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Effects of UV-B Radiation on Green Alga *Scenedesmus quadricauda*: Growth Rate, UV-B absorbing Compounds and Potential Respiration in Phosphorus Rich and Phosphorus Poor Medium

By

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With 5 figures

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Summary

GERM M., DRMAŽ D., ŠIŠKO M. & GABERŠČIK A. 2002. Effect of UV-B radiation on green alga *Scenedesmus quadricauda*: growth rate, UV-B absorbing compounds and potential respiration in phosphorus rich and phosphorus poor medium. – Phyton (Horn Austria) 42(1): 25–37, with 5 figures. – English with German summary

This contribution summarizes the effect of UV-B radiation on growth, amount of photosynthetic pigments, production of total UV screening compounds and terminal electron transport system (ETS) activity in green alga *Scenedesmus quadricauda* grown in phosphorus rich and poor media. An image Analysis System was used to estimate the growth rate of alga. The increasing UV-B radiation dose affected growth rate of alga. The production of total UV-B absorbing compounds as a protective screen was stimulated by increasing the UV-B dose. The amount of photosynthetic pigments showed a slight increase. Terminal electron transport system activity revealed enhanced potential respiration due to UV-B. The positive responses were more pronounced in alga growing in phosphorus poor medium.

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Zusammenfassung

GERM M., DRMAŽ D., ŠIŠKO M. & GABERŠČIK A. 2002. Auswirkung von UV-B auf die Grünalge *Scenedesmus quadricauda*: Wachstumsrate, UV-B absorbierende Komponenten und Atmung in Verbindung mit der Phosphorversorgung. – Phyton (Horn, Austria) 42 (1): 25–37, 5 Abbildungen. – Englisch mit deutscher Zusammenfassung.

In dieser Arbeit werden die Auswirkungen der UV-B-Strahlung auf Wachstum, Menge der photosynthetischen Pigmente, die Produktion von UV-abschirmenden Komponenten und die Aktivität des Elektronentransportsystems (ETS) bei der Grünalge *Scenedesmus quadricauda* von phosphatreichen und phosphatarmen Nährmedium zusammengefasst. Mit einem Bildverarbeitungssystem wurde die Wachstumsrate der Alge abgeschätzt. Mit steigender UV-B Strahlung wird das Wachstum der Alge beeinflusst. Die Produktion von UV-B absorbierenden Verbindungen als Schutz wurde mit steigender UV-B Rate angeregt. Die Menge photosynthetischer Pigmente nahm geringfügig zu, die Aktivität des terminalen Elektronentransportsystems deutete auf eine gesteigerte Atmung. Die positiven Reaktionen waren bei Algen aus dem phosphatarmen Nährmedium deutlicher ausgeprägt.

Introduction

The increased transmission of solar ultraviolet (UV) radiation as a consequence of stratospheric ozone depletion (MADRONICH & al. 1998) could exert harmful effects on living organisms (STAPLETON 1992, TERAMURA & SULLIVAN 1994, ROZEMA & al. 1995, SULLIVAN & al. 1996, JOHANSON 1997, ROZEMA & al. 1997). The direct dependence of primary producers on solar energy, that beside photosynthetic active radiation includes also harmful UV-B rays, results in disturbances in photosynthesis and lower production (FRANKLIN & al. 1996). In aquatic ecosystem the share of solar UV-B radiation within the euphotic zone is also increasing (HÄDER & al. 1998). Different studies report on the effects on primary production of plankton and altered relations in the biocenosis (CULLEN & LESSER 1991, BOTHWELL & al. 1993, HÄDER & al. 1998).

Phytoplanktonic organisms are key components in the productivity of aquatic systems. HÄDER & al. 1998 pointed out that prokaryotic organisms seem to be more sensitive to UV-B stress than eukaryotic algae because they suffer more DNA damage. It has been reported, that in cyanobacteria UV-B radiation affects processes such as growth, survival, motility, and also the enzymes of nitrogen metabolism and CO_2 fixation (DONKOR & HÄDER 1996). Photosynthetic pigments are also vulnerable targets (VINCENT & ROY 1993, SINHA & al. 1995). Eukaryotic algae are able to synthesise the pigments which strongly absorb in the UV-A and UV-B part of the spectrum (KARENTZ & al. 1991). The amount of these substances is strongly dependent on the group of organisms (NIELSEN 1996) and the level of UV-B radiation (HOLM-HANSEN & al. 1993). The aquatic environment is highly variable, concerning both physical and biological parameters (FALKOWSKI 1984) including the availability of nutrients which is crucial in relation to

different stresses. The combination of nutrient concentration and UV-B stress could change competitive relationships among algae.

This study was aimed to evaluate the effect of UV-B radiation on growth, pigment content and production of total UV-B absorbing compounds in the green alga *Scenedesmus quadricauda* cultured in phosphorus rich and phosphorus poor media.

Material and Methods

Plant material and growth conditions

Cell suspensions of *Scenedesmus quadricauda* (Turp.) were obtained from the National Institute of Biology collection. Alga were cultured in polyethylene (PE) open-top (200 ml) vessels in Jaworski medium where the concentration of phosphorus was either 100% or 25% of the full concentration. Algal suspensions were exposed to different UV-B radiation doses. The temperature in the growth chamber was 23 $\pm$ 2 °C, and GROLUX lamps provided 200 µmol $m^{-2}s^{-1}$ of photosynthetic active radiation (PAR, 12/12 hours, light/dark).

Q-Panel UV-B 313 fluorescent lamps were used as a UV-B source. Lamps were wrapped in cellulose diacetate filters to cut out UV-C radiation (wavelengths lower than 290 nm) and with Mylar foil to eliminate wavelengths below 320 nm (GEHRKE & al. 1996). Different doses were provided by evenly distributed perforations of Mylar foil and by varying the period of the irradiation from 3 to 6 hours. Due to the highly variable UV-B radiation in aquatic environment, different radiation doses were applied. They ranged from 0.8 to 6.7 kJ m^2/day of UV-B biologically effective radiation (UV-B_{BE}), which was calculated used the generalised plant action spectrum (CALDWELL 1968). UV-B_{BE} is usually used because different wavelengths do not affect biomolecules and biological processes to the same extent. Therefore a number of different UV-B action spectra or weighting functions have been developed. The result of weighting of each single wavelength with an action spectrum integrated over all relevant wavelengths give us the biologically effective UV-B dose (BJÖRN 1999). One of the most commonly used action spectra is the generalised plant action spectrum developed by Caldwell in 1968.

UV-B radiation and PAR were measured at the surface level of the cell suspension using the ELDONET dosimeter (European Light Dosimeter Network).

Algal growth

Counting of cells (Fig. 5) was made by Soft Imaging System, GmbH, analySIS 3.0, Münster, Germany. The Analysis System consists of Olympus microscope BX50, with a SONY 3CCD Colour Video camera DXC – 950 P connected to NOKIA 446Xpro screen. The software used for analysis is Soft Imaging System GmbH analySIS. The two-screen system allows to display images simultaneously in two image documents. Image analyser enables counting of objects, measuring size and area of objects, sharping the objects with different filters.

Growth rate (µ) was calculated as described in International standard (ISO 8692:1989 (E)).

Photosynthetic pigments

At the end of the experiment, we measured chlorophyll *a*, chlorophyll *b* and carotenoids. The amounts of chlorophylls and carotenoids were determined following

the methods described by JEFFREY & HUMPHREY 1975 and STRICKLAND & PARSONS 1972, respectively. Cell suspensions were filtered through Whatman GF/C filter. Afterwards filters were homogenised in extraction solution (90% (v/v) acetone) and centrifuged (10.000 Hz, 4 °C, 4 min) in top refrigerated ultracentrifuge (2K15, Sigma, Osterode, Germany). Extinction of supernatant was measured spectrophotometrically with UV/VIS Spectrometer System (Lambda 12, Perkin-Elmer, Norwalk, CT, USA). The pigment contents were calculated on a DM basis.

UV-B absorbing compounds

Cell suspensions were filtered through Whatman GF/C filter. Filters were homogenised in 5 ml extraction solution containing methanol : distilled water : HCl (37% v/v) (79:20:1 v/v/v), incubated for 20 minutes and centrifuged (4.339 Hz, 10 °C, 10 min) in top refrigerated ultracentrifuge (2K15, Sigma, Osterode, Germany). Extinction of supernatant was measured from 280 to 320 nm by 1 nm step with spectrophotometer Lambda 12 (Perkin-Elmer, Norwalk, CT, USA) as described by CALDWELL 1968. The values of extinctions were integrated for the determination of total amount of UV-B absorbing compounds. The relative amounts were expressed per dry mass (DM) of samples.

Terminal electron transport system activity

Terminal electron transport system (ETS) activity of mitochondria was performed as described by PACKARD 1971. Algae were homogenised in cold buffer (0 °C, 0.1 M sodium phosphate buffer pH = 8.4, 75 µM $MgSO_4$, 0.15% (w/v) polyvinyl pyrrolidone, 0.2% (v/v) Triton-X-100) in a mortar and with an ultrasound homogeniser (4710; Cole-Parmer, Vernon Hills, IL, USA) and centrifuged (10.000 Hz, 2 °C, 4 min) in top refrigerated ultracentrifuge (2K15, Sigma, Osterode, Germany). Homogenate was mixed with substrate solution (0.1 M sodium phosphate buffer pH = 8.4, 1.7 mM NADH, 0.25 mM NADPH, 0.2% (v/v) Triton-X-100), INT solution (2.5 mM 2-p-iodophenyl 3-p-nitrophenyl 5-phenyl tetrazolium chloride) and incubated for 40 minutes at room temperature. INT is used as the electron acceptor and throught its reduction by the transferred electrons formazan is formed, which absorption at 490 nm was determined with UV/VIS Spectrometer System (Lambda 12, Perkin-Elmer, Norwalk, CT, USA). ETS activity was calculated from the the rate of INT reduction, which was converted to the amount of oxygen utilised per g of alga dry mass (DM) per time unit as described by KENNER & AHMED 1975.

Statistical analyses

All measurements were conducted at the end of the exponential growth phase, and were carried out on 4–8 parallel samples. The significance of the differences among treatments was tested by two way Student's t-test. Relation between parameters was estimated with correlation coefficient.

Results and Discussion

The species studied thrives in eutrophic freshwaters (LAZAR 1975) at different depths where the penetration of UV-B radiation could be highly variable. In Fig. 1 and Tab. 1 the results of the influence of UV-B radiation dose on growth parameters are presented. The number of cells decreased

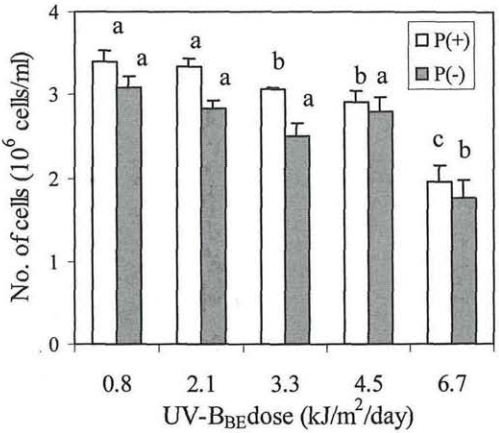

Fig. 1. Effect of different UV-B doses on concentration of cells of green alga *S. quadricauda* cultured in phosphorus rich (+P) and poor media (-P). a, b, c indicate significant differences (n = 4, $p < 0.05$).

Table 1.
The effect of UV-B dose on growth rate (μ) in green alga *S. quadricauda* cultured in phosphorus rich (+P) and poor (-P) media. a, b, c indicate significant different growth rates (n = 4, $p < 0.05$).

UV-B_{BE}dose (kJ/m^2day)	P(+) medium μ (per day) ± SE	P(-) medium μ (per day) ± SE
0.8	0.62 ± 0.01 a	0.61 ± 0.02 a
2.1	0.61 ± 0.01 a	0.59 ± 0.01 a
3.3	0.60 ± 0.01 b	0.58 ± 0.01 a
4.5	0.56 ± 0.01 b	0.56 ± 0.02 a
6.7	0.54 ± 0.02 c	0.50 ± 0.03 b

due to the enhanced dose; under the UV-B_{BE} radiation dose 6.7 kJ/m^2/day a 43% cell reduction was observed, in comparison to the lowest treatmment (0.8 kJ/m^2/day). The reduction was not dependent on the concentration of phosphorus in the medium as also revealed from growth rate (Tab. 1).

Even though the growth was evidently suppressed, UV-B radiation did not affect the amount of photosynthetic pigments (Fig. 2). The contents of chlorophyll *a* and *b* slightly but not significatly increased in *S. quadricauda* treated with higher UV-B doses. This increase could be due to lower cell density and consequently better light conditions (PAR) in the culture. But lower density also enables better penetration of harmful UV-B rays through the medium and therefore a negative effect would be expected. Data from literature are very controversial. UV-B – induced bleaching of

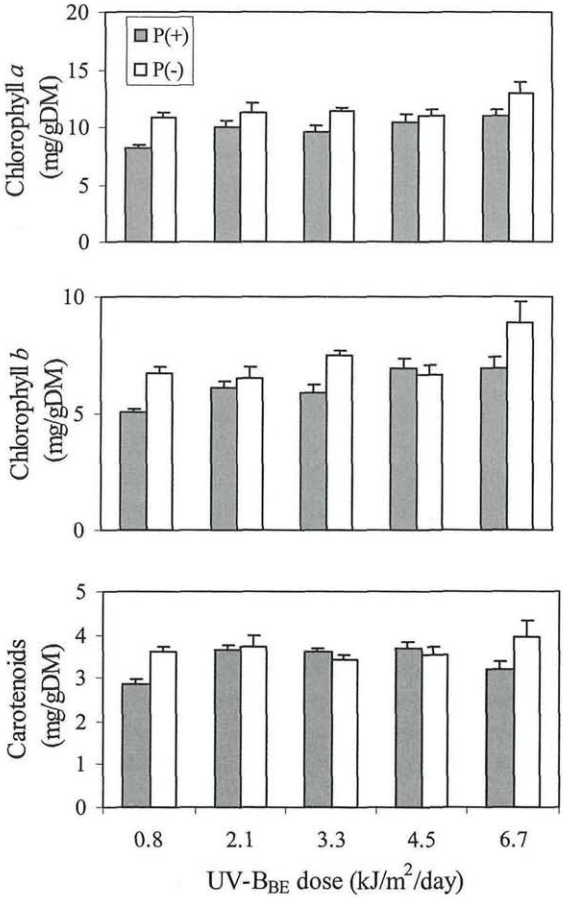

Fig. 2. Effect of different UV-B doses on chlorophyll *a*, chlorophyll *b* and carotenoid contents in green alga *S. quadricauda* cultured in phosphorus rich (+P) and poor (-P) media. No significant differences among treatments were obtained (n = 4–6, $p < 0.05$).

photosynthetic pigments has been reported (STRID & al. 1990, HOLM-HANSEN & al. 1993, BISCHOF & al. 1998), but different sensitivity among the pigments was also recorded. Even though accessory pigments appeared to be more sensitive than chlorophylls (TEVINI 1993), the destructive effect on chlorophyll was revealed by JANSEN & al. 1996 and OLSSON 1999. BORNMAN & VOGELMANN 1991 reported that Chl *a* is more sensitive than Chl *b* to UV-B. DEMMIG-ADAMS & ADAMS 1992 suggested that the decrease in Chl *a* is related to a kind of excess-light stress avoidance mechanism. On the contrary several researchers supported our results, reporting that UV-B radiation did not affect Chl *a* content (DAY & DEMCHIK 1996, ANTONELLI & al.

1997, TOSSERAMS & ROZEMA 1996), and in some cases the contents of Chl *a* even increased under UV-B radiation (BOGENRIEDER & KLEIN 1982, LIU & al. 1995). VEEN & al. 1997 found a stimulation of Chl *a* and *b* production in green alga *Selenastrum capricornutum*. BEARDALL & al. 1997 reported a negligible effect on Chl *a* in UV-B treated cells of *A. flos-aquae*. The fact that the production of Chl *a* is not depressed, but slightly stimulated, could also be explained as a protective strategy of cells. By "multiplication" of target sites, an organism avoids disturbances in activity. A similar phenomenon was reported for resistance to herbicides (PRASAD 1996). KARENTZ & al. 1991 also pointed out the protective role of chloroplasts in the cell. Their position in the cell could provide the protection of nucleus against strong radiation. Even though there is some evidence of the protective role of chlorophyll further researches are needed to elucidate this phenomenon.

The avoidance of damage due to harmful UV-B radiation in primary producers is also enabled by other mechanisms. According to the data from literature, synthesis of carotenoids is one of them (RAU & al. 1991, MIDDELTON & TERAMURA 1993). In our experiment UV-B radiation slightly stimulated the synthesis of carotenoids in *S. quadricauda*. The amount of carotenoids was in close relation with the amount of Chl *a* ($r = 0.83$, $p \leqslant 0.01$). This phenomenon was also reported by MIRECKI and TERAMURA 1984 for higher plants.

Another very efficient protective mechanism is the production of UV-B absorbing compounds (KARENTZ & al. 1991, HOLM-HANSEN & al. 1993), the amount of which depends on the UV-B dose (HOLM-HANSEN & al. 1993). In our study we measured an increase of these compounds at higher UV-B radiation doses only for *S. quadricauda* grown in phosphorus poor medium (Fig. 3). In phosphorous rich medium the increment was observed at 3.3 and 6.7 kJ/m^2/day $UV\text{-}B_{BE}$ doses. Higher production of methanol soluble UV-B absorbing compounds in *S. quadricauda* is not necessarily an advantage, because the production of secondary substances is costly (GULMON & MOONEY 1986). The disturbances in energetical status of alga could effect their proliferation. This fact is also supported by increased ETS activity under higher UV-B doses (Fig. 4). In phosphorus poor medium the potential respiration of alga was even higher. The increase of ETS activity in natural phytoplankton populations thriving under enhanced UV-B doses was also reported by FERREYRA & al. 1997.

Additional protection is provided by the organization of cells. *S. quadricauda* forms colonies of four cells (Fig. 5). XIONG & al. 1996 found out that species grown in colony or coenobia, are less vulnerable to UV-B radiation, because they form larger groups and in that way protect inner structures. Small size and filamentous species are much more exposed and vulnerable. Similar conclusion was made by KARENTZ & al. 1991, who reported that cells with lower area/volume ratio are more sensitive.

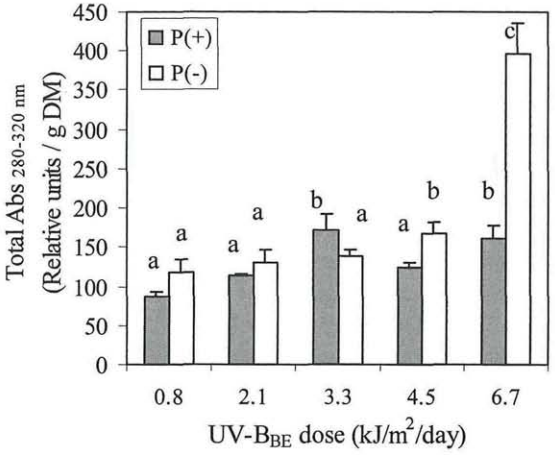

Fig. 3. Production of UV-B absorbing compounds at different UV-B doses in green alga *S. quadricauda* cultured in phosphorus rich (+P) and poor (-P) media. a, b, c indicate significant differences (n = 4-10, $p < 0.05$).

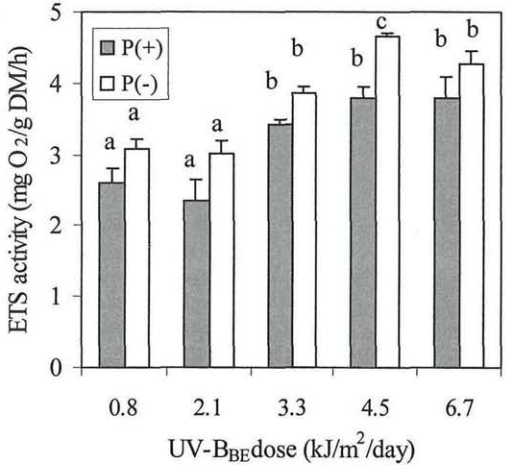

Fig. 4. Terminal electron transport system (ETS) activity in green alga *S. quadricauda* cultured in phosphorus rich (+P) and poor (-P) media. a, b, c indicate significant differences (n = 3, $p < 0.05$).

The reports on combined effects of enhanced UV-B radiation and shortage of nutrients are scarce. The researches on higher plants revealed the mitigation of UV-B damage under conditions of low nutrient supply. BOGENREIDER & DOUTE 1982 studied the influence of enhanced UV-B radiation on lettuce and found less disturbance in plants grown under nutrient poor conditions. Similar results were obtained by MURALI &

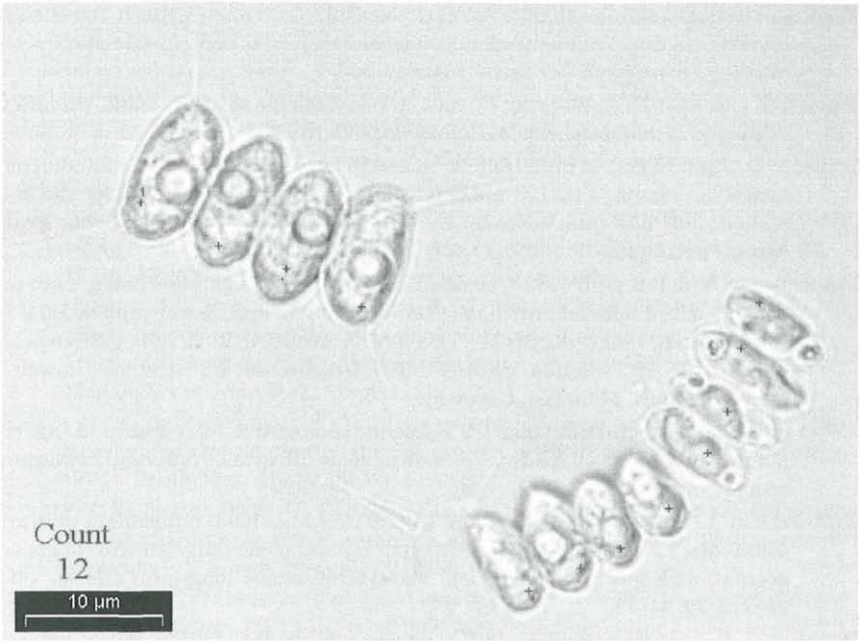

Fig. 5. Green algae *S. quadricauda*.

TERAMURA 1985a,b who studied the UV-B effects on soybean. Plants grown under optimal phosphorus supply were more sensitive than those grown under reduced phosphorus availability. Different phosphorus treatments in *S. quadricauda* showed a slight decrease in the number of cells and growth rate on one hand, but on the other hand an increased content of photosynthetic pigment, production of UV-B screening compounds and potential respiration.

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Recensio

SCHÜTTE Gesine, STIRN Susanne & BEUSMANN Volker (Eds.) 2001. Transgene Nutzpflanzen. Sicherheitsforschung, Risikoabschätzung und Nachgenehmigungs-Monitoring. – Gr. 8°, VI + 247 Seiten, kart. – Birkhäuser Verlag, Basel, Berlin, Boston. – ca. € 45. – ISBN 3-7643-6475-0.

Der vorliegende Band resultiert aus einem Gutachten an den Deutschen Bundestag zu Sicherheitsfragen (Untertitel !) und enthält demnach keine Beschreibungen transgener Nutzpflanzen. Im 1. Abschnitt wird u.a. auf Ausmaß des Anbaues gentechnisch veränderter Pflanzen und der Freisetzungsanträge eingegangen. „Das Riskio einer schädlichen Wirkung ergibt sich aus erwarteter Schadenshöhe und Eintrittswahrscheinlichkeit des Schadens" (p. 3). Auch auf das Problem des Verschweigens von Nicht-Wissen im Zusammenhang mit Risikoabschätzung wird kurz eingegangen. Der 2. Abschnitt behandelt den Stand der Diskussion zu grundlegenden konzeptionellen Fragestellungen. