CLINICAL EXPERTISE INSTITUTE

CONFIDENTIAL

REPORT

SPONSOR: **DNA HEALTH INSTITUTE, CRYOGENIC DIVISION**

IN VITRO STUDY: **CELLULAR GROWTH**

PHARMACOLOGICAL STUDY CONDUCTED
ON A NORMAL FIBROBLASTIC LINE OF
HUMAN SKIN, INTENDED TO ASSESS THE
EFFECT OF THE PRODUCT ON THE
CELLULAR GROWTH
(Dosage method of MTT conversion)

PRODUCT: EMBRYO EXTRACT

REPORT: No. R51208D of December 4, 1999

Study's Director:
J.R. CAMPOS
PhD in Cellular Biology
and Microbiology
I.E.C. Diploma in Dermocosmetology

25 pages document

HEALTH MINISTRY AUTHORIZATIONS

CLINICAL EXPERTISE INSTITUTE

REPORT

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EFFECT OF THE PRODUCT ON THE
CELLULAR GROWTH
(Dosage method of MTT conversion)

Sponsor (study requester):

Company name: DNA Health Institute, Cryogenic Division

Study's Supervisor: Dr. M. TORDUE

Biological Research Center:

Company name: I.E.C.

Study's Director: J.R. CAMPOS
PhD in Cellular Biology
and Microbiology
Diploma in Dermocosmetology

Target product: EMBRYO EXTRACT

Study request: protocol no. 71995D of November 6, 1999

Report: no. R51908D of December 4, 1999

Study's schedule:

- Start of trial : November 8, 1999
- End of trial : November 20, 1999
- End of study (final report signed by the study's Director) : December 4, 1999

SUMMARY

AUTHENTICATION

The study, object of this report, was conducted under my responsibility, in accordance with the experimental protocol, the procedures of the Biological Research Center and the Laboratory Good Practices regulations.

All the observations and numerical data obtained during this trial are reported in this document. After proofing, I certify these data as conforming to the real results.

Jean-Robert CAMPOS
Study's Director

I have read this report and I agree with its content.

Jean-Pierre GUILLOT
I.E.C. Director

LIST OF PERSONS INVOLVED IN THE STUDY

I.E.C. President
Name: J.P. GUILLOT, D.E.S.S., Pharmacologist – Toxicologist Expert

Executive Assistant
Name: Y. POHLMANN, D.U.E.L. in English

Study's Director
Name: J.R. CAMPOS, PhD in Cellular Biology and Microbiology
Diploma in Dermocosmetology

Technician
Name: L. AUFAURE, D.U.T in Applied Biology

Quality Assurance Manager
Name: N. GUILLOT, graduate from the "École Supérieure de Biologie et de Biochimie de Paris"

CLINICAL EXPERTISE INSTITUTE

SPONSOR: DNA Health Institute, Cryogenic Division

TARGET PRODUCT:
EMBRYO EXTRACT

ABSTRACT

CELLULAR GROWTH

PHARMACOLOGICAL STUDY CONDUCTED ON A
NORMAL FIBROBLASTIC LINE OF HUMAN SKIN,
INTENDED TO ASSESS THE EFFECT OF THE PRODUCT ON
THE CELLULAR GROWTH
(Dosage method of MTT conversion)

OBJECTIVE

Assess a product's effect on the growth of human skin normal fibroblasts, after contact time of 24, 72 and 144 hours.
The cellular growth was determined by dosage of the MTT conversion, which is metabolized only by living cells.

RELEVANCE OF THE STUDY

Among the various intrinsic skin ageing expression modes such as the quantitative diminution of collagen, elastin (Fazio et al., 1988), fibronectin (Yamada & Olden, 1981) and glycosaminoglycan, the functional action of the fibroblasts seem to play the major role. According to Lapière (1990), the rarefaction of the fibroblasts and the loss of their cohesion with the interstitium constitute the fundamental elements of ageing.

The dosage method of MTT conversion has enabled the profiling of human tumoral lines' cellular growth (Alley et al., 1988 ; Pagé et al., 1988 ; Vistica et al., 1991) during culture periods as long as 11 days.
This test has uncovered the proliferative effects of mitogen agents such as Concanavalin A, lipopolysaccharidic extracts (Mosmann, 1983) or of cytokines such as Interleukin IL-2 (Tada et al., 1986).

