



The New OECD Draft Test Guideline: The Performance of EST1000 for Skin Irritation Testing According to UN-GHS

B. Becker, J. Hoffmann, S. Hopf, D. Fuchs and H. W. Fuchs

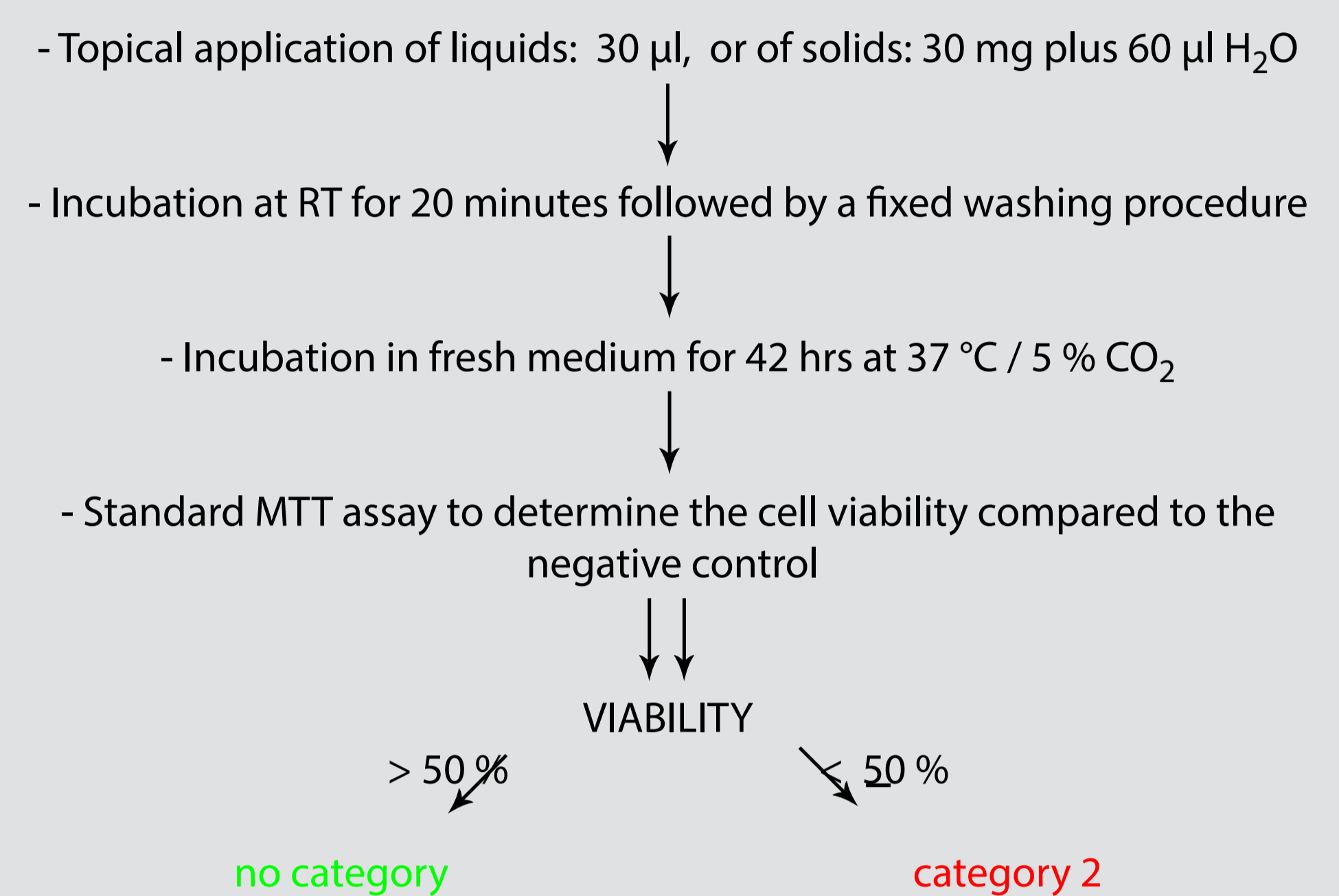
CellSystems® Biotechnologie Vertrieb GmbH, Hummelsbergerstr. 11, D-53562 St. Katharinen, Germany
jens.hoffmann@cellsystems.de

