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Skin applied mosquito repellent — Specification — Part 1: Lotions, creams, gels and ointments

EAST AFRICAN COMMUNITY

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Foreword

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in the East African Community. It is envisaged that through harmonized standardization, trade barriers that are encountered when goods and services are exchanged within the Community will be removed.

The Community has established an East African Standards Committee (EASC) mandated to develop and issue East African Standards (EAS). The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the public and private sector organizations in the community.

East African Standards are developed through Technical Committees that are representative of key stakeholders including government, academia, consumer groups, private sector and other interested parties. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the Principles and procedures for development of East African Standards.

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

The committee responsible for this document is Technical Committee EASC/TC 078, *Healthcare and medical devices*.

Attention is drawn to the possibility that some of the elements of this document may be subject of patent rights. EAC shall not be held responsible for identifying any or all such patent rights.

DEAS 1119 consists of the following parts, under the general title *Skin applied mosquito repellents — Specification*:

- *Part 1: Lotions, creams, gels and ointments*
- *Part 2: Sprays and roll-ons*
- *Part 3: Wipes*
- *Part 4: Bathing soaps*
- *Part 5: Bracelets, wristbands and patches*
- *Part 6: Jelly*

Skin applied mosquito repellent — Specification — Part 1: Lotions, creams, gels and ointments

1 Scope

This Draft East African Standard specifies the requirements, sampling and test methods for skin applied mosquito repellents in form of lotions, creams, gels and ointments.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EAS 346, *Labelling of cosmetic products — General requirements*

EAS 377 (all parts), *Cosmetics and cosmetic products*

EAS 846, *Glossary of terms relating to the cosmetic industry*

EAS 847-16, *Cosmetics — Analytical methods — Part 16: Determination of heavy metal content*

EAS 847-17, *Cosmetics — Analytical methods — Part 17: Physio-chemical test*

ISO 18416, *Cosmetics — microbiology — detection of candida albicans*

ISO 21149, *Cosmetics — Microbiology — Enumeration and detection of aerobic mesophilic bacteria*

ISO 22717, *Cosmetics — microbiology — detection of pseudomonas aeruginosa*

ISO 22718, *Cosmetics — microbiology — detection of staphylococcus aureus*

ISO 24153, *Random sampling and randomization procedures*

DEAS 1120-1, *Mosquito repellents — Performance test guidelines — Part 1: Skin applied repellents*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EAS 846 and the following apply. ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at <http://www.iso.org/obp>.

3.1

mosquito

blood-sucking dipterous insect of the family Culicidae. Aedes, Anopheles, Culex, Mansonia, and Stegomyia are genera containing most species involved in the transmission of protozoan and other disease-causing parasites

3.2

mosquito repellent

substance applied to deter mosquito from approaching or settling

3.3

natural repellents

repellents that contain, plant-based compounds

3.4

synthetic repellents

conventional repellents containing chemical compounds manufactured to imitate the natural compounds.

4 Symbols and/or abbreviated terms

DEET N, N-Diethyl-meta-toluamide or diethyltoluamide

IR3535 ethyl butylacetylaminopropionate

Picaridin 1-(1-methylpropoxycarbonyl)-2-(2-hydroxyethyl) piperidine or 2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester

5 Active ingredients

5.1 Natural repellents

5.1.1 Active ingredients used in natural repellents shall be plant based compounds which are able to deter mosquitoes from approaching or settling. Such shall be essential oils or any other plant extract approved by relevant authority as mosquito repellents.

5.1.2 The manufacturer shall provide adequate data on the repellence of such ingredients. Adequate data shall include laboratory studies showing estimation of effective dose (technical material) and estimation of complete protection time; and the outcomes of field trials showing efficacy and persistence of technical material; efficacy and persistence of formulated product.

5.1.3 The manufacturer shall have adequate data justifying the safety and proportion of ingredient(s) used in the product, for which claims are made.

