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### COMBINED *IN VITRO* ASSAY FOR 3T3 NRU PT ASSAY AND PHOTOHEMOLYSIS AS PART OF PHOTOTOXICITY TEST

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The aim of this study was to assess a possible alternative method as replacement for *in vivo* phototoxicity test. The 3T3 mouse fibroblast neutral red uptake phototoxicity assay (3T3 NRU PT assay) is a screening method for studying DNA or cellular damage. Photohemolysis assay is a mechanistic study for investigating oxygen-dependent membrane damage. The 3T3 NRU PT and photohemolysis assay were performed with several phototoxic substances (bithionol, anthracene, promethazine, thiourea, 8-methoxypsoraren, musk ambrette, bergamot oil, galaxolide) and non-phototoxic substances (pentalide, neo heliopan type E 1000, parsol MCX, parsol 1789, uvinul T 150). In a first assay, 3T3 cells were exposed to the substances and then irradiated with 5 J/cm<sup>2</sup> of UVA. The cell viability was measured by NRU assay. The phototoxic potential of test materials in this assay was assessed by determining the PIF (photoirritation factor) by using a cut-off value of 5. In another assay, human erythrocytes were exposed to the substances and then irradiated with 15 J/cm<sup>2</sup> of UVA. The phototoxic potential of test materials in photohemolysis was assessed by determining the hemolysis rate by using a cut-off value of 10%. The combined *in vitro* assay, 3T3 NRU PT and photohemolysis, showed a close pattern to *in vivo* response. This combined assay could be used to predict the phototoxicity.