# Cosmetic Safety: Proposal for the Replacement of *In Vivo* (Draize) by *In Vitro* Test

T.J.A. Pinto[1,*], T.I. Ikeda[2], L.L. Miyamaru[2], M.C. Santa[2], Bárbara R.P. Santos[2], A.S. Cruz[2]

[1]*Faculdade de Ciências Farmacêuticas-USP Bloco 15 Cidade Universitária-SP-Brazil and* [2]*Instituto Adolfo Lutz (Seção de Culturas Celulares e Seção de Cosméticos) Av. Dr. Arnaldo, 355-SP-Brazil*

**Abstract:** The procedures described by Draize have been criticized for ethical reasons. Thus, a comparative study was performed between ocular and cutaneous irritation tests, using rabbits and *in vitro* test through agar diffusion with the use of NCTC clone 929, FPC-IAL and SIRC cell lines. The results obtained revealed that the agar diffusion test positive samples, which presented up to degree 3 reactivity rate, according to USP 31, did not provoke ocular or cutaneous irritation. The samples which presented reactivity grade 4 also showed different degrees of ocular and cutaneous irritation, with the exception of two units of liquid soap for children use. According to the data, the diffusion agar method, using the American Pharmacopeia graduation, can be adopted as a sorting procedure in the evaluation of cosmetics. This is a result of its capacity of predicting irritation, what largely contributes for a decrease in the use of animals in tests.

## INTRODUCTION

The level of irritability of several substances and products for human use has been under evaluation since the 1940s, through experiments which use laboratory animals. Some of these assays, called ocular or cutaneous irritation tests, still adopted by official institutions, were originally described (Draize JPET 1944) [1].

Severe procedures which affect the safety of animals result in criticism and have been discussed by non-governmental entities. Therefore alternative methods have been investigated in an attempt to minimize this conflict.

Of all the *in vitro* methods described to evaluate cytotoxicity, the agar diffusion is the only one mentioned in the official bibliography, having been described (Guess JPS 1965) [2]. This same method, with the graduation of the American Pharmacopeia 31 (USP 31 2008) [3], was used in this study to evaluate the safety of cosmetics, aiming at its correlation with the ocular and cutaneous irritation *in vivo* method. This study was also performed with the intent to verify the relation between the origin of cell lines and the target tissue used in the *in vivo* test.

## MATERIALS AND METHODOLOGY

### Samples

This study was carried out using samples of cosmetics available in the market. They differed as to their colours, ingredients and/or brand. The quantity of samples analyzed was as follows: 78 units of lipstick with no sun protection factor, 16 units of blush, 15 units of compact powder, 15 units of make-up foundation, 33 units of eye-shadow, 15 pencils or eye-liners, 15 units of mascara, and 17 units of liquid soap, 4 of which for children use.

## EVALUATION OF BIOLOGICAL SAFETY

### *In Vivo* Evaluation

The methods which used *in vivo* tests varied according to their application. The ocular irritation test was applied to those products for the eye area. The primary cutaneous irritation method was used in the ones for the lips and face. The samples of liquid soap were evaluated both through cutaneous and ocular irritation tests.

As recommended by Food and Drug Administration (FDA), in the primary cutaneous irritation test 6 rabbits were used for each sample. The products were classified as irritant when the value of the primary irritation rate (IIP) varied from 1.0 to 8.0. Only 5 rabbits were used for each sample in the ocular irritation test, according to other authors, as it is rather aggressive (Springer FCT 1993) [4]. Products were classified as irritant whenever that rate was above 5.00. The procedures and the calculation of the irritation rate were based in the work carried out (Draize JPET 1944) [1].

### *In Vitro* Evaluation

It was performed through agar diffusion method using three cell lines. The NCTC clone 929 (ATCC-CCL1) cell line, belonging to the conjunctive tissue of mouse, cultivated in Eagle minimum medium, supplemented with 0.1 mM of non-essential amino acids, 1.0 mM of sodium pyruvate and 10% of fetal bovine serum without antibiotic (MEM 10% SFB), was used to evaluate all samples. SIRC cell lines (ATCC-CCL 60), from rabbit cornea, and FPC-IAL, fibroblastic rabbit skin cells, isolated through primary explants technique in Cell Culture Section of Adolfo Lutz Institute, cultivated in equal parts of Eagle minimum medium and

*Address correspondence to this author at the Faculdade de Ciências Farmacêuticas-USP Bloco 15 Cidade Universitária-SP-Brazil;
E-mail: tjapinto@usp.br

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Leibovitz medium number, 15 with 15 % of fetal bovine serum, without antibiotic (MEM + L15 15% SFB) were employed to evaluate the samples used in the eye and the skin area, respectively. Only liquid soap was tested in three line cells.