There are three reasons for the evaluation of the pharmacological substances' action on the human skin normal fibroblasts:

- work on the cellular model, which appears to play an important role in the genesis of skin ageing,
- use a cellular type, which maintains its differentiated character, so as to be able to extrapolate the activities recorded in situ at the organ.
- enable tests on human cells.

EQUIPMENT

The normal lines are commonly used since the works of Hayflick & Moorhead (1961). An advantage of this fibroblasts' culture is its ability to maintain, during successive subcultures, the genetic material of the original skin zone.

The human origin normal fibroblasts have been provided by the BIOPROTEC company (10 chemin des Cuers, 69570 Dardilly, France).
These are human origin normal lines, obtained from biopsies taken on healthy donors.

PROTOCOL

A preliminary cytotoxicity study, performed with the dosage of the MTT conversion, was conducted on the human fibroblasts, in order to select the range of concentrations, which could uncover the effect on cellular growth.

After inoculating the fibroblasts at a concentration of 10.10^3 cells/well, the plate was incubated for 24 ± 3 hours prior to adding the product under study.
Starting with the stock solution, 6 serial 1/2 in 1/2 dilutions were made in a culture medium lightly enriched with embryo serum.
The contact time Product-Cells was 24 ± 3 hours, before measuring the MTT conversion into formazan.

During the pharmacological study, the fibroblasts were inoculated into microplates at the rate of 5.10^3 cells/well.
After a 24 ± 3 hours incubation, the culture medium was eliminated and the cells were put in contact with 4 preselected concentrations of the product during approximately 24, 72 and 144 hours.

Following this contact period, 830 µg of MTT/ml was added in each well, followed by the incubation of the microplates during 3 hours ± 30 min in a CO2 incubator. During the research for the effect on the cellular growth, a positive control was performed with embryo serum (10%, 15%).

RESULTS AND CONCLUSION

In view of the experimental conditions used:

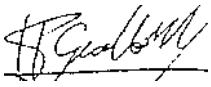
- the preliminary cytotoxicity has shown the following Cytotoxicity Index 50:
  90.50% < IC50 avg. = 92.85% < 96.20%
- the research for the activity of the "EMBRYO EXTRACT (batch no. 105956)" has shown:
  - a significant stimulation of the normal human fibroblasts' cellular growth as early as 72 contact hours
  - an increase in the cellular growth stimulation after 144 contact hours.
- a statistical analysis has enabled classifying the tested concentrations in function of contact time, and their effect on the cellular growth stimulation:

72 contact hours = 50% << 25% = 1.56% < 6.25% = 12.50%

144 contact hours = 50% << 1.56% = 1.56% < 6.25% = 12.50%

	EMBRYO EXTRACT CONCENTRATIONS (V/V)			
	1.56%	6.25%	12.5%	25%
Growth gain after 72 contact hours	17%	31%	35%	15%
Growth gain after 144 contact hours	32%	55%	71%	54%

In conclusion, the "EMBRYO EXTRACT" under study does not exhibit a significant cytotoxic effect on the mitochondrial activity of the normal human fibroblasts; after 72 contact hours, it has triggered a significant increase in the cellular growth, which translated into a stimulation of the mitochondrial activity, amplified after 144 contact hours.



Done
December 4, 1999

J.P. GU1LLOT
Pharmacologist – Toxicologist Expert
I.E.C. Director

J.R. CAMPOS
PhD in Cellular Biology
and Microbiology
Diploma in Dermocosmetology
Study's Director

QUALITY ASSURANCE

This study has been performed in conformity with the Laboratories Good Practices published by the French Health Ministry (Fasc. No. 84/17, 1984), with the Biological Research Center's standard procedures, with the I.E.C. general operating procedures and with the signed protocol.

The routine inspection of the "in-vitro" studies is ongoing; this is meant to insure, whenever possible, that all-important phases of a given test are inspected at least once a month. The inspection dates and the study type are shown below.

The results of these inspections have been reported to the Investigator and to Management.

Type of study	Dates of inspection	Report dates to Investigator	Report dates to Management
Identical test:	November 8, 1999	November 9, 1999	November 10, 1999

This report has been audited by I.E.C. Quality Assurance Department; it is a true report of the procedures followed and an exact recording of the original data generated by this study.

	Dates of inspection	Report dates to Investigator	Report dates to Management
Report (vs. Raw data):	November 30, 1999	December 1, 1999	December 1, 1999

Nicole GUILLOT
Quality Assurance Manager

Signature:

Date: December 4, 1999

1. 1. OBJECTIVE OF THE STUDY

Assess a product's effect on the growth of human skin normal fibroblasts, after contact time of 24, 72 and 144 hours.
The cellular growth was determined by dosage of the MTT conversion, which is metabolized only by living cells.

2. RELEVANCE OF THE STUDY

Among the various intrinsic skin ageing expression modes such as the quantitative diminution of collagen, elastin (Fazio and al., 1988), fibronectin (Yamada & Olden, 1981) and glycosaminoglycan, the functional action of the fibroblasts seem to play the major role. According to Lapière (1990), the rarefaction of the fibroblasts and the loss of their cohesion with the interstitium constitute the fundamental elements of ageing.

The dosage method of MTT conversion has enabled the profiling of human tumoral lines' cellular growth (Alley and al., 1988 ; Pagé and al., 1988 ; Vistica and al., 1991) during culture periods as long as 11 days.
This test has uncovered the proliferative effects of mitogen agents such as Concanavalin A, lipopolysaccharidic extracts (Mosmann, 1983) or of cytokines such as Interleukin IL-2 (Tada et al., 1986).

There are three reasons for the evaluation of the pharmacological substances' action on the human skin normal fibroblasts:

- work on the cellular model, which appears to play an important role in the genesis of skin ageing,
- use a cellular type, which maintains its differentiated character, so as to be able to extrapolate the activities recorded in situ at the organ.
- enable tests on human cells.

3. PRINCIPLE

In order to assess the product's effect on the cellular mitochondrial activity, a colorimetric dosage was executed with reference to the Mosmann method (1983) modified by Denizot et Lang (1986). However, the DMSO solvent was chosen because it better solubilizes formazan crystals (Alley et al., 1986; Twentyman & Luscombe, 1987).

This technique is based on the formation of formazan crystals under succinate deshydrogenase mitochondrial activity.
The MTT is a tetrazolium salt whose denomination is 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide.
It is reduced at the mitochondrial chain of electrons transfer level, site of the oxydative phosphorylation.
Only living cells, through their mitochondrial deshydrogenases, transform this pale yellow salt into a dark blue compound: the formazan.
The quantity of formazan is proportional to the number of active cells in the medium.

4. TARGET PRODUCT

4.1. Denomination: EMBRYO EXTRACT (batch no. l05956)

4.2. Study's Identification Code: IV 5096001

4.3. Presentation (galenic form and color): orange color liquid

4.4. Analytical Controls:

The Study's Supervisor was responsible for the determination of the identity, the physico-chemical characteristics and any other criteria enabling the identification of the product's batch.
For this type of study, no analytical dosage was performed and neither the stability nor the absorption of the product was evaluated by I.E.C.

4.5. Packaging: glass vial

4.6. Quantity provided and receipt date: 60 ml, received on November 7, 1999

4.7. Storage:

At a temperature of approximately + 4 °C.

A sample of the product shall be kept in our laboratories for 2 months as of the transmission date of the final report. At that time, unless otherwise instructed by the Study's Supervisor, we will destroy the product.

4.8. Vehicle:

The product has been solubilized in a culture medium.

4.9. Stability of Preparations:

The formulation requiring a dilution was used within 24 hours following the preparation.

5. EQUIPMENT AND METHODS

5.1. Normal Fibroblastic Line

The normal lines are commonly used since the works of Hayflick & Moorhead (1961). An advantage of this fibroblasts' culture is its ability to maintain, during successive subcultures, the genetic material of the original skin zone.

The human origin normal fibroblasts have been provided by the BIOPROTEC company (10 champ des Cuers, 69701 Dardilly, France).

5.1.1. Origin of Normal Line

This is a human origin normal line, obtained from biopsies taken on donors:

- sex : male
- age : newborn
- race : Caucasian
- anatomic location : prepuce.

5.1.2. Serological Screening of Biopsies

A serological screening was performed by the BIOPROTEC Company, which provided a certificate pertaining to the following research:

- HIV and HIV2
- Human leukemia virus (HTLV1 and HTLV2)
- Hepatitis A, Hepatitis C
- Hepatitis B, HbsAg, anti-HBs Ac, anti-HBc Ac
- Cytomegalovirus (CMV).

