps) can in principle be tested the same way as any other such repellent. The repellent should work against the most common wasp species occurring in each region. These are, e.g. *Vespula germanica* and *Vespula vulgaris*, in many parts of Europe. They appear as nuisance pests mostly in late summer/autumn, when nutrient demands of wasp colonies switch from proteins to carbohydrates. There may be protected species, e.g. *Vespa crabro* in Germany, as well. To our knowledge, no laboratory colonies of such social wasp species exist. Therefore, field tests are most suitable for efficacy evaluation.

A field test for repellents is described by Boevé et al (2016). The test seems suitable, but has, as the authors themselves write, the drawback that test and control areas were placed too close together (i.e. might influence each other). A modified test is described in the draft guidance (Appendix II) simulating a garden situation with two tables (one control, one test table), each with an attractant food source for wasps. One table is equipped with repellent fabric, the other without repellent. Video recordings are made from above to exclude any influence of human observers close to the tables. The number of wasp landings is recorded for each table. For a test to be valid, at least 20 landings per 30 min should occur in the control.

4.2.5 Treated articles to protect animals

There is a high number of products available intended to protect animals from parasites and nuisance pests. Most are designed for pets (particularly dogs) or horses, not excluding other pet or livestock species.

There are two types of articles with regard to AS: the first ones are based on a pyrethroid incorporated into fabric, claiming an insecticidal and/or repellent activity (e.g. dog vests, shirts, scarfs, blankets, sleeping mats or horse blankets). The second ones are based on "natural" ingredients like plant oils or certain compounds thereof (e.g. geraniol) claiming a repellent effect (primarily collars for dogs or horses). Many of the described articles claim efficacy against virtually all pest species affecting pets or horses including a "halo" effect, meaning that the whole animal (in case of horses even including the rider) should be protected.

Special cases in this context are dog collars treated with insecticide/acaricide that have already been marketed for decades. Different ASs (nowadays mostly pyrethroids) are deposited in the collar matrix that are slowly released during normal usage, spread over the whole body, and protect e.g. against ticks for weeks or several months. Such collars both prevent and cure an infestation by parasites and are thus regarded veterinary products. The same applies to ear clips releasing insecticide to protect cattle. Efficacy tests are regularly performed under the Veterinary Medicines Regulation (latest version: Regulation (EU) 2019/6). Examples for such studies are Fourie et al. 2019, Dantas-Torres et al. 2013, and Stannek et al. 2012. As these products are sufficiently regulated, we do not further deal with them in the following.

If only a repellent effect is claimed for products, then these clearly fall under the BPR. More difficult is the situation with respect to e.g. dog shirts treated with permethrin that might also have a curing effect. However, as long as such products are not claimed to cure a parasite infestation, they may be regarded as treated articles under the BPR.

Probably the most problematic aspect is the so-called "halo" effect considering the efficacy claimed for treated fabric, collars, etc. for animal use. From a mechanistic point of view, most pyrethroids, including permethrin, do not act over a distance, but primarily through direct contact.

An effect looking like a "halo" effect could, according to our opinion, nevertheless be achieved indirectly in two ways: (i) the pyrethroid could, through mechanical usage, be spread over the body of an animal (e.g. dog), or (ii) the target species may predominantly (in a statistical sense) come in contact with the treated fabric and be knocked-down or rendered unwilling to continue host-seeking before contacting uncovered body parts of the animal. The first way appears less likely, as most treated fabrics are designed not to lose much of the AS within a short time. The second possibility should highly depend on the design (size, colour, placement on the animal) of the treated article. If, for example, a treated dog vest covers most of the dog`s body, a tick, waiting for a host on vegetation, may by chance more often come into initial contact with that vest than with untreated body parts like head or feet. If, in a second example, horses grazing outside are covered by treated blankets, stinging or biting flies attracted by such horses may eventually come into contact with the blanket receiving a dose sufficient to KD. Over a longer period (hours, days) this may even lead to a transient local reduction of such fly species (particularly in places where there is no significant immigration of such flies from outside).

These considerations show that the label should clearly state how far the protective effect extends (e.g. the whole body) and it also should indicate how this effect is achieved (e.g.

spread of AS over the body). In addition, it should state whether the product repels and/or kills target species or reduces local abundance of pests, and how it does that. If there is any delay between application of the product and start of efficacy, this should also be mentioned (e.g. "…needs 48 h to reach full efficacy"). All such information is highly relevant to choose the right test conditions for product evaluation.

Concerning any test design with animals like dogs or horses, it has to be clarified beforehand with the local authorities whether the intended test procedure renders the test system an animal test. The BPR encourages to reduce animal testing. According to the EU Directive 2010/63/EU, animal tests are defined as "… any use, invasive or non-invasive, of an animal for experimental or other scientific purposes, with known or unknown outcome, or educational purposes, which may cause the animal a level of pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice".

This means, any efficacy test involving the bite of a parasite to an animal will most likely be regarded an animal test. If the proposed test is regarded an animal test according to the mentioned EU Directive, then an application must be made, and the test be supervised by the local authority. This greatly increases time and costs involved with a test.

In the following, mainly products to be used against flying insects and ticks occurring on horses and dogs are discussed with respect to efficacy testing. Most products found in the internet search and literature search deal with these species, although other possible target species can be a problem, e.g. lice (on horses, sheep, and other animal species).

4.2.5.1 Products to be used for horses (and cattle)

4.2.5.1.1 Flying insects

The species of biting and nuisance flies affecting horses and cattle are very diverse. The BPR (and revised PT19 document "Flies on grazing horses and cattle.pdf", dealing with repellents) gives a short but excellent overview of the complexity of this fauna. The biology of the different species involved is so different, that proving efficacy for one species can by no means be extrapolated to all other species. The document nevertheless suggests species to be tested as representatives for more general claims.

With the exception of few species (mosquitos, stable flies (*Stomoxys calcitrans*), and horn flies (*Haematobia irritans*)), to our knowledge none are maintained in laboratory cultures and thus would be available for any simulated-use tests. The authors of the above-mentioned document therefore conclude that the best way to prove efficacy would be field tests or semi-field tests. The same conclusion was drawn by Clark (2018) in his review on test systems for ectoparasiticides. Such tests can be adapted from the European Medicines Agency and could be used for treated articles, too. We recommend using the general principles as outlines for field tests described in the BPR and revised PT19 document.

Konovalenko & Magnér (2020) in their unpublished report describe a different field test based on Mottet et al. (2018). In this, the amount of fly annoyance is estimated from the frequency of specific avoidance behaviours of horses. We mention both in the draft guidance as practicable procedures to assess product efficacy.

A specific semi-field test against midges is described in Haanen & Jopin (2013) which could be a useful test procedure specifically for this parasite. In principle, the test design could be adapted to species other than midges, however, we are unaware, for which specific target species this test could be appropriate.

We are not aware of any simulated-use tests under controlled conditions for horses. Konovalenko & Magnér (2020) describe a test procedure resulting from an ECHA e-consultation (ECHA 2018) that is similar to a room test, but without live host. Target species are released in one room and heat and CO_2 are released as attractant stimuli in the other room. A blanket to be tested covers the attractant stimuli and KD of target species is recorded. We have no information on how well this set-up would attract any of the different target species. We therefore suggest evaluating KD rather in a cone test, and product efficacy in a field test.

4.2.5.1.2 Crawling arthropods

Besides biting and nuisance flies, ticks (*Ixodes ricinus*, *Dermacentor* spp, *Hyalomma* spp.) can be important parasites of horses. Although we are not aware of any simulated- use test with horses, we suggest adapting the simulated-use repellent test as used for humans and proposed for dogs (chapter 8.3.4.3.1) also for horses.

4.2.5.2 Products to be used for dogs

4.2.5.2.1 Flying insects

Flying insects like mosquitos are perhaps less of a problem for dogs in central and northern Europe, but sandflies transmitting *Leishmania infantum* (agent of canine Leishmaniosis) and mosquitos transmitting *Dirofilaria immitis* (agent of heart-worm disease) are important vectors in southern Europe.

We are not aware of any simulated-use tests with dogs against mosquitos.

4.2.5.2.2 Crawling arthropods

Among crawling arthropods, ticks and fleas are the most frequent parasites found on dogs in Europe.

Important tick species are *Ixodes ricinus*, *Dermacentor reticulatus* (vector of *Babesia canis*), and *Rhipicephalus sanguineus* (vector of *Ehrlichia canis*) and the most prevalent flea species are *Ctenocephalides felis* (despite its name, cat flea, the most prevalent flea species on dogs) and *C. canis*.

Any treated article with a plain surface can be evaluated with standard laboratory KD tests as described in chapter 4.2.1.2.

If treated articles like collars, scarfs, or vests are claimed to protect dogs against ticks, simulated-use tests are required. In the field, a tick can be picked up by a dog with virtually any body part, most probably by the head, breast or the feet. Treated articles usually do not cover all of these body parts and thus will only be able to protect the dog completely, if there is a distance effect extending to the uncovered body parts.

To test a repellent effect of e.g. collars, scarfs, or vests, we propose a slightly modified procedure to that described in the BPR (and revised document “TNsG_PT19_Ticks_Draft-DE_180815.pdf”) as simulated-use test for ticks.

To evaluate whether ticks actively climb a dog wearing a treated article, another set-up would be to place hungry ticks in a cage of suitable size (e.g. 2 x 2 m) and let the dog rest overnight in that cage as described in Fourie et al. (2013). The next day, the dog and the cage is screened for ticks. The number of attached and unattached ticks on the dog (dead or alive) and the number of living and dead ticks in the cage are counted. Percent protection is calculated with respect to an untreated control. At least 10 dogs, each in the test and the control, are

investigated. It has to be mentioned, however, that such a test procedure certainly is regarded an animal test.

Field tests with dogs are also possible. Test designs can be depicted from the European Medicines Agency (2016).

We are only aware of suitable simulated-use test with fleas as those described in the European Medicines Agency (2016).

4.2.6 Mosquito nets

We suggest using the existing WHO (2013b) guideline for any tests of mosquito nets. In this guideline, all test procedures are described in detail. Efficacy tests in this guideline are divided into phase I, phase II and phase III trials.

Phase I involves the cone test performed with different samples from bed-nets before and after a certain number of washes. This test determines the innate ability of bed-nets to knock-down or kill mosquitos. According to the calculation of Boyer et al. (2018), the proposed number of nets tested, and the number of cones used per test can be reduced (as compared to the guideline) without losing significant information. Furthermore, tunnel tests (chapter 4.2.1) are described. In order to reduce animal testing, however, we suggest not to use the WHO tunnel test whenever possible. In addition, procedures are described to determine washing resistance of mosquito nets and their regeneration time after washing. The latter is based on cone tests, again.

Phase II involves small field trials, also known as “Experimental hut studies” and Phase III involves large field trials. All of these are described in detail in the WHO (2013b) guideline.

In the BPR, field tests are generally not required, provided suitable simulated-use tests are available. Simulated-use tests with nets in that sense, however, are not described in the WHO (2013b) guideline. If such simulated-use tests are required for product authorization in the EU, we think a room test may be appropriate. Although no such test is described in the literature used for the current study, it may be performed similar to a room test as described for human clothes: A mosquito net with a person sitting or lying under it as an attractant source is placed in one of two rooms connected by a door. Mosquitos are released in the other room and the door opened. The number of mosquito landings on the net is recorded as well as mosquito KD (60 min after the test) and mortality (24 h after the test). If the number of landings decrease during the test and the number of knocked-down specimens and/or mortality increases compared to the control, this can be indicative for product efficacy. Such tests could be performed with fresh nets as well as with nets after a certain number of washings (simulating ageing), or after periods of ageing.

If, nevertheless, field tests or semi-field tests (experimental huts) are performed, they should be conducted in Europe or other climate regions according to label claim. Field tests should conform to the WHO 2013b guideline (Phase II and/or phase III field tests).

4.2.7 Overview of test systems, performance standards, and claims

Tables 1 to 6 give overviews of test systems, performance standards and possible claims according to product category and target organism.

Table 1. Overview of general KD tests suggested for efficacy evaluation of treated articles against target arthropods.

Abbreviations: contin: continuous; KD: knock-down; MO: Mortality; Ny: nymph; p.e.: post exposure.

Product type	Target organisms	Efficacy test	Performance standard	Reference
All treated fabrics with minimum (flat) surface area to allow cone test or KD test	Mosquitos	Cone test or ball test (contin. exposure)	100% KD within ≤ 71.5 min	WHO 2013b; 1998
	Mosquitos	Cone test (3 min exposure)	KD ≥ 95% at 1 h p.e., or MO ≥ 80% at 24 h p.e.	WHO 2013b
	Mosquitos	Tube test (3 min exposure)		WHO 1998
	Stomoxys calcitrans, *Phlebotomus papatasi*	Cone test (contin. exposure)	100 % MO after 24 h	Britch et al. 2018; Clark & Pearce 2018
	Ixodes ricinus (Ny)	KD test (contin. exposure)	Mean KD in ≤ 27.1 ± 8.5 min	TL 8305-0331
	Lepisma saccharina	KD test (contin. exposure)	Mean KD in ≤ 60.0 ± 21.0 min	TL 8305-0331
	Ticks (adults)	KD test (3 min exposure)	≥ 90% KD at 1 h p.e.	
	House dust mites	KD test (contin. exposure)	100 % MO after 24 h	Wongkamchai et al. 2005
	Human lice	KD test (contin. exposure)	100 % KD after 75 min.	Sholdt et al. 1989
	Human lice	KD test (2 min exposure)	100 % MO 24 h p.e.	Sholdt et al. 1989

Table 2. Overview of simulated- use tests and field tests suggested for efficacy evaluation of treated human apparel against target arthropods

Abbreviations: CPT: complete protection time

Product type	Target organisms	Efficacy test	Measured parameter	Validity criteria	Reference
All types of clothing (shirts, trousers, pants, jackets, socks, hats, etc.) that can at least in pieces be placed on a forearm	Mosquitos	Arm-in-cage test: defined test area on forearm fully or only partly covered by test fabric (if distance effect claimed)	CPT from bites (time to first confirmed landing)	≥ 20 landings/ min. in the control	BPR guidance, ECHA 2018
	Other flying insects (midges, horse flies, sand flies, blackflies, etc.)	Arm-in-cage test: defined test area on forearm fully or only partly covered by test fabric (if distance effect claimed)	CPT from bites (time to first confirmed landing)	≥ 20 landings/ min. in the control;	BPR guidance, ECHA 2018
	Mosquitos	Room test	Reduced bites on bare skin		Osborne et al. 2016
	Other flying insects (stable flies, sand flies, blackflies, etc.)	Room test	Reduced bites on bare skin		
	Ticks	Tick repellent test (adapted)	CPT (time until first confirmed tick crawls ≥ 3 cm upwards or stays ≥1 min on treated fabric)		BPR guidance, ECHA 2018
Clothing	Lice	Field test	Reduction of lice	--	Benkouiten et al. 2014
Shoes	Biting insects	No simulated-use test available			

Table 3. Overview of simulated- use tests and field tests suggested for efficacy evaluation of treated articles used close to the human body, or indoors, against target arthropods.

Abbreviations: CPT: complete protection time.

Product type	Target organisms	Efficacy test	Measured parameter	Performance standard	Reference
Wristlets (and other devices that can be placed on the arm)	Mosquitos	Arm-in-cage test: Test fabric is placed distant from test area on forearm	CPT		BPR guidance, ECHA 2018
Sleeping bags, tents, blankets, clip-ons, wristbands, stickers, tents	Mosquitos	Room test	Reduced bites on bare skin		Osborn et al. 2016
Curtains	Mosquitos	Room test (with alternative mosquito resting places)	Reduction of indoor mosquitos		
Stickers, clip-ons, wristbands, blankets, sleeping-bags	Ticks	Tick repellent test (adapted)	CPT (time until first confirmed tick crawls ≥ 3 cm upwards or stays ≥1 min on treated fabric)		BPR guidance, ECHA 2018
Fabric repellent barriers	Bed bugs	Three-chambers-test or simulated-room test	Number of bed bugs reaching the attractant (CO_2 and heat source)	≥ 90% efficacy	Vander Pan et al. 2019; Wang et al. 2013/ Todd 2011
Mattress liners	House dust mites	AATCC 194-2013 test method	Reduction of colony size	90 % reduction of mites compared to control	AATCC 194-2013
Mattress liners	House dust mites	Field test	Number of mites/g dust; amount of Der p1 allergen	< 100 mites/g dust and < 2 µg *Der p1* allergen/g dust	Cameron & Hill, 2002
Bracelets, hair tils, hairbands	Human lice	In-house repellent test	Distance lice crawl towards an attractant (heat)	≥ 90% repelled	Insect Services. unpublished

Table 4. Overview of simulated- use tests and field tests suggested for efficacy evaluation of treated articles for outdoor use against target arthropods.

Abbreviations: EU: European Union.

Product type	Target organisms	Efficacy test	Measured parameter	Performance standard	Reference
Insecticidal walls	Flying insects (outdoor)	Field tests (≥ 2 different eco-zones in the EU)	Percent local reduction of target species	>70% reduction of target species	Britch et al. 2018
Tick rolls	Ticks (*I. ricinus*)	Field tests (covering 2-3 years, at least 10 test sites and 10 control sites)	Reduced tick abundance as compared to control sites		Sonnenshein, Sonenshine 1993, Vogelsang et al., 2020, Drehman et al. 2018
Treated articles	Wasps	Field test	Reduced number of landing wasps as compared to control	≥ 90% repelled	Boevé et al. 2016

Table 5. Overview of simulated- use tests and field tests suggested for efficacy evaluation of treated articles to protect animals against target arthropods.

Product type	Target organisms	Efficacy test	Measured parameter	Performance standard	Reference
Devices (collars, blankets, etc.) to protect horses (cattle)	Biting and nuisance flies	Field test	Number of target species staying/landing on a host. Quantity of avoidance behaviour of the host.	> 80% reduction	Mottet et al. 2018
Blankets to protect horses	Midges	Semi-field test		> 80% reduction	Japin & Haanen, 2013
Treated fabrics (vests, shirts, collars) to protect dogs	Ticks	Simulated-use test”		CPT or ≥ 90% repelled	Draft guidance TNsG_PT19_Ticks_Draft-DE_180815.pdf

Table 6. Overview of simulated- use tests and field tests suggested for efficacy evaluation of mosquito nets against target arthropods.

Abbreviations: KD: knock-down.

Product type	Target organisms	Efficacy test	Measured parameter	Reference
	Mosquitos	adapted room test	Reduction of bites	Osborn et al. 2016
	Mosquitos	Large field test (phase II and III WHO)	Longevity of insecticidal activity of nets	WHO 2013b

4.3 Factors decreasing efficacy

Numerous factors might decrease the efficacy of treated articles, particularly washing (WHO 2013b; Banks et al. 2015; Richards et al. 2017), high temperature (Proctor et al. 2020; Mbando et al. 2018), and UV irradiation (Richards et al. 2017; Banks et al. 2015), but also physical abrasion during normal usage (Vaughn et al. 2014), ironing (Banks et al. 2015), and sweating (Mitchell et al. 2020).

Also, resistance of target organisms can profoundly affect efficacy.

4.3.1 Long-term efficacy and washing resistance

Washing of treated fabric probably exerts the greatest impact (i.e. decrease) on product efficacy. According to the impregnation method used, for example between 3 and 50% of the original permethrin content may be washed out already during the first washing (Faulde et al. 2006).

4.3.2 Efficacy at high temperatures

High temperatures may differently affect the efficacy of treated fabric. High ambient temperatures while wearing clothes may increase loss of AS from the fabric and concurrently dermal uptake of it (Proctor et al. (2020). It might also affect efficacy (e.g. KD times) against target organisms.

Ironing of fabric at 200°C can significantly reduce e.g. its permethrin content (Banks et al. 2015).

4.3.3 Ultraviolet (UV)-resistance

Exposure of treated fabric to natural sun light might reduce effectiveness of treated clothing (Banks et al. 2014; Mitchell et al. 2020), most probably caused by UV light. Laboratory tests must reflect the duration and intensity of UV irradiation most likely to occur in the field (e.g. Richards et al. 2017).

4.3.4 Resistance of target organisms

Insecticide or acaricide resistance in target organisms can profoundly affect product performance and even induce failure of the product. Resistance has been reported from populations of e.g. mosquitos (Dada et al. 2018), horn flies (Oyarzún et al. 2011), human lice (Durand et al. 2012), bed bugs (Dang et al. 2017), fleas (Rust 2016), and ticks (Rodriguez-Vivas et al. 2018). In ticks, resistance is mainly restricted to *R. microplus*, a species that stays on cattle throughout almost the whole of its life cycle.

4.3.5 The concept of complete protection time (CPT) for treated articles

Complete protection time is usually a parameter indicating duration of the protection by repellents directly applied on the skin. This concept can be adapted to treated articles to indicate their resistance towards washing and other environmental factors which may decrease efficacy. As washing is the most decisive factor influencing efficacy, resistance towards washing should be mandatory to demonstrate; otherwise, the label clearly has to state that efficacy is not guaranteed any more after washing.

4.4 Parameters relevant for risk assessment

4.4.1 Health risk

4.4.1.1 General consideration

According to WHO (2018, A generic risk assessment model for insecticide-treated nets – Revised edition), there are three steps of risk assessment:

1) Hazard assessment: Possible toxic effects and dosage levels are evaluated.

2) Exposure assessment: All relevant routes of exposure in a "realistic worst-case scenario" are evaluated, whereby accidental or voluntary misuse is excluded. Risks are estimated for adults, children (aged 6–11 years), toddlers (aged 12–24 months) and infants (aged < 12 months), as recommended by the European Human Exposure Expert Group (HEEG, 2013a). Exposure via mother's milk is estimated for infants and new-borns (birth to 1 month).

3) Risk characterisation: Exposure estimates are compared with acceptable exposure levels.

There are two WHO guidelines, the mentioned WHO (2018) for treated bed nets and the WHO (2019, Generic risk assessment models for insecticide-treated clothing, skin-applied repellents, and household insecticides), dealing amongst others with treated clothes. Both give excellent guidance on how to assess health risks, provide examples, e.g. how to calculate exposure, and give default values that may be used if there are no specific data available.

Aylward et al. (2018) lists values of acceptable daily intake (ADI) for several pyrethroids compiled from several organisations such as the FAO, the WHO, and the EU. They are all in the range between 10 and 70 µg/kg BW/day, with permethrin being at 50 µg/kg BW/d.

The US EPA, in a re-evaluation of permethrin (EPA 2009), considered permethrin likely to be carcinogenic to humans by the oral route. However, the cancer risk estimates are 1.2×10^{-6} and 3.6×10^{-6} for military personnel and garment workers, respectively, when wearing such clothes for 250 days/year. Thus, the risk was considered to be negligible.

4.4.1.2 Uptake by dermal contact

When using treated fabric, dermal contact is considered to be the main route of insecticide uptake, provided the fabric is treated with ASs with a low vapour pressure (like permethrin) (WHO 2019, 2018). Permethrin uptake can be estimated by measuring certain urine metabolite levels (Aylward et al. 2018). Uptake rates estimated by this way are < 4 µg/kg BW/day (Sullivan et al. 2019), 5-6 µg/kg BW/d (Appel et al. 2008), 0.3 to 14.7 µg/kg BW/d (Proctor et al. 2014), and 2.6 to 6.9 µg/kg/ BW/d (Proctor et al. 2020) in soldiers or forestry workers at work. These are all well below the ADI of 50 µg/kg BW/d for chronic oral uptake (Aylward et al. 2019). If dermal and oral routes of uptake do not result in different toxicities of permethrin, this suggests that even frequent usage of such clothes may be safe.

It has to be kept in mind, however, that the military usually adheres to a strict quality assurance system and that the initial permethrin content and daily release rates from BDUs are optimized to be well below the daily ADI but nevertheless provide sufficient protection against arthropod vectors. This may be different in clothes produced for the consumer market, and/or in fabric impregnated with different binding technology. Also, the manufacturer may produce fabric with inhomogeneous concentration of AS (Sullivan et al. 2019), possibly leading to locally different uptake rates.

Additionally, some studies show that individual uptake rates may increase by frequent hand-to-mouth contact (smoking) (Kegel et al. 2014) or increased ambient temperatures (Proctor et al. 2020). Possible causes for the latter may be an increased release of permethrin from fabric through sweating, or increased dermal absorption. On the other hand, showering after removal of treated clothing may reduce the uptake of permethrin (Proctor et al. 2014). Orsborne et al. (2016) measured permethrin residues on human skin being in the range of 2 to 5 µg/cm² skin at 0 to 60 min after removal of the fabric. This may be reduced by showering.