Most samples were evaluated in the following concentrations: 100% (without dilution (75%, 50%, 25% and 12.5%. Only liquid soap was tested in 100%, 10%, 1%, 0.1%, and 0.01% concentrations. The hydrosoluble samples were diluted in buffered saline solution (pH 7.2) and the remaining ones in cotton seed oil from Sigma (ISO 1999) [5].

### Agar Diffusion Method

Cell lines, in 3.0 x $10^5$ to 3.5 x $10^5$ cells/mL concentrations, were inoculated in Petri plates (60 x15 mm). The incubation was carried out for 48 h at 37 ºC in humid atmosphere containing 5% of $CO_2$. After the formation of cell monolayers, the culture medium was disposed of and 5 mL of overlay medium was added to each Petri plate. This medium is composed of equal parts of MEM twice concentrated and agar (BBL-Becton Dickinson) at 1.8 %, containing 0.01 % of neutral red (Merck), as vital dye. Discs of 0.5 cm in diameter, made of nontoxic filter paper, were soaked into the samples in different concentrations and placed over the agar layer before their complete solidification. The Petri plates were then again incubated (USP 31 2008, ISO 1999) [3, 5].

Latex fragments were used as positive controls and filter paper as negative controls, both 0.5 cm in diameter. The samples of each line cell were evaluated in triplicate.

The plates were then analyzed both macroscopic and microscopically and cytotoxicity detected due to the presence of a clear halo under or around the sample tested. The diameter of these halos was carefully measured by means of a calibrated pachymeter, their mean values being then calculated and used to evaluate their correlation with *in vivo* assays. The toxicity halos were also classified in reactivity grades (RG): from 0 to 4: 0 = absence of effect under the sample; 1 = cell alteration or degeneration under the sample; 2 = clear halo under the sample; 3 = halo between 0.5 and 1.0 cm around the sample; 4 = clear halo > 1.0 cm beyond sample (USP 31 2008) [3].

## RESULTS

### *In Vivo* Tests - Primary Cutaneous Irritation

Of all the samples evaluated, only liquid soap for adult use, identified as samples 1, 2, 3, 4, 5, 6, 7, 12, 13, 14, 15 and 16, showed positive result, with primary irritation index (P.I.I.) ranging from 1.0 to 3.5, when evaluated at a 100 % concentration.

### Ocular Irritation

All liquid soap samples, except those identified as numbers 8 and 11, showed positive results when evaluated with ocular irritation index ranging from 6.40 to 16.00 (Table **2**).

### *In Vitro* Cytotoxicity Through Agar Diffusion Method

Of 124 samples evaluated in NCTC clone 929 and FPC-IAL lines, only 31 showed toxicity at a 100% concentration, concerning, at least, one of the cell lines used (Table **3**).

From 63 samples evaluated in NCTC clone 929 and SIRC cell lines, only 11 showed toxicity at a 100 % concentration in at least one of the cell lines (Table **4**).

**Table 1. Edema and Erythema Values Used for the Calculation of Cutaneous Irritation Scores of Liquid Soap Samples Evaluated at a 100% Concentration**