Introduction

During the last years different skin models had been validated by ECVAM according to the former EU classification system. In December 2008 the EU adopted the UN Globally Harmonized System (UN-GHS) for Classification and Labelling which finally led to the publication of a draft proposal for the new OECD test guideline released March 20, 2009. According to the UN-GHS rules for skin irritation classification and labelling, the cut-off value to distinguish between no category (formerly "non irritant") and category 2 (formerly "R38") chemicals was shifted to an in vivo score of ≥ 2.3 from a value of 2.0 (former EU classification). Chemicals with an in vivo score between 2.0 and 2.3 were considered irritant (R38) under the former EU classification system and will be considered non-irritant (no category) under the UN-GHS classification system. As a consequence the list of reference chemicals was imbalanced and had to be adjusted.

The reconstructed epidermis model EST1000 has already proven its excellent performance in the field of in vitro skin corrosion and irritation testing. The new draft guideline finally made it necessary to adapt the established EST1000 test protocol for skin irritation testing according to the new UN-GHS classification. This includes incubation time and amount of the applied chemical. Here we present the final results obtained with the new test protocol and the updated list of reference chemicals.

Adapted Test Protocol



Results

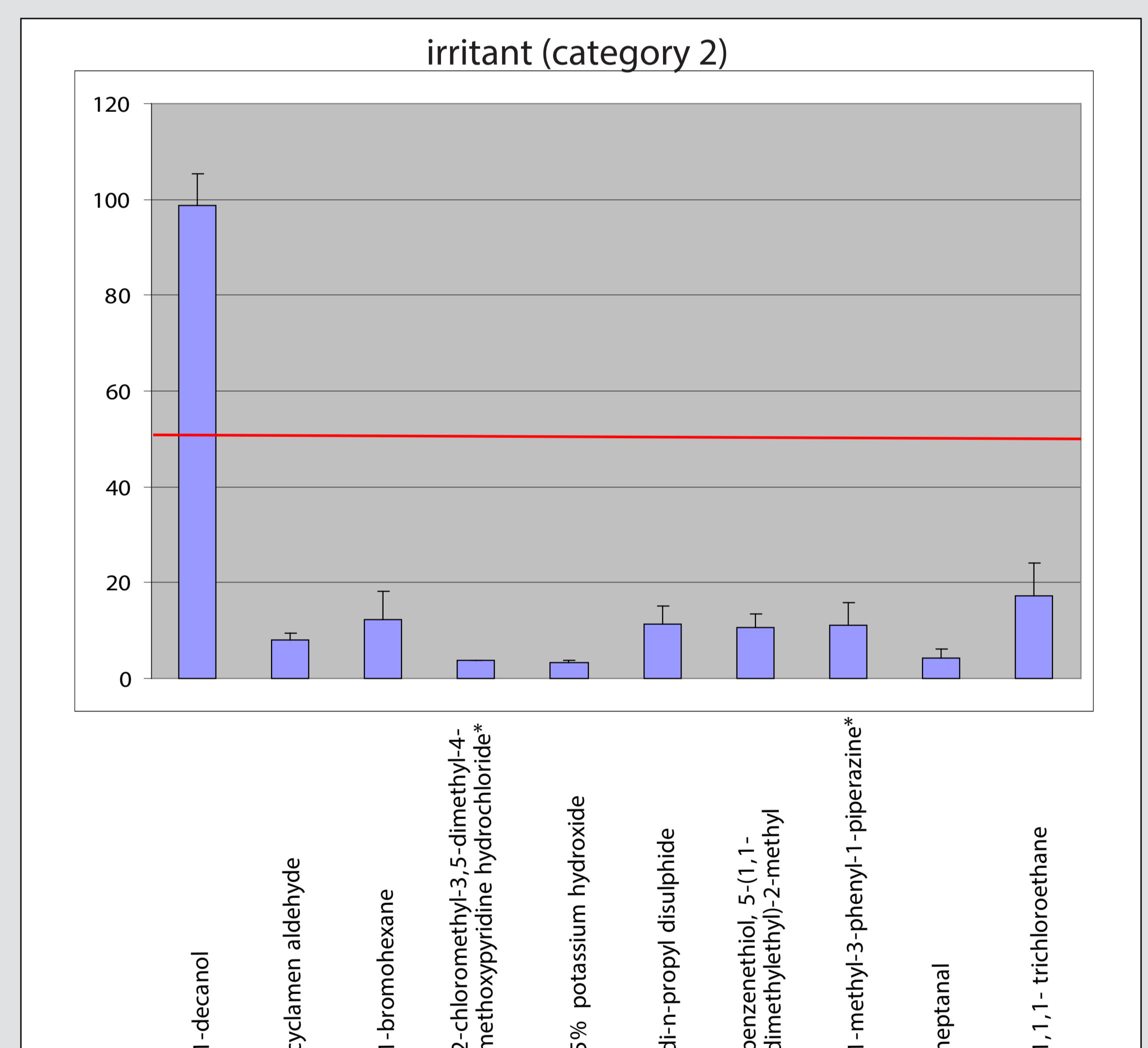
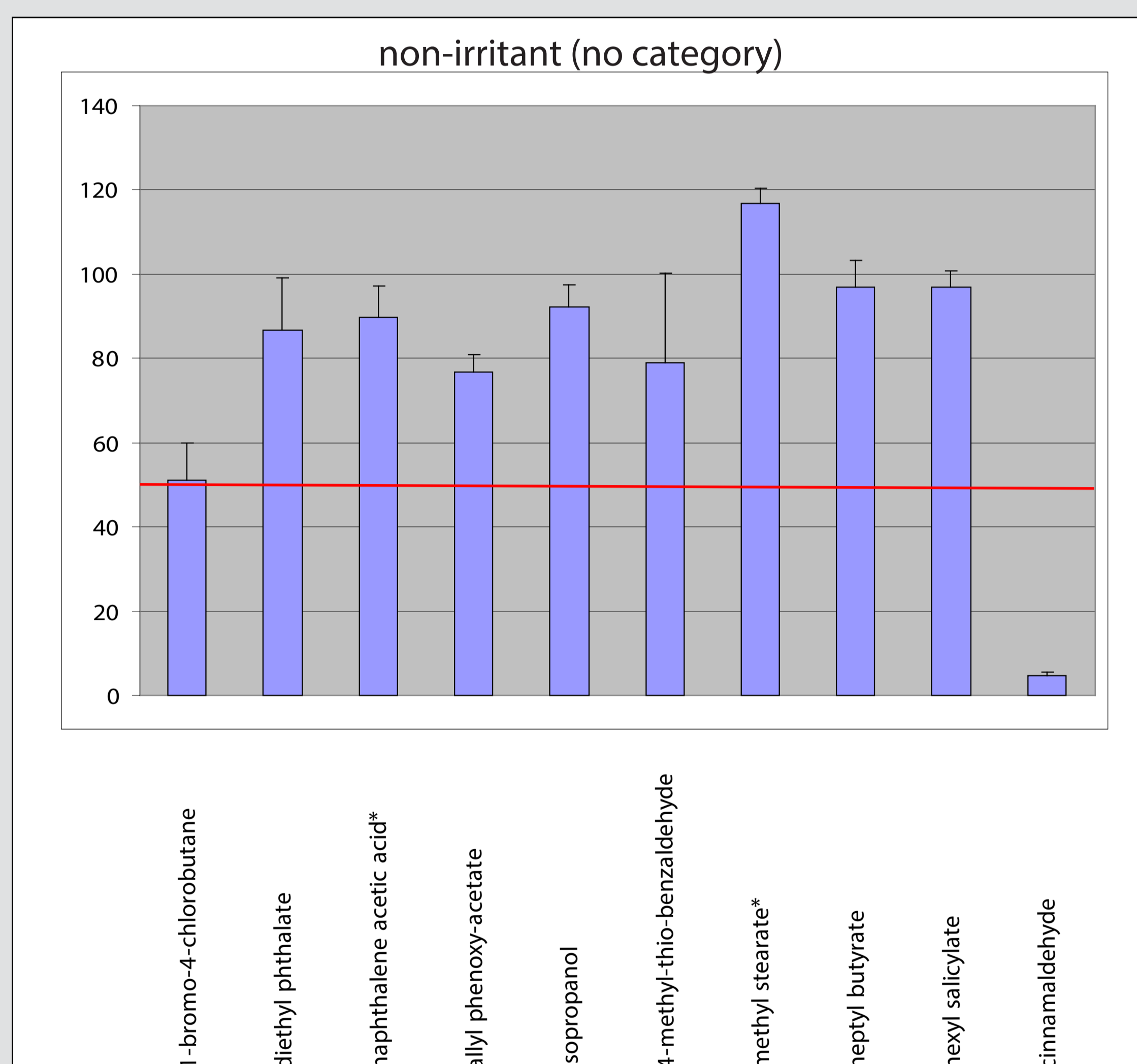