According to Appel et al. (2008), the release rate of AS from treated fabric could be determined using an artificial sweat solution. The method was established at the BfR (German Federal Institute for Risk Assessment) and described by Krätke & Platzek (2004).

4.4.1.3 Other routes of uptake

Other routes of uptake may be via air (inhalation). Because of the low vapour pressure of permethrin, this uptake route should be low and is considered low even for people sleeping under bed nets (WHO 2018). However, Faulde et al. (2006) found cross-contamination between treated and untreated fabric stored in the same room, even without direct contact to each other. Thus, uptake by inhaling dust (e.g. fabric fibres abrased during handling of treated fabric) may be considered.

If articles are treated with AS having higher vapour pressure and showing a (direct) spatial effect (e.g. transfluthrin, essential oils, repellents), uptake by inhalation of vapour may be relevant and the WHO (2019) guideline should be consulted.

Washing of treated clothing may release 3 to 50 % of the original permethrin content, depending on impregnation method, and high contents of AS may be transferred to untreated clothing when washed together with treated ones (Faulde et al. 2006). When washed in a machine, the health risk should be low (there is mainly an environmental risk), but if clothes are hand-washed, dermal uptake may occur.

These, and further consideration caused Appel et al. (2008) to recommend ”... manufacturers of impregnated clothing should provide data on concentrations, migration rates, homogeneity on impregnated fabrics, protective efficacy, and laundering resistance of the insecticide used for their products”.

4.4.1.4 Recommendations

In order to minimize any possible health risks, we recommend to:

a. Wear such clothes only when necessary (i.e. when staying in an area where vectors are prevalent)
b. Minimize contact of such clothing with bare skin (e.g. by using underwear)
c. Wash such clothes separately from untreated ones
d. Store such clothes separately from untreated ones, preferably in airtight bags to avoid contamination of the surrounding
e. Consider showering or washing of those body parts in contact with treated articles after usage or handling

We suggest mentioning at least recommendations a. and c. on the label of a treated article.

4.4.2 Environmental risk

4.4.2.1 General considerations

Compared to insecticides applied in a wide area, the usage of treated fabric has a relatively high target precision. It affects, in principle, only those target organisms that approach a host (e.g. a human wearing treated clothing) and get into contact with the treated fabric. The amount of AS released into the environment during normal outdoor usage should be comparatively low.

However, in special cases there may be non-target species killed if treated fabric is also attractive for them. This could be relevant, at least on a local scale, if e.g. insecticidal outdoor walls are used. The permanent outdoor deposition of treated material, e.g. tick rolls, may also lead to unforeseen contamination. It is beyond the scope of this study, though, to estimate the possible magnitude and impact of any such effect.

The probably most vulnerable environmental compartments are water bodies (lakes, ponds, rivers, etc.). At least pyrethroids are known to be in general highly toxic to cold-blooded water organisms. Most of the AS is released from treated fabric when washed (with detergent). Thus, washing water should be released into the wastewater system rather than into surface water. Whether swimming with treated articles in water bodies may release sufficient AS to thread freshwater organisms is also beyond the scope of the present study.

4.4.2.2 Recommendations

Based on these basic considerations and in order to minimize any possible environmental risks, it may be recommended:

- Not to swim in, or enter outside water bodies when wearing treated articles
- Not to allow animals (e.g. dogs) to swim or getting in contact with water bodies when wearing treated articles
- Not to hand wash treated fabric outside, thus releasing washing water into the environment

5 Discussion

There is a great variety of treated articles available on the market rendering it difficult to group them into meaningful categories for efficacy testing. Human apparel is a quite well-defined category, as are the categories mosquito nets and treated articles to protect animals. More difficult was the distinction between treated articles for outdoor use and articles to protect humans other than apparel. We found it appropriate to group all articles together that act close to the human body, regardless if they are used outdoors or indoors. Therefore, also sleeping bags and tents are included in this group, as well as all devices used indoors. A further category, treated articles for outdoor use, is restricted to those which do not come into close contact with humans. This grouping may facilitate both efficacy and exposure assessment.

Concerning efficacy testing, we propose to use existing test systems, only slightly modified for treated articles, whenever possible. KD tests can be performed either for flying insects, or for crawling arthropods almost without modification as compared to tests with liquid repellents. There are well defined performance standards available for mosquitos and ticks (*I. ricinus*) (WHO 2013b; TL 8305-0331, 2020). For many others (e.g. biting flies, bed bugs, lice, mites), however, no such evaluated end points are available. We tentatively suggest performance standards from literature data, if available. However, meaningful endpoints proving sufficient efficacy of treated fabrics may have to be gathered in future studies. For such studies we suggest to determine individual KD times of target organisms (to report mean ± SD KD times and the time to 100% KD) rather than exposing organisms for an arbitrary time (e.g. 2 min) to treated fabric and determining the percentage of mortality or KD after (arbitrary) fixed time points thereafter. This approach should give more precise and comparable results on the efficacy of tested articles (Gopalakrishnan et al. 2019) and can be used for virtually all treated fabrics.

For product authorisation, simulated-use tests or field tests should be mandatory. We suggest to adapt widely used tests for efficacy testing of treated articles, such as the arm-in-cage test and the room test using flying insects, and the tick repellency test for crawling parasites. Treated fabric claimed to protect the whole person or animal must regularly also protect body parts that are not covered by the article itself. To test this, the testing set-up has to be adapted accordingly (e.g. by measuring biting protection of the uncovered skin).

However, there may likely be also indirect effects contributing to a potential protection. Several studies show that already short exposure periods to fabric impregnated with pyrethroids, insufficient to induce immediate KD, can reduce the parasite's motivation to seek a host and/or bite. These include organisms as diverse as ticks (Eisen et al. 2017; Prose et al. 2018), bed bugs (Jones et al. 2015), and lice (Sholdt et al. 1989). The results of Osborne et al. (2016) suggest that this may also be true for mosquitos. This could partly explain the high efficacy of BDUs against ticks under field conditions (Faulde et al. 2015). The mentioned arm-in-cage tests and tick repellency tests, however, cannot account for this effect.

As a solution, room tests or field tests (with flying insects) may be performed. In such setups, target insects can choose to contact any body part and treated articles have to prove a significant reduction of bites (if not a significant reduction of landings) even on the uncovered parts of the body. In the case of crawling arthropods (ticks), no simulated-use test involving tick bites is available. The fingertip-assay described by Eisen et al. (2017) could in principle be a supplemental test to show if contact with a treated article reduces the tick's motivation to bite. However, further tests are necessary to prove that ticks unwilling to ascend a finger

really do not bite if offered a host. Thus, currently only field tests (Mitchell et al. 2020; Kime 2019; Faulde et al. 2015; Vaughn et al. 2014) involving tick bites are feasible.

Complete protection time is usually a parameter indicating duration of the protection by repellents directly applied on the skin. This concept can be adapted to treated articles to indicate their resistance towards washing and other environmental factors which may decrease efficacy. As washing is the most decisive factor influencing efficacy, we suggest to define CPT for clothing as resistance towards washing.

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7 Appendix I

Table A1: Results of the internet search for treated articles. The search covered the english and german language. The description column lists parts of the original label.

Key words	Product	Description	Link
Websites in English:			
Treated articles for humans			
insect repellent clothing	clothing, overalls, work wear, t-shirts, bandanas	Insect Shield® Repellent Apparel has been proven and registered to repel mosquitoes, ticks, ants, flies, chiggers, and midges. Insect Shield® Repellent Gear has been proven and registered to repel mosquitoes, ticks, fleas, and flies. The EPA requires extensive effectiveness data to prove a product's ability to repel insects. Many species and varieties of these insects have been tested, including those that can carry dangerous diseases. The repellency of Insect Shield apparel is EPA-registered to last through 70 launderings—the expected lifetime of a garment. This is also well beyond the life of most performance finishes commonly used in the technical-apparel industry	https://www.insectshield.com/
insect repellent bracelet	bracelet	"Enjoy the outdoors without being eaten alive by all the flying pests and the blood-sucking mosquitoes. The Great Outdoors™ bug repellent bracelet is an all-natural insect repellent made with essential oils, known to repel mosquitoes and other insects. KIDS & ADULT SAFE; LONG TIME OUTDOOR PROTECTION GUARANTEED; 100% NATURAL & DEET-FREE, ORGANIC DEET-FREE; Best mosquito repellent bracelet – Up to 300 hours of protection; 100% Natural No Toxic Chemical; & Deet Free;	https://www.great-outdoors.shop/product/mosquito-repellent-bands-12-pack/
insect repellent bracelet	Coleman® 7501 - Naturals™ Insect Repellent Snap Band	Naturals™ Insect Repellent Snap Band by Coleman®. The Coleman Citronella Snap Band provides long lasting protection from mosquitoes and can be snapped anywhere - wrists, ankles, backpacks, umbrella poles, tables, dog collars and more! Ingredients are stored in safety reservoir, so no contact with skin!	https://www.recreationid.com/coleman/repellent-bracelet-mpn-7501.html
insect repellent bracelet	Para'Kito	This type of wristband has a mesh pocket in which you place a replaceable repellent pellet. These little pellets are infused with seven different plant essential oils, which are claimed to together keep insects and other biting bugs at bay for 15 days. The wristband is also waterproof, so you can wear it in the pool, at the beach, or while trekking through marshlands. Para'Kito also produces refillable clips that work the same way, but can be attached to anything – your belt loop, your bag, your keyring, or anywhere else convenient.	https://www.canstarblue.com.au/health-beauty/mosquito-bands-really-work/
insect repellent bracelet	Swivel's Original Mozzie Band	Another pellet-based band, these waterproof neoprene wristbands use citronella, peppermint, geranium and lavender to deter mosquitoes. Each pellet is said to offer up to 15 days of insect protection. The lifespan of pellets can be prolonged by storing them individually in zip-lock bags while not in use. The company is Australian owned, with products available in camping and outdoors supply stores.	https://www.canstarblue.com.au/health-beauty/mosquito-bands-really-work/
anti-insect clothing	NosiLife jackets, trousers, shirts	The anti-insect treatment is built into our fabric and offers an outstanding defence against biting insects, lasting the lifetime of the garment. Designed and manufactured for travel. Our anti-insect treatment is built into our fabric and offers an outstanding defence against biting insects. Tested by an independent testing laboratory to offer a defence lasting the lifetime of the garment. Proven to defend against mosquitoes and other biting insects that can cause life threatening diseases such as Malaria.	https://www.craghoppers.com/technologies/nosilife /

Key words	Product	Description	Link
anti-insect clothing	NosiLife stretch	Un-rivalled anti-insect clothing with an increased range of motion. All the defence from Nosilife anti-insect clothing plus our Stretch fabric for extra comfort on the move.	https://www.craghoppers.com/technologies/nosilife-stretch/
insect protection blanket	blanket	Insect Repellent - High Warmth to Weight - Quick Dry - All Season Use, 100% Polyester, Dimensions: 56" x 68", Repels mosquitoes, ticks, flies and fleas including those that can carry dangerous diseases such as Lyme and malaria. Insect Shield can be used by the entire family with no restrictions for use. Built-in, odorless, invisible protection. No special care or storage required. Do not dry clean. Insect Shield converts clothing and gear into effective, long-lasting and convenient personal insect protection.	https://www.insectshield.com/Insect-Shield-Protection-Blanket-P1525.aspx
Treated articles for animals			
insect protection dog loop	bandana	Active ingredient: permethrin, polyester, light-weight, soft and elastic material, invisible, odourless insect protection, repels mosquitoes, ticks, flies, fleas and ants, long-term protection (for at least 25 washes), ideal for trips to the park, walks in the wood or for camping	https://www.brownspetrange.com/products/copy-of-insect-repellent-shield-dog-bandana-burgundy
insect protection blanket	blankets	Insect Repellent - High Warmth to Weight - Quick Dry - All Season Use, 100% Polyester, Repels mosquitoes, ticks, flies and fleas including those that can carry dangerous diseases such as Lyme and malaria. Insect Shield can be used by the entire family with no restrictions for use. Built-in, odorless, invisible protection. No special care or storage required. Do not dry clean. Insect Shield converts clothing and gear into effective, long-lasting and convenient personal insect protection.	https://www.insectshield.com/Insect-Shield-Protection-Blanket-P1525.aspx
insect protection blanket	clothing and others, like beds, blankets	Insect Shield for Pets permethrin dog clothes and other canine products keep insects away, repelling ticks, fleas, mites, and other bugs that can carry dangerous illnesses such as Lyme Disease and Heartworm. Choose from a variety of insect repellent gear for your dog, including permethrin-infused dog beds, blankets, neck gaiters, bandanas, and super stylish T-shirts, all with built-in Insect Shield technology.	https://www.insectshield.com/Pets-C108.aspx
insect repellent collar	equine collar	THE ALZOO HERBAL COLLAR; - Natural treatment for flies, mosquitos, horseflies, gadflies, midges, - For horses, ponies, donkeys and miniatures, - Repels pests at least 3 weeks, - Waterproof and sweatproof, - Comfortably worn around the neck, - Popular in the Southern U.S., Europe, West Africa, and other warm climates	https://www.alzooequine.com/
insect repellent collar	Insect Repellent Collar for Horses	The Insect Repellent Collar for Horses contains a blend of natural ingredients to protect the horse against flies and parasites, horseflies and midges. The collar is effective for 3-4 weeks depending on climate conditions. Once the active ingredient reaches the surface of the collar it then migrates across the horse. On the head. Adjust the length and attach using clips provided and then cut to size. The collar is waterproof and effective even when the horse is sweating. Contains geraniol. In rare cases the active ingredient may produce an allergic reaction.	https://totally-tack.com/insect-repellent-collar-for-horses/
insect repellent collar	Horse repellent collar	Soft, easy to wear and adjustable fly repellent collar with safety clips. Effective for 3 to 5 weeks for protection against biting and nuisance flies such as mosquitoes, horseflies and midges. The collar comes in 1 size (106cm) to fit most animals. Any excess can be removed. Contains a blend of natural ingredients, with known fly repellent properties. Once the active ingredient reaches the surface of the collar, it migrates across your horses` coat to give repellent protection.	http://www.horseflytrap.co.uk/product/insect-repellent-collar-for-horses/
insect repellent clothes dogs	insect repellent dog gear	Permethrin-Treated Clothing: Permethrin is an insecticide that is part of the pyrethroid family of synthetic chemicals. These chemicals act like natural extracts from the chrysanthemum flower. Permethrin affects insects if they eat it or touch it. It damages an insect's nervous system, causing muscle spasms, paralysis, and death. Permethrin has been used as an EPA-registered product since 1977. Insect Shield uses a proprietary formulation of permethrin in a system that results in permethrin tightly bound to the fabric fibres of each garment. The insect repellency is reported to last through 70 launderings.	https://pawsitivelyintrepid.com/insect-repellent-clothing-for-dogs-an-insect-shield-product-review/

Key words	Product	Description	Link
insect repellent clothes dogs	insect repellent dog clothing: shirt, hoodie, bandana	Insect Shield® Repellent Apparel and Insect Shield® Repellent Gear are revolutionary products designed to provide long-lasting, effective and convenient insect protection for your pets. Insect Shield apparel and gear, such as mosquito repellent clothing combine the Insect Shield process with a proprietary formulation of the insect repellent permethrin—resulting in effective, odorless insect protection that lasts the expected lifetime of the product, repelling mosquitoes, ticks, flies and fleas.	https://www.barkavenuedogboutique.com/insect-repellent-dog-clothing-c-130_268.html?view=all
insect repellent pillow	Insect Shield 23 by 16-Inch Insect Repellent Reversible Bed	Use in crates or outside to protect pets from harmful pests. Dual color options—slate grey side reverses to carrot orange. Removable cover for quick and easy cleaning. Cover can be machine washed up to 25 times. Effective against flies, fleas, ticks, mosquitoes, and midges (no-see-ums). Veterinarian approved by Dr. Katy.	https://www.amazon.com/Insect-Shield-Reversible-Protecting-Mosquitoes/dp/B00T06QRXE
insect repellent collar	Petvital Bio-Protective-Collar	Description, - Is effective on the basis of pure natural oils, - 35cm for cats and small dogs (with safety fastener), - 65cm for large dogs, - Totally innocuous also for young animals, - Protects repeated infestation for about 3 months, - Re-usable and environmentally-friendly	https://www.canina.de/en/Dogs/Petvital-Bio-Protective-Collar.html
Websites in German:			
Treated articles for humans			
Nosilife	Hose gegen Mücken	Die leichte NosiLife-Konstruktion wurde entwickelt, um vor Insektenstichen und UV-Strahlen zu schützen. In dieses Produkt ist als Insektenschutz der Wirkstoff Permethrin eingearbeitet. Permethrin ist ein Biozid. Biozidprodukte vorsichtig verwenden. Vor Gebrauch stets Etikett und Produktinformationen lesen.	https://www.campz.de/craghoppers-nosilife-pro-adventure-hose-herren-M118630.html?vgid=G1038314&_cid=21_1_-1_9_3960_1038314_431398592846_pla&ef_id=EAIaIQobChMI7Oewwtnb6gIVjKmyCh1aLwOfEAkYASABEgJe6PD_BwE:G:s&campaign_detail=shopping&gclid=EAIaIQobChMI7Oewwtnb6gIVjKmyCh1aLwOfEAkYASABEgJe6PD_BwE
Nosilife Kleidung	Cover Up (Tuch) gegen Insekten	Der NosiLife Sarong ist ein leichtgewichtiges, vielseitig einsetzbares und vor Sonne und Insekten schützendes Reise Essential mit vielfältigen Einsatzmöglichkeiten.	https://www.craghoppers.de/nosilife-sarong-sunset/?gclsrc=aw.ds&gclid=EAIaIQobChMI7Oewwtnb6gIVjKmyCh1aLwOfEAkYBSABEgJvdfD_BwE&gclsrc=aw.ds
Nosilife Kleidung	Longsleeve für Frauen (Insektenschutz)	Dabei wird diese Schutztechnologie schon bei der Produktion des Materials mit eingewebt. Diese innovative Technologie ist einzigartig in der Textilproduktion. Unabhängige Prüflabore haben die Schutzwirkung auf die gesamte Lebensdauer der Kleidungsstücke getestet. Diese Tests weisen auch auf, dass die NosiLife-Technologie von Craghoppers bis zu 90 % Schutz vor Mückenbissen und anderen Insektenstichen bietet und damit einen zusätzlichen Schutz vor lebensbedrohlichen Krankheiten wie Malaria bieten kann. Biozidprodukte vorsichtig verwenden. Vor Gebrauch stets Etikett und Produktinformationen lesen.	https://www.campz.de/craghoppers-nosilife-erin-ii-longsleeved-top-damen-M118991.html?vgid=G1038416&_cid=21_1_-1_9_3960_1038416_431422364603_pla&ef_id=EAIaIQobChMI7Oewwtnb6gIVjKmyCh1aLwOfEAkYCCABEgLk6vD_BwE:G:s&campaign_detail=shopping&gclid=EAIaIQobChMI7Oewwtnb6gIVjKmyCh1aLwOfEAkYCCABEgLk6vD_BwE
Nosilife Kleidung	Kleid für Frauen (Insektenschutz)	Aber was das Kleid tatsächlich von allen anderen abhebt, ist sein Gewebe: Der schnelltrocknende, vor Sonne schützende und Insekten abwehrende Ottomanstoff hemmt Gerüche und sorgt auch bei Hitze für ein frisches Gefühl.	https://www.craghoppers.de/nosilife-savannah-kleid-mid-khaki/?gclsrc=aw.ds&gclid=EAIaIQobChMI7Oewwtnb6gIVjKmyCh1aLwOfEAkYCyABEgIDe_D_BwE&gclsrc=aw.ds

Key words	Product	Description	Link
Nosilife Kleidung	Jacke (Insektenschutz)	Denn das Modell Lucca punktet mit einem geruchshemmenden Polyamidgewebe, das dank NosiLife-Technologie Insekten abwehrt und vor UV-Strahlen schützt.	https://www.craghoppers.de/nosilife-lucca-jacke-mid-khaki/?gclsrc=aw.ds&gclid=EAIaIQobChMI7Oewwtnb6gIVjKmyCh1aLwOfEAkYHyABEgIrg_D_BwE&gclsrc=aw.ds
Nosilife Kleidung	Sweatshirt (Insektenschutz)	Das Modell Tilpa besteht aus weichem und dehnbarem Baumwoll-Jersey mit NosiLife-Technologie und kann auf diese Weise Insekten abwehren und vor UV-Strahlung schützen. Dabei überzeugt es mit einem lässigen Stil und bequemer Passform – der perfekte Beistand, wenn die Temperaturen zurückgehen und die Mücken gierig werden.	https://www.craghoppers.de/nosilife-tilpa-crew-sweat-indian-yellow/?gclsrc=aw.ds&gclid=EAIaIQobChMIwP7T3dzb6gIVktCyCh0ufAilEAkYFyABEgKK1_D_BwE&gclsrc=aw.ds
Nosilife Kleidung	Socken (Insektenschutz)	Unsere beliebten Reisesocken mit praktischer Insektenabwehr sind jetzt auch als Einzelpaar in unterschiedlichen Farben erhältlich.	https://www.craghoppers.de/nosilife-reisesocke-einzelpackung-charcoal-soft-grey-marl/?gclsrc=aw.ds&gclid=EAIaIQobChMIwP7T3dzb6gIVktCyCh0ufAilEAkYGyABEgIq2fD_BwE&gclsrc=aw.ds
Nosilife Kleidung	Wüstenhut (Insektenschutz)	Gönnen Sie Ihrem Nacken den Sonnenschutz, den Sie sich wünschen, mit unserem beliebten Wüstenhut im Legionärsstil mit permanentem NosiLife-Insektenschutz.	https://www.trekkinn.com/outdoor-wandern/craghoppers-nosilife/136941349/p?utm_medium=afiliados&id_producte=7530164&country=DE&belboon=2007201133441540944&utm_source=487467
Nosilife Kleidung	Rock (Zecken- und Insektenschutz)	Miro von CRAGHOPPERS ist hergestellt aus dem permanent insekten- und zeckenschützenden NosiLife Polyamid Ottoman Material.	https://www.outdoorsports24.com/craghoppers-w-nosilife-miro-rock?number=18A-2991012835064&pup_e=6&pup_cid=62520&pup_id=18A-2991012835064
Nosilife Kleidung	Tuch (Mückenschutz)	Wenn du von Mücken umschwärmt wirst, hilft dir NosiLife bei der Abwehr. NosiLife: Exklusives permanent insekten- und zeckenabweisendes Material. Verhindert bis zu 90% aller Insektenstiche.	https://www.outdoorsports24.com/craghoppers-w-nosilife-florie-schal?number=20A-2991017726367&pup_e=6&pup_cid=62520&pup_id=20A-2991017726367
Nosilife Kleidung	Kurze Hose (Insektenschutz)	Die Hose schützt vor Insektenstichen, leitet Feuchtigkeit gut ab und trocknet nach dem Waschen schnell.	https://www.real.de/product/350820627/?utm_source=idealo&utm_medium=cpc&utm_content=de_01&utm_campaign=pricecomparison&utm_term=5998
Nosilife Kleidung	Top (Insektenschutz)	Allesa´s besteht aus leicht dehnbarem NosiLife-Jersey mit Insektenschutz, pflegeleichtem Finish und einer Auswahl hübscher Drucke oder Plains in frischen Tönen.	https://www.trekkinn.com/outdoor-wandern/craghoppers-nosilife-allesa-vest-top/137140938/p?utm_medium=afiliados&id_producte=8549631&country=DE&belboon=2007201214535900910&utm_source=487467
Schlafsack mit Insektenschutz	Pyjama (Insektenschutz)	Beim Backpacken, auf Fernreisen und selbst im Club-Urlaub nichts, ruiniert den Schlaf so gründlich wie Insekten. Wer den Travel Pyjama Insect Shield Cotton von Traveler's Tree im Gepäck hat beugt dagegen effektiv vor. Durch die Insect Shield® Imprägnierung hält der Schlafanzug Moskitos, Zecken, Ameisen, Flöhe, Mücken und anderes effektiv ab. Die Imprägnierung ist tief ins Gewebe eingearbeitet, komplett	https://www.bergzeit.de/traveler-039-s-tree-damen-travel-pyjama-insect-shield-cotton-s-brg/?gclid=EAIaIQobChMIlq2vgurb6gIV

