

Heppenheimer Andreas<sup>1</sup>, Poth Albrecht<sup>1</sup>, Hoffmann Jens

<sup>1</sup> Harlan Cytotest Cell Research GmbH, In den Leppsteinswiesen 19, D-64380 Rossdorf

<sup>2</sup> CellSystems Biotechnologie Vertrieb GmbH, Langelager Ring 5, D-53842 Troisdorf

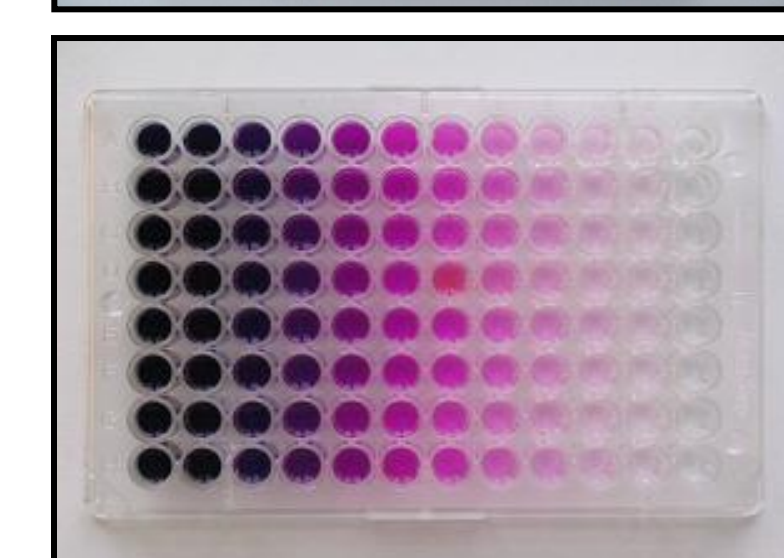
**Introduction:** Where substances are intended for use in personal care products applied to the skin an assessment of potential phototoxic hazard is required. The initial test is the measurement of a UV/visible absorption spectrum to identify absorption at relevant wavelength, followed by in an in vitro assay for phototoxicity, the Balb 3T3 neutral red uptake phototoxicity test (OECD 432). However, this test has its limitations, as non-hydrosoluble chemicals can be tested only at low concentrations due to their lack of aqueous solubility and also many complex mixtures or formulations cannot be tested. Consequently, it does not take into account the bioavailability of test chemicals topically applied to skin and in many cases e.g. after having a phototoxic effect in this test system, such information is required. To overcome these limitations, the use of reconstructed skin models is an interesting alternative and a useful follow-up test. In the present study the EST 1000 skin model (Cell Systems, Germany) was used and different chemicals, selected based on their results in the NRU phototoxicity test and their bioavailability to skin, were investigated in the human skin model.

### Material and Methods:

- 1) Balb 3T3 NRU Phototoxicity test: cell cultures are treated with different concentrations of a test compound for one hour in the dark; afterwards one culture gets irradiated with UVA light whereas a second identically treated culture is kept in the dark; the irradiation intensity is 5 J/cm<sup>2</sup>; after the irradiation period the test item is washed off the cell cultures, cultures are incubated overnight; evaluation of the possible cytotoxic effect is done with the NRU assay; the determination of the phototoxic effect is done by comparison of the irradiated cultures with the non irradiated cultures, the photo irritancy factor (PIF) calculated as quotient of the IC<sub>50</sub> value without irradiation and the IC<sub>50</sub> value with irradiation enables the classification of test compounds as non phototoxic and phototoxic, respectively
- 2) Phototoxicity test using the EST-1000 model: skin equivalents are treated with different concentrations of a test compound for 18 – 24 hours; skin equivalents are irradiated with UVA light at an intensity of 6 J/cm<sup>2</sup> whereas identically treated equivalents are kept in the dark; after irradiation the test compound is washed off the equivalents; equivalents are further incubated overnight; the evaluation of a possible cytotoxic effect is done with the MTT assay; the determination of the phototoxic effect is done by comparison of the irradiated equivalents with the non irradiated equivalents, a test compound is predicted to have a phototoxic potential if one or more concentrations of the irradiated equivalents reveal a decrease in viability exceeding 30% when compared with identical concentrations of the non irradiated equivalents



