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28th July 2020

CERTIFICATION OF TRANSLATION

TO WHOM IT MAY CONCERN

I, **Manoj CHAUHAN** competent to translate from French to English, certify to the best of my abilities that the translation of:

- **SGS Group- Test Report-No Estrogenic Activity**

is true and accurate translation of the original document from French to English.

This translation was processed by Lyric Technologies Pte. Ltd, (Reg. No : 201116568Z) an ISO 9001:2015 certified Translation Company.

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(Authorized Signature
on behalf of the translator)

(Manoj CHAUHAN)

(Name of the translator)

REFERENCES

Order: AS PER AGREED QUOTE
Quote: DR18-10125
Recd in Rouen 07/01/19
Applicant: Mr. GENEAU Serge
Client ID: DISINFECTANT
Description:
Nature: Cosmetic Product
Comment:

AQUAMA FRANCE SARL GEN EAUSELECTION
1 RUE DE LAREPUBLIQUE

69001LYON
FRANCE

Rouen,1 February 2019

TEST REPORT
RN19-00293.001 Page 1 / 2

Photo of the sample:

[Photo]

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Photo of the sample:

[Photo]

Parameters	Units	Results
Detection of estrogenic activity: molecular (2) (EMCDDA Molecular Method)		See Appendix

Results electronically validated by **Maïmiti BONNEL**
Project Manager Tel: 02 35 07 91 38

(Seal)

This validation has an electronic signature, it is carried according to the requirements of the ISO 17025 standard.

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STUDY REFERENCE	**EMCDDA-19/0008**
STUDY DATE	**14/01-21/01/2019**
ORDERING CUSTOMER	**SARL GEN'EAU SELECTION, AQUAMA France**
SGS REFERENCE	**RN19-00293.001**

1. OBJECTIVE OF THE STUDY

The In Vitro test for measuring the binding capacity between the human estrogen receptor, hERα, and estrogen allows the detection of endocrine disruptors with estrogenic activity in a product by a molecular testing. In case binding is demonstrated, a dosage expressed in estradiol equivalent is calculated according to the study model used.

2. TEST ELEMENTS

NAME	SOLUTION INDIGO
REFERENCE	SPRAY DECOUVERTE
BATCH	ND
PRESENTATION	PRODUCT PACKAGED IN SPRAY - MATERIAL: PLASTIC - DATE OF PACKAGING: 04/01/2019

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3. STUDY PRINCIPLE

This in vitro test is based on the binding competition of a fluorescent ligand to the human estrogen receptor hERα. The measured signal determines whether the fluorescent ligand is free or linked to the estrogen receptor.

In case of Liaison between an estrogenic-type endocrine disruptor (PE) and the estrogen receptor, the fluorescent ligand bound to the receptor is displaced in favor of the PE binding. This fluorescent ligand is extinguished by being free in solution and the measured fluorescence signal decreases. This decrease is proportional to the amount of unbound fluorescent ligand and thus to the amount of PE linked to the receptor. This test is therefore used to evaluate the presence of compounds with estrogenic activity in a sample

The amount of estrogenic-type disruptors is determined by measure of the decrease in signal of the fluorescent ligand correlated with the amount of receptor-disruptor (ER-PE) complex formed.

4. PROGRESS OF THE STUDY

Study Model: Molecular Testing

The standard dissociation curve between the fluorescent ligand and the non-fluorescent estrogenic ligand concentration is performed (Figure 2).

The measurements of the PE binding to the estrogen receptor were reproduced in triplicate independently for each concentration tested, 6 measurements per tube are performed.

Figure 1 shows the dissociation of the fluorescent ligand from the estrogen receptor by the sample (in the form of a histogram).

The results in terms of dissociation of the fluorescent ligand are normalized according to the following formula:

$$\text{Signal measured \%} = (S_{sample}-S_{min}) / (S_{max}-S_{min})$$

$$S_{min}=S_{\text{Free fluorescentligand}}$$

$$S_{max}= S_{\text{Bound fluorescentligand}}$$

In order to quantify the presence of estrogenic compounds in estradiol equivalent (contained in the tested sample), the standard curve is used (Figure 2). If the decrease in signal is not proportional to the amount of sample tested, then it is not possible to calculate an estradiol equivalent. We can deduced that the tested sample does not contain any estrogenic compounds under the experimental conditions used.