Figure 1: Viability of EST1000 after treatment with the 20 reference chemicals according to the adapted test protocol. Values were calculated from the MTT test using PBS as control (100%). Overall, 18 out of 20 chemicals were classified correctly. One chemical in each group (no category - left chart, category 2 - right chart) was classified incorrectly. SDs were in an acceptable range. A summary of the results which were obtained from 3 individual runs is displayed below.

Summary

test chemical	In vivo Score	Viability	SD	Classification
1-bromo-4-chlorobutane	0	51,10	8,90	no category
diethyl phthalate	0	86,64	12,45	no category
naphthalene acetic acid*	0	89,63	7,44	no category
allyl phenoxy-acetate	0,3	76,84	4,02	no category
isopropanol	0,3	92,10	5,30	no category
4-methyl-thio-benzaldehyde	1	78,84	21,42	no category
methyl stearate*	1	116,84	3,46	no category
heptyl butyrate	1,7	96,89	6,31	no category
hexyl salicylate	2	96,88	3,97	no category
cinnamaldehyde	2	4,64	0,96	category 2
1-decanol	2,3	98,70	6,60	no category
cyclamen aldehyde	2,3	8,03	1,47	category 2
1-bromohexane	2,7	12,17	5,95	category 2
2-chloromethyl-3,5-dimethyl-4-methoxy-pyridine hydrochloride*	2,7	3,67	0,21	category 2
5% potassium hydroxide	3	3,40	0,49	category 2
di-n-propyl disulphide	3	11,34	3,80	category 2
benzenethiol, 5-(1,1-dimethylethyl)-2-methyl	3,3	10,64	2,75	category 2
1-methyl-3-phenyl-1-piperazine*	3,3	11,18	4,58	category 2
heptanal	4	4,17	1,98	category 2
1,1,1-trichloroethane	4	17,36	6,79	category 2

* solid chemical bold: new chemical

Discussion

The 20 reference chemicals were tested with the adapted protocol for EST1000. The changes in the test protocol finally led to a correct classification of 18 chemicals. One chemical (cinnamaldehyde) was classified false positive and one chemical (1-decanol) was classified false negative. For 1-decanol there is reliable evidence that it is irritant in rabbits but non-irritant in humans. Cinnamaldehyde has also been classified incorrectly with other skin models

We conclude from these results:

- EST1000 distinguishes between no category and category 2 chemicals under UN-GHS
- The results exceed the defined accuracy values abundantly clear:

	requirements	EST1000
Sensitivity	80 %	90 %
Specificity	70 %	90 %
Overall Accuracy	75 %	90 %