Fig. 1: Picture 1: skin equivalents after MTT extraction; the two above rows represent the irradiated skin equivalents treated with CPZ whereas the lower two rows represent the non irradiated skin equivalents treated with CPZ  
Picture 2: Balb 3T3 cells in the NRU assay after neutral red extraction



### Results:

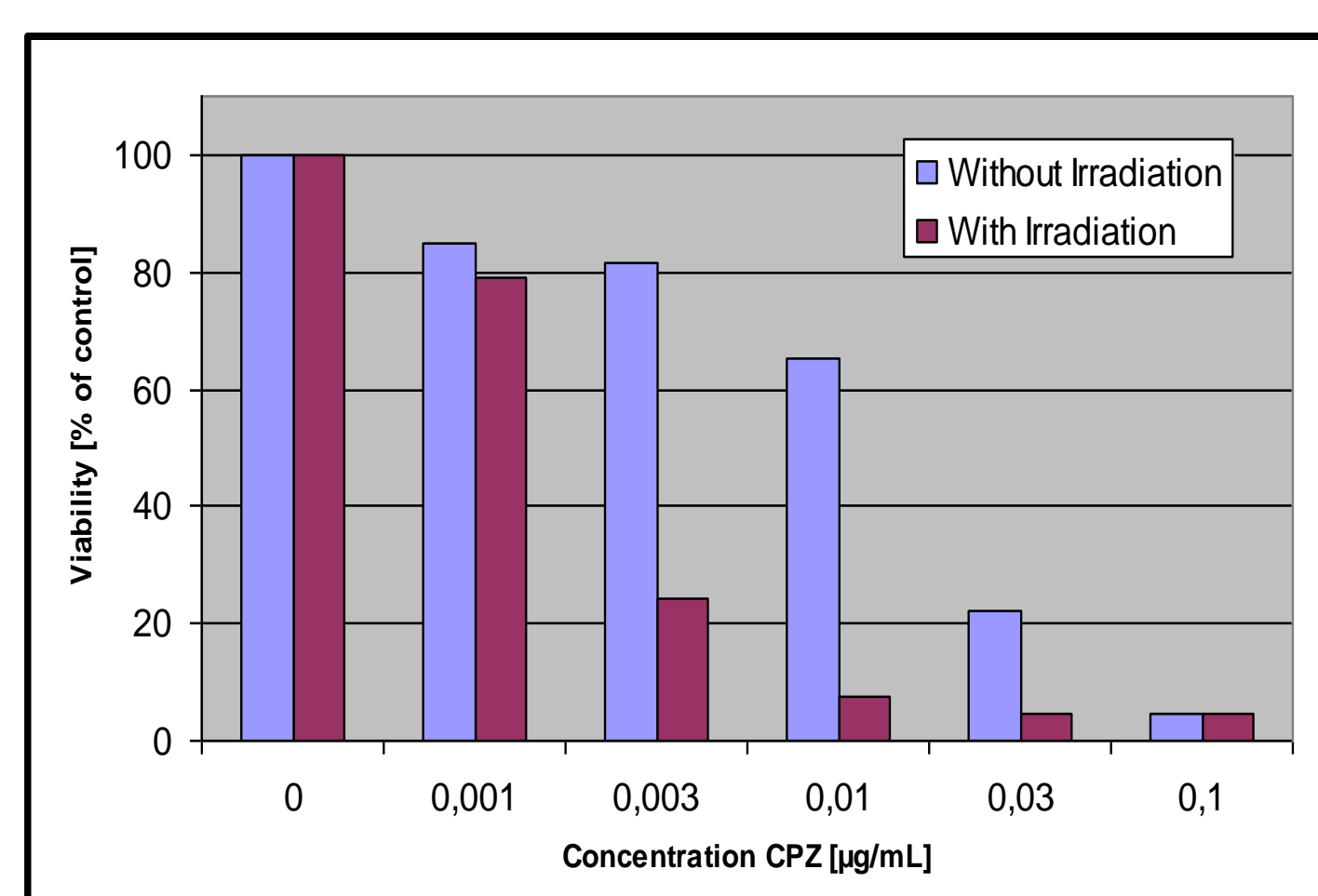


Fig. 2.: Results after treatment of the EST-1000 skin equivalents with chlorpromazine (CPZ); PIF = 8.3, relevant reduction in viability of the irradiated equivalents in comparison with the non irradiated equivalents at concentrations 0.003, 0.01, and 0.03 µg/mL

