WORK FLOW FOR INTEGRATION OF ANALYTICAL AND PHYSICAL-CHEMICAL METHODS FOR LN INTO RELATED CIPAC METHODS FOR CORRESPONDING ACTIVE INGREDIENTS

Scope

This document is intended to give guidance to data proposers planning to submit a data package for Long Lasting Insecticidal Nets to WHO and correspondingly for analytical methods to CIPAC. It does not cover the WHOPES efficacy tests but aims at filling the gap between the data requirements of the LN specification guideline and the guidelines of CIPAC for elaboration and validation of analytical methods for pesticides. It explains the clauses of the LN specification guideline and how the respective data requirements where collaboratively tested analytical methods are involved can best be met.

Introduction

Long lasting insecticide impregnated mosquito nets (LN) are becoming increasingly important as devices for prevention and control of vector borne diseases like malaria. The data requirements of these LN formulations which generally integrate a commercial insecticide with a fabric, the net, to provide the long lasting effects require internationally agreed standards for analytical and physical-chemical evaluation. In order to achieve a rapid knock-down with subsequent sufficiently high mortality of the target insects, pyrethroids like deltamethrin, permethrin, alpha-cypermethrin and lambda-cyhalothrin are the active ingredients which seem at the moment to be the best suited for combination with a net as a physical barrier.

The data requirements follow the specification guideline for LN as described in the FAO and WHO Manual on pesticides specifications, (currently March 2006 revision of the first edition). The analytical procedures and physical-chemical methods for characterizing the properties for active ingredient on LN material need to be CIPAC adopted methods or equivalent, whereas the physical test methods are established ISO methods.

The clauses involved where CIPAC methods will come into the play are:

- **Determination of the content of active ingredient** in the net including suitable identity tests and other methods which do not necessarily need collaborative validation like for relevant impurities and determination of stereoisomer ratio, where required
- **Release rate or retention index**. This property is required for a slow release formulation like a LN. Release and retention index are counterparts, being the proportion of active ingredient that is, and is not, removed from an LN product by each wash. Direct measurement of active ingredient release has been shown to be costly and problematic producing results which are more variable than those from measurement of retention. A harmonized method for determination of active

ingredient retention index is currently under development by CIPAC and includes standardized wash steps using a phosphate free detergent. This work was among other initiated by the Recommendations of the 11th WHOPES Working Group Meeting, where the need for a better standardized wash method was expressed. In contrast to earlier work, where attempts to determine a "surface concentration" by solvent washes were found to lead to largely variable results and are therefore strongly disrecommended, this harmonised method calculate a retention index based on the determination of the active ingredient content in the unwashed and washed LN. The use of sequential wash steps with an aqueous detergent is expected to lead to similar results as with the WHO wash method (actually used for WHOPES laboratory efficacy trials), with the advantage of using a standardized soap instead of a commercial product (Savon de Marseille). A draft method is expected to be available in late 2009.

- Accelerated storage test and subsequent determination of total content, release rate or retention index and stereoisomer ratio if required together with physical tests.

Special care and attention should be paid to sampling of the net for analysis. We recommend adhering to the sampling protocol as outlined in the LN draft Guideline in the Manual: different sections of netting are sampled to collect e.g. 5 pieces of 100 cm^2 or 25 cm x 25 cm to make up a pooled sample and subsequent subsampling. The sampling, subsampling and analysis should be done in such a way that the error introduced is ≤ 5 % and hence smaller than the tolerance of the average active ingredient content in the net (usually 25 %, in line with the general requirement of the Manual for non-homogeneous formulations having a concentration of the active ingredient in the range of up to 25 g/kg) and also smaller than the possible degradation of the insecticide in the accelerated storage test.

As LNs will include a range of polymers for the yarn, as well as different coating or integration technologies of the active ingredients, it is highly recommended to develop product group specific procedures. Even if harmonized analytical methods, as provided by CIPAC, are used as general test procedures, technological variations within LN types may be too large to cover them with one method. On the other hand, evaluation of a specified CIPAC LN method for each and every LN type may become virtually impossible to handle, and are not in agreement with the standardization approach of CIPAC. A good compromise has therefore to be found between the need to harmonise analytical methods for quality control purpose and the need to establish accurate specifications suitable for each type of product.

A modular approach

Instead of handling each LN as a formulation of its own and file them individually into CIPAC work flow, it may be better to develop a modular procedure. Following our present experience, two types of polymer (polyethylene or polyester fiber material) are used to produce the yarn and the net or netting. Whereas polyethylene is primarily used with the insecticide incorporated, polyester fibers are used with insecticide coated on the surface of the yarn.

