Comparison of the sensitivity algae vs. duckweed
A simulation study

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The toxicity of substances or mixtures is based on the interaction of the substances and the respective organisms. As organisms can be structured quite differently their different sensitivity is just logic consequence. The sensitivity different organisms must be compared if substances should be detected with a maximum of sensitivity or biotest batteries should assess the hazards of environmental samples. For this reason quite a lot of comparative studies between algae and daphnids have been done. In many cases the EC-values were calculated from inhibition values based on area under the growth curve (agc) or final biomass. But EC-values of almost exponentially growing systems, calculated from final biomass or age depend on test duration and the absolute growth rate of the controls (Nyholm 1985, Nusch1982). Figures 1 and 2 show the dependence of the EC20 and EC50 for growth rates typically found with duckweed (0.275 d-1; 0.325 d-1; 0.375 d-1) and single cell green algae (0.9 d-1; 1.4 d-1; 1.9 d-1) on test duration. EC-values based on growth rates drastically change with the test duration while the EC-values depending on growth rates remain constant. The longer the test and the higher the growth rate the higher is the EC-value based on agc and final biomass.

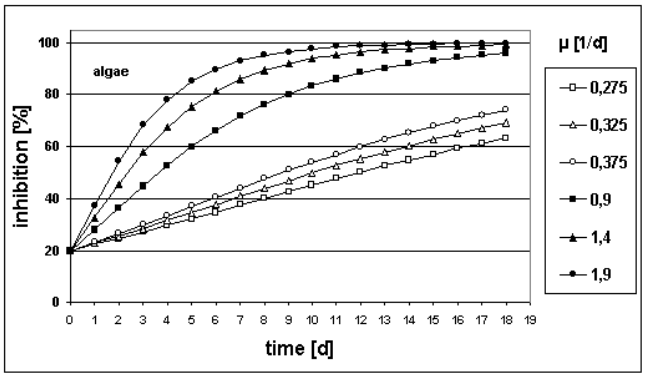

Fig. 1: Influence of the absolute growth rate of the control and of the test duration on the inhibition of final biomass for 20 percent inhibition of growth rate.

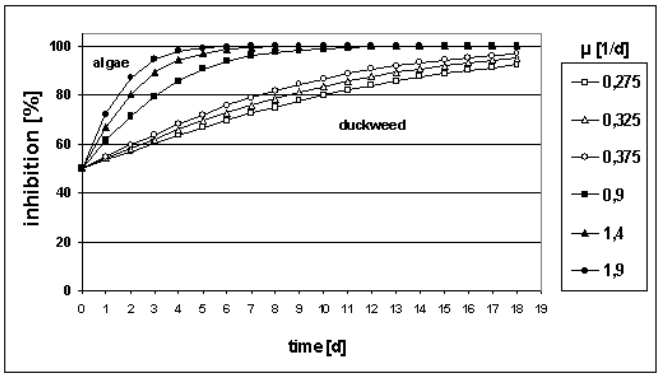

Fig. 2: Influence of the absolute growth rate of the control and of the test duration on the inhibition of final biomass for 50 percent inhibition of growth rate.

To get a quantitative impression of this phenomena and its consequences on the evaluation of comparative studies, the basic scheme of some studies was used for numerical simulations. The aim of the work was to answer of the question in how far comparisons on the basis of the more sensitive calculation method final biomass results in different EC-values than the less sensitive but ecologically more relevant growth rate. Three scenarios of comparison were chosen from literature. Some US-studies (Fairchild et al. 1997) used a test duration of 4 days for algae and duckweed (scenario D4A4), in the context of a screening test battery for pesticides (Grossmann et al. 1992) a one day test with algae was compared to a 7 days test with duckweed (scenario D7A1) and a third scenario used a 3 days test with duckweed (OECD, ASTM). In all simulations only the values based on final biomass were calculated as agc calculation result in relatively similar values if it is done on a non logarithmic basis (Nyholm 1985). Figure 3 shows the dependence of inhibition values on the absolute value of the growth rate for the D4A4-scenario.