5.1.4 The essential oils used and other plant extracts in natural repellents shall be, but not limited to:

- a) cedarwood oil;
- b) tea tree oil;
- c) geranium oil;
- d) rosemary oil;
- e) lemongrass oil;
- f) citronella oil;
- g) eucalyptus oil;
- h) cinnamon oil;
- i) neem oil;

- j) lavender
- k) cloves
- l) basils
- m) jasmine; and
- n) pyrethrum

5.1.5 The proportion of single or blended active ingredient (s) in natural repellent shall be set by the manufacturer in accordance with the relevant standard and records shall be availed.

5.1.6 Pyrethrum extracts such as pyrethrins shall be considered in natural repellents. The limits of pyrethrins in natural repellents shall not be less than 0.5% w/w, when tested in accordance with annex F.

5.2 Synthetic repellents

5.2.1 Synthetic repellents shall contain synthetic chemical compounds which are able to deter mosquitoes from approaching or settling on the surface.

5.2.2 If a synthetic active ingredient is blended with other active ingredient (s), either natural or synthetic, the proportion shall be set by the manufacturer based on scientific research and records shall be availed.

5.2.3 Synthetic repellents and their active ingredients shall be approved and registered by relevant authority before being released to the market.

6 Requirements

6.1 General requirements

6.1.1 The product shall constitute a mosquito repellent that is formulated as lotion, cream, gel or ointment and shall be essentially a product which has active ingredient (s) added to a certain level.

6.1.2 It shall be primarily composed of water, surfactants, fatty alcohol, fragrance, oil and other emollients. All ingredients shall meet the requirements of EAS 377.

6.1.3 When applied to the skin, the product shall have the benefit of repelling mosquitoes and shall not cause harmful effect to the skin.

6.2 Specific requirements

6.2.1 Active ingredients and their content in synthetic repellents shall meet the requirements prescribed in Table 1.

Table 1 — Active ingredients content for synthetic repellents in form of lotions, creams, gels and ointments.

S/N	Characteristic	Requirement	Test method
i	DEET, % w/w.	4 – 30	Annex A
ii	Picaridin, % w/w.	5 – 20	Annex B
iii	IR3535, % w/w.	7.5 – 20.07	Annex C

6.2.2 The product shall comply with the specific requirements given in Table 2 when tested in accordance to the methods described therein.

Table 2— Specific requirements for skin applied mosquito repellents in form of lotions, creams, gel and ointments

S/N	Characteristic	Requirement	Test method
i	Thermal stability	To pass test	EAS 847-18
ii	pH	3.5 – 8.5	EAS 847-17
iii	Total fatty substance content, % m/m, min	5	Annex E
iv	Total residues, % m/m, max	40	Annex D

6.3 Heavy metal requirements

The product shall comply with the heavy metal requirements given in Table 3 when tested in accordance with the test methods specified therein.

Table 3— Heavy metal requirements for skin applied mosquito repellent in form of lotions, creams, gel and ointments

S/N	Heavy metal	Limit, mg/kg, max	Test method
i	Lead,	10	EAS 847-16
ii	Arsenic	2	
iii	Mercury	2	
The total amount of heavy metals as lead, mercury and arsenic, in combination in the finished product shall not exceed 10 mg/kg			

6.4 Microbiological requirements

The product shall comply with the microbiology limits specified in Table 4 when tested in accordance to the methods described therein

Table 4— Microbiology limits for skin applied mosquito repellent in form of lotions, creams, gel and ointments

S/N	Characteristic.	Requirement	Test method
i	Total viable count, cfu/g, max • Products for children below three years • Other products	100 1000	ISO 21149
ii	Staphylococcus aureus (per g)	Not detected	ISO 22718
iii	Pseudomonas aeruginosa (per g)	Not detected	ISO 22717
iv	Candida albicans (per g)	Not detected	ISO 18416

6.5 Biological efficacy

When tested in accordance with DEAS 1120-1, the product shall repel 100% of the mosquitoes from approaching or climbing on that surface, within protection time indicated by the manufacturer.

7 Packaging

The product shall be packaged in suitable well-sealed containers that shall protect the contents and shall not cause any contamination, deterioration or reaction with the product.