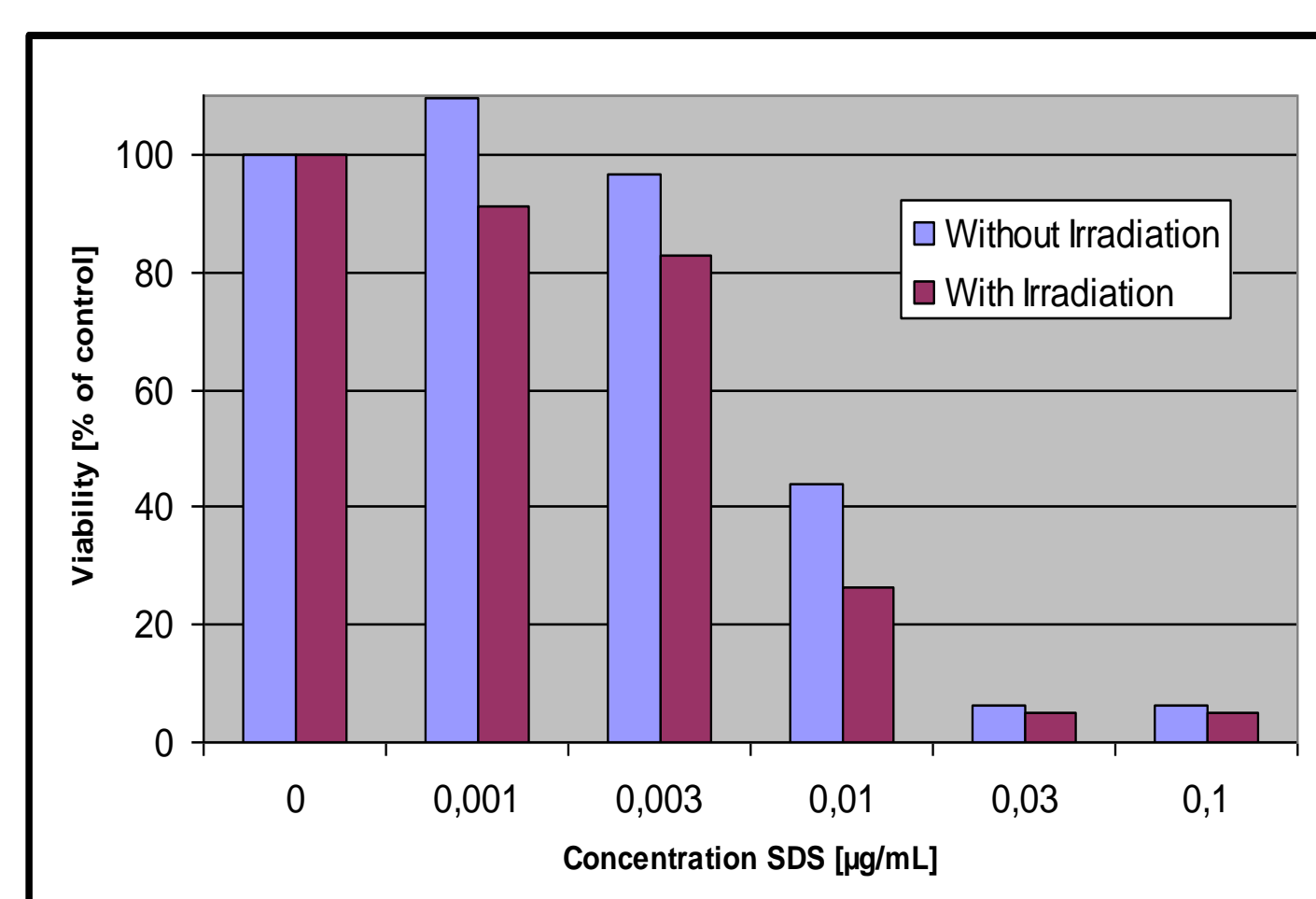


Fig. 3: Results after treatment of the EST-1000 skin equivalents with sodium dodecyl sulfate; PIF = 1.3, no relevant reduction in viability of the irradiated equivalents in comparison with the non irradiated equivalents