If, on the contrary, the signal decrease is proportional to the amount of sample tested, an estradiol equivalent can be calculated. The latter incorporates a precautionary principle and a notion of threshold.

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Postulate of the study

The product is completely absorbed by the skin and diluted in the volume of blood circulating in the body (about 5L). The molecules present in the product are not metabolized by humans into other more or less toxic molecules.

Notion of threshold – potential risk

The amounts of estradiol E2 circulating naturally in "humans" are expressed below:

- In postmenopausal women/men: $4.0 \times 10^{-11} - 2.0 \times 10^{-10}$ mol.L^{-1}
- In non-menopausal women (excluding ovulation): $1.0 \times 10^{-10} - 6.0 \times 10^{-10}$mol.L^{-1}
- In women (ovulation): 2.0×10^{-9} mol.L^{-1}

According to the study model, a value is considered critical when it is equal to or greater than half of the average estradiol level circulating in women in ovulation, i.e., 1.0×10^{-9}mol.L^{-1}.

Molecular test protocol

1. Preparation of the fluorescent hERα-Ligand complex in a study buffer:
   [hERα]=5 10^{-7}M and [Ligand fluorescent]=1.7 10^{-9}M

2. Preparation of the test sample:
   Dilution of the sample in the Study Buffer (1ml in total volume of 2ml)
   Stirring for 20h at 30°C
   The sample was diluted 1 time

3. Subsequently, different concentrations of the sample are prepared in a fluorescent ligand solution complexed with hERα

Sample	1	2	3	4	5	6
Sample volume (µl)	0	5	10	20	40	90
Fluorescent ligand solution volume complexed with	800					

4. Measurement of the fluorescence signal at 20°C.

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5. RESULTS

The results of fluorescence measurements to assess the presence of estrogenic compounds in the tested sample are presented below.
Up to 2.501g/100ml, the measured signal is independent of the content of this sample in the solution. The measured signal is not significantly altered by the content of the tested sample. We can deduce an absence of interaction between the estrogen receptor and the compounds present in the solution obtained after extraction.

Tested sample	Standard curve E2
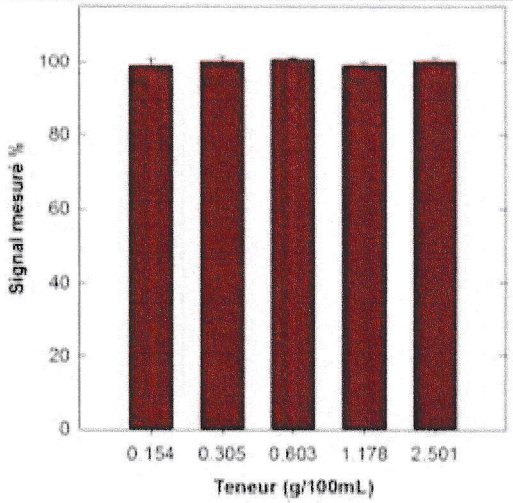	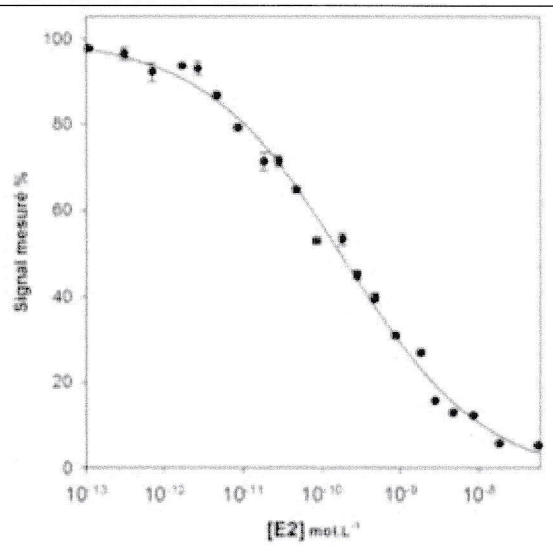
Figure 1	*Figure 2*

6. CONCLUSION

Under experimental conditions, up to 2.501g/100ml, the product **SOLUTION INDIGO packaged in plastic spray on 04/01/2019, reference SPRAY DECOUVERTE,** does not bind to the estrogen receptor, therefore does not show any estrogenic activity.

Véronique LE TILLY /BIOLOGIST
23/01/2019,
[SIGNATURE]

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