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Especially inhibition of final biomass of algae can rise between different tests if growth rate rises from the minimum allowed level (0.9 d-1) to the high but common value of (1.8 d-1) from 57 to 77 percent for algae (EC20). This results in EC-value differences of about factor 1.5 for steep (like 3,5 DCP) and about factor 3 for shallow (like K2Cr2O7) concentration-response-curves (crc). For duckweed the difference of ECbiomass-values for different growth rates (0.275 d- and 0.375 d-1) is only 3 percent for EC20 and 4 percent for EC50. If both organisms are compared the inhibition of final biomass for EC20 of algae is 35 percent higher (30 percent for EC50) than the value of duckweed if medium growth rates (0.325 d-1 for duckweed and 1.4 d-1 for algae) are considered. 35 percent differences in inhibition result in an ECbiomass-value for duckweed about factor 2 higher than that of algae if a steep concentrations-response-curve is presumed. For shallow curves the difference of EC-value can reach factor 7 for the same EC-value based on growth rate.

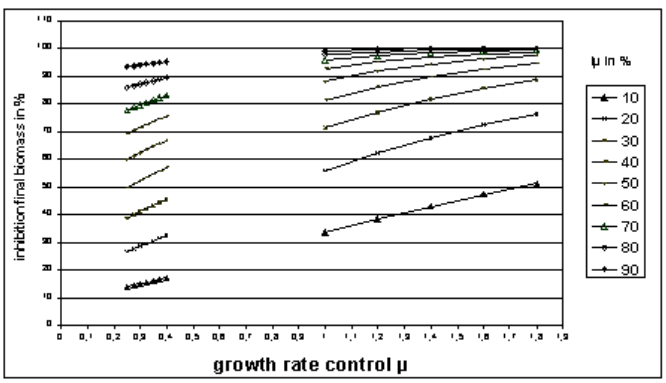

Fig. 3: Dependence of the inhibition of final biomass from growth rate of the controls for duckweed (left) and algae (right) for the scenario D4A4 (test duration duckweed 4 days, algae 4 days)

This little example already shows that only inhibition values calculated on growth rates allow a scientifically solid comparison of different organisms sensitivities. Studies based on areas under the curve or final biomass can only demonstrate that one test design has a larger tendency to produce smaller EC-values than another test design. In addition the scenarios with algae at different growth rates compliant to standards show high ostensible differences in sensitivity. This could explain some test to test or interlaboratory differences finally based on different but not documented growth rates. Generally the scenario D4A4 structurally tends to evaluate toxicity towards algae much higher than toxicity towards duckweed. But in a comparative study of 16 herbicides with selenastrum capricornutum and lemna minor calculated on final biomass the duckweed system led to smaller EC50 values for 8 substances (Fairchild et al. 1997). Going beyond that again a rectilinear comparison of sensitivity of a 4 days duckweed test, 4 days tests with different algae and a 14 day test of submersed rooted macrophytes is not appropriate to measure sensitivities of test species. If the test design is a 7 days duckweed test compared to a 1 day test with algae (scenario D7A1), duckweed seems to be more sensitive if final biomass is the basis of calculation.

Figure 4 shows that for a given growth rate inhibition and medium absolute growth rates inhibition duckweed has an inhibition between 2 and 10 percent higher than algae. The fact that in real tests duckweed leads for most of the tested substances to EC-values equivalent or lower than that for algae just illustrates that the result is more a product of the test design than one of different sensitivities.