| Samples | 24 Hours Scores | | | | 72 Hours Scores | | | | IIP* |
|---|---|---|---|---|---|---|---|---|---|
| | Intac Skin | | Abraded Skin | | Intac Skin | | Abraded Skin | | |
| | Edema | Erythema | Edema | Erythema | Edema | Eryhtema | Edema | Eryhtema | |
| 1 | 1.00 | 2.00 | 1.00 | 2.00 | 0.00 | 1.00 | 0.00 | 1.00 | 2.00 |
| 2, 5,6,7 and 16 | 0.00 | 1.00 | 0.00 | 1.00 | 0.00 | 1.00 | 0.00 | 1.00 | 1.00 |
| 3 and 4 | 1.00 | 1.00 | 1.00 | 1.00 | 0.00 | 1.00 | 0.00 | 1.00 | 1.50 |
| 8and 11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 9 | 0.00 | 1.00 | 0.00 | 1.00 | 0.00 | 0.33 | 0.00 | 0.33 | 0.66 |
| 10 | 0.00 | 0.66 | 0.00 | 0.33 | 0.00 | 0.16 | 0.00 | 0.16 | 0.33 |
| 12 | 1.00 | 2.00 | 2.00 | 2.00 | 1.00 | 2.00 | 1.00 | 2.00 | 3.25 |
| 13 | 2.00 | 2.00 | 2.00 | 2.00 | 1.00 | 2.00 | 1.00 | 2.00 | 3.50 |
| 14 | 0.00 | 2.00 | 0.00 | 2.00 | 0.00 | 1.00 | 0.,00 | 1.00 | 1.50 |
| 15 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 2.00 |
| 17 | 0,00 | 1,00 | 0,00 | 1,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,50 |

*PII – Primary irritation index – each value obtained represents the mean scores of 6 rabbits.
Interpretation: values from 0.00 to 0.90 = non – irritant; values above 1.00 = irritant.

**Table 2. Irritation Values in the Different Tissues and Ocular Irritation Values Obtained According to Draize Scale, after 24 Hours of Liquid Soap Samples Instillation, Evaluated at a 100% Concentration**

| Samples | Cornea | Iris | Conjuntivae | Ocular Irritation Index * |
|---|---|---|---|---|
| 1 | 5.00 | 2.00 | 6.00 | 13.00 |
| 2, 3, 4 ,5 ,6 and 15 | 5.00 | 0.00 | 6.00 | 11.00 |
| 8 | 0.00 | 0.00 | 0.00 | 0.00 |
| 9 | 5.00 | 2.00 | 6.40 | 13.40 |
| 10 | 2.00 | 0.00 | 4.40 | 6.40 |
| 11 | 0.00 | 0.00 | 0.00 | 0.00 |
| 12, 16 and 17 | 5.00 | 5.00 | 6.00 | 16.00 |
| 13 | 5.00 | 4.00 | 5.60 | 14.60 |
| 14 | 5.00 | 4.00 | 6.00 | 15.00 |

*Each irritation value represents 5 rabbits on average.
Interpretation: values from 0.00 to 5.00 = non-irritant; values above 5.00 = irritant.

**Table 3. Diameter of Toxicity Halos (cm) Obtained at Different Concentrations from Samples of Lipstick (with or without Sun Protection Factor), Blush, Compact Powder and Make-Up Foundation, Concerning NCTC clone 929 and FPC-IAL Cell Lines**

| Samples | N° | Diameter (cm) of Toxicity Halos | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NCTC Clone 929 | | | | | FPC-IAL | | | | |
| | | 100% | 75% | 50% | 25% | 12.5% | 100% | 75% | 50% | 25% | 12.5% |
| | 3 | 1.92 | 0.70 | – | | | 2.00 | 0.70 | – | | |
| | 4 | 0.50 | – | | | | 0.70 | 0.70 | 0.50 | – | |
| | 7,22,24, 46 and 47 | – | | | | | 0.50 | – | | | |
| | 9 | 2.10 | 1.30 | 0.70 | – | | 2.22 | 1.30 | 0.70 | 0.50 | – |
| | 10 | 2.20 | 1.10 | 0.70 | 0.50 | – | 2.10 | 1.30 | 0.90 | 0.70 | – |
| | 11 | 2.20 | 1.30 | 0.90 | 0.50 | – | 2.16 | 1.30 | 1.10 | 0.70 | – |
| **Lipstick** | 12 | 0.70 | 0.50 | – | | | 0.84 | 0.70 | 0.70 | – | |
| | 13 | 1.90 | 0.70 | 0.50 | – | | 1.90 | 0.70 | 0.50 | – | |
| | 14 | 1.50 | 0.90 | 0.50 | – | | 1.50 | 0.90 | 0.50 | – | |
| | 23 | 0.50 | – | | | | 0.50 | 0.50 | – | | |
| | 25 | – | | | | | 0.70 | – | | | |
| | 60 | – | | | | | 1.10 | 0.90 | 0.70 | – | |
| **Lipstick with** | 7 | 0.50 | 0.50 | – | | | 1.90 | 1.10 | – | | |
| **SPF** | 8 | – | | | | | 0.70 | 0.70 | 0.50 | 0.50 | – |
| | 1 | – | | | | | 0.70 | – | | | |
| Blush | 14 | – | | | | | 0.50 | 0.50 | – | | |
| Com-pact | 7 | 0.70 | 0.70 | – | | | 0.50 | 0.50 | – | | |
| | 15 | – | | | | | 0.70 | 0.50 | – | | |

**(Table 3) contd…**

| Samples | N° | Diameter (cm) of Toxicity Halos | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NCTC Clone 929 | | | | | FPC-IAL | | | | |
| | | 100% | 75% | 50% | 25% | 12.5% | 100% | 75% | 50% | 25% | 12.5% |
| | 7 | 0.70 | 0.50 | 0.50 | – | | 1.26 | 1.02 | 0.86 | – | |
| | 8 | 1.00 | 0.80 | 0.50 | – | | 1.80 | 1.70 | 1.52 | 1.42 | 1.30 |
| Make-up | 9 | 0.50 | – | | | | 1.16 | 1.16 | 1.00 | – | |
| **Foundation** | 10 | 0.70 | – | | | | 1.10 | 0.90 | 0.70 | – | |
| | 11 | 0.50 | – | | | | 1.56 | 1.30 | 1.10 | 0.90 | – |
| | 12 | 0.70 | – | | | | 0.50 | – | | | |
| | 13 | 0.50 | – | | | | 0.90 | – | | | |
| | 14 | 0.70 | 0.50 | 0.50 | – | | 1.40 | 1.02 | 1.02 | 0.72 | 0.50 |
| | 15 | 0.70 | 0.50 | – | | | 0.70 | 0.50 | 0.50 | – | |

- Without toxic effect

**Table 4. Diameter of Toxicity Halos (cm) Obtained at Different Concentrations from Samples of eye -Shadow, Mascara for Lashes and Eyeliner, Concerning NCTC clone 929 and SIRC Cell Lines**

| Samples | N° | Diameter (cm) of Toxicity Halos | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NCTC Clone 929 | | | | | SIRC | | | | |
| | | 100% | 75% | 50% | 25% | 12.5% | 100% | 75% | 50% | 25% | 12.5% |
| | | 0.50 | 0.50 | – | | | 0.70 | 0.50 | – | | |
| | | 0.50 | 0.50 | 0.50 | 0.50 | – | 0.70 | 0.70 | 0.70 | 0.50 | – |
| **Shadow** | 30 | 0.50 | – | | | | 0.50 | 0.50 | – | | |
| | 1 | 0.84 | – | | | | 0.84 | – | | | |
| **Mascara** | 7 | 0.70 | 0.50 | 0.50 | – | | 0.50 | – | | | |
| | 10 and 13 | 0.50 | – | | | | – | | | | |
| | 3 | 1.10 | 0.70 | 0.50 | – | | 1.50 | 1.10 | 1.06 | 0.50 | 0.50 |
| **Eye** | 4 | 1.50 | 0.70 | 0.70 | – | | 1.10 | 0.82 | 0.50 | – | |
| **Liner** | 8 | 0.90 | 0.90 | 0.50 | – | | 0.70 | 0.70 | 0.50 | – | |
| | 12 | 0.70 | 0.50 | 0.50 | 0.50 | – | 0.50 | 0.50 | 0.50 | 0.50 | – |

- Without toxic effect.

The toxicity halo diameters of 17 samples of liquid soap are shown in Table **5**. All the samples showed toxicity at a 10% concentration concerning the three line cells used. Sample number 11 was the only exception, as it didn't show any effect at 10% concentration, relatively to SIRC cell line.

The reactivity grades were determined using the measures of toxicity halos diameters obtained in the samples (USP 31 2008) [3], whose criteria was also followed relatively to polymers, that is, samples which showed up to grades 2 were considered non-reactive. Also following USP, all the measures of diameters converted into toxicity halos, whose values lie between 0.10 and 0.49 cm, were also considered grades 2

Table **6** shows the whole scores of reactivity grades obtained in all the groups of samples evaluated in this study. Of the 204 samples tested, 31 do not meet the criteria of acceptance, recommended by (USP 31 2008) [3].