5.2. Test Procedure

5.2.1. Preliminary Study of General Cytotoxicity

Before researching the product's proliferative effect on the fibroblastic line, a preliminary study of general cytotoxicity, performed from the MTT conversion dosage, has allowed narrowing the range of the product's concentrations.

5.2.1.1. Inoculation

Prior to any study involving a spectrophotometric microtitration, it was necessary to determine the cellular concentration to be distributed in each well, in order to homogenize the various inoculations specific to the line and to each colorimetric technique.

Starting with the cellular suspension, the fibroblasts were spread into a microplate with a multichannel pipette at the rate of 100 µl/well (i.e. a cellular concentration of 10.10^3 cells/well).
After inoculation, the microplate was incubated for 24 ± 3 hours at 37 °C ± 0.5 °C in a humid atmosphere containing 5 ± 0.5% CO2.

5.2.1.2. Solubilization of the Product

During preliminary product solubility tests, various concentrations were tested in an aqueous medium. From the selected stock solution, which did not exhibit any precipitate, 6 serial 1/2 in 1/2 dilutions were made.

5.2.1.3. Addition of the Product

The product was added 24 ± 3 hours after inoculation, in order to avoid any interaction between the product and the cells attachment and to allow recovery of the cells' membranous integrity after trypsinization.

The product was diluted in a culture medium containing a low concentration of embryo serum. A low content of embryo serum avoids interaction of the plasmatic proteins.

The inoculation medium was removed by suction through a vacuum pump system, and then replaced by either:
- the product under study at 7 concentrations
- the negative control

The volume processed was identical to the inoculation volume, namely 100 µl.

Regardless of the xenobiotics' nature (control, product under study), 4 points per concentration were realized.

5.2.2. Research of Effect on Cellular Growth

5.2.2.1. Inoculation

Starting with the cellular suspension, the fibroblasts were spread into the microplates with a multichannel pipette at the rate of 100 µl/well.

With the testing lasting approximately 24, 72 and 144 hours, the inoculation's cellular concentration adopted for this test was 5.10^3 cells/well.

After inoculating the cells in a complete culture medium, the microplates were incubated at 37 °C ± 0.5 °C, in an atmosphere composed of 5 ± 0.5% of CO2 and 95 ± 0.5% of saturated humid air.

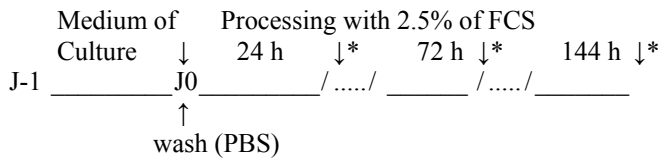
5.2.2.2. Positive Control

The embryo serum is an element, which substantially promotes the growth of various cellular types. The generally accepted concentration for normal cells culture is approximately 10%.
In view of its importance in cellular proliferation, this factor has been used as positive reference at 10 and 15% concentrations.

5.2.2.3. Processing

Following the preliminary cytotoxicity test performed with the MTT test, the range has been narrowed around concentrations, which have not exhibited a significant cytotoxicity.
The product under study was serially diluted from a stock solution, and 6 points per concentration were obtained.

The product and the positive control were added 24 ± 3 hours after inoculation, in order to avoid any interaction between them and the cells attachment and to allow recovery of the cells' membranous integrity after trypsinization.
After removal of the inoculation's medium and washing with PBS, 100 µl of medium containing the product at 4 concentrations were distributed in each well, and 6 points per concentration were obtained.
The product was diluted in a medium containing 2.5% of embryo serum in order not to hide a potential effect of the product on the cellular growth.
After inoculation, the plates were incubated in the incubator for periods determined in accordance with the following diagram:



↓* = dosage of MTT conversion)

6. STATISTICAL ANALYSIS

In function of the generated information, a statistical analysis was performed on the various tests (Dagnelie, 1970, 1973):

- Factorial analysis with 1 or several factors,
- Least Significant Difference Method

The samples being independents, the series under study were not paired.

6.1. Variance Analysis, fixed model with one classification criteria

Variance analysis with one classification criteria, or one factor, allowed comparing the means of the various product's concentrations and of the positive control.

The various states being well defined (product's nature, concentrations), this is a fixed model whose mathematical representation is given by the following formula:

$$x = \mu + a + e$$

where: μ is the means of the population,

a corresponds to the factor's action,

e represents the population's intrinsic variability.