8 Labelling

In addition to the labelling requirements given in EAS 346, the package shall be legibly and indelibly labelled in English and/or any other official language (French, Kiswahili, etc.) used in the importing East African Partner State with the following information:

- a) name of the product; as “skin applied mosquito repellent”
- b) form of product as “lotion”, “cream”, “gel” or “ointment”
- c) instructions for use;
- d) active ingredient (s) content;
- e) protection time;
- f) age group and/or health condition for which use is prohibited;
- g) storage conditions;
- h) disposal instructions;
- i) expiry date;
- j) date of manufacture;
- k) batch number; and
- l) precaution/warning

9 Sampling

Sampling shall be done in accordance with ISO 24153.

Annex A (normative)

Determination of DEET content

A.1 General

The sample is dissolved in carbon disulfide and the difference in absorbance at 14.18 μm and at 14.48 μm is determined. The quantity of meta-isomer is obtained from this value by means of a calibration curve prepared by the use of a reference standard.

A.2 Apparatus

A.2.1 Double-beam infrared spectrophotometer

A.2.2 Two equivalent infrared absorption cells, with sodium chloride windows and a path length of approximately 0.4 mm.

A.3 Preparation of calibration curve

A.3.1 Weigh (to the nearest 0.1 mg) into four volumetric flasks sufficient amounts of the reference DEET standard of known purity to give concentrations of approximately 20, 40, 60 and 80 g/L when dissolved in carbon disulfide.

A.3.2 Fill the reference cell with carbon disulfide and the sample cell with each of the standard solutions in turn, and record the spectra. The spectrum may be scanned rapidly, except for the region 12 – 15 μm , where a normal speed should be used. Carry out a blank measurement with carbon disulfide to correct for any inequality in the paired cells and to determine whether a cell correction is required.

A.3.3 Measure the absorbance at 14.18 μm and at 14.48 μm and calculate the difference between these values, ΔA , for each of the solutions. Plot the values of ΔA against the concentration (mg/L) of the meta-isomer.

A.3.4 If a cell correction is required, the value of ΔA is determined from the formula:

$$\Delta A = [A_{14.18} - A_{14.48}]_{\text{ref.}} - [A_{14.48}]_{\text{blank}}$$

Where ref. = determination with reference standard

blank = determination on CS₂ blank

A.4 Procedure

Weigh (to the nearest 0.1 mg) about 0.5 g of the sample, transfer quantitatively to a 10 mL volumetric flask, and make up to the mark with carbon disulfide. Measure the infrared absorption at 14.18 μm and 14.48 μm using the same conditions as described in clause A.3. Determine the concentration of meta-isomer by comparing this value with the calibration curve. A standard sample should be run each day to check the calibration of the instrument.

A.5 Calculation

DEET content (g/kg)

$$= \frac{(C_1 \times P)}{C_2} \text{ where,}$$

C₁ concentration (g/L) of standard DEET found from calibration curve

C₂ concentration (g/L) of sample taken

P purity (g/kg) of the reference standard

Annex B (normative)

Determination of ethyl butylacetamidopropionate (IR3535).

B.1 Outline of method

B.1.1 Ethyl butylacetamidopropionate is determined by capillary gas chromatography using flame ionisation detection and internal standardisation.

B.1.2 The retention time of the ethyl butylacetamidopropionate peak of the sample solution should not deviate by more than 2 % from that of the calibration solution.

B.2 Reagents

B.2.1 Acetonitrile

B.2.2 Ethyl butylacetamidopropionate, standard of known purity

B.2.3 Methyl tridecanoate, internal standard

B.2.4 Calibration solution.

B.2.4.1 Weigh (to the nearest 0.1 mg in duplicate) into volumetric flasks (10 ml) about 100 mg ethyl butylacetamidopropionate standard (s mg) and about 100 mg methyl tridecanoate (r mg).

B.2.4.2 Dissolve in acetonitrile and fill to the mark with acetonitrile (solutions C1 and C2). The solutions are stable for one week at room temperature.

