

## Abstract

The aim of the presented doctoral thesis was to use the sludge worm *Tubifex tubifex* as a model for biological activity testing. In the presented study, the *Tubifex* worm was used to evaluate photosensibilisation, i.e. sensibilisation of an organism towards UVA radiation (365 nm; radiant flux density of 350  $\mu\text{W}/\text{cm}^2$ ) after contact with certain substances.

The tested substances included proven photosensibilizing agents – rose bengal, khellin and xanthotoxin; isolated natural substances – thiophene polyacetylene umbelliferon, scopolin, and scopoletin; synthetic compounds – salicylanilides and thiosalicylanilides; and penicillin drugs Augmentin and Ospamox and their active substance standard amoxicillin. The final evaluation of photosensibilizing properties of the substances was carried out according to the Commission Directive 2000/33/EC PHOTOTOXICITY – *IN VITRO* 3T3 NRU PHOTOTOXICITY TEST.

The test was performed in 24-well plates where one set of plates with the photosensibilizing agent was irradiated and the second set was placed in an incubator ( $20\pm 2^\circ\text{C}$ ) as a dark control without any light presence for the whole period of the experiment. The plates were evaluated immediately after the given period of irradiation (15, 30, 45, 60, 120, and 240 minutes). Mortality and percentual number of damaged individuals were the proposed endpoints

From among the proven photosensibilizing agents, rose bengal showed the highest PIF factor (5.72) in mortality evaluation after 30-minute irradiation. Rose bengal was chosen as positive control for other experiments.  $\text{PIF} < 5$  was reached after longer irradiation (60, 120, and 240 minutes). The highest PIF factor of all the tested substances was found in thiophene polyacetylene. When evaluating mortality after 60-minute and 120-minute irradiation,  $\text{PIF} = 28.15$  and  $\text{PIF} = 57.5$ , respectively, were reached. When calculating the percentual number of damaged individuals, lower values were acquired:  $\text{PIF} = 7.50$  and  $\text{PIF} = 18.57$  for 60- and 120-minute irradiation, respectively. Other tested substances showed markedly lower photoirritation factor. In khellin,  $\text{PIF} < 2$  for mortality and percentual number of damaged individuals was found. Percentual number of damaged individuals only and  $\text{PIF} < 1.5$  was found in xanthotoxin. In umbelliferon and scopolin, neither mortality nor damage were observed. In scopoletin only, 33 % of damaged individuals were observed after 120-minute irradiation. 4',5-dibromo-salicylanilide showed mortality only with  $\text{PIF} < 2$ . Mortality only and  $\text{PIF} < 2$  were found in the basic thiosalicylanilide. In various thiosalicylanilide substitutions (4'-methyl-thiosalicylanilide, 5-chloro-thiosalicylanilide, 3',4'-dichloro-thiosalicylanilide, 3,5-dichloro-4'-chloro-thiosalicylanilide, 3,5-dichloro-4'-bromo-thiosalicylanilide),  $\text{PIF} < 1$  was found in mortality evaluation. The *Tubifex* worm damage was observed in 3,5-dichloro-4'-chloro-thiosalicylanilide and 3,5-dichloro-4'-bromo-thiosalicylanilide only. The active substance standard (amoxicillin) showed mortality only after 30-minute irradiation and  $\text{PIF} = 1.14$ . In medicinal products Augmentin and Ospamox,  $\text{PIF} = 0.35$  and  $\text{PIF} = 0.82$ , respectively, were found for mortality; no damage was observed.

The presented results show that *Tubifex tubifex* is a suitable testing organism for the studies of potentially phototoxic substances. It is sufficiently sensitive to the activity of the photosensibilizing agents, especially to singlet-oxygen generating substances.