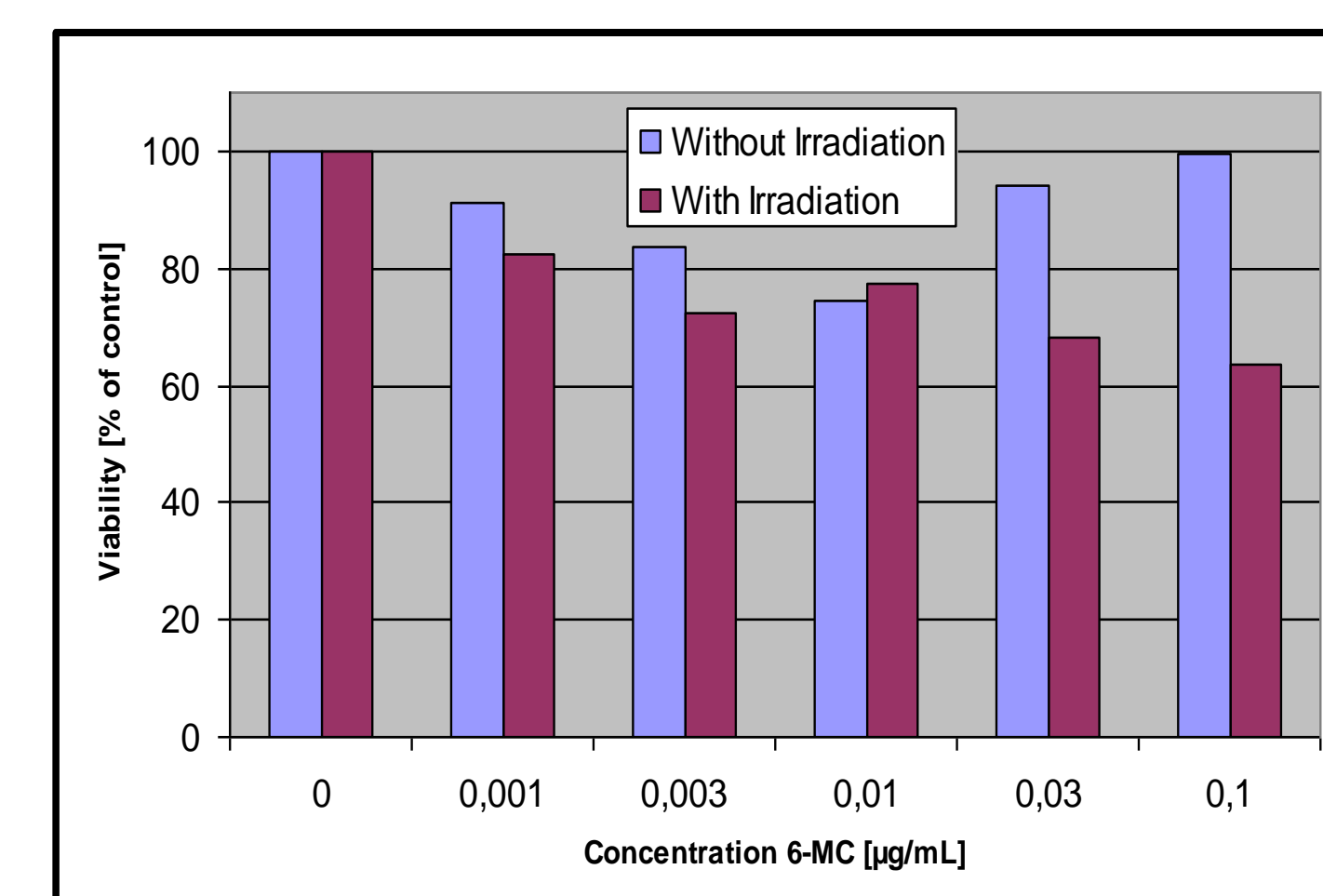


Fig. 4: Results after treatment of the EST-1000 skin equivalents with 6-methylcoumarin (6-MC); PIF = not applicable since no IC<sub>50</sub> values can be calculated, relevant reduction in viability of the irradiated equivalents in comparison with the non irradiated equivalents at the highest tested concentration of 0.1 µg/mL