B.3 Apparatus

B.3.1 Gas chromatograph equipped with a split/splitless injection and a flame ionisation detector

B.3.2 Capillary column fused silica, 25 m × 0.32 (i.d.) mm, coated with CP-Sil 5 CB, film thickness: 1.2 µm

B.3.3 Electric integrator or data system

B.4 Procedure

B.4.1 Chromatographic conditions (typical)

B.4.1.1 Column fused silica, 25 m × 0.32mm (i.d.), film thickness: 1.2 µm, coated with CP-Sil 5 CB

B.4.1.2 Injection system

B.4.1.2.1 Injector: split injection

B.4.1.2.2 Split ratio:1:50

B.4.1.2 Detector: Flame ionisation

B.4.1.3 Temperatures

B.4.1.3.1 Injection port: 300 °C

B.4.1.3.2 Detector: 310 °C

B.4.1.3.3 Oven program

B.4.1.3.3.1 initial: 120 °C

B.4.1.3.3.2 Program rate: 10 °C/min

B.4.1.3.3.3 Final: 260 °C

B.4.1.4 Injection volume: 5 µl

B.4.1.5 Gas flow rates

B.4.1.5.1 Helium 1.1 ml/min

B.4.1.5.2 Helium (make up) 45 ml/min

B.4.1.5.3 Hydrogen 40 ml/min

B.4.1.6 Retention times

B.4.1.6.1 ethyl butylacetamidopropionate: about: 10.4 min

B.4.1.6.2 methyl tridecanoate: about: 10.9 min

B.5 Preparation of sample.

B.5.1 Weigh in duplicate (to the nearest 0.1 mg) into volumetric flasks (10 ml) sufficient sample to contain about 100 mg ethyl butylacetamidopropionate (w mg) and about 100 mg methyl tridecanoate (q mg).

B.5.2 Dissolve in acetonitrile and fill to the mark with acetonitrile (solutions S1 and S2). The solutions are stable for one week at room temperature.

B.6 System equilibration

Inject 0.1 µl portions of the calibration solution and repeat the injections until retention times and calibration factors vary by less than 0.1 % of the mean of three successive injections.

B.7 Determination.

B.7.1 Inject 0.1 µl portions of the calibration solution and sample solutions in the following sequence: C₁, S₁, C₂, S₂,...etc.

B.7.2 Determine the peak areas and calculate the mean response factor (*f*) of the calibration solution injections bracketing the injections of the sample solutions.

B.8 Calculation

B.8.1 Individual response factor f_i is calculated as follows:

$$f_i = \frac{I_r \times S \times P}{H_s \times r}$$

B.8.2 Ethyl butylacetamidopropionate shall be calculated as follows:

$$\frac{H_w \times f \times q}{I_s \times w}$$

where:

f_i individual response factor

f mean response factor

H_s peak area of ethyl butylacetamidopropionate in the calibration solution

H_w peak area of ethyl butylacetamidopropionate in the sample solution

I_r peak area of the internal standard in the calibration solution

I_q peak area of the internal standard in the sample solution

s mass of ethyl butylacetamidopropionate standard in the calibration solution (mg)

r mass of internal standard in the calibration solution (mg)

q mass of internal standard in the sample solution (mg)

w mass of sample taken (mg)

P purity of ethyl butylacetamidopropionate standard (g/kg)

Repeatability r 18 g/kg at 1002 g/kg active ingredient content

Reproducibility R 18 g/kg at 1002 g/kg active ingredient content

Annex C (normative)

Determination of icaridin

C.1 outline of method

Icaridin is determined by capillary gas chromatograph using internal standardisation and flame ionisation detection.

C.2 Reagents

C.2.1 Icaridin reference standard with known content

C.2.2 Dimethyl Phthalate internal standard

C.2.3 Propan-2-ol

C.2.4 Calibration solution.

Weigh (to the nearest 0.1 mg) about 100mg icaridin reference substance (*s* mg) and 100 mg dimethyl phthalate (*r* mg) into a volumetric flask (20 ml). Fill to the mark with propan-2-ol and homogenise.