The samples being equal and small, the homoscedasticity's importance was relatively secondary. The null hypothesis will be H0 : m1 = m2 = ...= mp.

The rejection of the null hypothesis, relative to the fixed model, has raised the issue of knowing which means are differing from one another. This issue has been resolved with the Least Significant Difference Method.

6.2. Least Significant Difference Method

Since the variance analysis was performed later, it was advantageous to use the global estimate provided by the residual mean square.

The least significant difference is equal to $\Delta = t\ 1\text{-}\alpha/2\ (2.Cmr/n)^{1/2}$ with the number of degrees of reedom of the distribution t which is always the number of degrees of freedom of the mean square or variance which served as comparison basis.

7. RESULTS

7.1. Serological Screening of Biopsies

The serological screening of biopsies gave negative results (see appendix).

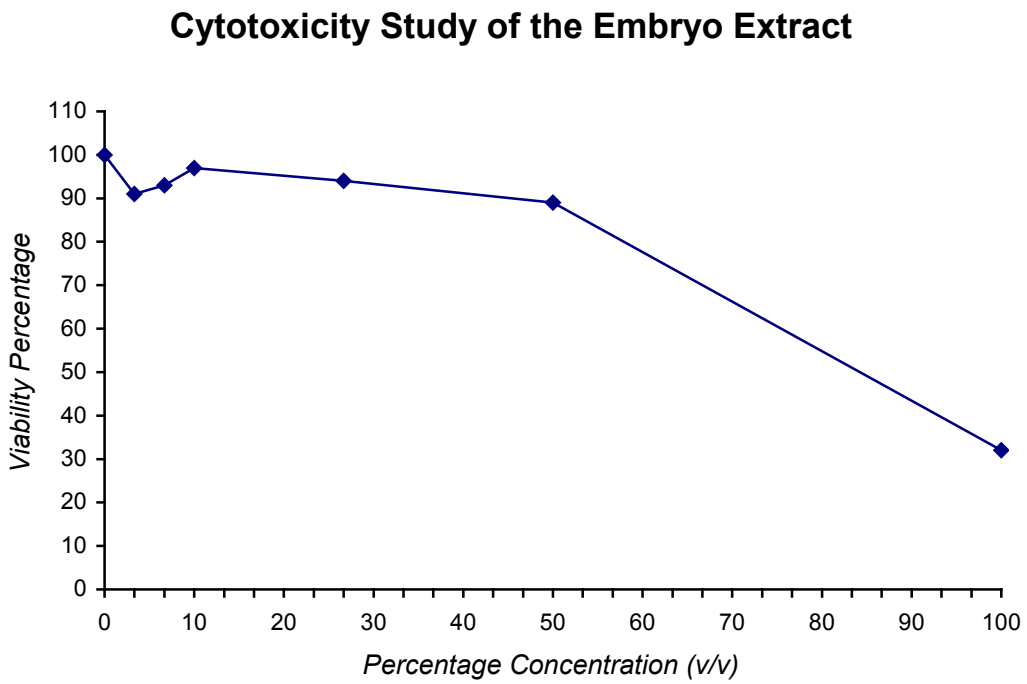
7.2. Preliminary Study of Cytotoxicity

The range of concentrations, falling between 3.12% and 85% (V/V), did not show any cytotoxic effect of the Embryo Extract. On the other hand, a decrease in the mitochondrial activity was observed when the Embryo Extract was tested pure (see Graph p. 21).

The preliminary cytotoxicity study has shown the following Cytotoxicity Index 50 (I.C.50):

	LOWER INTERVAL	IC 50 AVERAGE	UPPER INTERVAL
EMBRYO EXTRACT	90.50%	92.85%	96.20 %

The confidence intervals were calculated with an error likelihood of 5%.



7.3. Research of Effect on Cellular Growth

7.3.1. Influence of Embryo Extract

After a 24 hours contact, the Embryo Extract does not show any significant positive effect with respect to a negative control, regardless of the tested concentration (Graphs p. 23 and p. 24).

On the other hand, at 72 contact hours, a stimulating effect is noticeable for concentrations between 1.56% and 25%. This effect is further amplified at 144 contact hours (Graphs p. 23 and p. 24).

	EMBRYO EXTRACT CONCENTRATIONS (V/V)			
	1.56%	6.25%	12.5%	25%
Growth gain after 72 contact hours	17%	31%	35%	15%
Growth gain after 144 contact hours	32%	55%	71%	54%

7.3.2. Variance Analysis

Since the variance analysis with 1 fixed model has shown that the observed F was much larger than the theoretical F (see appendix), it is possible to reject the null hypothesis of the means equality. This rejection of the null hypothesis has raised the issue of knowing which means are differing from one another.

7.3.3. Least Significant Difference

Classification of the various Embryo Extract's concentrations and of the positive control (FCS) after 24 contact hours:

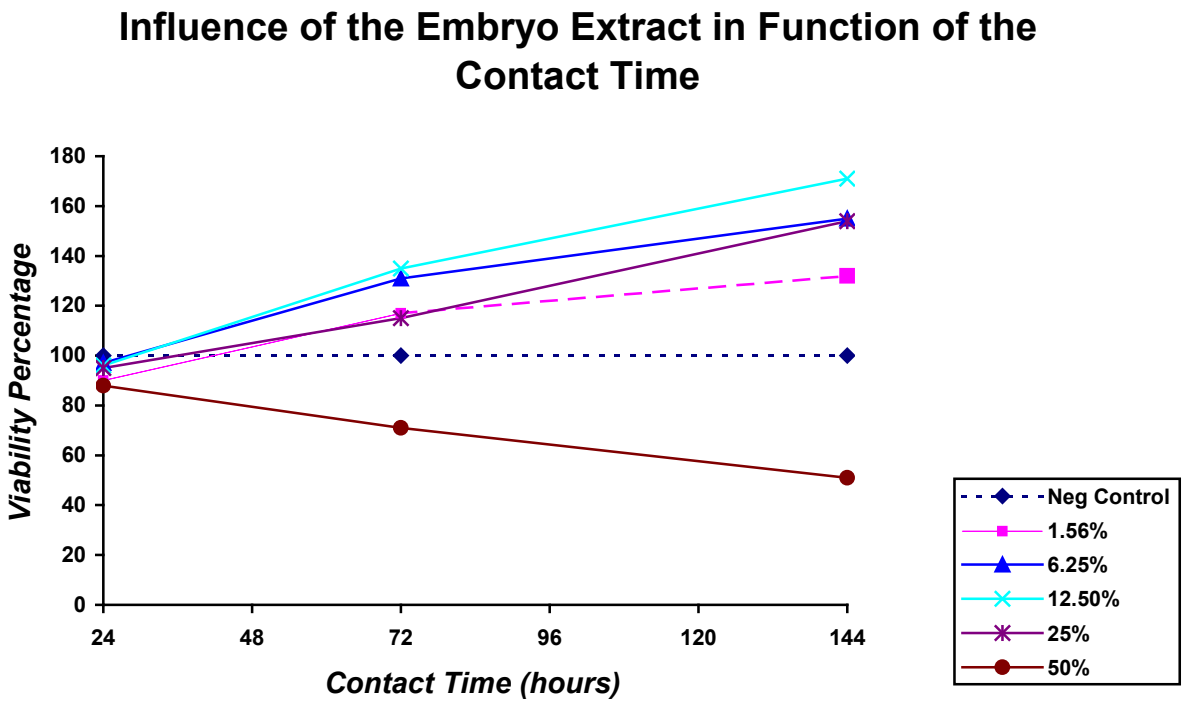
50% = 1.56% < 25% = 12.50% = 6.25% = Control 15% FCS = Neg. Control = Control 10% FCS
but 25% < Neg. Control = Control 10% FCS and 12.50% = 6.25% < Control 10% FCS.

Classification of the various Embryo Extract's concentrations and of the positive control (FCS) after 72 contact hours:

50% << Neg. Control << 25% = 1.56% < Control 10% FCS = Control 15% FCS = 6.25% = 12.50%
but Control 10% FCS < 12.50%.

Classification of the various Embryo Extract's concentrations and of the positive control (FCS) after 144 contact hours:

50% << Neg. Control << 1.56% < Control 10% FCS < 6.25% = 25% << Control 15%. FCS < 12.50%.



Influence of the Embryo Extract in Function of the Tested Concentrations

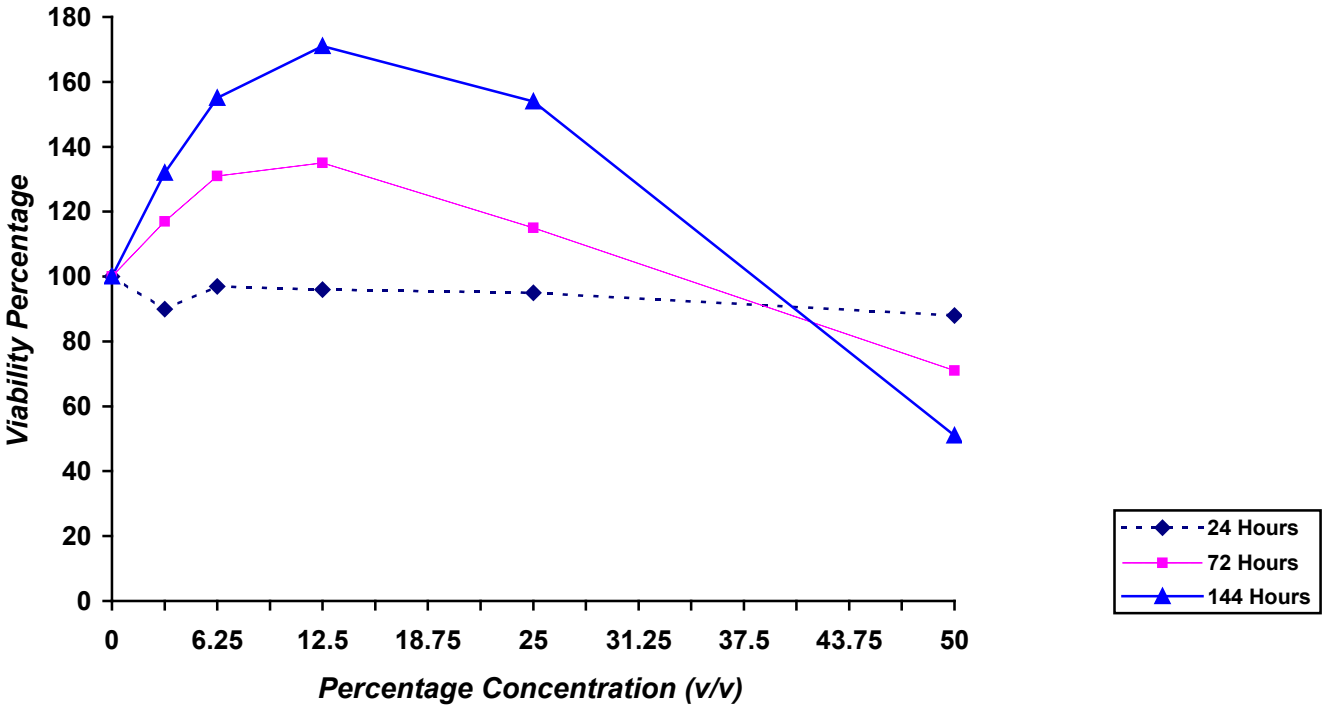


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TABLE I

RAW DATA OF THE PRELIMINARY CYTOTOXICITY STUDY

	D.O.1	D.O.2	D.O.3	D.O.4	D.O. average	Standard deviation	Viability %
Negative Control	1.111	1.136	1.107	1.083	1.109	0.022	100
3.12%	1.058	0.970	0.996	1.033	1.014	0.039	91
6.25%	1.061	1.060	0.979	1.018	1.030	0.039	93
12.5%	1.097	1.072	1.093	1.050	1.078	0.022	97
25%	1.085	1.054	1.003	1.049	1.048	0.034	94
50 %	0.950	1.030	1.010	0.978	0.992	0.035	89
85%	0.913	0.934	0.877	0.883	0.902	0.027	81
100%	0.278	0.300	0.456	0.395	0.357	0.083	32

TABLE II

RAW DATA FROM THE RESEARCH OF THE EFFECT ON GROWTH AFTER 24 CONTACT HOURS

	D.O.1	D.O.2	D.O.3	D.O.4	D.O.5	D.O.6	D.O. average	Standard deviation	Viability %
Negative Control	0.865	0.923	0.903	0.903	0.948	0.932	0.912	0.029	100
10% ES.	0.896	0.910	0.918	0.900	0.991	1.036	0.942	0.058	103
15% ES.	0.925	0.908	0.691	0.932	0.970	1.016	0.907	0.113	99
1.56%	0.717	0.819	0.766	0.827	0.886	0.928	0.824	0.077	90
6.25%	0.836	0.884	0.840	0.877	0.942	0.957	0.889	0.051	97
12.50 %	0.888	0.914	0.786	0.857	0.903	0.918	0.878	0.050	96
25.00%	0.875	0.848	0.829	0.788	0.913	0.964	0.870	0.063	95
50.00%	0.802	0.775	0.763	0.765	0.839	0.872	0.803	0.044	88

ES = Embryo Serum ; Conc. = Percentage concentration (V/V).