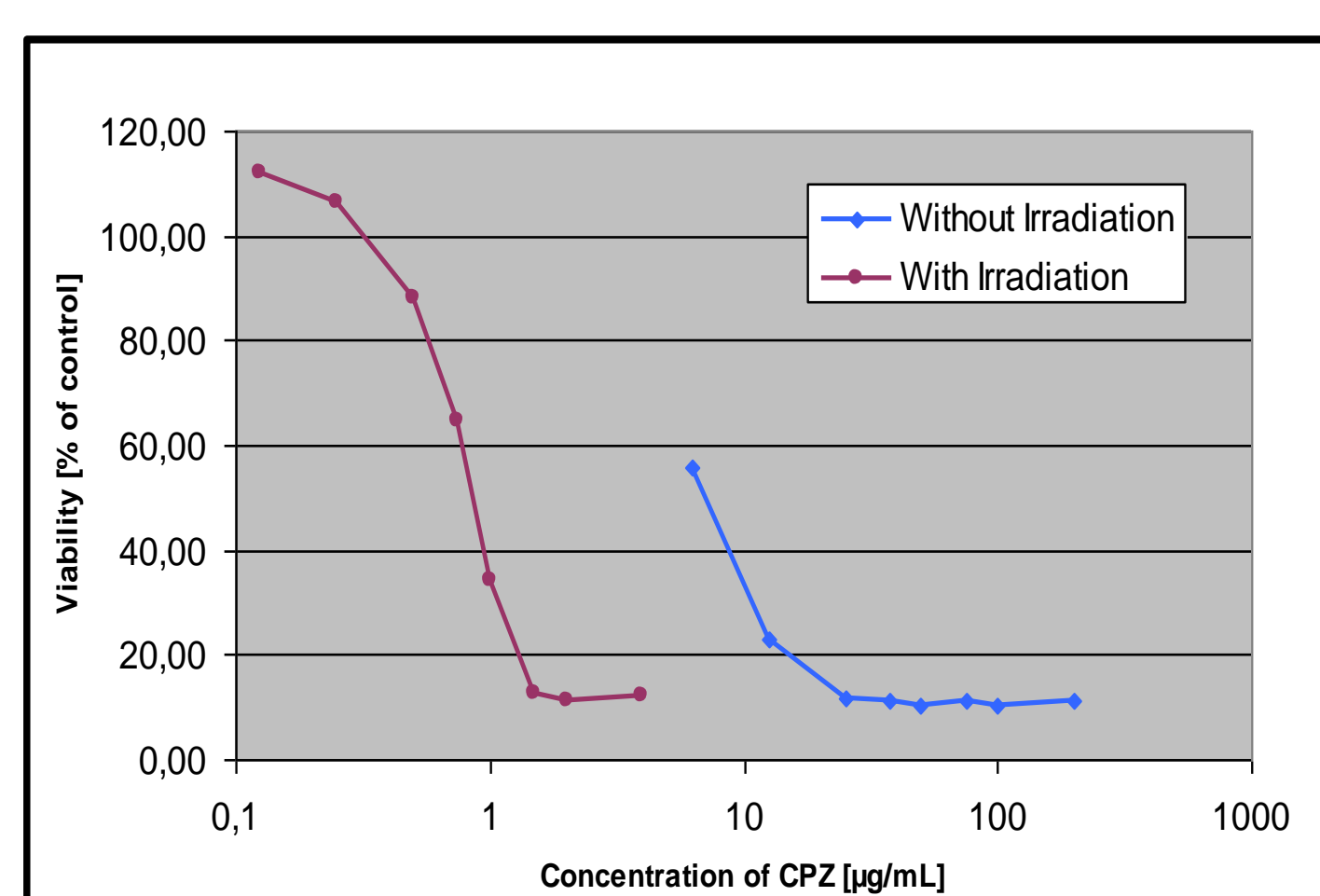


Fig. 5: Results after treatment of the Balb 3T3 cells with CPZ; PIF = 8.5

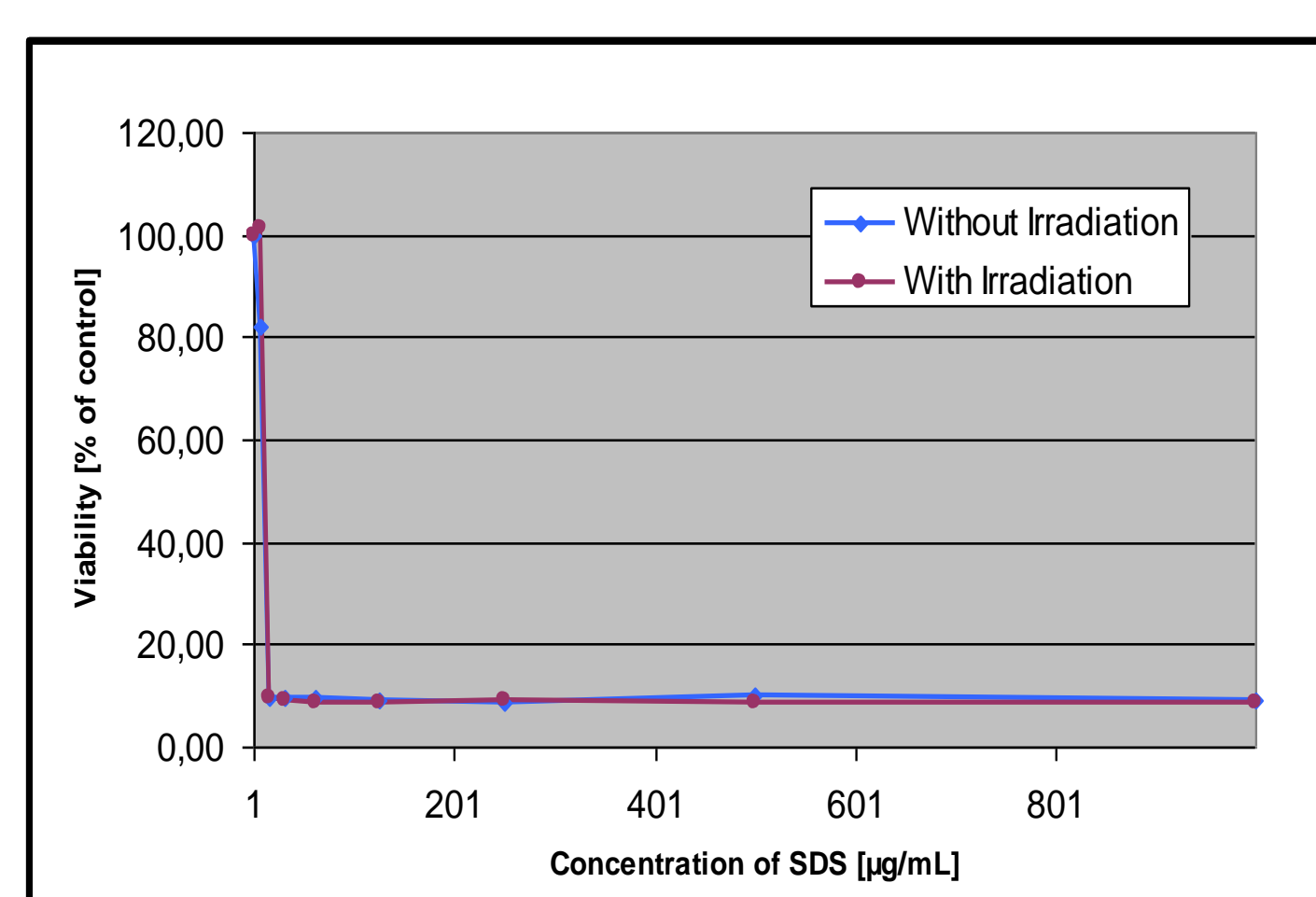


Fig. 6: Results after treatment of the Balb 3T3 cells with SDS; PIF = 0.9

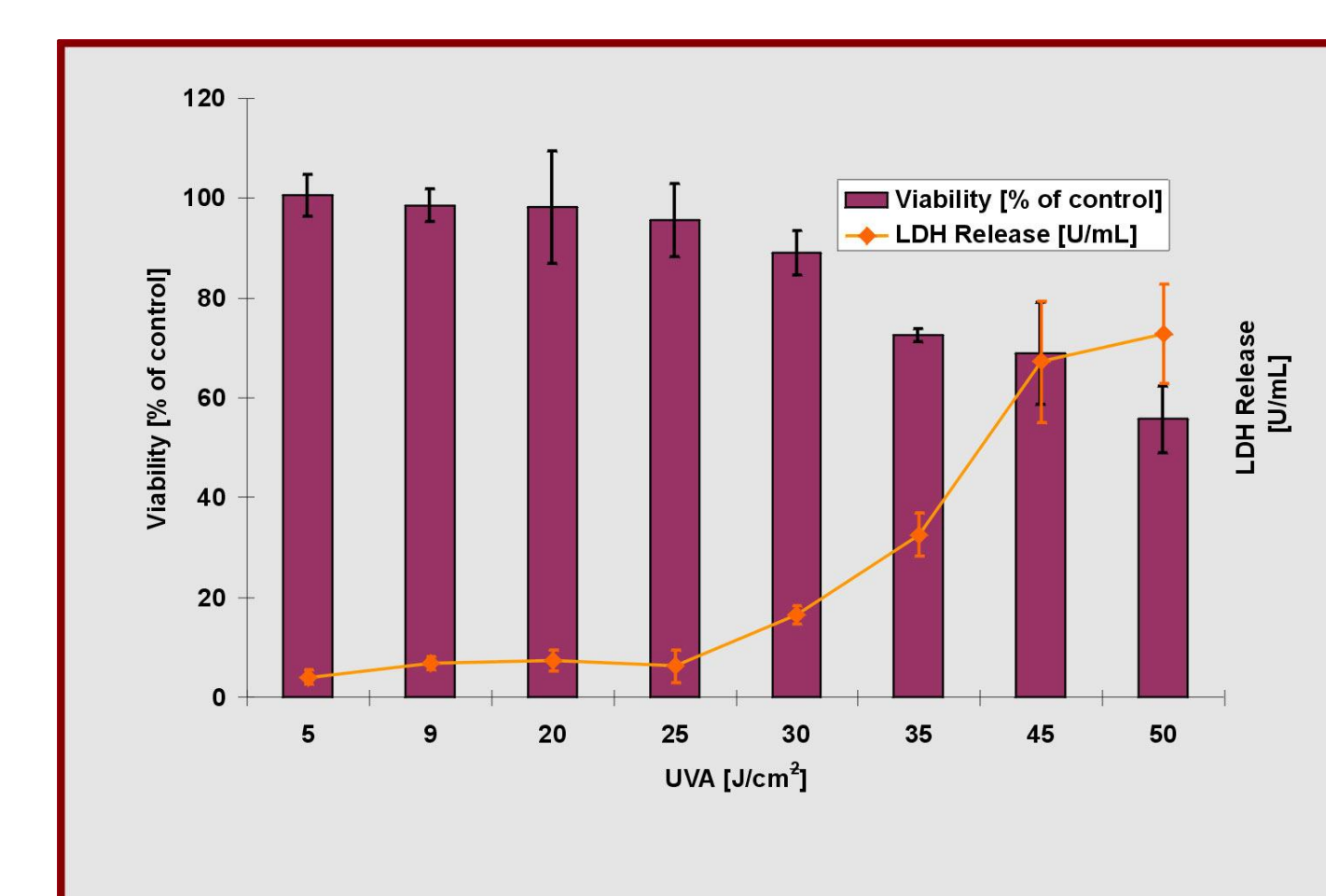


Fig. 7: EST-1000 were exposed to different intensities of UVA reaching from 0 to 35 J/cm<sup>2</sup>. The tissue viability was monitored by using a standard MTT assay and measurement of the LDH activity; Results provided by CellSystems

As basis for phototoxicity testing the photo sensitivity of the test system needs to be determined in preliminary experiments. The photo sensitivity of the EST-1000 model is given in figure 7. The results clearly show a great resistance against UVA irradiation. No relevant effects can be observed up to an irradiation intensity of 30 J/cm<sup>2</sup>. Only slight effects can be observed up to an intensity of 50 J/cm<sup>2</sup>.