**Table 5. Diameter of Toxicity Halos Obtained at Different Concentrations from Samples of Liquid Soap, Concerning NCTC Clone 929, FPC – IAL and SIRC Cell Lines**

| Samples | Diameter (cm) of Toxicity Halos | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NCTC Clone 929 | | | | FPC-IAL | | | | | SIRC | | | |
| | 100% | 10% | 1% | 0.1% | 100% | 10% | 1% | 0.1% | 0.01% | 100% | 10% | 1% | 0.1% |
| 1 | 6.00 | 3.40 | 1.26 | – | 6.00 | 6.00 | 2.26 | 1.10 | – | 6.00 | 1.20 | 0.50 | – |
| 2 | 4.00 | 2.60 | 1.36 | – | 6.00 | 3.30 | 1.00 | – | | 3.54 | 2.30 | 0.50 | – |
| 3 | 4.30 | 3.06 | 1.10 | – | 4.34 | 4.10 | 1.32 | – | | 3.74 | 3.40 | 0.72 | – |
| 4 | 5.44 | 3.00 | 1.10 | – | 6.00 | 4.30 | 1.64 | 0.70 | – | 3.40 | 1.40 | – | |
| 5 | 6.00 | 3.36 | 0.85 | – | 6.00 | 4.60 | 1.85 | 0.70 | – | 3.64 | 1.50 | – | |
| 6 | 5.44 | 2.60 | 0.70 | – | 6.00 | 3.80 | 0.70 | 0.50 | – | 3.60 | 2.16 | – | |
| 7 | 5.44 | 3.20 | 0.96 | – | 6.00 | 3.90 | 1.40 | – | | 5.00 | 1.74 | – | |
| 8 | 6.00 | 3.20 | – | | 6.00 | 3.90 | – | | | 4.74 | 2.00 | – | |
| 9 | 6.00 | 2.94 | 0.70 | – | 6.00 | 3.90 | 1.10 | – | | 6.00 | 2.34 | – | |
| 10 | 4.36 | 2.16 | – | | 6.00 | 3.40 | 1.40 | – | | 3.40 | 1.40 | – | |
| 11 | 4.50 | 1.12 | – | | 6.00 | 1.20 | 0.70 | – | | 3.84 | – | | |
| 12 | 4.04 | 3.00 | 0.50 | – | 6.00 | 4.04 | 0.70 | – | | 3.90 | 2.50 | – | |
| 13 | 4.50 | 2.54 | 0.70 | – | 6.00 | 3.10 | 1.04 | 0.84 | – | 4.20 | 1.90 | 0.50 | – |
| 14 | 4.24 | 3.06 | 0.50 | – | 6.00 | 5.60 | 3.30 | 1.00 | – | 4.20 | 2.04 | – | |
| 15 | 6.00 | 3.24 | 0.70 | – | 6.00 | 4.24 | 3.36 | 1.06 | – | 3.74 | 1.94 | – | |
| 16 | 5.04 | 3.70 | 0.70 | – | 6.00 | 6.00 | 2.82 | 1.00 | – | 3.42 | 2.90 | – | |
| 17 | 6.00 | 2.16 | 0.70 | – | 6.00 | 4.04 | 1.92 | 0.86 | – | 4.00 | 1.40 | – | |

-without toxic effect.

**Table 6. Cytotoxicity of All the Samples According to USP 31**

| Samples | Reactivity Grades | | | | |
|---|---|---|---|---|---|
| | 0 ou 1 | 2 | 3 | 4 | Total of Samples |
| Lipstick with and without sun protection factor | 60 | 11 | 7 | - | 78 |
| Blush | 14 | 2 | - | - | 16 |
| Compact powder | 13 | 2 | - | - | 15 |
| Make-up foundation | 6 | 7 | 2 | - | 15 |
| Eye-shadow | 30 | 3 | - | - | 33 |
| Mascara | 11 | 4 | - | - | 15 |
| Eye-pencils | 11 | 2 | 2 | - | 15 |
| Liquid soap | - | - | - | 17 | 17 |
| Total | 145 | 31 | 11 | 17 | 204 |

Fig. (**1**) summarizes the positive and negative results obtained in *in vivo* and *in vitro* tests of all the samples used in this study. The samples were grouped according to the place of application. The results of *in vitro* tests were achieved through both classifications: the toxic effect and the USP (USP 31 2008) [3] criteria. The percentage of negative results of *in vivo* tests varied from 81 % to 91.5%, being these between 65% to 66% relatively to *in vitro* tests, which only took the toxic effect into consideration. When these same results were classified in reactivity grades, the percentages varied from 76.3% to 81.6%.