C.3 Apparatus

C.3.1 Gas chromatograph capable of operating in the range 150 °C to 330 °C, fitted with a flame ionisation detector, a split injector and an autosampler

C.3.2 Column quartz, 30 m × 0.25 mm (i.d), coated with dimethyl polysiloxane/ diphenyl polysiloxane 95/5% (e.g. DB5), film thickness 0.25 µm

C.3.3 Electronic integrator or data system

C.4 procedure

C.4.1 Chromatographic conditions (typical)

C.4.1.1 Column: Quartz, 30 m × 0.25mm (i.d.), coated with dimethyl polysiloxane/ diphenyl polysiloxane 95/5% (e.g. DB5), film thickness 0.25 µm

C.4.1.2 Injection system

C.4.1.2.1 Injector: split injection

C.4.1.2.2 Split flow: 40ml/min

C.4.1.2 Detector: Flame ionisation

C.4.1.3 Temperatures

C.4.1.3.1 Injector: 240 °C

C.4.1.3.2 Detector: 330 °C

C.4.1.3.3 Oven program: 150°C hold for 2 min, gradient: 10°C/ min to 330 °C, hold for 3 min.

C.4.1.4 Injection volume: 1 µl

C.4.1.5 Gas flow rates

C.4.1.5.1 Helium (carrier) 1.5 ml/min (100kpa)

C.4.1.5.2 Hydrogen: about 30ml/min

C.4.1.5.3 Air: about 300ml/min

C.4.1.5.4 Nitrogen (makeup): about 25ml/min

C.4.1.6 Run time: about 25 min

C.4.1.7 Retention times

C.4.1.6.1 Dimethyl Phthalate: about 3 min

C.4.1.6.2 Icaridin: about 4.5 min

C.5 Preparation of sample

Weigh (to the nearest 0.1 mg) into a volumetric flask (20 ml) sufficient sample to contain about 100 mg icaridin (w mg) and about 100 mg dimethyl phthalate (q mg). Fill to the mark with propan-2-ol and homogenise.

C.6 Equilibration of the system

Inject 1 µl portion of calibration solution and repeat the injections until retention times and the icaridin to the internal standard peak area ratio vary by less than 0.5% of the mean for successive injections.

C.7 Determination

C.7.1 Inject in duplicate 1 µ portions of the calibration solution (C_1 and C_2) and of the sample solution (S_1 , S_2 , etc.) in the following sequence: C_1 , S_1 , S_2 , ... C_2 .

C.7.2 Determine the peak areas and calculate the response factor (f) from the calibration solutions bracketing the injections of the sample solutions. Calculate the content of the sample solutions.

C.8 Calculation

C.8.1 Response factor f is calculated as follows:

$$f = \frac{I_r \times S \times P}{H_s \times r}$$

C.8.2 Icaridin content shall be calculated in g/kg as follows:

$$\frac{H_w \times f \times q}{I_s \times w}$$

where:

f mean response factor

H_s peak area of icaridin in the calibration solution

H_w peak area of icaridin in the sample solution

I_r peak area of dimethyl phthalate in the calibration solution

I_q peak area of dimethyl phthalate in the sample solution

s mass of icaridin in the calibration solution (mg)

r mass of dimethyl phthalate in the calibration solution (mg)

q mass of dimethyl phthalate in the sample solution (mg)

w mass of sample taken (mg)

P purity of icaridin standard (g/kg)

Repeatability r 12 g/kg to 14 g/kg at 986 g/kg active ingredient content

Reproducibility R 14 g/kg at 986 g/kg active ingredient content

Annex D (normative)

Determination of residue

D.1 Procedure

Weigh accurately about 5 g of the material in a weighed, clean and dry squat form weighing bottle and dry to constant mass at $105^{\circ}\text{C} \pm 1^{\circ}\text{C}$. Cool in desiccators and weigh.

D.2 Calculation

The residue, expressed as percent, shall be calculated as follows;

$$\frac{M_1}{M_2} \times 100$$

where,

M_1 mass in g of the residue; and

M_2 mass in g the material taken for test

Annex E (normative)

Determination of total fatty substance content

E.1 Outline of the method

The emulsion is broken with dilute mineral acid and the fatty matter is extracted with petroleum ether. It is weighed after removal of the solvent.