The results clearly show a good correlation between the phototoxicity test using the EST-1000 skin model and the standard Balb 3T3 NRU phototoxicity test. The PIF values of the test compound chlorpromazine (CPZ) and sodium dodecyl sulfate (SDS) are comparable. The PIF value for CPZ in the EST-1000 phototoxicity test was 8.3 and therefore only slightly below the PIF of 8.5 for CPZ obtained in the Balb 3T3 assay. The PIF value for SDS obtained in the EST-1000 test was 1.3 whereas the PIF value for SDS obtained in the Balb 3T3 assay was 0.9. These values state the good correlation between the three-dimensional skin model and the monolayer cell cultures. As for 6-methylcoumarin (6-MC) only experiments with the skin model were performed. This compound is known to possess a phototoxic potential in vivo but it does not show effects in vitro (Liebsch et al., 1995). The results presented in figure 4 show a distinct difference in cell viability at the two highest tested concentrations of 0.03 and 0.1 µg/mL. At the highest tested concentration of 0.1 µg/mL the cell viability of the irradiated cultures is decreased by 36% in comparison to the non irradiated equivalents. This is an indication of a phototoxic potential of 6-MC. The reason for this difference may be the difference in the cell type. The EST-1000 model consists of human epidermal keratinocytes whereas the Balb 3T3 cells are a fibroblast cells. The different cell types offer different ways of absorption, distribution, metabolism, and excretion (ADME). Especially the difference in metabolising the photodegradation products may be responsible for the different results.

### Conclusions:

- The phototoxicity test using the EST-1000 model correlates very well with the standard Balb 3T3 NRU phototoxicity assay
- The EST-1000 model shows a great resistance against UVA irradiation
- In vivo phototoxic compounds not detected in the Balb 3T3 assay may be detected in the EST-1000 model
- The phototoxicity assay using the EST-1000 skin model offers a promising alternative for topically applied test compounds and can be used as a follow-up test to the Balb 3T3 phototoxicity test

### References:

- Liebsch M, Doring B, Donnelly TA, Logemann P, Rheins LA, Spielmann H, Application of the human dermal model Skin 2 ZK 1350 to phototoxicity and skin corrosivity testing, Toxicology In Vitro, 1995; 9: 557 – 562