Key words	Product	Description	Link
		geruchlos und dauerhaft (hält 70 Wäschen). Die Haut selbst kommt dabei mit dem Wirkstoff gar nicht in Kontakt.	WODtCh1JxgvQEAQYDyABEgLsYPD_BwE
Schalfsack mit Insektenschutz	Schlafsack mit Insektenschutz	- mit Insect Shield Insektenschutzimprägnierung. Cocoon Insect Shield TravelSheets sind extrem leichte und geräumige Leicht-Reiseschlafsäcke oder rechteckige Innenschlafsäcke mit Insektenschutzimprägnierung- Die Insect Shield Technologie eröffnet neue, wirkungsvolle Wege in der Bekämpfung von Insekten. Die Insect Shield Linie von Coccon wird mit diesem dauerhaften, effzienten und praktischen Insektenschutz versehen Insektenschutz von Insect Shield wird in einem speziellen Verfahren ins Gewebe der Cocoon eingebaut und muss daher nicht – wie andere Insektenschutzmittel – direkt aufgesprüht werden.	https://www.amazon.de/Cocoon-Anti-M%C3%BCcken-Baumwollschlafsack-Insect-Shield/dp/B01AT4IFSK/ref=sr_1_4?dchild=1&keywords=Schlafsack%2BMit%2BInsektenschutz&qid=1595248384&sr=8-4&th=1
Moskitoschutzbe-kleidung	Bluse (Zecken- und Mückenschutz)	Maul bringt bereits bei der Fertigung einen dauerhaften Insektenschutz in das Material mit ein, damit ist es nahezu mückendicht und es reduziert sich der Gebrauch von Insektenschutzmitteln und deren Verpackung.	https://www.outdoor-renner.de/maul-hochalm-damen-lange-krempel-outdoor-bluse-mueckenschutz.html
Kleidung mit Mückenschutz bei Renner XXL	Outdoor Decke (Zecken- und Mückenschutz)	Vielseitig einsetzbare Outdoor-Decke mit permanentem Mückenschutz / Insektenschutz. Schützt wirksam und nachhaltig vor allen Insekten (Zecken, Mücken, Fliegen...)	https://www.outdoor-renner.de/mueckenschutz-insektenschutz-outdoor-decke.html
Zelt mit Insektenschutz	Moskitozelt	Imprägniertes Moskitonetz mit zusätzlichem Schutz durch eine rein pflanzliche Wirkstoffkombination aus ätherischen Ölen und Geraniol. So werden Mücken vom Netz ferngehalten und stechen nicht durch. Noch nach 5 Monaten Gebrauch mit über 95 % Wirksamkeit. Waschen mindert den Effekt. Die Imprägnierung (nach Öko-Tex Standard 100) kann mit dem Greenfirst®-Spray erneuert werden. Schützt gemäß der Biozid-Regulation 528/2012 und ist für den EU-Markt zugelassen.	http://vi.raptor.ebaydesc.com/ws/eBayISAPI.dll?ViewItemDescV4&item=174349917465&category=65965&pm=1&ds=0&t=1595250143976
ebay- Zelt mit Insektenschutz	Armband (Mückenschutz)	Unter Verwendung hochwertiger Materialien, langlebiges Mückenschutzmittel Hergestellt aus sicherem Silikonmaterial, ungiftig und harmlos für den menschlichen Körper. Natürlicher Extrakt, keine Nebenwirkungen, sicher für Ihr Kind. Dieses Produkt kann Mücken effektiv abtöten und ist ideal für Outdoor-Aktivitäten. Geeignet für die ganze Familie, besonders für Kinder.	https://www.ebay.de/itm/5PCS-Muckenschutz-Armbander-Anti-Mucke-Silikon-Zelten-Schadstofffrei/353130414634?hash=item523836822a:g:gIEAAOSwaOpe8Fx-
Zeckenschutz-kleidung	Beinstulpen gegen Zecken	In diesen neuen Baumwoll- Stulpen sind Micro-Kapseln mit Eukalyptus verarbeitet, deren aromatischer Duft eine sichere Abwehr gegen Zecken leisten. Zecken und alle stechenden und beißenden Insekten hassen diesen Duft und suchen das Weite, ehe es zu den gefährlichen Bissen oder Stichen kommen kann.	https://www.otto.de/p/fussgut-beinstulpen-zeckenschreck-stulpen-set-2-teilig-1100694391/#variationId=1100695581
protection clothes insects	Spannleintuch für Reisen	Dank der Insect Shield Behandlung schützt der Bezug vor lästigem und beißendem Getier in und auf der Matratze wie Moskitos, Zecken, Ameisen, Fliegen, Flöhe, Sandflöhe und Mücken.	https://www.bergzeit.de/cocoon-insect-shield-protection-spannleintuch-natural-single/?gclid=EAIaIQobChMIv-jPv8Hg6gIVDeh3Ch1i4AfKEAkYASABEgJ7-fD_BwE
protection clothes insects	Matratzenüberzug	Dank der Insect Shield Behandlung schützt der Bezug vor lästigem und beißendem Getier in und auf der Matte wie Moskitos, Zecken, Ameisen, Fliegen, Flöhe, Sandflöhe und Mücken.	https://www.bergzeit.de/cocoon-insect-shield-ueberzuege-fuer-thermomatten-olive-green-black-large/?gclid=EAIaIQobChMIv-jPv8Hg6gIVDeh3Ch1i4AfKEAkYBSABEgLosvD_BwE
Protection clothes insects	Multifunktionstuch	Das BUFF UV Insect Shield Protection Multifunktionstuch mit einem UV-Schutz und Insektenschutzmittel, ist ideal für deine Outdoor Aktivitäten. Ein leichtes, nahtloses Schlauchtuch zum	https://www.sportdeal24.de/BUFF-UV-Insect-Shield-Protection-Multifunktionstuch-sunset-

Key words	Product	Description	Link
		Schutz vor Sonnenstrahlung und Insekten. Insektenschutz zum Abhalten von Insekten. Maschinenwäsche.	multi?rg=2&gclid=EAIaIQobChMIv-jPv8Hg6gIVDeh3Ch1i4AfKEAkYCSABEgJNJ_D_BwE
Nosilife	Stiefel	Sein starkes Obermaterial aus NosiLife-Wildleder und Mesh und der innovative Crawler Guard halten stechende Insekten auf Abstand, während der hoch geschnittene Schaft mit Ghillie-Schnürung für zusätzlichen Halt sorgt.	https://www.craghoppers.de/salado-hi-boot-rubble/
Moskitonetze Nosilife	Reise Moskitonetz	Die besonders feine Maschenstärke verhindert dabei, dass die Moskitos durch die Maschen schlüpfen können, während eine Imprägnierung mit dem Cocoon Mückenschutz Insect Shield das Übrige tut. So kommen Stechmücken erst gar nicht in die Nähe des Netzes und man kann erholsam schlafen	https://www.bergfreunde.de/cocoon-insect-shield-travel-mosquito-netmoskitonetz/?aid=c53d425a7ce2f26e96f9ceae9471a67d&pid=10004&gclid=EAIaIQobChMIndjfkvvg6gIVENZ3Ch2zEg5oEAQYECABEgJ6rPD_BwE&wt_mc=de.pla.google_de.1595625054.60348417796.302374332685
Mückenschutz Armband	Anti-Mücken Armband	**Natürlicher Wirkstoff:** Ihr Armband wirkt durch natürliche Öle mit Zitronenduft. Dadurch, dass der Wirkstoff nicht direkt auf den Körper aufgetragen wird, ist das Armband auch für sensible Haut geeignet. Hinweis: Armband vorsichtig verwenden. Vor Gebrauch stets Etikett und Produktinformationen lesen. Enthält Citronellol, Geraniol und Citral. Biozidprodukt vorsichtig verwenden. Vor Gebrauch stets Etikett und Produktinformation lesen.	https://www.pearl.de/a-NX7057-5110.shtml;jsessionid=i73FC144FE363FE61EE7B92CD70C12898?vid=917&wa_id=40&wa_num=1102&utm_source=googleps&utm_medium=cpc&gclid=EAIaIQobChMIi4-SzZi76gIVk813Ch1exg9fEAkYASABEgJP__D_BwE
Treated articles for animals			
Decke gegen Zecken	Decke für Haustiere	Innovative Decke, die hilft zu schützen gegen Insekten, geeignet für Hunde und Menschen. Breites Spektrum Schutz: zuverlässig schützt gegen stechende Insekten wie Mücken, Zecken, Ameisen, Fliegen, Milben und Flöhe. Mit Permethrin behandelt: harmlos für Hunde, aber sehr effektiver Wirkstoff. Eine synthetische Variante eines Insektenschutzmittel, die Auftritt natürlich in bestimmten Chrysanthemum Arten. Die neue, patentierte Technologie aus den USA: Insect Shield ist das Ergebnis von Jahren der Forschung und bietet patentierte, bewährte Schutz vor Insekten. Schutz: reduziert das Risiko von Infektionen mit von Insekten übertragene Krankheiten. Bis zu 24 Wäschen.	https://www.amazon.de/Outdoor-Decke-sch%C3%BCtzt-Insekten-Camping/dp/B06X93B2C6
Halstuch gegen Zecken bei Tieren	Hundehalstuch gegen Flöhe,Zecken,Ameisen, Mücken und Fliegen	Wirkstoff: Permethrin. Unsichtbarer, geruchloser Insektenschutz gegen Mücken, Zecken, Fliegen, Flöhe und Ameisen. Lange wirksamer Schutz (bis zu einer Entfernung von 30 cm und für mindestens 25 Wäschen). Das mit Permethrin behandelte Halstuch ist für Tiere völlig unbedenklich. Waschbar bei 60° C. Hinweis: Die Form der Aufbereitung in Insect Shield® ist auch für Katzen unbedenklich, obwohl Permethrin verwendet wird. Es liegen bisher jedoch keine Studien über die Verwendung von Insect Shield® für Katzen vor. Deshalb empfehlen wir, das Produkt nicht dauerhaft mit ihnen in Berührung zu bringen.	https://www.meintierdiscount.de/Hunde-Trixie-Insect-Shield-Dog-Loop-bordeaux-Groesse-L,51774-61789,58p.htm?kk=a4c62ee-17329307924-1c9ca3&gclid=EAIaIQobChMI7enqtYy76gIVkLh3Ch1NgASFEAkYDiABEgLBEPD_BwE&&utm_source=Kelkoo&utm_medium=Cost-per-Click&utm_campaign=Preisvergleiche
Keramik Halsband für Hunde gegen Zecken	Keramik Halsband gegen Zecken für Hunde mit Mikroorganismen	100% natürlicher Zeckenschutz durch EM Keramik und effektive Mikroorganismen	https://www.ganzoo.de/paracord/zeckenschutz/fertige-zeckenhalsbaender/1367/zeckenschutzhals

Key words	Product	Description	Link
			band-em-keramik/xs-l?gclid=EAIaIQobChMIysb0h8rl6gIVy513Ch0iKAOhEAYYASABEgIPMPD_BwE
Kleidung gegen Zecken Pferde	Insekten-Abwehr-Band für Pferde gegen Fliegen und Insekten	Funktionen: - zum Schutz des Pferdes vor Fliegen und Insekten Zusatzinformation: - 4-6 Wochen wirksam, Einfach um den Hals legen und schon ist das Pferd geschützt. Natürlicher Wirkstoff. Schützt das ganze Pferd vor Insekten!	https://www.reitshop24.de/fliegenhalsband-fuer-pferde-hkm-pferde-insektenschutzhalsband-3384-von-hkm?number=H4800-0000_0&gclid=EAIaIQobChMIsrqY6JC76gIVmOd3Ch25hQKnEAQYBiABEgJIC_D_BwE
Bayer Hund Insektenschutz	Hundekissen mit Insektenschutz	Die Hauptfunktion ist der Schutz des Gewebes selbst vor Insekten. Die sekundäre Aufgabe besteht darin, Insekten, die sich bereits auf dem Tier befinden, effektiv zu töten. Der Wirkstoff Permethrin ist für Mensch und Tier harmlos, aber für Insekten tödlich. Es ist eine künstlich hergestellte Version eines natürlichen Abwehrmittels, das in bestimmten Chrysanthemenblütenarten zu finden ist. Die Blume produziert dieses Insektizid zum Eigenschutz. Insect Stop schützt zuverlässig vor Flöhen, Zecken, Moskitos, Ameisen, Fliegen und Laufmilben. Und das bis zu 25 Waschungen.	https://www.frankonia.de/p/2007546?kk=a4c6327-1732e8a4251-21298c&navCategoryId=63234&campaign=PSM/KEL/Home&lmEntry0=PSM&lmEntry1=KEL&lmEntry2=Home&fdcampaign=feed/de/60308/kelkoo/2007546
Bayer Insektenschutz für Tiere	Auriplak (Gegen Mücken und Fliegen bei Kühen)	Die Ohrclips schützen Ihre Rinder gegen alle häufig vorkommenden Weidefliegen. Auriplak ist über die ganze Saison (4 Monate) wirksam. Die Täfelchen sorgen für eine sichere und einfache Bekämpfung von Fliegen und Mücken. Virbac Auriplak kann ganz leicht an der Ohrmarke befestigt werden. Die Clips schützen Ihr Vieh gegen die meistverbreiteten Weidefliegen wie die Kopffliege und die Weidestechfliege. Wirkstoff: Permethrin	https://www.medpets.de/auriplak/?channable=e1693.NDU1OA&gclid=EAIaIQobChMIvI3h4N696gIVCM13Ch2x0gi1EAkYASABEgLjTfD_BwE
Hundeweste gegen Insekten	Insect Shield Hundeweste Insektenschutz	InsectShield Hundeweste hält Zecken, Mücken und Flöhe fern. Wirkstoff: Permethrin. Unsichtbarer, geruchloser Insektenschutz.	https://www.tiierisch.de/produkt/insect-shield-hunde-weste-insektenschutz?ref=froogle&utm_source=googleshopping&utm_campaign=googleshopping%7Ccpc&utm_medium=cpc&utm_term=Hundeweste&utm_content=30405&gclid=EAIaIQobChMItePTm_HR6gIVEJOyCh0UeQ1AEAQYDyABEgLydvD_BwE
Zeckenschutz	Zeckenrollen für Mensch und Haustier	Zeckenrollen sind: eine wirksame Methode, mit dem lokalen Ökosystem und mithilfe der Maus (Hauptwirte der Zecken) Zecken aktiv und zielgerichtet zu töten. Zeckenrollen arbeiten langfristig und unterbrechen strukturell den Lebenszyklus der Zecke. Sie bestehen aus biologisch abbaubarem Zellstoff, der Baumwollelemente enthält. Diese Baumwolle ist mit einem Zecken-tötenden Wirkstoff behandelt. Sie brauchen nur die Zeckenrollen in Ihrem Garten auslegen, und können so die Zecken rund um Ihr Eigenheim beseitigen. Zecken durchleben vier Lebensstadien: Ei – Larve – Nymphe – adulte Zecke. Die Parasiten suchen sich Wirtstiere – vorzugsweise Mäuse – und Mäuse kommen sowohl auf dem Land wie in der Stadt vor. Mäuse sind extrem neugierig, finden die ausgelegten Zeckenrollen und krabbeln hinein. Die behandelte Baumwolle dient zum Nestbau. So kommt das Fell der Maus mit der Zecken-tötenden Substanz in Berührung und die Zecken im Fell der Maus sterben ab. Die Maus wird gegen Zecken immun und zu einem sehr wirksamen Zeckenvernichter.	https://www.amazon.de/Ixogon-Zeckenrollen-Mittel-Zecken-Garten/dp/B004OJ05MQ/ref=sr_1_167?adgrpid=71466541859&dchild=1&gclid=EAIaIQobChMItePTm_HR6gIVEJOyCh0UeQ1AEAMYASAAEgLlL_D_BwE&hvadid=391552419576&hvdev=c&hvlocphy=9043100&hvnetw=g&hvqmt=b&hvrand=2848447134462842602&hvtargid=kwd-297961682551&hydadcr=27930_1978092&keywords=insektenabwehr&qid=1594907431&sr=8-167
Zeckenschutz	Hunde- Katzenhalsband	Canina Petvital Bio Schutzhalsband. Kokosöl, Glyzerin, Geraniol. Das Band locker um den Hals schnallen. Überlänge abschneiden und ggfs. am Schlafplatz des Tieres oder an einer anderen befallenen	https://www.fuetternundfit.de/canina-petvital-bio-schutzhalsband.html?c=265

Key words	Product	Description	Link
		Stelle ablegen. Je nach der Größe des Tieres dauert es 6 bis 48 Stunden, bis sich die Wirkung voll entfaltet. Das Bio-Schutz-Halsband kann und soll permanent getragen werden.35cm für Katzen und kleine Hunde (mit Sicherheitsverschluss).65cm für große Hunde	
Decke gegen Zecken	Bernsteinkette gegen Zecken für Hunde und Katzen	Die in den fossilen Harzen enthaltenen ätherischen Öle haben eine abweisende Wirkung auf Parasiten. Reibt die Bernsteinkette am Fell von Hund und Katze, führt dies außerdem zu einer Reibungselekrizität. Das Fell lädt sich durch Abgabe von Elektronen an das Halsband positiv auf. Diese statische Aufladung wird von Zecken und Flöhen wahrgenommen und gemieden.	https://www.premiumpetshop.de/PetLove-Bernsteinkette-fuer-Hunde-und-Katzen-55cm

Table A2: Results of the literature search for test methods to evaluate efficacy of treated articles, and for risk assessment.

Reference	Use type	Article category	Intended use/ Claim	Target species	Active substance	Mode of action	Test system/purpose of publication	Efficacy level/Results	Efficacy parameters	Exposure parameters	Non-target effects	Miscellaneous
Mosquito nets												
Wuletaw et al. 2020	Human apparel	Mosquito nets	Protection against mosquitos	Mosquitos	1.8mg/kg Deltamethrin	Contact; Rep./Insecticide	Field trail in Ethiopia with 330 households, the distributed nets were inspected for the presence of bedbugs once every month consecutively for a total of four rounds and the infestation status was recorded during 4 months.	Consistent decline in the number of nets in use. Proportion of nets infested by bed bugs increased (81.8%, 270/330; 93.3%, 308/330; 92.1%, 304/330; 94.5%, 312/330; during rounds 1, 2, 3 and 4, respectively);				Bed bugs infestation in the nets probably forced users to discard even newly distributed nets within the first six months.
Mulatier et al. 2019	Mosquito net	Mosquito net	Protection against mosquito vectors	*Anopheles gambiae*	Deltamethrin (25 mg/m²); DEET (500 mg/m²)	Contact; Rep./Insecticide	Influence of infection of mosquitos (KdrKis strain, resistant to pyrethroids) with *Plasmodium falciparum* on success to find a hole and pass a treated mosquito net and blood-feed. WHO tunnel test (WHO 2013b)	Deltamethrin nets: Mosquito passing rate, blood-feeding rate and mortality not influenced by infection. Mosquito blood-feeding rate lower after deltamethrin contact. DEET nets: higher mortality of infected than uninfected mosquitos				
Janko et al. 2018	Mosquito net	Mosquito net					Analysis of 33 Demographic health survey and malaria indicator surveys in 21 countries sub-Sahara including >169.000 children (< 6 years old)	Children sleeping under nets with 21% lower odds of acquiring malaria. Nets less than 1 year old had the strongest effect. No difference between A.I. in nets (deltamethrin, permethrin, unknown)				
Boyer et al. 2018	n.A.	Mosquito net		*A. arabiensis*			Statistical considerations to reduce the number of mosquitos to be used in efficacy tests.	Percentage levels are given for accuracy, specificity and sensitivity to determine >80 mortality or >95% KD in cone tests using 1, 2, 3, or 4 cones for a test trial. Authors recommend a sample size of 40 nets and tests with two cones per net measuring mortality only.				

Reference	Use type	Article category	Intended use/ Claim	Target species	Active substance	Mode of action	Test system/purpose of publication	Efficacy level/Results	Efficacy parameters	Exposure parameters	Non-target effects	Miscellaneous
Abai et al. 2017	Mosquito net	Mosquito net		*A. stephensi*	Different pyrethroids	Contact; Rep./Insecticide	Ball test WHO (1997), 11 mosquitos per test and 10 replications.	Individual KD times				
WHO 2013b	Mosquito net	Mosquito net	Protection from mosquito vectors	*Anopheles* spp. (2-5 d old)			WHO guideline. Cone test: Test species: *Anopheles* mosquito, full susceptibility proven every 6 months. Test conditions: 27 ± 2°C and 75 ± 10% RH. Test procedure: 5 non-blood-fed female mosquitos are exposed to each piece of net (25 x 25 cm) under standard WHO cones for 3 min, thereafter held for 24 h with access to sugar solution. Pieces from 4 different nets should be tested with 10 cone tests with 5 mosquitos each (=50 mosquitos per net, 200 in total). Controls tested on untreated net at the same day before and after test trials. Tests are invalid if mortality of controls is >10% on a given day.	Cone Test: ≥ 80% mortality (corrected according to Abbot), or ≥ 95% knock-down must be achieved. KD recorded 60 min and mortality 24 h after test. Mosquitos considered alive if they can both stand up and fly in a coordinated manner. A mosquito is moribund if it cannot stand, cannot fly coordinated of takes off but immediately falls. A mosquito is dead if it cannot stand, or is immobile or shows no signs of life.				Washing procedure: Net samples (25 cm x 25 cm) placed individually into 1-l beakers containing 0.5 l deionized water, with 2 g/l soap (pH 10–11) fully dissolved. The beakers are shaken at 30 °C (water bath) for 10 min at 155 movements per min. Samples removed and rinsed twice in deionized water for 10 min, dried (room temperature) and stored (30°C darkness).
WHO 2013b (continued)	Mosquito net	Mosquito net	Protection from mosquito vectors	*Anopheles* mosquitos, 5-8 d old, non-blood-fed			WHO guideline. Tunnel test: Test species: *Anopheles* mosquito, full susceptibility proven every 6 months. Test conditions: 27 ± 2°C and 75 ± 10% RH. Test setup: glass tunnel 25 x 25 cm, 60 cm long, extended on both sides by 25 x 25 x 25 cm netting cage. At two-third of the tunnel, the test net (20 x 20 cm with 9 holes, 1 cm diam.) is placed inside a cardboard frame. Behind the net, a live host (guinea pig or rabbit) is placed as a bait. Test procedure: 100 female mosquitos released inside the cage distant to the bait. Mosquitos touch the test net and find the holes in the net to reach the live host. After 12-15 h, the mosquitos are collected from the different compartments and mortality and blood-feeding rates recorded. The test is invalid if mortality in the control is >10% and blood-feeding in the control is <50%.	Tunnel test: Blood-feeding inhibition assessed by comparing blood-fed females (dead or alive) between test and control. WHO criteria: ≥80% mortality, or ≥ 90% blood-feeding inhibition.				The wash resistance index (w) can be determined by chemical analysis and is expressed as a percentage by the following formula: $w = 100 \times n\sqrt{(tn/t0)}$, where, n = number of washes, tn= total active ingredient content (in g/kg) after n washing cycles; t0 = total active ingredient content (in g/kg) before washing of nets (no washing).

Reference	Use type	Article category	Intended use/ Claim	Target species	Active substance	Mode of action	Test system/purpose of publication	Efficacy level/Results	Efficacy parameters	Exposure parameters	Non-target effects	Miscellaneous
WHO 2013b (continued)	Mosquito net	Mosquito net	Protection from mosquito vectors	Mosquitos			WHO guideline. Phase I (laboratory) tests: Test material: 4 nets from 2 production batches: 14 pieces (25 x 25 cm) are cut out each net according to Figure 1. a) Evaluation of regeneration time after washing (net kept at 30°C after washing): 4 unwashed and 4 washed pieces are tested in the cone test and KD and mortality evaluated at days -1, 1,2,3,4,5,7 (and longer if necessary) after washing. b) Evaluation of wash resistance: Pieces are washed at intervals including regeneration time and cone tests performed on 4 pieces each after 1,3,5,10,15,20, and 25 (4 x 7 = 28 tests, or more if claimed) washes. The remaining pieces are stored for chemical analysis. c): if efficacy falls below cut-off level: tunnel test can be performed (with nets after 20 washings).	Phase I a): Efficacy curves of 60 min knock-down and 24 h mortality. The number of days to reach a plateau = regeneration time. b) KD and mortality of mosquitos is plotted against number of washes. Cut-off level: ≥80% mortality after 24 h or ≥95% KD after 60 min.	No. of washes			
WHO 2013b (continued)	Mosquito net	Mosquito net	Protection from mosquito vectors	Mosquitos			WHO guideline. Phase II (small scale field tests): nets meeting the requirements in phase I studies can undergo phase II studies using experimental huts that differ according to geographical region. Ethical considerations must apply. Six nets from different production batches (and huts) are used for each treatment arm (e.g. untreated net, unwashed test net and control net, 20 x washed test net, and positive control net). One net and pieces of the others are retained for chemical analysis. Tests are performed in Latin square rotations of treatments, nets, and sleepers. Phase III (large prospective field studies covering 3 years): to determine the duration of insecticidal activity, net survivorship or attrition, the fabric integrity of candidate LNs and user acceptability over 3 years. Usually, at least 400 to 500 nets are necessary.	Phase II: reduction in mosquito hut entry (Poisson or neg binomial regression) and blood-feeding (Percentage personal protection: logistic regression or generalized linear mixed models); Killing effect (acute and delayed mosquito mortality) Phase III: Net attrition (misuse, damage, etc.), net survivorship, fabric integrity (holes in net), Insecticidal activity (cone tests). At least 80% of nets should be effective in WHO cone tests or tunnel tests after 3 years.	No. of washes, fabric integrity			

Reference	Use type	Article category	Intended use/ Claim	Target species	Active substance	Mode of action	Test system/purpose of publication	Efficacy level/Results	Efficacy parameters	Exposure parameters	Non-target effects	Miscellaneous
Faulde et al. 2012	Mosquito net	Mosquito net	Protection from mosquitos	*Ae. aegypti*	Deltamethrin, cyfluthrin, permethrin etofenprox, all polymer coated.	Contact; Rep./Insecticide	Laundering according to ISO 6330:2000 20 times. Cone test with 10 female mosquitos, 3 replicates. Arm-in-cage test (cage 40 x 40 x 60 cm), 400 mosquitos/test at 27°C. Tape-fastened test fabric covered forearm. Test duration: 5 min.	Landing and biting of mosquitos not prevented by the net.		Laundering decreased pyrethroid content		ISO washing protocol is considered more stringent than the WHO protocol of washing. ADI-values according to Bundesinstitut für Risikobewertung (2010): 0.003 mg/kg body weight, in cyfluthrin, 0.01 mg/kg in deltamethrin, 0.03 mg/kg in etofenprox, and 0.05 mg/kg in permethrin.
WHO 2008	Mosquito nets		Protection from mosquitoes	Mosquitos	Netprotect / Dawaplus: 63mg/m^2 / 40mg/m^2 Deltamethrin; Duranet: 261mg/m^2 α-Cypermethrin; Iconmaxx: 50mg/m^2; λ-Cyhalothrin	Gas phase; Rep./Insecticide	WHO cone test, wire-ball tests and field studies in huts in 2 African countries. Treatment kit to impregnate mosquito nets	Netprotect: WHOPES Phase I criteria of >95% KD after 20 washes met. Mortality <80% after 15 washes (WHOPES main efficacy criteria of Phase II studies). DuraNet: Criteria of 95% KD after 20 washes met, despite mortality <80% after five washes. DawaPlus: Criteria of >95% KD after 20 washes met. Mortality consistently <80%. Iconmaxx: Criterium of >95% KD after 20 washes met. Unexpected variation in mortality (13% to 89%).		No of Washings		DawaPlus: Mortality consistently <80% demonstrateing unexpected variability, perhaps due to variability in initial deltamethrin content. Iconmaxx: Unexpected variation of mortality from 13% (unwashed net) to 89% (net washed 5 times), perhaps caused by variation in initial cyhalothrin concentration.
WHO 2005	n.A.	Mosquito net	Protection from mosquitoes	Mosquitos			Older guideline to test mosquito nets (now replaced by guideline 2013). Cone test and tunnel test with guinea pig as host					

Reference	Use type	Article category	Intended use/ Claim	Target species	Active substance	Mode of action	Test system/purpose of publication	Efficacy level/Results	Efficacy parameters	Exposure parameters	Non-target effects	Miscellaneous
Hougard et al. 2003	Mosquito net	Mosquito net		Resistant and susceptible strain each of: *A. gambiae, C. quinquefasciatus*	α-cypermethrin, cyfluthrin, deltamethrin, λ-cyhalothrin, etofenprox, permethrin, bifenthrin	Contact; Rep./Insecticide	Determination of efficacy of 7 pyrethroids when sprayed on mosquito net evaluated by WHO cone tests and tunnel tests. Irritancy test: time from landing to first take off in cone test.	KD_{50} after 4-12 min according to pyrethroid in *A. gambiae* and two times as long with *C. quinque-fasciatus*. Mortality much lower or zero in resistant strains particularly *C. quinquefasciatus*, but >80% in all but a few pyrethroids tested with *C. quinque-fasciatus*. Irritancy level was by far least with bifenthrin (*A. gambiae*), In tunnel tests <95% feeding inhibition in susceptible strains.				Overall best insecticide: α-cypermethrin, followed by bifenthrin.
WHO 1998	n.A.	Mosquito net		Mosquitos			WHO guideline. Insect susceptibility test (= WHO tube test): plastic tubes (125 mm length, 44 mm diameter) held vertically during test. Test mosquitos: unfed females 24-48 h post emergence. Test conditions: 25±2°C (max 30°C) and 70-80% RH. 4-5 repetitions with 20-25 mosquitos giving a total of at least 100 specimens. Exposure time: 60 min. WHO cone test: Cone attached to bed nets. 3 min exposure of 5 mosquitos per cone. 10 replicates to give at least 50 specimens plus the same number of untreated controls.	WHO tube test: Percentage knock-down at 10, 20, 30, 40, 50, and 60 min. (if <80% knock-down: Percentage knock-down at 80 min (in untreated tube). KD_{50} and KD_{95} (Probit analysis). If control mortality (after 24 h) is >5 and <20%, correction by Abbot`s formula. Cone test: mortality after 24 h (knock-down may also be measured)				Age and physiological status of mosquitos influence outcome of test, also the temperature during the test. Cone Test: there is a risk that mosquitos rest on the cone surface and not on the test surface. A list of suppliers for testing material is given.
Flying insects: non-mosquitos												
Weeks et al. 2019	n.A.	Repellent	Personal protection (skin)	*Phlebotomus papatasi* (5 -7 d old, unfed)	IR 3535	Contact/gas phase; Rep.	Arm-in-cage: 20 x 20 x 20 cm. Test area: 3 x 4 cm on human hand.	CPT; Protective efficacy (Abbot)		Dermal contact; inhalation	Water organisms (swimming)	Pre-screening of volunteers by sensitivity test against sandflies
Eyupoglu et al. 2019	Outdoor use	Bee repellent	To protect from		DEET, different	Gas phase; Rep.	Distribution of bees (n=100) inside a test box (wood and glass: appr. 30 x 30 x 30 cm) was photographed every 30 min and	None		Inhalation		Very preliminary test system with respect to biological efficacy.

Reference	Use type	Article category	Intended use/ Claim	Target species	Active substance	Mode of action	Test system/purpose of publication	Efficacy level/Results	Efficacy parameters	Exposure parameters	Non-target effects	Miscellaneous
		fabric	bee stings		terpenoids, microencapsulated		videotaped for 1 min every 10 min. Test box size: test duration: 2 h. Test material was deposited in a corner of the box in a petri dish.					
Mottet et al. 2018	Animal use	Leg bands	To protect against flies	*S. calcitrans*	leg bands: 54% citronella, 40% phenyl ethyl propionate	Gas phase; Rep.	Outdoor test. 6 horses (with ear and face masks) in 5 x 5 m enclosures (without gras) for 2 h/day, 5 d/week over 6 weeks (between 12:30 and 14:30). Fly annoyance behaviour counted in 4 30 min intervals: tail swishes (for 5 minutes), shoulder twitches (for 5 minutes), and hoof stomps and head-backs (simultaneously for 20 minutes) for a total of 2 hours. Count of stable flies at 0, 30, 60, 120 min on horse`s front limbs. 5 protected and one unprotected (control) in Latin square design.	Frequency of hoof stomps and head backs sig. reduced by leg bands. Increase of fly density in second hour (accumulation of flies after test start).				
Britch et al. 2018	Outdoor use	Military protective wall (HESCO geotextile)	To reduce vector abundance	*Phlebotomus papatasi, C. quinquefasciatus, M. domestica, S. calcitrans*	λ-cyhalothrin	Contact; Insecticide	Field test: "wall" unit appr. 2.5 m high, 2.7 m wide, 1.8 m deep. 4 treated and 1 untreated unit covered by fabric (treated, untreated). Fabric strips sampled throughout several months and cut into 1 x 5 inch. Put in glass tubes together with test species: mortality after 24 and 48 h.	Arbitrary benchmark: 90% mortality. Time course of efficacy for up to 142 days.			All flying insects could potentially be affected.	
Zhu et al. 2018	Animal use	Repellent	To protect from fly bites	*S. calcitrans; Haematobia irritans*	Coconut fatty acids	Contact/gas phase; Rep.	In a field test, 18 heifers were treated with test material and the number of biting flies on all 4 legs and belly counted between 13:00 and 16:00 at predetermined intervals	Best result is close to 90% repellency				
Haanen & Japin 2013	Animal	Horse blanket (Ivanhoe	To protect against bites of midges	*Culicoides*			Study on 4 farms (Netherlands) with two horses each. Each horse stood in a tent with one side open for two hours a day and 4 days sampling time, thereby wearing an insect blanket for 1 h in a rotating manner. Collection of midges after trapping time.	Between 124 and 12536 midges, unfed and fed, caught per farm. Number of trapped unfed midges similar between groups, but less blood-fed midges in horses				

Reference	Use type	Article category	Intended use/ Claim	Target species	Active substance	Mode of action	Test system/purpose of publication	Efficacy level/Results	Efficacy parameters	Exposure parameters	Non-target effects	Miscellaneous
		Horse equipment)					Blanket was individually fit to horses and included head and neck parts.	with blankets. The chance of horses being bitten is 2.27 times higher without blanket.				
Boeve et al. 2016	Outdoor	Repellent	To deter wasps from tables (outside)	Wasps	Icaridin	Gas phase; Rep.	Field test: Washed artificial skin (10 x 10 cm, purchased from Amazon) was treated with test substance and 1 ml of four-berry jam (sugar content: 60%) placed in the centre as an attractant. Up to six such skin plates (5 pre-treated at different time points to evaluate efficacy period, and one untreated as a control). The number of landing wasps and wasps flying over the plate was video-recorded for 1 h. 18 replications. Weather conditions and ambient temperature were recorded.	Approx. 70% of wasps observed were feeding and 30% flying. Wasp-free time was higher on treated skins than on untreated ones.		Inhalation		Test sheets should be placed apart from each other to avoid a common repellent cloud. Lure should not be in direct contact with the repellent surface. Weather conditions possibly can influence tests.
Mosquitos												
Vatandoost et al. 2019	Human body	Insecticidal blanket (Skintex)	To reduce mosquito bites when sleeping	*An. stephensi*	Permethrin, microencapsulated	Contact; insecticide	Washing procedure according to WHO 2013b. WHO cone test with 50 female mosquitos/cone giving a total of 100 mosquitos per test.	KD and mortality rates increased up to 6 washings, then decreased.	Usage may increase efficacy	Dermal contact		Perhaps, microcapsules are physically crushed during usage and thereby release AS (efficacy may increase during usage).
Gopalakrishnan et al. 2019	n.A.	Treated fabric		*Ae. aegypti*; *An. stephensi* (2 - 5 d old (27°C), unfed)	Permethrin	Contact; insecticide	To compare test methods: WHO cone tests: a): continuous exposure; b): 3.min exposure	a) 100% KD time (effective if ≤71.5 min); b) Percentage mean KD (1 h post exposure: effective if ≥ 95%) and mortality (24 h post exposure: effective if ≥ 80%)		Retention of AS 85.1%, 56%, and 36.2% after 1, 5 and 10 washings		Continuous exposure test recommended: results correlate better with permethrin residues and the 3-min test failed to show sufficient efficacy in almost all samples.
Halbkat et al. 2019	Human apparel	Fabric for clothing	Personal protection	*Ae. aegypti* (starved	Botanicals	Gas phase; Rep.	Arm-in-cage test: 30 x 30 x 30 cm; test duration 10 min. Test area: 33 x 150 mm on forearm	Repellency = Percentage of landing (video camera) and Protection = percentage of	time (gasing off)	Inhalation		Repellency of the same compound much higher on white fabric than on black one.

Reference	Use type	Article category	Intended use/ Claim	Target species	Active substance	Mode of action	Test system/purpose of publication	Efficacy level/Results	Efficacy parameters	Exposure parameters	Non-target effects	Miscellaneous
			against mosquitos	for ≥2 hours)				blood-feeding (squeezing of mosquitos)				
Akila & Ekong 2019	Human body	Bracelet	Protection against mosquitos	Mosquitos	geraniol, citronella	Gas phase; Rep.	Study on 10 households in Nigeria over ten days. Mosquito number evaluated by Pyrethrum spray on day 1, 6 und 11 of study.					No control, no statistics. "Decline" can be merely due to usage of Pyrethrum spray.
Mbando et al. 2018	Outdoor use	Eave ribbon	Decrease no. of mosquitos entering house	*An. arabiensis*	Transfluthrin (0.25; 2.47; 18.5; 61.7 g/m²)	Contact/gas phase; Rep./insecticide	1. Tests in semi-field facility (Tanzania) (9.6 x 21 m) with small livestock and vegetation inside and two experimental huts (3.1 x 2.7 m). 500 female mosquitos released at 18:00, start of test 30 min later. Treated fabric: three-layered woven sisal fibres, 15 cm wide and 1 or 2.5 m long. Fabric soaked in solutions of transfluthrin to give different concentrations and adhered after drying to the eave space under the roof of house. Baseline mosquito activity, human landing catches, 2 persons) determined during first 5 nights, then test for 10 nights. Mosquito mortality assessed with 100 mosquitos in a cage close to the hut during night. 2. Field experiment with experimental huts in Tanzania according to WHO.	Decrease of indoor and outdoor biting-rate >99% with at least 0.2% transfluthrin (0.25 g/m²)	Increasing temperature increases evaporation	Increasing temperatures should increase inhalative uptake		
Tangena et al. 2018	Human apparel; Outdoor	Overall (short and long pant legs), portable insecticide coils	Personal protection against mosquitos	*Ae. albopictus, Ae. aegypti*	Permethrin (0.05 mg/cm² (Insect Shield); para-menthane-diol (PMD), Metofluthrin	Contact; insecticide, and gas phase (Metofluthrin, PMD)	Field study (Lao) with Latin square design: Test of overall (Permethrin); short pant legs (PMD), coil (0.015% metofluthrin. 1. Outdoor human landing catches 12:00-18:00 or 17:00 - 23:00 according to village for 45 min each hour. 14 volunteers from each of two villages rotated places during tests, 14 test days. 2. Cone test with laboratory mosquitos according to WHO.	Appr. 13.000 female mosquitos caught. From 92.3% protection with mosquito coils to 0% in permethrin-treated short overalls and untreated overalls. Cone test: Only about 25% mortality of susceptible mosquitos with clothing (permethrin), less than that recommended for mosquito nets.	Field use (2 weeks) reduced KD effect	Inhalation (portable coil; PMD)		No "halo" effect of permethrin-treated clothing in field study.
Richards et al. 2018	Human	Clothing		*Ae. albopictu*	Permethrin	Contact;	Comparison of cone test and petri dish test. Petri dish assay (EPA 2009): 3-10	Slightly higher KD and mortality rates in petri dish		washing		Washing: 27°C, 39 min/wash and drying

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	apparel	(Insect Shield)		*s, Ae. aegypti*		insecticide	female mosquitos per petri dish lined with test fabric. After 2 min exposure, mosquitos were chilled for 45 s, taken out and KD and mortality recorded after 2 and 24 h. Cone test according to WHO 2013b with the same times to determine mortality/KD.	assay compared to cone test (not significant). More forced contact in petri dish (mosquito cannot fly away)				(30 min at 50°C), 59 ml fragrance free detergent/wash.
Richards et al. 2017	Human apparel	Clothing	Personal protection against mosquitos	*Ae. albopictus*	Permethrin (125 µg/cm²)	Contact; insecticide	Test of clothing after different no. of washings, kept at different temperatures, simulated sunlight (in incubators at 18 or 32°C with xenon lamps). Cone test (WHO 2013b) with 12 mosquitos per test for 3 min. KD: 2 h post exposure, mortality: 24 h post exposure. Likelihood of mosquito KD predicted by multinomial logistic regression.	Washing and light exposure sig. reduced mosquito KD (washing: p<0 .0001, 37–60% reduction; light: p = .009, 7% reduction) and/or mortality (washing: p< .0001, 24–35% reduction; light: p < .0001, 12% reduction). Permethrin content, but not mosquito KD, varied by fabric type. Temperature without effect.	Washing, light significant!	Washing, light		Permethrin content affected by fabric type and no. of washings as well as interaction of light and washings, and light and type of fabric. Correlation of permethrin content and mosquito mortality and KD.
Richards et al. 2017	Human body	Sonic device, clip-on (leg), bracelet (arm), aerosol	Personal protection against mosquitos	*Ae. aegypti*, 1.5 to 2 weeks old, deprived of food	Methofluthrin, citronella, and others	Gas phase; Rep.	11 repellents tested to inhibit attraction of mosquitos using a taxis assay inside a wind tunnel with 50-125 female mosquitos each. Taxis cage: 3 chambers, the middle one separated from the others by a funnel with a 5 cm opening during tests inside a wind tunnel (1.2 x 1.2 x 14.6 m; speed 2 m/s). Two volunteers served as attractant upwind, mosquitos released in middle chamber. Test time: 15 min. Tests invalid if attraction in control (without repellent) <80%. The test system is regarded better than arm-in-cage test by authors.	No significant reduction of mosquito attraction with sonic device, all bracelets and the citronella candle. Only OFF!clip-on (methofluthrin) was effective.		Inhalation		

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Most et al. 2017	Human apparel	BDUs	Personal protection against mosquitos	Field study: primarily *An.*; Laboratory study: *Ae. aegypti*	cis:trans-permethrin 25:75 at 1.300 mg/m²	Contact; Rep./insecticide	Field test in French Guiana. Machine or hand washing of uniforms. Additional KD test according to TL 8305-0331.	Field test: N=25 persons (9.5 person-months) in malaria area experienced no malaria. N=125 control persons (30.5 person-months) experienced 11 cases of malaria (36.1 per 100 exposed person-months). TL8305-0331: Mean of 25 launderings per uniform. KD_{99} was 54 ± 50 min. Mean remaining permethrin content in uniforms: 732 ± 321 mg/m²	Abrasion, weathering: Permethrin loss larger than expected from no. of washings			Cited effective dosages of permethrin on fabric: 25-200 mg/m² against sand flies (Burgess et al. 1988); 80 - 100 mg/m² against *Anopheles* species (Darriett et al. 1988); 62 - 250 mg/m² against *Am. americanum* (Schreck et al. 1982). Sublethal doses may stimulate attachment in *H. dromedarii* and *D. reticulatus* (Fryauff et al. 1996; Buczek et al. 2015). -> Clothing should be used with ≥200 mg/m² permethrin.
Faulde et al. 2016	Human apparel	Clothing and BDUs	Personal protection against mosquitos	*Ae. aegypti*, *An. stephensi C. pipiens*	Permethrin	Contact; insecticide	Efficacy of 4 commercial clothing types and 1 BDU was compared. Washing of fabrics according to EN ISO 6330:2000. Cone test (WHO 2013b): 10 mosquitos per cone: time to 99% knock-down (up to 6 h). 10 replications per test.	Biocidal efficacy of BDUs was sig. higher than with all other products except Labonal socks with all mosquito species. None of the commercial products would meet the licensing conditions of the TL 8305-0331. *Ae. aegypti* showed highest sensitivity, followed by *An. stephensi* and *C. pipiens.*	Bioactivity on fibre is more important than content of permethrin.			Initial permethrin concentrations and % loss after 100 launderings 4300 mg/m² (loss: 58.1%) for Labonal socks, 4000 mg/m² (loss: 85.8%) for Sol's Monarch T-shirts, 1310 mg/m² (loss: 78.6%) for the BDUs, 1300 mg/m² (loss: 98.5%) for Insect Shield T-shirts, and 870 mg/m² (loss: 95.4%) for ExOfficio T-shirts. Initial content of permethrin may be too high to be toxicologically safe, exceeding maximum concentrations by the US EPA (1250 mg/m²) and the GFIFRA (1300 mg/m²) (Appel 2008). These proved effective

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												also against sand flies, fleas, and ticks (cited literature).
Orsborne et al. 2016	Human apparel	Treated cloth (Insect Shield) Shirts, trousers, shorts	Personal protection against mosquitos	*Ae. aegypti* (resistant and susceptible), 3-7 d old, unfed.	Permethrin	Contact; insecticide	1) Arm-in-cage tests with arm fully and partially covered by test and control fabric and bare arm. Landing pressure: 10 landings / 30 s. Test duration: 90 s. 1 participant. 10 replicates (300 mosquitos per test). 2) Flight room experiment in 10 m^3 room. 2 rooms connected by door.	Arm-in-cage test: Best protection with full coverage: 58% landing reduction, after 10 washes: 18.5%. Bite protection was >97%. Room test: landing was not sig. reduced, but blood feeding by >90% (full covered arm). KD and mortality >80% at all time points and >90% at 1 h and 24 h after exposure.		Permethrin content on skin 0.002 to 0.005 mg/cm^2 (0-60 min post removal of fabric)		Clothes washed in a machine at 30°C, 800 rpm, 25 ml detergent in 59 l water.
Toledo et al 2015	Hum n body	Treated curtain (PermaNet)	to reduce indoor *Aedes* abundance	*Aedes* mosquitos.	Deltamethrin coated by (UV) protectant	Contact; insecticide	Randomized controlled trial in Cuba to evaluate the *Aedes*-reducing effect of insecticide-treated curtains in 6 test and control clusters with almost 7.000 households. Max 3 curtains per household in bedroom or door. WHO tube assay (held vertically, exposure time: 3 min) to determine deltamethrin resistance in mosquitos. Immature mosquitos collected monthly in all households and determined (quality inspectors).	Mosquitos were susceptible to deltamethrin and mortality after 1 year of usage was 73.1% and 59.1% in unwashed and washed curtains				

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Banks et al. 2015	Human apparel	Long- and short leef shirts		*Ae. aegypti*	Permethrin, factory and self-applied	Contact; insecticide	Mosquitos 3-5 (WHO cone test) and 5-7 (arm-in-cage test) old. Arm-in-cage test: Test fabric wrapped around the forearm and taped in place. Controls: Untreated fabric on arm, bare arm, and 20% DEET on arm. 30 Mosquitos per test, predetermined biting pressure: 10 landings within 30 s. Test duration: 90 s, count of mosquito landings and counting of bites on the arm. UV-radiation of clothes at 12.5 cm distance by a 300 W Ultra Vitalux lamp (equivalent to 16 times mid-sun day irradiation) for 20 to 1200 min. Ironing at 200°C for 1 to 18 times for 30 s each.	Cone test: KD between 51% and 98% according to impregnation method. KD decreased below threshold level after 10 hand washings and 15 machine washings, in line with a decreased permethrin content determined. Arm-in-cage test: biting protection between 65% and 91%, landing protection between 23 and 50%. No significant reduction of permethrin content with UV-light alone.	Washing, ironing and combined washing/ironing/UV light decreased permethrin content.	Exposure decreases with repeated washing, ironing.		Washing procedure of clothing according to WHO (handwash) or in a washing machine (30 min at 30°C, 25 ml of unscented soap in 59 l water).
Kitau et al. 2014	Human body	blanket	Personal protection against mosquito bites	*An. gambiae, An. arabiensis* (3-4 d old)	Permethrin 1130 mg/m²	Contact; insecticide	Cone test (WHO) with 10 mosquitos each on blankets washed 0, 5, 10, 15, 20 times for 3 min (4 replications). KD determination after 10 min and mortality after 24 h. Ball test according to WHO (better contact than cone test): wire ball frames attached to blankets and mosquitos released inside for 3 min (4 replications) KD determination after 60 min and mortality after 24 h Arm-in-cage test: cage 30 x 30 x 30 cm. Tests performed if 10 landings within 30 s on untreated arm. Test time: 90 s for control and test arm (blanket placed on it). 3 replicates with different volunteers. Experimental hut test according to WHO 2013b with 7 volunteers in Tansania.	Cone test: >80% KD. Rapid decrease after washings. Ball test: somewhat higher mortality and KD than cone test. Arm-in-cage test: 100% protection after 20 washings: probably due to thick material preventing biting. Protection against landing declined after 5 washes. Hut tests: nets reduced biting rate of mosquitos significantly stronger than blankets. Body coverage by blankets was estimated at 80%	Washing; thickness of fabric	Washing, percentage body coverage by blankets?		Washing procedure according to WHO (2005)
Banks et al. 2014	Human apparel		Bite protection; reducing arthropod population;	Mosquitos	Permethrin and other pyrethroids, DEET	Contact/gas phase; Rep./insecticide	This review recommends cone test (WHOPES 2005) for evaluation of KD and mortality and arm-in-cage test after simulated weathering and washing. No WHOPES guidelines for insecticide treated clothing available. Bite protection: best when using insecticide-treated clothing plus repellent on untreated areas (skin). Intervention trials to reduce			Sunlight, washing, type of fabric, AS binding method		Review article; Extensive list of effectiveness of clothing against arthropods. Review on permethrin safety.

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							pathogen incidence (Malaria): Overview of field trials is given.					
Revay et al. 2013	Human body	Vitamin B patch sonic device, repellent wristbands, diffusors	Protection against mosquitos for 8 to 200 h according to product	*Ae. albopictus; C. pipiens*, 5 d old and starved for 24 h	Metofluthrin, essential oils, terpenoids,	Contact/gas phase; Rep./insecticide	8 volunteers (entomologists). Semi-field tests according to EPA (1999) with minimum biting pressure of 1 bite/min in greenhouses (80 x 30 x 3 m; not solid, but fine mesh) in Israel. Groups of 1500 females of either mosquito species released in greenhouse at 17:00. Test start 3 h later. First trial: Number of landing, probing and biting mosquitos counted on the arm opposite to the repellent treated arm or side. 16 repetitions with rotating volunteers for each product and mosquito species. Second trial: comparison of the best two products and recording landing, probing, biting on the leg with repellent on the arm with 3 volunteers.	Only two products achieved significant reduction in mosquito attack (<90%): Two clip-ons (with metofluthrin)				Increased mosquito attack when using the sonic device or a wristband with citronella.
Britch et al. 2010	Outdoor	Treated camouflage screening	Reduce outdoor mosquitos in military camps	*C. tarsalis* (outdoor)	Bifenthrin	Contact; insecticide	Outdoor study in California, USA. A total of four 3 x 3 m frames, 2 m high, placed at least 50 m apart from each other in a semidesert field site and sprayed with bifenthrin. Mosquitos collected using light traps the day bevor and after construction and at days 7, 14, 21, and 28.	Reduction of mosquitos by appr. 60% upon start of study declining to 20% after 28 d. The own aim is a >50% reduction in mosquito number as a standard.			Non-target species may be killed	
Mosquitos and ticks (and chigger mite)												
TL8305-0331 (Feb. 2020)	Human apparel	BDUs	Personal protection from vectors	Ticks, mosquitos	Permethrin (1300 mg/m², max. 1600	Contact; Rep./insecticide	Test animals: *Ae. aegypti* females (from governmental breeding establishment), *I. ricinus* nymphs (field-collected, used immediately or after a max 4 d storage period at 10°C). Test system: WHO cone test (*Ae. aegypti*) and WHO tube test (*I.*	Endpoint after 100 washings: *Ae. aegypti*: time to 100% KD. The mean of (at least) two 100% KD times must be <71.5 min. *I. ricinus*: Determination of individual	No. of washings;	Fabric must be washed separately		Washings of test fabric according to DIN EN ISO 6330, Appendix B, procedure no. 6M, with following deviations: 2 kg of fabric, 1 m wide,

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					mg/m²); After 100 washings: >200 mg/m² (or less if sufficient efficacy		*ricinus*). Each test: 10 test animals. At least two test repetitions and two untreated control tests. Bioactivity of unwashed fabric and after 100 washings.	KD times. Calculation of the mean of a test (10 animals). The mean of the mean KD times of (at least) three repetitions must be <27.1 min		from untreated fabric (cross-contamination possible).		no additional load. Per washing use of 25 g Taxat Color (Fa. Ecolab; detergent without optical brightener). Washing machine: Typ A (Wascator FOM 71 CLS, Electrolux) with software according to DIN EN ISO 6330. No drying between washings.
Sullivan et al. 2019	Human apparel	Clothing (Insect Shield). Pants and socks	Personal protection from vectors	*Ae. albopictus* (susceptible) and *Ae. aegypti* (resistant), *I. scapularis* nymphs	Permethrin	Contact; Rep./insecticide	13 outdoor workers in North Carolina, USA, used long-lasting impregnated clothing for 3 months. Urinary samples taken to monitor permethrin uptake. Tick mortality assessed by exposing ticks for 3 min on fabric (horizontally) and determine mortality after 24 h (Eisen et al. 2017). Mosquito KD (2 h post exposure) and mortality (24 h post exposure) assessed by petri dish assay (Richards et al. 2018) with 2 min exposure and 45 s chilling period at -20°C before and after test to transfer mosquitos.	Most socks and pants induced >85% tick mortality still after 3 months. No effective KD or mortality in mosquitos after three months. Calculated permethrin uptake was <4µg/kg BW per day.		Calculated permethrin uptake was <4µg/kg BW per day.		Permethrin content 14.2 µg/cm² in socks and 48.5 µg/cm² in pants after three months. High variability in permethrin content between individual clothes may be due to inhomogeneous manufacturing. A calculation is given to compute daily uptake of permethrin from urinary metabolites measured.
Vaughn et al. 2014	Human apparel	Treated clothing (socks, shirt, pants, hat)	Personal protection against tick bites	*A. americanum*, Chigger mites, mosquitos	Permethrin, administered by Insect Shield LLC.	Contact; Rep./insecticide	Double-blinded placebo-controlled randomized control trial among outdoor workers (n=127 in first year; n=101 in second year) in North Carolina, USA, over two tick seasons. Primary outcome: tick bites. Secondary outcome: tick encounters (ticks crawling on clothing or subject)	Protective effectiveness (tick bites): year 1: 82% (95% CI: 66-91%); year 2: 34% (95% CI: -64-74%). Overall: 65% protective effectiveness (95% CI: 29-82%) based on a total of 1045 tick bites. Protective effectiveness (tick encounters): reduction in tick encounters (581 vs 286 ticks), chigger bites (Risk ratio = 0.66 to 0.71 (year 1 and 2) and mosquito bites (Risk ratio = 0.66 to 0.56	Perhaps extensive usage (socks)	No adverse events related to treatment of participant`s clothing reported.		Participants used other protective measures like DEET, or self-administered permethrin (also in the treatment group) which could have confounded the results. There were fewer than 70 washings during the study and the loss of efficacy was higher than expected, perhaps due to environmental conditions.

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								(year 1 and 2) in treatment group.				
Faulde et al. 2006	Human apparel	BDUs	Personal protection against vector bites	*Ae. aegypti*; *I. ricinus* (field-collected)	Permethrin (1300 mg/m²)	Contact; Rep./insecticide	Field testing (Afghanistan)	KD time (100% KD; mean KD time). Washing: 3 to 50% of original permethrin content washed out depending on impregnation method		Washing (ISO 6330); Cross contamination	Water organisms	Cross contamination: 6 months storage: 32 to 400 mg/m² when washed together and 10 to 65 mg/m² when stored together, and <0.2 to 2 mg/m² when stored not in contact to each other, according to impregnation method.
Ticks												
Mitchell et al. 2020	Human apparel	Socks, shirt, pants, hat	Personal protection against tick bites	Primarily *I. scapularis* ticks (field test)	Permethrin, administered by Insect Shield LLC.	Contact; Rep./insecticide	Double-blinded placebo-controlled randomized control trial among outdoor workers (n=82 persons in first year; n=51 in second year) in Rhode Island and Massachusetts, USA, over two tick seasons. Primary outcome: tick bites. Secondary outcome: tick encounters (ticks crawling on clothing or subject)	Protective effectiveness (tick bites): year 1: 65% (95% CI: 45-78%); year 2: 50% (95% CI: 27-66%). Overall: 58% protective effectiveness (95% CI: 43-69%) based on a total of 226 tick bites. Protective effectiveness (tick encounters): year 1: 36% (95% CI: 31-42%); year 2: 46% (95% CI: 40-51%) Overall: 41% (95% CI: 37-44%)	Perhaps: temperature, UV exposure, or perspiration	No adverse events related of participants reported.		Participants used other protective measures like DEET, or self-administered permethrin (also in the treatment group) which could have confounded the results.
Kime 2019	Human apparel	BDU		Ticks			Literature review on field studies of impregnated uniforms against ticks					
Fourie et al. 2019	Animal	Dog collar	To protect against tick bite and disease transmission	*D. reticulatus*			Parallel, randomized, negative controlled efficacy study according to good clinical practice. 9 test dogs and 32 control dogs, caged. Monthly tick challenges up to 8 months: 50 *D. reticulatus* adults (8% infection rate with *Babesia canis*) and the dog were place in an infestation crate for 1 h. Tick count on dog after 48 h.	Acaricidal efficacy (corrected for control) and percentage prevention of transmission efficacy.				
Prose et al. 2018	Human	T-shirts	Personal	*I. scapulari*	Permethrin	Contact;	Contact irritancy test: Playing card (64 x 89 mm) at 45° angle, with test textile sewn	Contact-irritancy test: % of dislodged ticks and ticks with	"Fuzzy"			Laboratory and field-collected *I. scapularis*

Reference	Use type	Article category	Intended use/ Claim	Target species	Active substance	Mode of action	Test system/purpose of publication	Efficacy level/Results	Efficacy parameters	Exposure parameters	Non-target effects	Miscellaneous
	apparel	, pants, long-sleaved shirts, socks (Insect Shield)	protection against tick bites	*s; A. americanum; D. variabilis*		Rep./insecticide	on it. 5 to 10 tick nymph) placed in centre and number of remaining ticks counted every min for 5 min. Mortality: 24 h later. Toxicity test: 5 to 10 nymphs or 5 adults in continuous contact with textile for 1, 2, or 5 min. Mortality: 24 h later Classification of ticks: 1) completely motionless 2) movement, but unable to right itself or walk 3) right itself, but uncoordinated movement or no orientation to stimulus 4) normal movement and response to stimulus. Only ticks in 4) further "finger ascension assay" to determine of ticks ascend to a finger	different KD classifications (see Test system). Toxicity test: % of ticks able to move normally after 1 min exposure (1 h after exposure); Exposure time to induce loss of normal movement 1 h after test. Recovery rate 24 h later. After 5 min all ticks lost ability to move normally 1 h post exposure. Tick species differently susceptible: *I. scapularis* nymphs > *A. americanum* nymphs > *I. scapularis* females > *D. variabilis* females > *A. americanum* females	surface of fabric (socks) can prevent ticks from dislodging from a vertical (45°) surface.			showed the same results, but field-collected nymphs with better climbing behaviour? Only minor differences between types of fabric fibres.
Eisen et al. 2017	Human apparel	Unwashed Insect Shield textiles		*I. scapularis* nymphs 4-5 months post moult	Permethrin, DEET (pos. control)	Contact/gas phase; Rep./Acaricide	Four scores for tick vigour: 1. completely motionless, 2. leg movements but unable to right up, 3. able to stand up, but uncoordinated movements, 4. normal walking. Finger assay: ticks placed in front of a finger placed vertically on a glass surface. The first finger phalanx is untreated (tick introductory zone), the second wrapped by test or control fabric (25 mm wide): insufficient numbers of ticks climbed onto control fabric. Playing card (64 x 89 mm) assay: textile on card and test textile (13 mm wide) on the upper half (card held vertically at 45° angle). A finger on top served as stimulus for ticks placed on the bottom side to climb up. Horizontal petri dish assay with one half treated and untreated zone (filter paper) and introductory zone (untreated piece of filter paper, 15 x 15 mm) in the middle. One finger each placed 10 mm away from this zone on untreated and treated side.	Primary effect: contact irritancy rather than spatial repellency. In vertical assays, ticks dislodged within <5 min. Tick exposure time longer on treated surfaces at an 45° angle compared to a 90° angle. Control ticks after exposure of 120 s on vertical untreated fabric: 29 of 30 climbed an untreated finger. Directly after contact times of 10 to 120 s, test ticks displayed normal behaviour, but did not climb the finger (n=120 ticks). Willingness to ascend finger only 1-2 hours after exposure restored (only in ticks with normal movement). After 7 d, ticks either dead or showing normal movement and ascend the finger (120 s exposure). After 10 s				Continuous contact assays (as in papers by Faulde et al) may produce better standardized data, but do not show effects already evident after exposure times too short for KD effect to occur.

Reference	Use type	Article category	Intended use/ Claim	Target species	Active substance	Mode of action	Test system/purpose of publication	Efficacy level/Results	Efficacy parameters	Exposure parameters	Non-target effects	Miscellaneous
								exposure, ticks recovered after 1 d.				
Rossbach et al. 2016	Human apparel	Work clothes from different suppliers, cut-resistant or not.	To protect against tick bites	*I. ricinus* (outdoor)	Permethrin (1250 to 1500 mg/m² according to supplier)	Contact; Rep./insecticide	16-week case-control study in German forestry workers to bio-monitor permethrin uptake when wearing treated pants (n=82 persons) versus untreated ones (n=82). Washing was done privately according to washing instructions. Permethrin metabolites were measured in urine. Urinary elimination half-life of permethrin in human body: 30-40 h. acceptable daily according to WHO 1999: 50 mg/kg body weight.	Test group with higher metabolite excretion than control group (even before start of test). Metabolite levels highest in the first week of tests, declining thereafter. Differences between distributors of pants, and higher excretion in cut-protected pants. Mean daily permethrin uptake calculated as 1.9 µg in the control and 27.5 µg in workers using treated pants (20 time higher). But uptake was 100fold lower than ADI.		washing, distributor of fabric, wearing underwear		Most uptake of permethrin should be dermal, inhalative uptake is negligible. Cited: the calculated risk for cancer is 3 cancer cases in one million population when wearing the pants a lifetime during working hours.
Faulde et al. 2015	Human apparel	BDU (blouses and trousers)	Personal protection against tick bites	*I. ricinus* (outdoor)	Permethrin 1300 ± 300 mg/m²	Contact; Rep./insecticide	Field trial: Determination of tick density at all training sites and study years (very useful to determine risk of tick-bite) revealing mean tick densities between 28.9 and 106.5 ticks/100 m². Washing according to EN ISO 6300:2000.	Tick bite incidence 2009 (untreated uniforms; 8.8%, 262 tick bites, n= 2977 military personnel, 66.679 tick-exposure days (TED), 0,39% tick bites/TED). Tick bite incidence 2010 (treated uniforms used; 0.035%, n= 2885 military personnel, 63.571 TED, 0.0016% tick bites/TED) and 2011 (0.078%, n= 1289 military personnel, 0.0056% tick bites/TED). Protective effectiveness 2009 to 2010: 99.6%, and 2009 to 2011: 98.6%. Overall effectiveness 2009 to 2010/2011: 99.4%.		No. of washings, training conditions		Decline in effectiveness from 2010 to 2011 was very low (despite long usage of BDUs).
Richards et al. 2015	Human apparel	Clothing (Insect		ticks	Permethrin	Contact; Rep./insecticide	Field trial (Appalachian region, USA)	Cited in Kime 2019: Sample size of 34 participants was too low to detect any statistical difference between				

Reference	Use type	Article category	Intended use/ Claim	Target species	Active substance	Mode of action	Test system/purpose of publication	Efficacy level/Results	Efficacy parameters	Exposure parameters	Non-target effects	Miscellaneous
		Shield)						treatment group and untreated group.				
Ticks, mites, fleas												
Wilder-Smith et al. 2014	Human apparel	Treated clothing, BDUs, nets	Protection from mites and ticks	Biting arthropods	Permethrin	Contact; Rep./insecticide	Review. In most publications, the cone test (WHO) and arm-in-cage tests were used.					Further studies are needed to define the most appropriate guidelines for testing insecticide-treated clothing
Dantas-Torres et al. 2013	Animal	Dog collar	Protect against bites of ticks, fleas, pathogens	*R. sanguineus* group ticks. C. *felis* fleas	Imidacloprid/Flumethrin	Contact; Rep./insecticide	Randomized controlled field study with 122 dogs (≤6 months old, caged) in southern Italy (July 2011 to April 2012). Monthly examination of dogs for ticks and fleas.	Efficacy against tick attachment and fleas: 99.7% and 100%, respectively				
Stannek et al. 2012	Animal	Dog collar	To protect against diverse arthropods	*C. felis, R. sanguineus, I. ricinus, I. scapularis, D. reticulatus, D. variabilis*, *Sarcoptes scabiei, Trichodectes canis* (biting louse)	Imidacloprid/Flumethrin	Contact; insecticide	Controlled indoor study with dogs. Infestation with ticks/fleas before, and at monthly intervals after using the collar. Larvicidal efficacy (fleas): Dogs (treated or untreated) rested on fleecy polyester blankets placed on the bottom of a transport box for 3 h for 3 consecutive days. Blankets were frozen at -20°C for 24 h, thawed and inoculated with 50 one-day old flea eggs plus 0.5 g flea rearing medium. Samples were incubated at 26 ± 2°C and 75 ± 8% RH for 4 weeks and emerged fleas counted. Effect of shampooing or immersion in water was tested by shampooing dogs every month or immerse them in water for 5 min monthly. Efficacy against mites: naturally infested dogs were treated. Efficacy against lice: naturally infested dogs were treated and number of lice counted before and after initiation of treatment.	Efficacy compared to the control (adult ticks and fleas). Percentage larvicidal efficacy (fleas). Mites: Treatment was counted successful when all of the following applies: no live mites (skin scrapings), complete resolution of skin papules and crusts, >90% improvement of body areas with hair loss at day 90 after start to wear collar.	Shampooing, immersion in water		Swimming in water	Immersion in water reduced longevity of efficacy against fleas: Imidacloprid more water soluble than Flumethrin.
Vaughn et al. 2011	Human	Clothing (sock	Personal protecti	Ticks	Permethrin, admini	Contact; Rep./in	A nonrandomized open label pilot study conducted among 16 outdoor workers (North Carolina) under actual field	68 tick bites in the control (N=7 persons; 9.7 bites per subject), and 6 tick bites in				Subjects in the control group spent a total of 1164 outdoor work

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	apparel	s, shirt, pants, hats and boots)	on against ticks		stered by Insect Shield LLC.	secticide	conditions. Participants completed questionnaires at the start of follow-up (March, 2008) and at the end of follow-up (September, 2008), and tick bites and outdoor work hours were reported on weekly tick bite logs for the entire follow-up period.	treatment group (N=9; 0.7 bites per subject). 57 (83.8%) of reported bites were work related (control group), but only one tick bite (16.7%) work related in treatment group. Subjects in control group: 62 (91.2%) tick bites while wearing self-applied repellent. Of the 6 tick bites in the treatment group, 1 was acquired while wearing Insect Shield–treated clothing, the other five while wearing either self-applied repellent only, or no repellent.				hours during the study period, compared to 1732.5 outdoor work hours spent by subjects in the treatment group.
Mites												
Kim 2017	n.A.	Essential oils on fabric	Protect against house dust mites	*D. farinae*	Microencapsulated Eucalyptus oil	Gas phase; Acaricide	AATCC test method 194–2007: Treated fabrics (5 0 mm diam.) and 50 mites placed together with 50 mg nutrient mixture into a petri dish (100 mm diam.). The rim of the dish was coated with sticky gel and the petri dish covered by a mesh with <50 µm pore size. Test duration: 72 h at 25°C and >65% RH. Three replicates.	98% mortality in test and 0% in negative control.				
Jeon et al. 2017	n.A.		Protect against house dust mites	*D. pteronyssinus, T. putrescentiae*	Essential oils	Gas phase; Acaricide	Mite mortality test: Fabric disc and filter paper assay. Fabric impregnated with test substance, dried, and 20 mites added for 24 h at 26±1°C. 3 Repetitions. Negative and positive controls: acetone and benzyl benzoate.	Determination of LD_{50} and LD_{90} by probit analysis.				
Nechita et al. 2015	n.A.		Protect against poultry red mite	*D.gallinae* (poultry red mite)	Essential oils	Rep.	Repellency test: a circular rubber ring (OD: 45 mm) was placed on filter paper of the same size and both encased by two pieces of glass. A small arena (0.81 cm diam.) on the filter paper inside the ring was treated with test substance (2 µl) and a single mite introduced and video recorded for 30 min	Percentage of time spent on treated area <20%.				

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Steidle et al. 2014	n.A.		To bait house dust mites	*D. pteronyssinus* (European house dust mite)	Pentadecane, neryl propionate, (Z)-8-heptadecene	Attractant	Describes a possible test system for long range (1 m) attractants for house dust mites.					Long-range attractant test.
Rahel et al. 2013	n.A.		Protect against indoor mites	*D.pteronyssinus, D. farinae, T. putrescentiae, Acarus siro*	Chitosan/metal on fabric	Acaricide	Mite mortality test: Filter paper (10 mm diam.) plus test fabric placed in a 25 ml vial. 100 µl water added to increase moisture. 10 adult mites added, the vial closed and mortality determined after 24 h using a dissecting microscope. 10 repetitions. Control: untreated fabric and fabric with Chitosan only (without Ag)	Control mortality: 15%. Test mortality: ≥80%. Adding other metals like Cu or Zn caused no, or lower mortality.				Ag+ ions responsible for mite mortality with efficacy close to acaricides like benzylbenzoate or permethrin.
Mahakittikun et al. 2009	Human body	Mattress liner		*D. pteronyssinus*	Acaricide (not specified)		53 mite-proof covers from 10 countries tested, mainly physical barriers, but 2 with acaricides. Test of mite/allergen penetration with heat escape method: Ten adult mites placed on the inner or outer surface of test fabric stretched over a 50 ml beaker filled three quaters with water. A 60 W light bulb placed over the mites and illuminated for 15 min. Continuous observation whether mites penetrate the fabric to escape the heat. Three repetitions for each side of the fabric (=60 mites in total)	Percentage of fabric penetrated by mites. Authors think that only tightly woven fabrics can provide sufficient barrier against mite and allergen penetration.				
Wongkamchai et al. 2005	n.A.			*D. pteronyssinus*	Different pyrethroids	Contact; Acaricide	Mite mortality test (basically an adaptation of the tick larval package test): treated filter paper (2.5 x 2.5 cm) placed on a glass slide (5 x 5 cm) and an o-ring (2 cm diam.) placed on it. 20 adult mites placed on the filter paper and another treated filter paper placed on top as well as a plastic slide (5 x 5 cm with a central 2 cm hole). Clips keep the system together. Incubation at room temperature and 75% RH for 24 h. Mite mortality thereafter determined	No mite escaped and no mortality in the control was observed. New test setup very successful. LD50 values for different pyrethroids were determined.				