Basically all pyrethroids can be used with this textile material with the limitation of a sufficient thermal stability of the insecticide in the spinning and stretching process of the polyethylene to achieve the necessary tensile strength.
In order to determine the active ingredient content, nets with the insecticide incorporated call for a total dissolution of the polymer or a complete migration of the active ingredient into the extraction solvent to achieve an acceptable recovery, whereas the coated type cannot be dissolved into a solvent but need a total extraction of the active ingredient from the coating.

This approach can be based on a product specific sample preparation step followed by an approved CIPAC active ingredient method as analytic module. This modular approach should allow make best use of prior work and adopted CIPAC methods by merely adding those elements, where additional methods are necessary.

1. Sample generation module for total content and release/retention index

Each proposer will develop a robust, reproducible sample preparation procedure that is able to generate representative samples regarding the target molecule being analyzed using a given CIPAC method.

Total active ingredient content. CIPAC is aware of the fact that the dissolution of the polyethylene based nets, with incorporated insecticide, calls for solvents or combinations of solvents which may or may not be compatible with the subsequent analytical technique. The coated type nets usually allow for more flexibility in the use of solvents, which may be chosen for compatibility with the analytical technique of the adopted CIPAC method (HPLC, capillary GC). The sample preparation shall be based on standard technologies and a validation report according to the CIPAC Method Extension Guideline is to be provided by the proposer. Any sample preparation must be designed in a way that a **given CIPAC method may be used later on without any change in procedure approved and published.** Data shall be generated by a qualified laboratory and be validated, where necessary, by an independent laboratory.
The sample generation module shall be related to a given CIPAC active ingredient method and either linked into the main method or one of the formulation related procedures whichever is best suited regarding the active type (formulation or product used to produce the LN) and content to be expected.

Release/retention index. The **retention index** calls for determination of the total content in net samples with a different washing history and is therefore very similar to the determination of the total content. A harmonized CIPAC wash method based on a provisional CIPAC Method and the WHO wash method is under development. The wash method is used to produce samples of a net representing a well defined wash history and allows to indirectly determining the amount given off in the wash step. This new draft CIPAC wash method applicable for coated and incorporated type of nets is expected to be available by late 2009 under www.cipac.org

2. Analytical module

Once a standardized sample extract is produced the corresponding active ingredient related CIPAC method is used to characterize the material both in qualitative and quantitative terms. The sample shall be evaluated according to the published procedure for the given CIPAC method (including injection and calculation procedure). The identity test will usually be based on chromatographic methods like retention time of the pyrethroid as non-destructive methods like infrared technique are expected to fail. It will be essential that only the least modifications or deviations of the CIPAC method will become necessary. Depending on deviations from the CIPAC method, a tiered approach for a possible evaluation is foreseen.

Practically the work flow proposed mean:

A. Sample preparation module.
Prior to undertaking development work, the proposer should answer the following questions:

1. Sampling protocol: adhere to the sampling procedure as outlined in Note 6 of the LN Specification Guideline and deviate only in cases where results show that a different sampling protocol needs to be followed.
2. Does a CIPAC method for the active ingredient in TC, to be combined with the net and certain formulations exist? If yes, all measures should be taken so that a LN method can be developed as a method extension to this existing CIPAC method.
3. Is the LN under consideration a coated type or incorporated type? The incorporated type net has to be completely dissolved or the active ingredient has to migrate completely into the extraction solvent in order to achieve an acceptable recovery. The coated type net cannot be dissolved into a solvent but need a total extraction of the active ingredient from the coating. Both the coated type and the incorporated type call for a suitable determination of the recovery and other parameters if applicable.
4. Do the solvent systems seem to provide sufficient recovery compatible with the subsequent analytical technique? If not, sufficient effort should be made to achieve the compatibility before a new method is considered.
5. Are the conditions of extraction of the insecticide and/or solubilization of the polymer compatible with the active ingredient to prevent introduction of artifacts (degradation, epimerization, volatilization, chromatographic interferences, etc.).

B. Release/retention index module
As mentioned above, the retention index relies on the total active ingredient content in samples after different washing steps. The new harmonised CIPAC wash method is recommended both for coated and incorporated LN.

C. Analytical module

6. Whereas the analytical method should remain essentially the same for all formulations, the identity test or tests and the range of concentrations are mostly different for LN. With LN, the concentration range is a few g of insecticide per kg of net or netting. Adaptations in the calibration process are therefore necessary.

7. Furthermore, the inert ingredients, stabilizers or even the dissolved polymer may interfere, therefore the chromatographic conditions have to be optimised to achive a good separation
8. All these aspects have to be covered in the validation process as outlined in the CIPAC Guideline “Extension of the Scope of Methods” available as download from the CIPAC website (http://www.cipac.org/document/extenmet.pdf). The method extension and the validation data need to be presented at the CIPAC Meeting for discussion and possible adoption.

Prepared on behalf of CIPAC

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