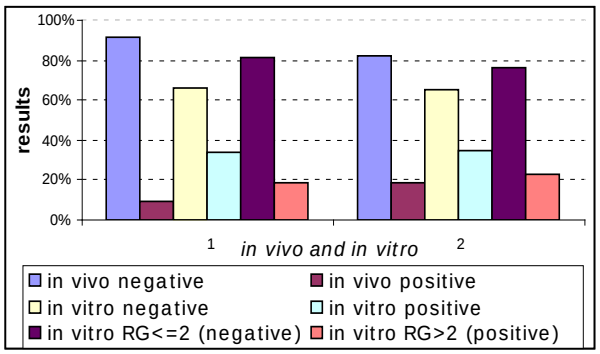


**Fig. (1).** Results obtained from *in vivo* and *in vitro* tests according to the place of application of samples: 1) cutaneous and 2) ocular. RG= reactivity grade.

The evaluations of Pearson correlation coefficient between the cell lines used resulted in high values ranging from $r$=0.94 (p=0.0001) to 0.881 (p=0.001). As for the agar diffusion method and the *in vivo* tests, the evaluation of the correlation was performed solely in liquid soap samples, the only ones which presented positive results in the animals. This method showed some significant correlations, both in ocular tissue individual rates and total ocular irritation rate, whose values varied from $r$=0.482 (p=0.050) to $r$=0.785 (p=0.0002).

The sensitivity and specificity of the agar diffusion method were calculated so that the samples graded up to 2 were considered non irritant and, above this rate, irritant. The results obtained demonstrated 100% of sensitivity in the *in vivo* tests. As for specificity, the values varied from 89% to 91%, in the cutaneous irritation test, and reached 95% in the ocular irritation test.

## DISCUSSION

The use of animals in tests which evaluate cutaneous and ocular irritation caused by cosmetics has been causing polemic. Thus this has become a crucial matter, especially in the European Community, whose population is strongly in favour of new initiatives and cruelty-free cosmetics (Barrela CR 2002) [6].

Toxicological methods are important, among these, the agar diffusion method is one of the pioneers of *in vitro* test. Due to its reproducibility it was adopted by the International Organization for Standardization (ISO) and by the United States Pharmacopeia (USP), as an official method for evaluation of plastic and medical devices (USP 31 2008, ISO 1999) [3, 5]. Some institutions recommend the use of any mammal cell line, others specify NCTC clone 929 cell line, adopted due to its stability and easy handling.

This study adopted not only NCTC clone 929, but also FPC – IAL and SIRC cell lines. This strategy results from the analyses of the close correlation between ocular irritation and *in vitro* cytotoxicity tests using cornea cells, as well as the cutaneous irritation test using human skin fibroblast (Lee TIV 2000, Watanabe TIV 1989) [7, 8].

This study tested 204 samples, of which 141 were evaluated using primary cutaneous irritation test and 80 using ocular irritation test. It was observed that 12 (8.5%) samples showed cutaneous irritation and 15 (19%) ocular irritation, when evaluated without dilution (Tables **1**, **2** and Fig. **1**).

The number of samples revealing toxic effects proved to be higher concerning the *in vitro* test, after comparing the results of samples tested through *in vitro* and cutaneous and ocular irritation tests (Tables **3- 5**). The data confirm the results obtained by other authors and also corroborate cell lines higher sensitiveness relatively to laboratory animals (Rougier TIV 1994, Wilhelmus SO 2001) [9, 10].

Still comparing *in vivo* and *in vitro* results (Fig. **1**), it was verified that the percentage of positive samples in the cutaneous and ocular irritation tests was lower than the one observed in the *in vitro* test, when only taking into account the presence of toxic effect. However, this difference is reduced when the USP 31 criteria is adopted. As for the negative results, this difference is about 10% relatively to *in vivo* tests. Thus, the adoption of this criteria and the consideration that all non-reactive samples of *in vitro* tests eliminate the necessity of an *in vivo* evaluation, can reduce the number of animals used in about 90%.

According to Table **6**, from 1861 animals used, 1527 rabbits could have been spared if the underlying observations were taken into account, as the samples which did not show effect in the cell lines or toxicity halo up to 1.0 cm (RG 3) did not cause ocular or cutaneous irritation in animals. Only the samples graduated as RG 4, with toxicity halos higher than 1.5 cm or a diameter of 3.4 cm, caused cutaneous or ocular irritation in rabbits. This was verified in samples of liquid soap, which contain surfactant agents, known as irritant substances.

Studies carried out by other researchers showed correlations among several established and newly isolated lines, without significant differences (Pinto JAOACI 2000) [11]. This study followed the same procedures when it compared FPC – IAL and SIRC cell lines with NCTC clone 929 ones.

FPC-IAL and SIRC cell lines, when employed in the evaluation of liquid soap, did not reveal significant correlation. However, FPC – IAL line showed a higher number of positive results, as well as larger diameters in the toxicity halos (Table **5**). This observation may be related to the studies of Cornelis (Cornelis TIV 1992) [12], who, after comparing human skin fibroblasts with keratinocytes, concluded that the former are more sensitive. They interpreted the fact based on the similarity between keratinocytes and cornea cells as to the epithelial origin, being keratinocytes main function to act as a protective barrier.

The correlation between *in vitro* and *in vivo* methods was calculated using toxicity halos diameter values, since these measures, according to other authors, provide better results when compared to ocular irritation tests (Earl TIV 1995, Harbell FCT 1997, O´Brien TIV 1994) [13-15].

The values of the correlation rates obtained in the cell lines and in the *in vivo* test did not indicate a close relation between the origin of the cell line and the target place of the *in vivo* test, contrary to conclusions of other studies (Lee TIV 2000, Watanabe TIV 1989) [7, 8].

The agar diffusion method has been previously used by other authors in the evaluation of cosmetics or their raw-materials, as a substitution method for the cutaneous and ocular irritation assays (Combrier ATLA 1992, O´Brien TIV 1990, Wallin IJTCOT 1987) [16-18]. The results of the ocular irritation tests were encouraging, according to these authors, what was confirmed by the present study, in which a higher number of samples and different cell lines were used.

The best significant correlation values between the agar diffusion and ocular irritation test were obtained, in this study, when the samples of liquid soap were evaluated at concentrations of 10% and 1%. Similar data were observed by other authors when they evaluated products containing surfactant agents, at a concentration of 10%, using NCTC clone 929 cell line (Earl TIV 1995, O´Brien TIV 1994) [13, 15]. However, Rougier (Rougier TIV 1994) [9] did not obtain significant correlations when they used this method with lung fibroblast cells, to evaluate ocular irritation caused by cosmetics.

The percentages of sensitivity and specificity were high, relatively to all the samples and different cell lines used both concerning ocular and cutaneous irritation tests. According to Gettings (Gettings FCT 1996) [19], such results can guarantee that the agar diffusion method is efficient to evaluate the safety of products.

Courtellermont (Courtellermont TIV 1999) [20] emphasized that the toxicity of manufactured products rarely reproduces the total effect of all their ingredients, what makes the development of formulae safe testing models prioritary. This is a consequence of the fact that there are several reports of products which cause irritation mainly in the skin and eye area (Draelos CD 2001, Wolf CD 2001) [21, 22].

According to the comments of different authors (O´Brien TIV 1994, Wallin IJTCOT 1987, Ohno TIV 1999) [15, 18, 23], and as result of a comparative evaluation of *in vitro* and *in vivo* methods results, it is possible to state that, although it often does not show high correlations, the former can be safety used in the sorting of cosmetic products.

The *in vitro* test is therefore a significant contribution to the evaluation of acute toxicity of such products, reducing or even eliminating *in vivo* tests, especially if the American Pharmacopeia acceptance criteria were, in this case, expanded from 2 to 3 degree of reactivity.

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