TABLE III

RAW DATA FROM THE RESEARCH OF THE EFFECT ON GROWTH AFTER 72 CONTACT HOURS

	D.O.1	D.O.2	D.O.3	D.O.4	D.O.5	D.O.6	D.O. average	Standard deviation	
Negative Control	0.938	1.001	1.016	1.140	1.137	1. 146	1.063	0.089	100
10% FCS.	1.467	1.357	1.309	1.374	1.360	1.328	1.366	0.055	128
15% FCS.	1.470	1.504	1.367	1.420	1.425	1.277	1.411	0.080	133
1.56%	1.248	1.231	1.195	1.313	1.250	1.221	1.243	0.040	117
6.25%	1.463	1.371	1.371	1.345	1.383	1.430	1.394	0.044	131
12.50 %	1.528	1.490	1.204	1.375	1.476	1.514	1.431	0.124	135
25.00 %	1.272	1.263	1.190	1.194	1.208	1.200	1.221	0.037	115
50.00 %	0.810	0.753	0.690	0.705	0.796	0.779	0.756	0.049	71

TABLE IV

RAW DATA FROM THE RESEARCH OF THE EFFECT ON GROWTH AFTER 144 CONTACT HOURS

	D.O.1	D.O.2	D.O.3	D.O.4	D.O.5	D.O.6	D.O. average	Standard deviation	
Negative Control	0.989	1.101	0.962	1.043	1.187	1.170	1.075	0.093	100
10% ES.	1.573	1.479	1.384	1.468	1.500	1.423	1.471	0.065	137
15% ES.	1.909	1.662	1.780	1.782	1.736	1.794	1.777	0.081	165
1.56%	1.490	1.441	1.321	1.383	1.457	1.403	1.416	0.060	132
6.25 %	1.717	1.618	1.616	1.719	1.720	1.580	1.662	0.064	155
12.50%	1.967	1.911	1.811.	1.776	1.855	1.732	1.842	0.087	171
25.00 %	1.725	1.658	1.658	1.804	1.608	1.511	1.661	0.100	154
50.00 %	0.827	0.836	0.812	0.833	0.903	0.836	0.841	0.032	51

ES = Embryo Serum ; Conc. = Percentage Concentration (V/V).

TABLE VIII

LEAST SIGNIFICANT DIFFERENCE (24 CONTACT HOURS)

Δ 24 h= $1.83 \cdot 10^{-2}$

	-Δ	AVERAGE	+ Δ
Negative Control	0.893	0.912	0.930
Control 10% FCS	0.923	0.942	0.960
Control 15% FCS	0.888	0.907	0.925
1.56%	0.806	0.824	0.542
6.25 %	0.870	0.889	0.907
12.50%	0.860	0.878	0.896
25.00 %	0.851	0.870	0.888
50.00 %	0.784	0.803	0.821

TABLE IX

LEAST SIGNIFICANT DIFFERENCE (72 CONTACT HOURS)

Δ 72 h= $1.83 \cdot 10^{-2}$

	-Δ	AVERAGE	+ Δ
Negative Control	1.042	1.063	1.083
Control 10% FCS	1.345	1.366	1.386
Control 15% FCS	1.390	1.411	1.431
1.56%	1.222	1.243	1.263
6.25 %	1.373	1.394	1.414
12.50%	1.410	1.431	1.451
25.00 %	1.200	1.221	1.241
50.00 %	0.735	0.756	0.776

TABLE X

LEAST SIGNIFICANT DIFFERENCE (144 CONTACT HOURS)

Δ 144 h= $1.83 \cdot 10^{-2}$

	-Δ	AVERAGE	+ Δ
Negative Control	1.062	1.075	1.088
Control 10% FCS	1.458	1.471	1.484
Control 15% FCS	1.764	1.777	1.790
1.56%	1.403	1.416	1.429
6.25 %	1.649	1.662	1.675
12.50%	1.829	1.842	1.855
25.00%	1.648	1.661	1.674
50.00 %	0.828	0.841	0.854