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							using a dissecting microspcope. Three replicates and untreated controls.					
Cameron & Hill 2002	Treated article	Mattress liner	Reduce number of house dust mites	House dust mites	Permethrin 450 mg/m²	Contact; Acaricide	Placebo-controlled blind field trial. Volunteers collected dust samples before and at certain intervals after test start to obtain mite counts and allergen levels. Method of mite recovery from dust samples: 100 mg dust plus 45 ml water in a baker were inverted 20 times and frozen at -20°C for 1 h. The upper layer of the ice cube contains the mites which are counted in a petri dish. N=18 volunteers with sufficient mite infestation received a treated or placebo mattress liner.	Number of mites per gram dust was significantly lower for 27 months, whereby at 5 months only 1% of mites were collected compared to the placebo control. From a medical point of view: mite level should be <100 mites/g dust and allergen level (Der p1) < 2µg/g dust.		Should be: body heat, sweat, very close contact to liner.		Mite sampling per unit surface might be more appropriate than per weight unit of dust (discussed in literature). No information on whether or not the liners were washed during study
Nordenfors et al. 2001	Animal	Acaricide clip put on chicken or in stable	Releases AS when in contact to hens for two years	*D. gallinae*	Permethrin strip: weight: 6.5 g, with 10% permethrin and 6% PBO	Contact; Acaricide	Field test in two chicken farms (Sweden). Site A: 1 strip for 5 hens who could contact the strip (500 hens plus control). Site B. strips placed in the stable outside the reach of hens. Mites trapped and counted during the study.	Mite numbers declined during the first weeks, but then remained stable. Test was confounded by increasing acaricide resistance of mites.				
Fleas												
Su et al. 2014				*C. felis*		Rep.	Repellency assay: two filter paper strips, one impregnated with test substance in ethanol, the other with ethanol only were glued together, placed in a vial and a cat flea added and the distribution of fleas on each half recorded.	>90% repellency in certain substances.				
Bed bugs												
Van der Pan et al. 2019	Human body		To protect from bed bugs	*C. lectularius*: several strains (resistant/ susceptible). Adults of	α-cypermethrin; bendiocarb	Contact; insecticide	Novel simulated-use test: 3-compartment test system: a container (30 cm diam., 20 cm height) serves as release site for bed bugs. It is connected through a pipe (5 cm diam.), to an acrylic box (10 x 10 x 20 cm) the floor of which is lined with test fabric. This, in turn is connected via a further pipe (5 cm diam.) to a steel container (35 x 35 x	3-compartment test: >80% of bedbugs crossed the test surface resulting in >96% mortality (α-cypermethrin). Bendiocarb: 54-72% of bedbugs crossed the surface resulting in 28-46% mortality. No-choice test:				3-cmpartment test suitable to test barrier efficacy of treated articles. Contact time (time to cross the barrier) may also be determined under red light. Test system may

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				uniform age (no more difference than 7d) at 7-9 d after their last blood-meal.			50 cm) at a height of 20 cm. Below the end of the pipe, a trap container (glass aquarium) catches any bed bugs that walked through the pipes and traversed the test fabric. CO2 (0.75 l/min) as attractant is introduced into the steel container, and 300 ml of water in an Erlenmeyer flask heated to 80°C to heat and moisturize the air. Air is sucked out at the release container. 100 bed bugs (50 females and males each) are transferred within a harbourage to the release container and released after 15-30 min. Bedbugs are removed after 24 h and mortality determined immediately and further for up to 7 d. Three replications with a total of 300 bedbugs. As a control 50 females and males each are stored in petri dishes in direct proximity to the test apparatus. No choice surface test: 6 female and 6 male bed bugs placed on test fabric and held in place by a glass ring (2 cm height, 8 cm diam.) for 2 h. Three replicates and controls. 24-well filter paper contact bioassay: 1.6 cm diam. test fabric placed on the bottom of wells of a 24-well plate. A bed bug is introduced into each well (18 test wells and 6 controls for each plate) for 24 h and mortality determined (for up to 7 d). Five replicates (90 test and 30 control animals) for each test fabric (or test concentration).	100% mortality (α-cypermethrin) and <56% mortality (bendiocarb). 24-well test: Determination of EC_{50} for each bedbug strain.				be used to determine repellency (percentage of bedbugs crossing the barrier). After contact with bendiocarb (simulated-use test): mortality in male bedbugs was significantly higher than in female ones.
Kells & Hymel 2016	Human body	Mattress liner	To protect against bed bug bites	*C. lectularius* (fed 7 days prior to test)	Permethrin	Contact; Rep./insecticide	Adsorption of permethrin by bed bugs as a function of time and distance walking on mattress liner. Test insects: bed bugs, held individually in clean cage 24 h before test. Test arena: 9 cm diameter modified petri dish. Bed bug walking speed and distance recorded with infrared video recording. Exposure time was pre-set. After exposure, bed bugs were analysed for permethrin uptake by gas chromatography.	The distance moved was unaffected by treatment and increased up to 50 min test time. ANVOVA: exposure time was a significant class effect and distance walked a significant covariable. Permethrin uptake was 15.1, 21.0, 42.0 and 55.0 ng/insect after 1, 10, 50, and 200 min of exposure, respectively.				Distance walked and time of exposure significant parameters for permethrin uptake for bed bugs walking on treated fabric.

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								Further uptake after 50 min was not significant.				
Jones et al. 2015	Human body	Mattress liner (Active Guard)		*C. lectularius* (5 strains)	Permethrin	Contact; insecticide	Feeding success and fecundity (number of eggs laid) after a brief exposure (10 min) to treated fabric was investigated in pyrethroid resistant and susceptible bed bugs. Virgin females individually placed into petri dishes lined with fabric for 1 or 10 min and thereafter offered a blood-meal (artificial feeding) and weighed before and after feeding period (30 min). N=40 bedbugs/strain. Each female was mated with a fed male and egg production and egg hatch observed.	Proportion of feeding bedbugs was significantly lower after 10 min exposure on treated fabric (predicted probability to feed: 0.87 vs 0.17 according to logistic regression analysis). Odds ratio for feeding attempts. This applied to all strains. Blood meal size sig smaller after 10 min exposure to treated fabric and only one female produced eggs.				Short exposure, insufficient to induce KD nevertheless significantly reduced blood-feeding.
Shikano et al. 2015	Human body	Mattress liner (Active Guard) plus spores		*C lectularius*	Permethrin plus fungal spores	Contact; insecticide; biological	Petri dish assay: 15 min contact time, forced contact. Tests after different times after application of spores.	Bedbug mortality and survival time. Spore survival time.				Fungal spores may act on pyrethroid resistant bedbugs.
Wang et al. 2013	Treated article	Repellent barrier		*C. lectularius* (4 strains)	Different Ais	Rep.	Petri dish assay (filter paper, treated, untreated, exposure time 2 and 24 h (in dark cycle). Arena repellency assay: a stool (26 x 26 cm) placed on an arena (80 x 75 x 5 cm, paper as walking substrate, rims to prevent escape). Under each chair leg, an interceptor was placed, its outer side (height: 2.2 cm) lined with test fabric. Bedbugs, inside a harbourage, placed in the centre of the arena held in place by a plastic ring (13.3 cm diam., 6.3 cm heigh). After 1 h, bed bugs were released and pressurized CO2 released at 100 ml/min on top of chair. Bed bug number in interceptors determined after 2-3 h (one interceptor untreated as control). Room	Petri dish assay: only 5% DEET caused 100% repellency after 2 and 24 h. Arena assay: 50 to 80% of bed bugs were trapped in the control and only <25 in tests according to substance tested.				

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							ventilated to maintain low ambient CO2 levels.					
Wang et al. 2013	Treated article	Band sprayed with insecticide	Part of IPM against bedbugs.	*C. lectularius*	Cyfluthrin	Contact; insecticide	Laboratory study: interceptor coated with fluoropolymer to prevent bedbug escape. A wooden rod (16.5 cm tall, 3.5 cm diam.) placed on it, wrapped with a 3.8 cm wide band of sports layer (bedbugs can walk on it) treated with cyfluthrin dust (5 mg/cm²) and a 2 cm band of smooth tape. A 3.7 cm plastic dish containing bedbugs placed on top. Bedbugs walking down cross the insecticide band and may fall into interceptor. The smooth band prevents re-entry of bugs. Four interceptors plus rod (2 untreated, 2 treated) placed in an arena (80 x 75 cm) and a CO2-source (100 ml/min, 3 h per day) placed in the middle. 4 Arenas were used (8 replicates). 15 adult and 15 nymphal bedbugs starved for 1 week conditioned inside the dish for 24 h before test. Field study: apartments with at least 9 bedbugs/2 weeks/interceptor were selected. Application of insecticide bands around legs of furniture, sofas, etc. compared to control. Bedbug counts with interceptors.	Lab trial: bedbug mortality compared to control. Field study: decline of bedbug counts within three months of operation.				Bed bugs did not avoid insecticide-treated bands.
Jones et al. 2013	Treated item	Mattress liner (Active Guard)		*C. lectularius*	Permethrin	Contact; Rep./insecticide	Test of resistant and susceptible bedbug populations. Contact test: plywood panel (15.2 x 15.2 cm) covered by test fabric. A ventilated petri dish (10 cm diameter; 2.5 cm high), the inner wall coated with Fluon, kept bedbugs in place. Bedbugs assessed after 1 h, 4 h, 1, 3, 6, and 10 d. Repellency assay: Petri dish assay with one half of bottom treated and untreated fabric. Individual bug released in centre and its movement recorded (video: 2 samples/s) over 12 h in the dark phase. N= 24-36 replications. Feeding inhibition assay: 10 bedbugs placed in a cylindrical chamber (5.5 cm dia.; 3.8 cm height), aperture covered by test or control fabric	Contact test: % mortality (corrected) >90% already after 1 d, except resistant strain. Repellency assay: time spent on treated/ untreated area: there was no difference, hence no repellency in all strains tested. However, distance travelled was always larger on control surfaces than on treated ones (except resistant strain), while walking speed was lower on treated surfaces. Feeding inhibition assay: feeding success				Feeding rate in bedbugs probably depends on laboratory strain used. Field-collected strains may feed at much lower rates on artificial feeding system.

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							and placed on parafilm-covered blood-filled feeding device (placed on the side) for 30 min. Count of fed bedbugs and their condition assessed after 1, 2, 4, and 7 d. Five replicates. A test is valid if control mortality ≤15%.	approx. 80% or more on untreated fabric and significantly lower (between 2 und 50%) on treated ones. Mortality after feeding between 4 and 83% according to strain.				
Moore and Miller (2006)				*C. lectularius*	Insecticides (0.02% to 0.06%) applied until run-off	Contact; Rep./insecticide	Petri dish assay: no-choice and choice (repellent) assays with susceptible strain.	Time to 50% mortality (less than 60 min in λ-cyhalothrin, bifenthrin, deltamethrin and permethrin). No repellent effect in any of the substances.				
Lice												
Benkouiten et al. 2014	Human apparel	T-shirt, socks, underpants	To cure from pediculosis	*P. humanus humanus*	Permethrin	Contact; Rep./insecticide	Double-center, randomized, double-blind, placebo-controlled trial. A commercial 8% permethrin solution was used according to instructions: clothes soaked in solution for 15 min. Participants received underwear on day 1, 15, and 45. Lice: PCR screening for three mutations responsible for pyrethroid resistance.	Significantly more (28%) persons free of body lice in the treatment group than in the control group (9%) on day 15. No such difference at day 45.		Permanent contact		The percentage of resistant lice increased during study
Sholdt et al. 1989	Human apparel	BDU	To protect against lice	*P. humanus humanus*	Permethrin 0.125 mg/cm²	Contact; Rep./insecticide	Test 1: Field collected lice (Peru; 10-20 per test) placed on sheets of treated fabric (inside petri dish). KD observed at 5 to 15 min intervals until all lice immobilized. Observation of lice 12 h later. 5 replicates with 20 lice each. Test 2: Laboratory tests with lab lice and fabric washed several times. Lice: 0, 15, 30, and 60 s exposure on treated fabric and observation at 0.5, 1, 6, and 12 h post exposure. Effect on feeding behaviour: 50 lice exposed to fabric for 60 s and allowed to feed on rabbit.	KD_{50} and KD_{100} in 45 and 75 min, respectively. 100% KD even after 15 s exposure (6 h later). Effect on feeding behaviour: 15 min after a 60s exposure, no louse was able to grip the rabbit and all were dead after 24 h.	Washing			
Risk assessment												
Proctor et al. 2020	Human	BDU			Permethrin at 0.073		Studies with military personnel to evaluate dermal permethrin uptake (uniforms) in high- and low temperature conditions.	High temperature conditions resulted in significantly higher (2-3 times) permethrin		Dermal uptake; high		Cited: US Army requires permethrin contents of unwashed

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	apparel				to 0.096 mg/cm²		N=32 volunteers in 4 groups: continuous wear time (33 h), and 3 days 8 h wear time, both at high (35 °C, 40% RH at day, and 30 °C, 50% RH during night) and low (3 °C, 80% RH at day, or 13 °C, 60% RH during night) temperatures. All groups performed standardized physical activity and were medically monitored. Only hand, feet, and face washing allowed during study.	uptake. Possible causes: more release of permethrin from uniform through sweating, or increased dermal absorption. No acute health impacts or difference in cognitive performance between groups. Calculated exposure levels 7-15 times below ADI (50 µg/kg/d) for permethrin.		temperature		uniforms between 0.095 mg/m² and 0.135 mg/m² leading to 99-100% bite protection for up to 50 launderings.
WHO 2019	Human apparel	Insecticide treated clothing					Generic risk assessment model for insecticide treated clothing, skin applied repellents, and household insecticides. Estimates: clothes are used every day. Arms, legs, feet and trunk are in contact with clothing, no underwear. Risk assessment for these products must reflect the highest dose of active ingredient that could be used in practice, based on realistic behaviour. Calculation of daily dermal exposure (Box 1). Oral route: hand to mouth transfer (Box 2) and direct suckling (infants; see Box 3). Dermal exposure during washing (Box 4) and oral exposure during washing (Box 5). An example is given for treated clothes in part 4. Part 6: Risk assessment for self-administration to clothes.	Insecticide treated clothing: dermal route of exposure most relevant. Unless insecticide has a high vapour pressure, inhalation route is negligible. Typical dislodgeable fraction (dermal route) is 0.8% (rabbit model). If no data are available a default of 6% is assumed. Body surfaces are given for several age groups of humans. The wash-resistance index describes the amount of insecticide available for transfer.		Dermal route		Three steps of risk assessment: Hazard assessment (possible toxic effects and dosage levels), Exposure assessment (all relevant routes of exposure on a "realistic worst-case scenario", misuse excluded, but e.g. no protective gloves used, etc.), Risk characterisation (comparison of exposure estimates with acceptable exposure levels)

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WHO 2018	Mosquito net	Mosquito net					Generic risk assessment model for insecticide treated mosquito nets. Exposure assessment for: 1. Sleeping under net (inhalation, contact, chewing (infants)) when net is used every night (Box 1) 2. Dermal contact to net assuming that one third of body surface may be in contact with net, using known dermal penetration rates or defaults (Box 2). 3. Oral exposure, (Box 3 and Box 4). Washing of the net (adults and children) (Box 5 and Box 6). 4. Exposure via breast milk, (Box 7 and Box 8). Exposure scenarios for self-treating nets (Box A1 to A6).	Dermal route of exposure is most relevant. Default dermal uptake of pyrethroids (if no data available): 10%.				Three steps of risk assessment: Hazard assessment (possible toxic effects and dosage levels), Exposure assessment: Risks estimated for adults, children (aged 6–11 years), toddlers (aged 12–24 months), and infants (aged < 12 months). Exposure via mother's milk estimated for infants and new-borns. Risk characterisation: comparison of exposure estimates with acceptable exposure levels.
US EPA 2009							Re-evaluation of permethrin. Assumption: wearing permethrin-impregnated clothing for 250 days/year.	Permethrin considered likely to be carcinogenic to Humans by the oral route. The cancer risk estimates are 1.2×10^{-6} and 3.6×10^{-6} for military personnel and garment workers, respectively when wearing impregnated clothes.				A lot of toxicological data for humans and non-target organisms are presented.
Aylward et al. 2018							The analysis presents a tiered screening approach to the interpretation and assessment of urinary biomonitoring data for 3-phenoxybenzoic acid (3-PBA). Daily urinary output for adults assumed as 24 mg/kg body weight (conservative estimation).					ADI-values for different pyrethroids given. Values are given for estimated chronic urinary excretion rates per gram pyrethroid incorporated.

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Kegel et al. 2014	Human apparel	BDU			Permethrin (0.13 mg/cm²)	Contact; Rep./insecticide	Permethrin uptake by wearing BDUs (treated and untreated) compared between personnel in Germany and Afghanistan (median: 90 and 56 day of wearing uniform in study I and II, respectively). Three urinary metabolites were measured.	Control subjects: median metabolite sum between 0.18 to 0.24 µg/L with no difference between location in Germany or Afghanistan, comparable to the general public in Germany. Test subjects` metabolite sum: 23.67 µg/L. Smokers and subjects in Afghanistan showed higher metabolite levels.		Washing; Smoking (hand to mouth contact); Transpiration?	.	Cited: permethrin labelled as group 3 (‘not classifiable as to its carcinogenicity to humans’) by the International Agency for Research on Cancer. The longer the wearing period, the lower the permethrin uptake (probably because of several washings).
Proctor et al. 2014	Human apparel	BDU			Permethrin 0.101 to 0.125 mg/cm² after one washing.		Studies with military personnel to evaluate dermal permethrin uptake when wearing uniforms. N=6 volunteers with weartime 31 h (study A) continuously and n=11 volunteers with weartime 8 h daily for 3 days (study B).	Calculated daily dose was 0.31 to 14.17 µg/kg and 1.05 to 3.37 µg/kg in Study A and B, respectively.		Showering may reduce exposure levels		Initial detection of urinary metabolites beginning at 6-10 h after putting on the uniform.

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Appel et al. 2008	Human apparel	BDU			cis:trans-permethrin 25:75 at 1300 mg/m²		Two field studies with German soldiers in Germany and Afghanistan wearing untreated and treated BDU (the same study as in Kegel et al. 2014) measuring urinary metabolite levels. An overview of toxicological aspects of permethrin is given.	Test subjects ≈ 200fold higher metabolite levels than control subjects (their metabolite levels the same as in the general public of Germany). Soldiers in Afghanistan had higher metabolite levels than soldiers in Germany. Test subjects more effects like redness, itching of skin, swelling, rash. Maximum internal exposure estimated as 5-6 µg/kg BW (= 5-fold below the values an ADI would produce). Assuming a dermal absorption of 2%, an exposure of 250 µg/kg/day was calculated (=18.75 mg/day for a 75 kg person). A uniform-skin contact area of 1.5 m² leads to an exposure of 1.25 µg/cm²/day, still 100-fold below the value of 130 µg/cm² causing paraesthesia (Flannigan & Tucker, 1985). From this, a release rate of 1% per wearing event is calculated.				Permethrin cis-isomer is more toxic than trans-isomer. Oral LD_{50} in rats: 6000 mg/kg BW in the 20:80 cis:trans-isomer and 220mg/kg in the 80:20 cis:trans-isomer (WHO, 1990, 1999). Half-life in human: 5 d (trans-isomer), 10 d (cis-isomer) (single oral dose). Dermal absorption through skin is rate-limited. An ADI of 0.05 mg/kg BW suggested by WHO (1999).

Reference	Use type	Article category	Intended use/ Claim	Target species	Active substance	Mode of action	Test system/purpose of publication	Efficacy level/Results	Efficacy parameters	Exposure parameters	Non-target effects	Miscellaneous
Macedo et al. 2007	Human apparel; mosquito net	Self-impregnate uniforms and nets	Protection from mosquitos	Mosquitos	alpha-cypermethrin, cyfluthrin, lambda-cyhalothrin, d-Phenothrin, Permethrin, Resmethrin, Piperonylbutoxid	Dermal contact, dermal exposure, inhalation; Rep./insecticide	Quantitative risk assessment of human health risks associated with mosquito management tactics focused on acute, sub-chronic, and chronic exposures after insecticide application in different scenarios. Acute exposures were defined as single-day exposures after a single application or use of the chemical. Sub-chronic exposures were defined as the daily exposure over 180 d with multiple spray events. For chronic exposures, it was assumed that personnel might be deployed for 250 d/yr for 10 yr.	Dermal exposures through contact with BDUs: 0.066 mg/kg BW/d (acute, sub-chronic), and 0.045 mg/kg BW/d (chronic). The greatest cancer risk estimate was 8.64 x 10^{-6}. Potential dermal exposures through contact with bed nets ranged from 3 x 10^{-4} to 0.177 mg/kg BW/d (acute), 1*10^{-4} to 0.059 mg/kg BW/d (sub-chronic), and 8.06*10^{-5} to 0.04 mg/kg BW/d (chronic). Potential inhalation exposures from sleeping under the nets ranged from 2.45 to 5.87 * 10^{-6} mg/kg BW/d (acute), 1.02 to 2.44 * 10^{-7} mg/kg BW/d (sub-chronic), and 6.99*10^{-8} (chronic).		Dermal exposure, inhalation		Because a tier-1 risk assessment uses very conservative assumptions and parameters are overestimated, the resulting quantitative risk values typically are conservative and err on the side of safety.
Krätke & Platzek 2004		Clothing					Measuring the release of substances from fabric: wash 0.5 g fabrics in 25 ml artificial sweat at 40°C for 60 min at 90 rotations/min. Use acid and alkaline artificial sweat according to DIN 54020. Analyse and quantify at 1 g or 1 cm² of fabric. The highest values are used for exposure calculation.	After 28 wash cycles, the migration rate out of the textile is less than 10% compared to the first washing cycle. Textile dyes have a worst-case penetration rate through skin of 1%, when sweating occurs ≈2%.				Substances >700 molecular weight and/or water/octanol coefficient log <1 and >6 unable to penetrate skin. Examples are given to calculate exposure based on migration of dye from the textile and dermal absorption through the skin.

8 Appendix II – Draft guidance

8.1 General introduction

This draft guidance deals with articles that are treated with insecticides/acaricides, repellents or attractants in order to protect humans or animals from arthropods. These articles are grouped into five categories which are taking both exposure and efficacy into account. These categories will be treated separately in this guidance:

1. Human apparel, including all types of clothes and shoes,

2. Articles for human use, including all articles and devices other than clothes or mosquito nets that are either used close to the human body, or used indoors,

3. Articles for outdoor use,

4. Articles to protect animals,

5. Mosquito nets.

The efficacy studies should normally be performed according to established guidelines. These may be international, EU or national guidelines. If no specific guidelines for the treated article are available, tests should preferably be adapted from existing guidelines describing test systems for a similar usage, particularly existing tests in PT18 and PT19 of the BPR. For example, repellents to be used on human skin, effective against mosquitos, can be tested by the arm-in-cage test, a well described test method. The same test system, with only minor modifications, may be used for treated clothes with a repellent effect against mosquitos.

If no guidelines are available that can be adapted, the applicant may use elements of their own methods (intra-company Standard Operating Procedures, Test Protocols or Study Plans), provided, however, that the study plan and report are scientifically robust, well reported and provide clear and scientifically based results. The test methods and the test conditions applied must clearly and fully be described and must address the efficacy claim appearing on the SPC.

Due to ethical reasons, for products applied on humans or animals, field trials are not required, particularly for ticks that can act as vectors. It is therefore not recommended to perform field trials with treated articles against ticks, as the infective status of these cannot be known in the field., For other organisms however, field trials can serve as additional information. If suitable simulated-use tests are not available, field trails should be conducted. They should be conducted in an area with high target organism density, and at a time when the relevant species is active, preferably within Europe. As true replication is almost certainly impossible in field trials, a full description of any factors that might be expected to influence product performance should be given. These are intended to provide the authorities with information to assist with the interpretation of the results obtained.

8.2 Claims

A clear label claim should be submitted. The label claim should precisely describe the effect of the treated article on the target organism and on the user.

It is required that the claim describes how and where exactly the product will work. If there is no spatial effect, it should be written on the label, that protection extends only to the area covered by the product and not to other areas (e.g. uncovered body parts of humans or animals).

If there is any delay between application of the product and commencement of full efficacy, this must also be mentioned (e.g. "…needs 48 h to reach full efficacy").

In addition, the label should state whether the product repels and/or kills target species. If only a repellent effect is claimed, no enhanced mortality after the efficacy test in comparison to the control is expected.

For specific claims (e.g. efficacy is claimed when humans or animals are under physical exercise), efficacy must be demonstrated in the relevant situations.

In addition, impregnation of fabric may result in an inhomogeneous distribution of active substances (AS) in the fabric (Sullivan et al. 2019; Rossbach et al. 2016). To show the range of AS concentration within the fabric, the applicant should provide suitable analytical data from different parts of the fabric and/or from different batches (e.g. WHO 2013b).

8.2.1 Spatial effect ("halo" effect)

If a spatial or "halo" effect is claimed (i.e. the product protects areas distant from the treated article) this has to be proven in a simulated-use test or a field test. The label ought to describe how far this effect reaches (e.g. "protects the whole (human or animal) body", or "protects an area of 9 m² around the product", or "protection extends up to 30 cm beyond the product"). The label should also indicate by which means the spatial effect is achieved. This can be e.g. by evaporation of the active substances (AS), or by (mechanical) transfer / diffusion of the AS to uncovered (human or animal) body parts, or indirectly by local reduction of host-seeking activity of target species.

8.2.2 Long-term efficacy and washing resistance

To account for how long the article will remain effective under realistic conditions of use, the concept of complete protection time (CPT), known from repellents applied to the skin, should be adopted. The minimum parameter tested should be the number of washings the articles tolerates without losing its efficacy. CPT is then defined as the number of washings until a first confirmed event (e.g. landing for mosquitos or crawling upwards more than 3 cm for ticks) occurs. For example, if fabric is tested after 0, 10, 50 washings, and the first confirmed event occurs after 50 washings, then the CPT of the product can be claimed for 10 washings. Depending on the purpose of the article and the claim, CPT can be extended to other wear and tear factors like UV or abrasion.

Generally, efficacy throughout the lifetime of the clothes is assumed. The following life expectance may be assumed: Trousers, pants, and robust shirts may be used for 2 years (e.g. on 30 weekends/year, washed after each weekend). Thus, as a default, 60 washings (trousers, pants, robust shirts) should be carried out for the assessment of CPT. For jackets a three-year usage is assumed including a monthly washing during the outdoor season, resulting in an efficacy to be proven after 21 washings. Thin shirts are assumed to be used for 20 outdoor weekends, resulting in sufficient efficacy to be proven after 20 washings. Mosquito nets are probably used for no more than 3 years. The WHO (2013b) guidance assumes a number of 20 washes during that time. If nothing is stated on the label, then these default number of washings apply. Deviations from the number of washings have to be justified.

8.2.2.1 Recommended washing method

There are two washing methods available. The DIN EN ISO 6330 is recommended as the standard method for efficacy evaluation of treated clothes. Alternatively, a hand-washing procedure (WHO 2013b) may be used, if applicants can provide convincing evidence, that the

respective article will be only hand-washed by the user, e.g. bed-nets used in some tropical areas. Delicate fabric like bed-nets may also be gently washed inside a laundry bag in a washing machine.

8.2.2.2 Regeneration time after washing

After a washing cycle the superficial layer of AS may be washed off the fabric fibre and the efficacy of the fabric may be diminished until sufficient AS from inside the fabric fibres diffuses out and becomes bioavailable. This is known from mosquito nets and the WHO (2013b) guideline recommends to determine the so-called regeneration time for bed-nets. The phenomenon may likely also occur in fabrics other than mosquito-nets and it is therefore recommended to determine the regeneration time in clothes, too. This is done by performing daily KD-tests after washing. When efficacy increases and reaches a plateau (which may last several days), the time until beginning of the plateau is taken as regeneration time. This regeneration time (if applicable) must be stated on the label (e.g. “after washing it needs 48 h to restore full activity”).

8.2.3 Efficacy at high temperatures

High temperatures may differently affect the efficacy of treated fabric. High ambient temperatures while wearing clothes may increase loss of AS from the fabric and concurrently dermal uptake of it (Proctor et al. (2020). It might also affect efficacy (e.g. KD times) against target organisms. If an efficacy at high temperatures is claimed, this must be proven under the respective temperatures, e.g. at 30°C for tropical areas.

Ironing of fabric at 200°C can significantly reduce efficacy. The label should therefore advise to avoid ironing of treated clothes. If treated clothes are claimed to be resistant to ironing, the respective efficacy must be proven after a number of ironings reflecting the lifetime of the fabric.

8.2.4 Ultraviolet (UV)-resistance

Exposure of treated fabric to natural sun light might reduce efficacy of treated clothing (Banks et al. 2014; Mitchell et al. 2020), most probably caused by UV light. If a UV-resistance is claimed, this must be proven in a suitable test. Laboratory tests must reflect the duration and intensity of UV irradiation most likely to occur in the field (e.g. Richards et al. 2017). The efficacy must be proven after irradiation with an UV dose equivalent to that the respective product would receive throughout its lifetime under field conditions.

8.2.5 Claims for local reduction of target organisms

Products for outdoor use or the protection of cattle and horses claiming a local reduction of target organisms (e.g. tick rolls, mobile insecticidal walls, horse blankets) must be tested in the field. Tests should be performed in well-defined climate and eco-zones and the label should indicate in which climate regions (e.g. temperate, Mediterranean, or tropical) the device is effective. Tests should be performed during seasons when the target organisms are abundant. Methods for monitoring the abundance of target organisms must be scientifically sound. If applicable, also the abundance of possible non-target organisms ought to be monitored in parallel. A precise description of the habitat and monitoring of the abiotic conditions during the test is essential to judge the outcome of the test.

The number of field sites must be sufficient to allow statistical comparison between test and control sites, or between pre- and post-intervention abundance of target organisms. The mean

of the reduction of target organisms should be reported (accompanied by measures of e.g. variance, confidence limits) and this should be stated on the label.

8.2.6 Resistance of target organisms

Insecticide or acaricide resistance in target organisms can profoundly affect product performance and even induce failure of the product. Resistance has been reported from populations of e.g. mosquitos (Dada et al. 2018), horn flies (Oyarzún et al. 2011), human lice (Durand et al. 2012), bed bugs (Dang et al. 2017), fleas (Rust 2016), and ticks (Rodriguez-Vivas et al. 2018). In ticks, resistance is mainly restricted to *R. microplus*, a species that stays on cattle throughout almost the whole of its life cycle.

Efficacy of biocides is usually tested on susceptible target organisms. If, however, efficacy against resistant populations is claimed, then the efficacy of treated articles should be proven in tests with resistant individuals with known resistance level.

8.3 Efficacy tests

8.3.1 Human apparel

Human apparel includes all type of clothes like shirts, blouses, trousers, jackets, including shoes. It can be equipped with insecticidal, acaricidal, or repellent properties and may typically protect against mosquitos and ticks, not excluding other blood-sucking arthropods.

8.3.1.1 Mosquitos

8.3.1.1.1 Test species

For authorisation of treated clothing against mosquitoes, testing should be performed with *Culex* spp., for example *Culex quinquefasciatus* and an *Aedes* spp., for example *Ae. aegypti*.

8.3.1.1.2 Laboratory tests

Clothes should normally be tested in the laboratory with WHO cone tests or tube tests (WHO 2013b, 1998) using female mosquitos. The tests are well described in the WHO guidelines and involve tests of mosquitos of defined age, nutritional status, and insecticide resistance status. Controls should be performed on the same day with an equal number of specimens using untreated clothing. A test is valid if no more than 10% mortality occurs in the control.

Cone test (WHO 2013b)

Cones are placed on test fabrics, and five mosquitoes introduced and exposed to the fabric for 3 minutes (Figure 1). Test conditions: 27±2°C and 75%±10% RH. Ten replicates giving a total of 50 mosquitos should be conducted. Determination of knock-down (KD) at 1 h after exposure and mortality at 24 h after exposure of mosquitoes. The cut-off point is ≥ 95% KD and/or ≥ 80% mortality. A test is valid if no more than 10% mortality occurs in the control.

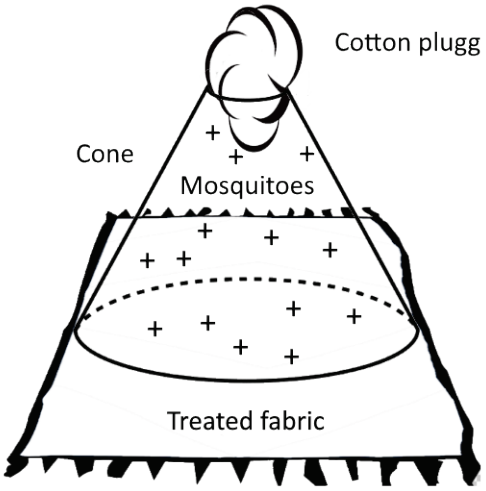

Figure 1. Set-up of a cone test (courtesy of J. Magnér, Swedish Chemicals Agency, 2020).

Alternatively, mosquitos are continuously exposed until 100% KD occurs and the mean ± SD times until KD is recorded. Controls should be kept on untreated fabric for the same time as needed in the test to achieve 100% KD.

The cone test can be used for all clothes that can be laid on a plain surface. There is currently no protocol for a laboratory test with shoes available.

Tube test (WHO 1998)

The tube test according to WHO guidelines can be performed with pieces of cloth lining out the inner wall of a test tube (125 mm length, 44 mm diameter). 20 to 25 mosquitos are introduced and exposed to test fabric for 60 min. Ideal test conditions are 25±2°C (max 30°C) and 70-80% RH with 4-5 repetitions giving a total of at least 100 test individuals. Percent KD is determined after 10, 20, 30, 40, 50, and 60 min, and mortality after 24 h.

Tubes should be kept vertically or horizontally during tests according to the species. For example, using *Ae. aegypti*, test tubes should be kept horizontally, because the species tends to fly upward and rest on the gauze covering the opening of the tube if kept vertically. When *Anopheles* spp. are tested (under dim light), vertically kept tubes are recommended, as these readily rest on the walls of the tube. For other species, a pre-test should determine whether a vertical or horizontal position of the tube induces more mosquitos to rest on the wall.

Cut-off point is ≥98% mortality. A test is valid if no more than 20% mortality occurs in the control.

8.3.1.1.3 Simulated-use tests

A simulated-use test is recommended for product authorisation. This may preferably be an arm-in-cage test, simulating the worst case in terms of biting pressure of mosquitos. If a product claiming a “halo” effect (i.e. protection of body areas not covered by clothing) shows insufficient efficacy in that test, it may be further tested in a room test. If large parts of the body are covered by treated clothing, most free-flying mosquitos may first land on clothing. When contacting such clothing only for a short time, insufficient to induce knock-down, mosquitos may still have acquired a dose rendering them unwilling to bite.

Arm-in cage test

Arm-in-cage tests, or arm-to-cage tests are well described in WHO guidelines, and in the BPR guidance (and revision document TNsG_PT19_Mosquitoes-Repellent-Sim-use-Test_Draft_DE_180821.pdf). The general conditions for arm-in-cage tests as described in the BPR guidance (treatment of human subjects, test conditions, etc.) should be met. Instead of repellent applied to the forearm, this forearm is covered with test fabric and tested in the same way (Orsborne et al. 2016). The number of mosquitos inside a cage must be high enough to provide a minimum number of 20 mosquito landings per min.

A forearm covered by test fabric is inserted into a cage containing mosquitos. Depending on the label claim, the arm can be fully or partly covered by test fabric, leaving additional uncovered skin exposed. It is recommended to standardize the test area on the forearm by covering the arm with material not penetrable for mosquitos, leaving a test area of defined size free (e.g. 5 x 15 cm, see figure 2). The skin of the test area is covered by test fabric or only partly covered if a "halo" effect is claimed and has to be evaluated. The test fabric should be close to the skin to enable mosquitos to reach the skin with their mouthparts. Test conditions (temperature, relative humidity, light conditions, photophase (light/dark cycle), etc.) must be suitable for the test species.

Controls are performed using the other arm of the same test subject. The control arm should be prepared identically as the test arm, except that untreated fabric of the same material or at least the same penetrability (thickness) for mosquitos is used. The control is performed first and should show at least 20 landings/min on the test area. If this is proven, the same test subject can proceed with the test using the other arm. At least 10 subjects (males/females) should be tested. Test duration is 3 min.

Tests are repeated depending on the label claim. For example, if an efficacy period of 6 months or an efficacy after a certain number of washings is claimed, tests may be repeated with fabric worn/weathered for 6 months or after a certain number of washings as described on the label.

As a default, complete Protection Time (CPT) with regard to the number of washings (see 8.2.2) should be assessed.

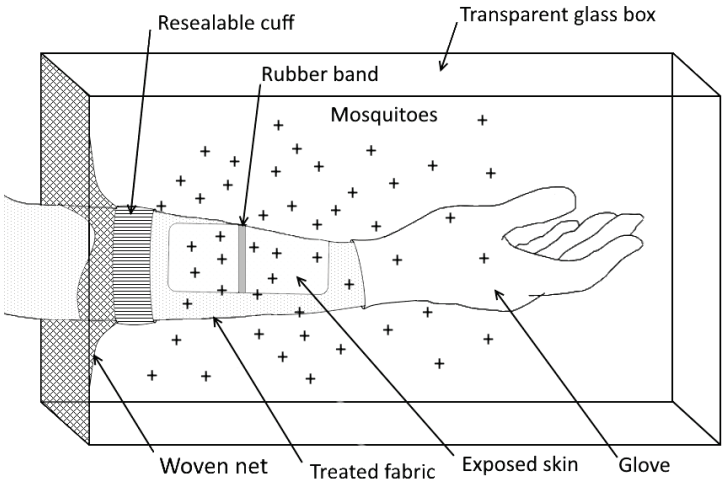

Figure 2. Set-up (schematically) of an arm-in-cage test (courtesy of J. Magnér, Swedish Chemicals Agency, 2020).

Room test

The room test is performed in two adjacent rooms connected by a door. Rooms should be of the size of a Peet-Grady chamber each (1.80 x 1.80 x 1.80 m) or larger, bright coloured to easily monitor mosquitos, and of surfaces easy to clean (e.g. tiles, metals, see figure 3, see also Orsborne et al. 2016). Ventilation should be possible in order to clear off room air from any volatile repellents or insecticides.

A total of 30 female mosquitoes, or more if required, pre-selected for their sufficient motivation to seek a host are used per trial and released in the mosquito room. A test person is sitting in the adjacent room (on the floor or on a chair) wearing the test clothes and some underwear to prevent mosquito bites through the clothes, but leaving some parts of the body uncovered, e.g. the lower legs. For ease of test, a head-net may be worn. A test starts when the door to the mosquito room is opened. Then, the number of landings on the treated clothes is recorded, e.g. by two persons observing mosquitos through two windows from outside to see either side of the person. Mosquito landings on the bare skin are counted by the test person inside the room, and any landing mosquito is aspirated. If reduction of bites has to be evaluated, a mosquito is aspirated after biting. Test duration is 15 min. At the end of the test, all remaining mosquitos are collected and kept at appropriate conditions for survival with access to a 10 % sugar solution. The percentage of knocked- down individuals is counted after 60 min and mortality after 24 h is determined. The test should be repeated 10 times, i.e. with 10 persons, preferably 50 % females and males. Controls are performed under identical conditions but with untreated clothes instead of treated ones.

As a default, complete Protection Time (CPT) with regard to the number of washings (see 8.2.2) should be assessed.

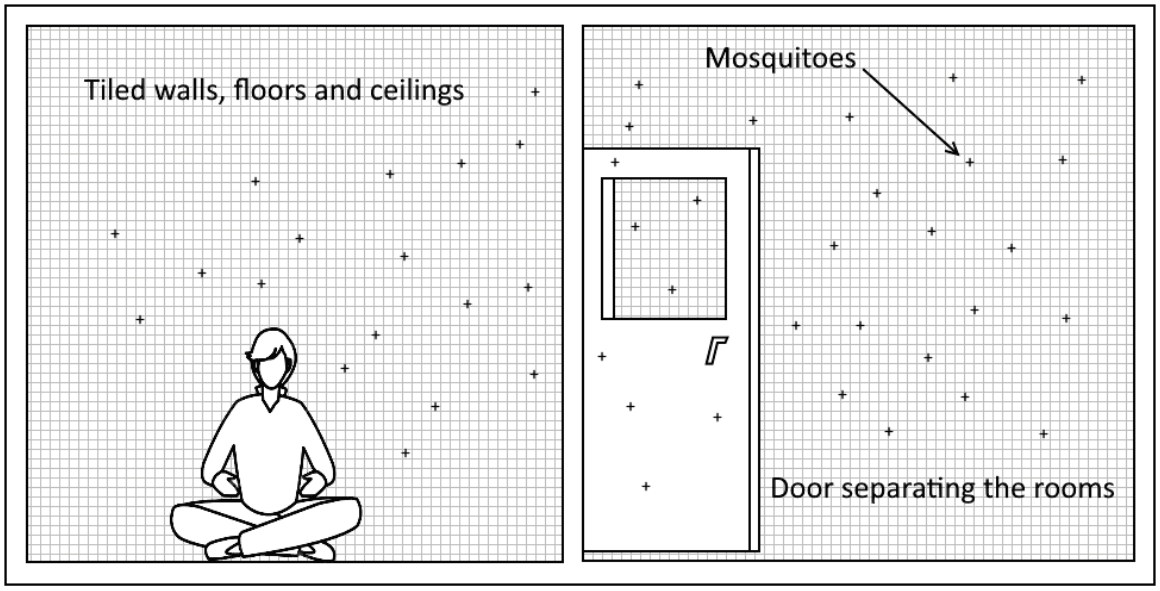

Figure 3. Schematic set-up of the room test (courtesy of J. Magnér, Swedish Chemicals Agency, 2020).

8.3.1.1.4 Field tests

Field tests are not mandatory, except for specific claims that cannot be sufficiently proven in simulated-use tests.

8.3.1.2 Sand flies and other blood-feeding flies

Efficacy against sandflies can be evaluated in an arm-in-cage test according to Weeks et al. (2019). Also, a room test according to 8.3.1.1.3 may be adapted to the species.

For other blood-feeding flies like midges, horseflies, and blackflies, also the arm-in-cage test or room test is suggested. The test conditions have to be adjusted to the needs of the particular species providing optimal conditions for their host-seeking.

8.3.1.3 Ticks

8.3.1.3.1 Test species

Products should be tested either on *Ixodes ricinus* or on *I. scapularis* (Büchel et al. 2015). If a specific species is claimed, tests have to be performed with this species.

For a general claim against ticks, *I. ricinus* and at least a second species from a different genus (e.g. *Rhipicephalus sanguineus* or a European *Dermacentor* species) should be tested.

There are further *Rhipicephalus* species difficult to differentiate from *R. sanguineus*. Therefore, the species of the test organism should be well-defined and the origin of the organisms specified.

When efficacy in the tropics is claimed, *Hyalomma marginatum* or *Amblyomma variegatum* should be tested. *H. marginatum* behaves differently than *I. ricinus* and *I. scapularis*, since it actively seeks the host and moves quickly on the ground.

Sufficient efficacy of the product should be proven against adults. In certain species, where the nymphal stage is the most relevant one transmitting pathogens to humans (e.g. *I. ricinus*), testing of nymphs can be sufficient. This, however, should be stated on the label. The nymphs (and larvae) of certain species (*Hyalomma* spp., *Dermacentor* spp.) do not bite humans. Here, adults only have to be tested.

8.3.1.3.2 Laboratory tests

For KD testing, between 5 and 10 ticks are placed on a piece of cloth (e.g. 15 x 15 cm) and held in place by a glass ring of suitable size (e.g. 10 cm diameter, height 5 cm). The inner wall of the glass ring should be coated to prevent ascension of the ticks (e.g. Fluon) and it should tightly fit to the fabric to prevent escape of the ticks. Time to KD of individual ticks is recorded. The mean (± SD) knock-down time is calculated. In total, 50 tick individuals should be tested. A test is valid if no more than 10% KD occurs in the control. Mean KD time for *I. ricinus* nymphs should be ≤ 27.1 min (according to TL 8305-0331 (2020)).

For repellent testing, the Moving Object Bioassay (MOB) showing results very close to those of simulated-use test with humans (Dautel et al. 2013) can be used. In short, a single tick on a glass rod approaches a heated, slowly rotating vertical drum. It is attracted by the warmth of the drum and changes to the moving surface of the drum. The attachment site is covered by treated fabric and the repellent effect can be detected either by (i) a reduced number of ticks approaching the drum, (ii) a reduced number of ticks transferring to the drum or (iii) an increased number of ticks falling off the drum surface compared to untreated controls. It is thus possible to discriminate between contact repellents and substances acting over short distance.

8.3.1.3.3 Simulated-use tests

A simulated-use test is recommended for product authorisation. The efficacy of repellent products can be performed as described in detail in the BPR guideline and the revised document TNsG_PT19_Ticks_Draft-DE_180815.

Briefly, the efficacy of clothes or fabrics must be proven by covering the test arm of a test subject with a treated fabric and the control arm with untreated fabric. Three markings are placed on the fabrics (control and treated): Moving from the wrist in direction to the elbow, a "release line" in the untreated area on skin 3 cm below the border to the fabric, a "boundary line" (between untreated area and fabric) and a 3 cm marking above the "boundary line". Arms are kept vertically (the hand is placed on a flat surface, e.g. a table) and a single tick is released in an untreated area. A fabric tape connects skin and textile, allowing the tick a barrier-free transition to the treated fabric. Ticks may not, when walking upwards, enter the treated fabric, or, if they enter such fabric, may not walk a distance of > 3 cm upwards or remain on the treated fabric for more than 1 min. Ticks to be used are pre-screened for sufficient walking activity on the control arm shortly before usage. At least 20 ticks are tested per volunteer. A tick is defined as repelled when it does not cross the treated cloth or when it crawls onto the treated fabric but turns back or falls off (without walking a distance of at least 3 cm) within 3 min. Tests should be performed with a minimum of 10 human subjects (50 % females and males).

The efficacy period is defined as CPT. The CPT covers the period from unworn/unwashed fabric to the time/number of washings when the first confirmed event (two ticks are not repelled) occurs. For example, if fabric is tested after 0, 10, 50 washings, and the first confirmed event occurs after 50 washings, then the CPT of the product can be claimed for 10 washings.

8.3.1.4 Lice

8.3.1.4.1 Test species

Tests should be performed with a susceptible strain of the human body louse (*Pediculus humanus humanus*).

8.3.1.4.2 Laboratory tests

The efficacy of clothes (unwashed or washed) against human lice can be evaluated using a KD assay as described for ticks. Between 5 and 10 lice are placed on a piece of cloth (e.g. 15 x 15 cm) and held in place by a glass ring of suitable size (e.g. 10 cm diameter, height 5 cm). The inner wall of the glass ring can be coated to prevent ascension of the lice (e.g. Fluon) and it should tightly fit to the fabric to prevent escape of the lice. Time to KD of individual lice is recorded. The mean (± SD) KD time is calculated. In total, 50 louse individuals should be tested. A test is valid if no more than 10% KD occurs in the control. At continuous exposure, 100% of the lice should be knocked-down within 60 min. (Sholdt et al., 1989).

As an alternative test comparable to ticks or mosquitos, lice can be exposed to test fabric for 3 min, and the mortality determined 24 h after the test should be 100%.

8.3.1.4.3 Simulated-use tests

There are no evaluated simulated-use tests available.

8.3.1.4.4 Field tests

Field tests to evaluate the efficacy of treated clothes against body lice can be adapted from Benkouiten et al. 2014. They must be carefully designed, scientifically robust and comply with general ethical principles.

8.3.2 Articles used close to the human body or indoors

This product category includes all treated articles for human use other than clothes and mosquito nets. The articles are either kept close to the human body (e.g. wristbands, clip-ons, hairbands, mattress liners, sleeping bags, blankets, tents, etc.) or used indoors (e.g. insect barriers, curtains) and thus are likely to come into contact with the human body.

8.3.2.1 Mosquitos

8.3.2.1.1 Laboratory tests

Articles should be tested in the laboratory with WHO cone tests or tube tests (WHO 2013b, 1998), if applicable (see chapter 8.3.1.1.2).

8.3.2.1.2 Simulated-use tests

Products to be used on or at a human arm should be tested in an arm-in-cage test (chapter 8.3.1.1.3.) using a defined bare test area on the forearm. This test area should be distant from the test article (i.e. if the product is kept at the wrist, the test area should be on the upper part of the forearm and not directly at the wrist) to show (a small) distance effect.

Products used elsewhere at the body (e.g. dispensers or portable insecticide coils clipped to the belt, stickers attached to clothes or skin, etc.) should be evaluated in a room test as (chapter 8.3.1.1.3.) for human apparel.

Sleeping-bags, blankets, tents, or curtains could also be evaluated in a room test.

8.3.2.2 Ticks

8.3.2.2.1 Laboratory tests

If products consist of fabric, like tents, sleeping-bags, or blankets, the efficacy can be tested according to the KD tests described in chapter 8.3.1.3.2.

8.3.2.2.2 Simulated-use tests

Products to be kept on or at the human body claiming to protect humans from ticks through a repellent effect can be tested according to the test procedure as described in chapter 8.3.1.3.3 (human apparel), with slight modifications.

If the repellent effect is claimed to be of a short range, i.e. extends only slightly beyond the article (e.g. wristband), this article is placed on the test arm. The arm is held vertically in a well aerated room by placing the hand on a plain surface (e.g. a table). Ticks are placed on the forearm on a release line 3 cm below the device. Ticks are pre-screened for sufficient walking activity by the same person before start of the test using a wristband without active ingredients or a comparable common wristband in a separate room to prevent influence of the test device. Ticks are sufficiently active if they walk 3 cm upwards past the untreated device within 3 min. Pre-screened ticks are then observed whether they, within a 3 min test period, enter the test device and either walk 3 cm upwards past the device or remain on the device for at least 3 min.

For products claiming a repellent effect covering the whole body (e.g. clip-on fastened at a belt), efficacy can be evaluated using the same test. The product should prevent ticks from crawling on the body (e.g. the leg) during a test time of e.g. 3 minutes.

Products covering large parts of the body like sleeping-bags or blankets can be tested as clothing (chapter 8.3.1.3.3).

8.3.2.3 House dust mites

8.3.2.3.1 Claims

If an acaricidal activity of mattress liners or other fabric is claimed, a laboratory mortality test should be conducted to determine the innate acaricidal efficacy of the fabric. If it is claimed that mattress liners cannot be penetrated by house dust mites, this should be proven by a penetration test.

Further, the established AATCC method may be used.

If a field test is performed, it should be conducted in Europe in households with high numbers of house dust mites.

8.3.2.3.2 Test species

Products should be tested on the European house dust mite *Dermatophagoides pteronyssinus* or another species (*D. farinae*) if relevant or claimed.

As house dust mites are very susceptible for desiccation, it is important to keep the relative humidity at ≥ 75% during the test.

8.3.2.3.3 Laboratory tests

Mortality test (Wongkamchai et al. 2005): Treated fabric (2.5 x 2.5 cm) is placed on a glass slide (5 x 5 cm) and an O-ring (2 cm diameter) placed on it. 20 adult mites are placed on the fabric and another treated fabric placed on top as well as a plastic slide (5 x 5 cm with a central 2 cm hole to allow for gas and moisture exchange). Clips keep the system together at 20 to 25°C and 75% RH for 24 h. Subsequently, mite mortality is determined using a dissecting microscope and compared to an untreated control. Five replicates should be conducted for treatment and control, each. Test mortality should be ≥ 90% and control mortality ≤ 10%.

Penetration test (Mahakittikun et al. 2009): Ten adult mites are placed on the inner or outer surface of the test fabric stretched over a 50 ml beaker filled three quarters with water. A 60 W light bulb is placed over the mites and illuminated for 15 min. It is continuously observed whether mites penetrate the fabric to escape the heat. Five repetition are performed for each side of the fabric (=100 mites in total). No mites should penetrate the fabric.

AATCC long-term test: This test is precisely described in the AATCC 194-2013 guideline. Briefly, 25 pairs of house dust mites from a healthy colony are placed on a 10 cm diameter piece of test fabric inside a petri dish covered by mite-proof mesh. 50 mg of ground food is evenly added and the setup is incubated at 25 ± 1°C and 73-76% RH for 6 weeks. The resulting mites are then extracted by the heat escape method (a fine mesh and adhesive tape is placed on top of the fabric and placed on a 50°C source for 5 h.). The percent reduction of mite numbers compared to a control is calculated. The control is performed identically, but with untreated fabric. To be valid, the control must show “normal” increase of the population. The test is performed with 3 replicates, in the control and the test. Mite reduction in the test should be ≥ 90% compared to the control.

8.3.2.3.4 Field tests

Field tests (Cameron & Hill, 2002) should be placebo-controlled and blind for the user. Dust samples (e.g. from mattress) are collected before and at certain intervals after test start to obtain mite counts and allergen levels. Method of mite recovery from dust samples: 100 mg dust plus 45 ml water in a baker are inverted 20 times and frozen at -20°C for 1 h. The upper layer of the ice cube contains the mites which are counted in a petri dish. The number of mites per gram of dust are calculated and the allergen level in house dust is determined. At least ten households each with sufficient mite infestation numbers should receive either a treated or placebo mattress liner (minimum total of 20 households). From a medical point of view, the mite level should be < 100 mites/g dust and the allergen level (Der p1= the main allergen of the mite) should be < 2 µg/g dust.

8.3.2.4 Bed bugs

8.3.2.4.1 Test species

Tests should be conducted with adult bed bugs (*Cimex lectularius*, or the tropical bed bug *Cimex hemipterus* according to claim) unless nymphs are specifically targeted. Bed bugs should be tested five to ten, preferably seven days after their last blood meal.

Tests should be conducted during the natural bed bug activity time, i.e. during the dark phase (in darkness or under red dim light).

8.3.2.4.2 Claims

If the treated article is claimed as a barrier for bed bugs preventing access of bedbugs across the treated article, efficacy must be proven in a simulated-use test. If barriers of different width are to be authorized, then the test barrier must be no wider than the smallest barrier to be authorized. The label should clearly state that barriers are not effective if cut smaller.

For product authorisation, the efficacy of repellent products should be proven in a simulated-use test as described below.

8.3.2.4.3 Laboratory tests

Laboratory KD tests can be performed as described above (chapter 8.3.1.3.2).

8.3.2.4.4 Simulated-use tests

There are two tests systems available – a closed three-chambers-system (Vander Pan et al. 2019) and an open test inside a room (Todd, 2011; Wang et al. 2013). The three-chambers-system should be preferably used as it is a worst-case test providing standardised conditions. If specific claims have to be tested and the three-chambers-system is not suitable, also the open test or a modification thereof may be used.

Three-chambers-system: The test system consists of three closed chambers joined by connector tubes. In the first chamber (harbourage chamber), a sealed harbourage, a bag made of e.g. tissue paper, containing 50 to 100 bed bugs is placed. After a minimum of 1 h of acclimatization the harbourage is opened. In the middle chamber (test chamber) the treated surface is placed. The test chamber is connected to a third chamber (host chamber) containing a CO_2 source and a heat source. The connector tube between test- and host chamber should protrude approximately 10 cm into the host chamber. A collecting vessel is placed under the open end of the connecting tube. This vessel contains filter paper as a harbourage and the inner walls should be treated with a substance that prevents bed bugs from escaping (e.g. Fluon). Efficacy is evaluated by counting the number of bed bugs which have crossed the surface in the test chamber and fall into the vessel of the host chamber.

A CO_2 source releases CO_2 (e.g. 0.75 l/min) into the host chamber that is additionally heated to 37 ± 2°C. A suction pump connected to the harbourage chamber pumps out air through all three chambers creating a constant airflow from host- through test- and harbourage chamber preventing CO_2 saturation in the system. The connector tubes should be lined with material (e.g. masking tape or paper) which is not slippery for bed bugs. Test duration should be 8 h to cover the natural bed bug activity over night. Within this period, 8 h darkness or red light is obligatory.

A minimum of 5 independent replicates should be performed (each treatment and control).

The repellent effect of the product is determined by comparing the number of bed bugs trapped in the collection vessel of the test system with the number of bed bugs trapped in the collection vessel in the control without treated fabric.

Open Test: A chair or stool (or miniaturized bed) with four legs is placed in the centre of a test arena (at least 0.6 m²) lined out with paper or other material enabling normal movement of bed bugs. Onto that simulated furniture, a CO_2 source (minimum release rate: 100 ml/min) and a heat source is placed to mimic a human host. Under each leg (of the furniture) a bed bug interceptor is placed to trap bed bugs. A bed bug interceptor can be a commercial bed bug trap or a custom-made double-wall trap. The treated fabric is applied on the outer wall of the interceptor. If the test surface is wider than the interceptor is high, the interceptors should be placed onto the treated surfaces and the distance the bed bugs have to walk over the treated fabric should be the same as the intended width of the barrier. Bed bugs, inside an artificial harbourage, are placed in the centre of the arena right under the furniture. After an acclimatization of at least 1 hour, the harbourage is opened.

Tests should be conducted with 50 to 100 bed bugs (equal number of both sexes). Test duration should be 8 h to cover the natural bed bug activity over night with 8 h darkness or under dim red light.

A minimum of 5 independent replicates should be performed (each treatment and control).

The repellent effect of the product is determined by comparing the number of bed bugs collected in the interceptor in the test arena with the number of bed bugs in the control arena having the identical set-up, but untreated fabric as barrier.

If prevention of bed bug bites is claimed, 100% of the bed bugs should be prevented from crossing the barrier compared to the control. If a reduction of bed bug bites is claimed, at least 90% of the bed bugs should be prevented from crossing the barrier compared to the control.

8.3.2.4.5 Field tests

Field trials must not be conducted for product authorisation. If field trials are conducted, they must take place in buildings with an appropriate bed bug density. These tests should preferably take place in Europe or other relevant regions according to the claims (e.g. tropical regions).

8.3.2.5 Human head lice and body lice

8.3.2.5.1 Claims

If the treated article is claimed as a barrier for lice preventing access of lice across the treated article, efficacy must be proven in a simulated-use test.

If the treated article is claimed to prevent entry of lice onto the human body, this must be proven in a simulated-use test.

8.3.2.5.2 Test species

Human head lice (*Pediculus humanus capitis*) and body lice (*Pediculus humanus humanus*) are regarded to be the same species (Light et al., 2008) and can either be used as test species for each other. Tests should be conducted with adult lice, preferably within 1 d after their last blood meal. If claimed separately, also juvenile stages should be tested. If efficacy against eggs is claimed, eggs of an age of 0-1 d and 4-5 d may be used to test efficacy on eggs with and without developed nerve cells. Typical conditions to keep all stages of lice are about 32°C and 76% RH.

8.3.2.5.3 Laboratory tests

Laboratory tests can show the inherent efficacy of test products. For treated fabric, standard KD tests can be performed to determine the KD times of lice before and after certain usage times (or washings). In continuous KD tests, 100% KD should be reached within 75 min.

The efficacy of test products like bracelets, hair tils or hairbands against human lice can be evaluated using a choice test (In-house test, IS Insect Services GmbH). The test product is fixed on a vertical cylinder covered with filter paper (Figure 4). The bottom temperature of the cylinder is kept at approx. 37 °C. The setup is based on the natural behaviour of lice entering a host and searching for the warm skin surface, in this case to walk downwards (thermotaxis).

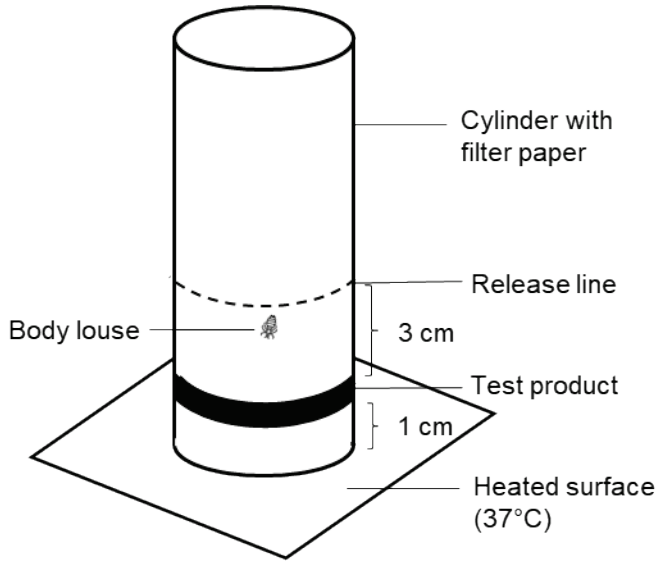

Figure 4. Schematic drawing of the laboratory test setup with lice.

A single louse is placed on the release line on the cylinder surface above the test product. The total observation time for each individual is a maximum of 2 minutes. After the test duration of 2 min it is determined whether the louse is above or below the test product. Lice that remain above are considered repelled, lice that crossed the test product in direction to the heated bottom are considered as not repelled.

In total, at least 30 louse individuals should be tested, one after another. For technical repetition, the test product is changed after every 10th louse and replaced by a new one. Control runs are performed either with test products without AS or only with filter paper with a border line. A test is valid if > 70% of the lice in the control move towards the heating plate proving sufficient activity of the lice.

The repellent effect is evaluated by the percentage of lice repelled in the test in comparison to the control. Temperature of the test room should be between 20-23°C (higher temperatures influence the thermotaxis). At least 90% of the lice should be prevented from crossing the treated article.

8.3.3 Articles for outdoor use

8.3.3.1.1 Devices to reduce local arthropod abundance (e.g. portable wall with treated fabric to reduce mosquito or biting fly abundance)

8.3.3.1.2 Claims

If the label claims the local reduction of certain species or species groups (e.g. midges, mosquitos, sandflies, etc.), this must be proven in a field test.

The label should also indicate in which geographic regions (e.g. temperate Europe, southern Europe) the device is effective.

8.3.3.1.3 Laboratory tests

Standard KD tests provide valuable supplementary data on the basic efficacy of the device against target species. These should be performed in parallel to field tests to show sufficient efficacy against the local target species at the beginning and in the course of tests throughout the efficacy time of the product (e.g. on a monthly basis for devices being effective for several months).

8.3.3.1.4 Field tests

Field tests (Britch et al. 2010, 2018) should be performed in at least two field sites within different climate zones of Europe, or in other field sites outside Europe if claimed. The field sites should provide sufficient numbers of target species during the tests. The abundance of target species is estimated by suitable traps set out before and after placement of the devices. This can be done for up to several months, or longer, depending on the claim. In parallel, samples of fabric material are taken at regular intervals of outdoor use to monitor any decrease of KD efficacy caused e.g. by rain, sunlight, wind, etc.

In parallel, suitable traps to monitor non-target flying insect species (e.g. yellow traps or flight traps) should be set out to evaluate any reduction of non-target species.

To be sufficiently effective, a reduction of target species abundance of $\geq 70\%$ compared to the pre-treatment number should be proven against each target species group (mosquitos, midges, or others) claimed.

8.3.3.2 Tick rolls

8.3.3.2.1 Claims

If the label claims the local reduction of certain species, this must be proven in a field test.

If local reduction of ticks functions via host-targeted devices (e.g. tick rolls), field tests in at least 10 test areas and an equal number of control areas should be performed to account for local differences in the tick host fauna. In addition, efficacy should be monitored over at least two years, as effects may not appear before the second or third year of usage of tick rolls.

The label should also indicate in which geographic regions (e.g. temperate Europe, southern Europe) the device is effective.

8.3.3.2.2 Laboratory tests

Standard KD tests can provide valuable supplementary data on the basic efficacy of the device against target species.

8.3.3.2.3 Field tests

The test design can be similar to Drehmann et al. (2018). When tick density is measured by flagging a defined area for ticks, pairs of control/test gardens should be flagged preferentially at the same day (tick activity can vary considerably according to weather conditions). Such flagging may be performed e.g. three times a year (April; May/June; September) at days when weather conditions are good covering an area of appr. 100 m² (e.g. 10 x 10 m²) or more, if the test area is large enough. More frequent flagging could influence density of questing ticks by itself. Also, the transects to be flagged should be chosen by chance (and not always be exactly the same). The percentage reduction (mean ± SD) of host seeking ticks of the test areas compared to the control areas should be recorded.

8.3.3.3 Wasp repellent devices

8.3.3.3.1 Test species

The repellent should work against the most common wasp species occurring in a respective region. These are, e.g. *Vespula germanica* and *Vespula vulgaris*, in many parts of Europe.

8.3.3.3.2 Simulated-use tests

This test can be performed like the field test described below. Wasp nests are transferred into the lab and placed in a separate room with connection to the test room with the tables.

8.3.3.3.3 Field tests

In field studies the efficacy of outdoor area repellents against naturally occurring wasps can be tested (in Europe e.g. *Vespula vulgaris*, *Vespula germanica*) from summer to autumn. Depending on the type of data recording required, this can be achieved by direct observation or video evaluation. The study design corresponds to Boevé et al. (2016).

The test apparatus is set up outdoors. Common garden tables (e.g. Ø 90 cm) can be used for this trial: one for the test product and one for an untreated control (Figure 1). In case of the simultaneous use of a reference product a third table can be used. In the latter case the tables will be set up in a triangular fashion with even spacing of at least 2 m in-between tables.

On each table, four glass dishes (e.g. Ø 9 cm) are evenly distributed in 25 cm distance to the glass dish/test product in the centre of the table (outer edge petri dish). Each of the four dishes contains a bait that naturally attracts wasps (e.g. of boiled ham (early season) or berry jam (late season)). The total amount of bait on each table will be the same throughout the trial. Depending on the type of application, the treated article is placed in the centre of the table or the whole table covered with treated fabric.

The tests will be carried out preferably on sunny days to ensure high levels of wasp flight and foraging activity. Three replicates on different days but at the same location should be conducted. The table order should be rotated on each replication to avoid table location bias. To establish sufficient wasp activity the tables are fitted with bait alone for at least one hour (but better 1-2 days) prior to the start of the test until a sufficient and equally distributed number of wasp landings is observed on all tables (e.g. at least 20 landings within 30 minutes).

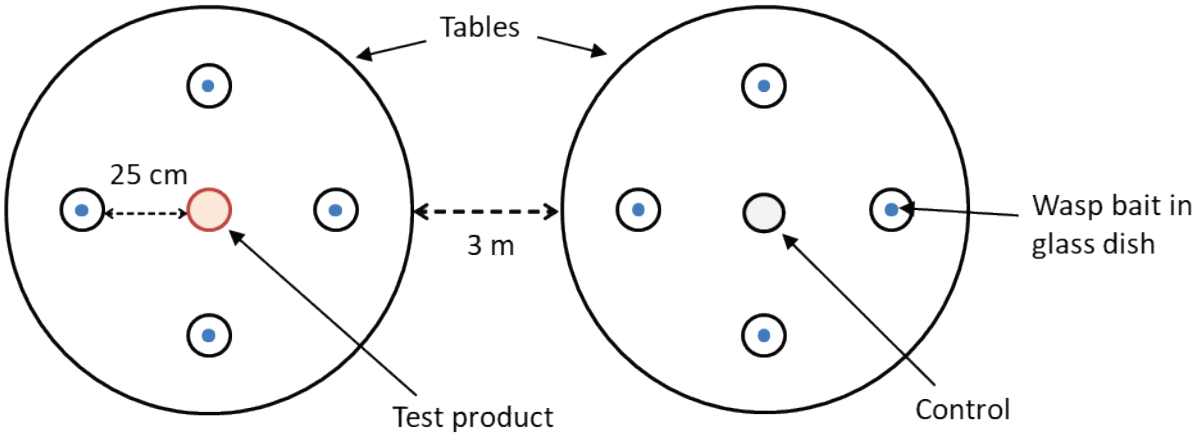

Figure 5. Experimental setup: Two tables with four glass dishes with baits. In the centre of each table either the test product or the control is placed. One camera per table is recording the wasps' behaviour from above.

At test start the test product is placed on one of the tables. The total observation time should be at least 2 hours or according to the product label claim. The baits are renewed as necessary for each table if depleted or dried out.

Video clips are analysed every minute or every 10 minutes (depending on the duration of observation) to determine the number of wasps present at each table and the wasp free time. The repellent effect is evaluated by the mean number of wasps on and above the test table and the wasp free time, both compared to the control table.

8.3.4 Articles to protect animals

8.3.4.1 General introduction

This product category includes all treated articles intended to protect animals from parasites or nuisance pests. These include all treated articles that are permanently or temporarily used close to the animal body (e.g. collars, scarfs, vests, sleeping mattress, blankets, etc.).

Laboratory tests (KD tests) are useful to monitor the basic efficacy of the articles to be tested. For product authorization, however, simulated-use tests or field tests are required. Field tests should only be performed when there are no suitable simulated-use tests available. If field tests are performed, they must be conducted in at least two sites in Europe at a season, when target organisms are prevalent. The presence of target organisms (and the species composition) should be demonstrated before and after a field test, e.g. by catching or collecting target organisms from the host. Alternatively, suitable traps can be set up, or, in the case of ticks, by flagging of the vegetation. Here, care must be taken, not to sample the whole active population, which may easily occur e.g. when collecting adult *Dermacentor* ticks from the same site that will thereafter serve as field test site.

8.3.4.2 Articles to protect horses (and cattle)

8.3.4.2.1 Biting flies

Test species
Products intended for use on cattle or horses should be tested using the claimed host. Products intended for use against specifically claimed fly species must be tested with these species. Results from one fly species may not be extrapolated to other species.

Products intended as a general fly protectant claimed for use on grazing cattle must be tested against *Haematobia irritans* (horn flies), *Hydrotaea irritans* (head flies) and *Haematopota pluvialis* (horse fly). Treated articles claimed for use on horses must be tested against *Hy. irritans* and *H. pluvialis* (see revision document "PT19 - Flies on grazing cattle and horses").

Laboratory tests
Laboratory tests provide data on the basic efficacy of the treated article at the beginning, and throughout the claimed efficacy time of the product. For flying insects, the cone test (WHO 2013, see chapter 8.3.1.1.2) or a similar test design should be performed according to target species.

Simulated-use tests
To our knowledge, there are currently no established simulated-use tests available. If such a test is designed, it should be scientifically robust and specifically adapted to the target species to be used.

Field tests
Field tests should be performed as proposed in the revision document "PT19 - Flies on grazing cattle and horses" of the BPR. Using this test procedure, the efficacy of a test product is evaluated by counting the target species staying on the animal (horse). Alternatively, the efficacy can also be estimated by counting specific avoidance behaviour of the horses (e.g. tail swishes, shoulder twitches, hoof stomps, head-backs) as described in Mottett et al. 2018. It must be ensured, however, that the abundance and composition of target species is recorded (by direct counting or catches from the host) at least at the beginning and at the end of the test.

Semi-field tests as described in Jopin & Haanen (2013) can be used to evaluate the efficacy of e.g horse blankets against midges. Horses are kept in outdoor tents for 2 h/day for 4 days and all midges entering the tent are vacuumed and counted (unfed and fed midges counted separately). The main criterium is the reduction in number of fed midges. This test design may be adapted to other fly species, if appropriate.

8.3.4.2.2 Ticks

Test species
Products intended for use as effective against specifically claimed tick species must be tested with these species. Products claimed to be effective against ticks in general must be tested against *Ixodes ricinus* and at least another species from another genus prevalent on the host (e.g. *Dermacentor reticulatus* (ornate cow tick), or *Hyalomma marginatum*).

Laboratory tests
Laboratory tests provide data on the basic efficacy of the treated article at the beginning, and throughout the claimed efficacy time of the product. For ticks, the KD test (chapter 8.3.1.3.2) should be performed.

Simulated-use tests
To our knowledge, there are currently no established simulated-use tests available.

8.3.4.3 Articles to protect dogs (and cats)

Among crawling arthropods, ticks and fleas are the most frequent parasites found on dogs and cats in Europe.

To our knowledge, there are currently no established simulated-use tests against mosquitos available.

8.3.4.3.1 Ticks

Test species
Products claimed effective against specific tick species must be tested with these species. Products claimed to be effective against ticks in general must be tested against *I. ricinus* and at least another species from another genus prevalent on the host (e.g. *D. reticulatus* (ornate cow tick), or *R. sanguineus* (brown dog tick)).

Laboratory tests
Laboratory tests provide data on the basic efficacy of the treated article at the beginning, and throughout the claimed efficacy time of the product. For ticks, the mentioned KD test should be performed.

Simulated-use tests
Two simulated-use tests are available, which may be either performed.

A simulated-use repellent test on dogs for ticks is described in the BPR draft document "TNsG_PT19_Ticks_Draft-DE_180815.pdf". The procedure is designed for repellents and can be modified for testing products like repellent fabrics (e.g. vest) used on dogs. The test product is applied according to the label claim. The tick walking on a blunt rod is held onto the test product placed on the dog (e.g. the lateral area of the thorax). Ticks are attracted by the body warmth and chemical host cues in direction of the dog. During observation time of 3 minutes a tick is repelled when not crawling onto the treated fabric. If surrounding uncovered body parts are claimed to be protected by the test product, a tick is repelled when it does not crawl onto the fur. Thereby, the rod is kept at a distance to the treated fabric equivalent to the maximum distance claimed (e.g. if it is claimed that body parts up to 50 cm distant to the treated fabric are protected, the rod is kept at a distance of 50 cm). In controls with untreated fabric (before test start or on the other lateral side of the test animal) sufficiently locomotive ticks are selected and subsequently used for tests. To be sufficiently active, a tick needs to walk on the untreated fabric or to the fur within 3 min observation time. Biting can be prevented by permanent observation of the tick.

Alternatively, a simulated-use test as described in Fourie et al. (2013) can be conducted. Hungry ticks (n= 30 to 50 pairs) are placed in a cage of suitable size (e.g. 2 x 2 m) and a dog introduced to rest overnight in that cage. The next day, the dog and the cage is screened for ticks. The number of attached and unattached ticks on the dog (dead or alive) and the number of living and dead ticks in the cage are counted. Percent protection is calculated with respect to an untreated control. At least 10 dogs, each in the test and the control, are investigated.

Field tests
If field tests are conducted, they should orientate on test designs as described by the European Medicines Agency (2016).

8.3.4.3.2 Fleas

Test species
Products should be tested with the common flea species prevalent on the hosts in question. In Europe, *Ctenocephalides felis* (cat flea), or *C. canis* (dog flea) are most commonly found on dogs.

Laboratory tests
Laboratory tests provide data on the basic efficacy of the treated article at the beginning, and throughout the claimed efficacy time of the product. For fleas, the KD test should be performed.

Simulated-use tests
Simulated-use tests with fleas can be performed as described in the European Medicines Agency (2016).

Field tests
Field tests with fleas can be performed as described in the European Medicines Agency (2016).

8.3.5 Mosquito nets

Mosquito nets should be tested according to existing guidelines, preferably according to WHO (2013b). All test procedures are described in detail in this guideline. Tests include the cone test performed with different samples from bed-nets before and after a certain number of washes. This test determines the innate ability of bed-nets to knock-down or kill mosquitos.

In order to reduce animal testing, the WHO tunnel test should be avoided, whenever possible.

Field tests are not required. However, if field tests or semi-field tests (experimental huts) are performed as supplementary data, they should be performed in Europe or other climate regions according to label claim. Field tests should conform to the WHO 2013b guideline (Phase II and/or phase III field tests).

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