E.2 Reagents

E.2.1 Dilute hydrochloric acid 1:1 (v/v)

E.2.2 Petroleum ether, B.P. 40 °C to 60 °C

E.2.3 Methyl orange indicator solution—Dissolve 0.1 g of methyl orange in 100 mL of water.

E.2.4 Sodium sulphate, desiccated

E.3 Procedure

E.3.1 Weigh accurately about 2 g of the material into a conical flask; add 25 ml of dilute hydrochloric acid, fit a reflux condenser into the flask and boil the contents until the solution is perfectly clear.

E.3.2 Pour the contents of the flask into a 300 ml separation funnel and allow it to cool to 20 °C and rinse the conical flask with 50ml of petroleum ether in portions of 10 ml.

E.3.3 Pour the ether rinsings into the separation funnel shake the separation funnel well and leave until the layers separate.

E.3.4 Separate out the aqueous phase and shake it out with 50mL portions of ether twice. Combine all the ether extracts and wash them with water until free of acid (when tested with methyl orange indicator solution).

E.3.5 Filter the ether extracts through a filter paper containing sodium sulphate into a conical flask which has been previously dried at a temperature of 60 °C ± 2 °C and then weighed.

E.3.6 Wash the sodium sulphate on the filter with ether and combine the washings with the filtrate. Distil off the ether and dry the material remaining in the flask at a temperature of 60 °C ± 2 °C to constant mass.

E.4 Calculation

The total fatty substance, expressed as percent, shall be calculated as follows;

$$= \frac{M_1}{M_2} \times 100$$

where,

M_1 is the mass, in grams, of the residue, and

M_2 is the mass, in grams, of the material taken for the test.

DEAS for enquiry stage

Annex F (normative)

Determination of total pyrethrins

F.1 General

The active ingredients in pyrethrum extract may be determined using a HPLC system first by injecting a solution of the analyte into the chromatograph, followed by the separation and comparison of peaks areas of the analytes in the sample with that of an external standard containing a known amount of the analytes. The peaks are eluted in the following order: Cinerin II, Pyrethrin II, Jasmolin II (total Pyrethrins II) and Cinerin I, Pyrethrin I, Jasmolin I (total Pyrethrins I).

F.2 Reagents

F.2.1 World pyrethrum standard, 50 %

F.2.2 Acetonitrile, HPLC grade

F.2.3 Water, HPLC grade

F.3 Apparatus

A liquid chromatography System equipped with an auto-sampler, a Variable Wavelength Detector (or equivalent) and a Column {Phenomenex, 250 x 4.6 mm Luna Phenyl-Hexyl 5 μ Reverse Phase (or equivalent).

F.4 Operating conditions

F.4.1 Flow rate: 1.5 ml/min

F.4.2 Composition: 40:60 (% , v/v water/acetonitrile)

F.4.3 Elution: Isocratic

F.4.4 Oven temperature: 40 °C

F.4.5 Wavelength: 240 nm

F.4.6 Injection Volume: 15 μ l

F.4.7 Stop time: 22 min

F.4.8 Post time: 1 min

F.5 Preparation of the standard

Weigh 20 mg of the pyrethrum standard to the nearest 0.0001 g in a 100 mL volumetric flask and dilute to volume with acetonitrile and label it. Transfer a small portion to a sample vial and label it accordingly.

F.6 Sample preparation

In a 100 ml volumetric flask, weigh 20 mg to the nearest 0.0001 g of the sample to be analyzed and dilute to volume with Acetonitrile. Sample this solution using a vial and label it accordingly.

F.7 Procedure

After the chromatograph is stable, make a minimum of three injections for the standard solution as well as for the analyte and average the area counts. The relative standard deviation between injections should be within 2 %.

F.8 Calculation of the % total pyrethrins (active ingredient)

The total pyrethrins, expressed as percent shall be calculated as follows;

$$\frac{\text{(Average sample area X weight of standard X Purity of the standard (in \%))}}{\text{(Average standard area X Weight of sample)}}$$

Bibliography

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DEAS for enquiry stage