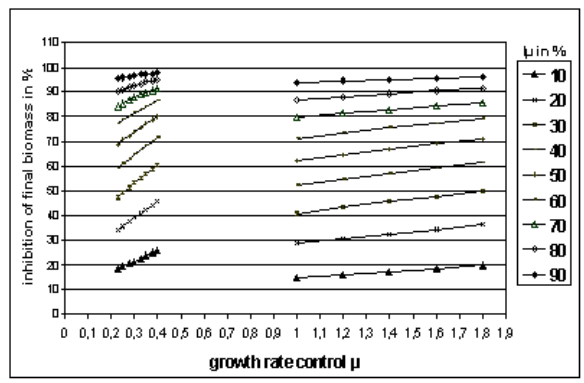

Fig. 4: Dependence of the inhibition of final biomass from growth rate of the controls for duckweed (left) and algae (right) for the scenario D7A1 (test duration duckweed 1 days, algae 1 days)

In the third scenario D7A3 a 7 days duckweed test is compared with a 3 days test with algae. In this case the test design for algae is 2 - 18 percent more sensitive than that for duckweed (Figure 5).

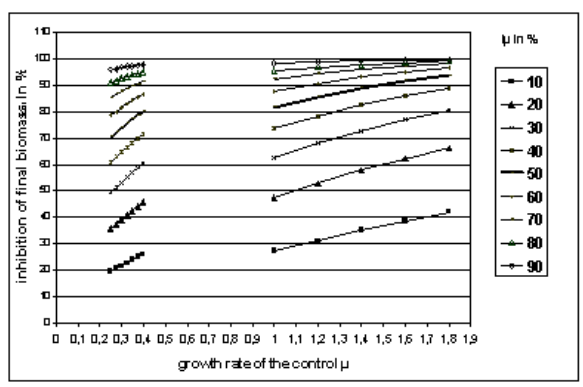

Fig. 5: Dependence of the inhibition of final biomass from growth rate of the controls for duckweed (left) and algae (right) for the scenario D7A3 (test duration duckweed 7 days, algae 3 days)

Depending on the slope of the concentration-response curve 18 percent difference correspond to EC-values differing between factor 1.5 for steep crc and factor 4 for flat crc. Future research projects, standardisation procedures and comparative studies should take these connections into consideration. As a consequence the allowed range of growth rate for standard tests with algae should be more limited than it is today if final biomass and area under the curve should be used. The more consequent solution however would be to calculate at least additionally EC-values based on growth rates. Only growth rate based data are a valid basis for comparisons between different organisms. As Fig. 6 shows, data based on final biomass (or area under the curve) do not provide any basis for an appropriate comparison.

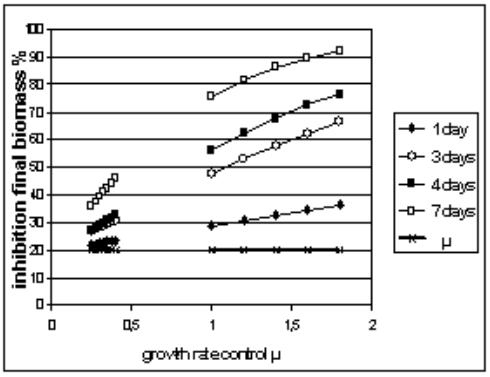

Fig. 6: Dependence of inhibition of final biomass for EC20 (left) and EC50 (right) from the growth rate of the controls for duckweed and algae.

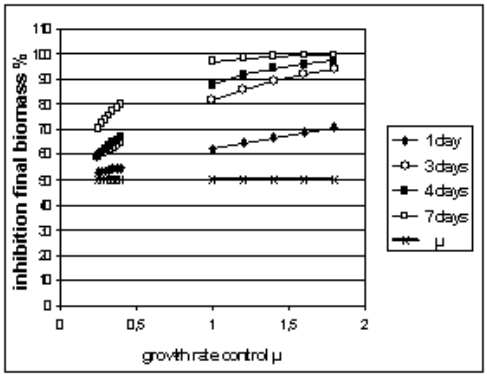

Fig. 7: Dependence of inhibition of final biomass for EC20 (left) and EC50 (right) from the growth rate of the controls for duckweed and algae.

Waiting for a broad consensus for the appropriate method of data calculation test results should be presented with all calculation methods or the raw data should be easily accessible.

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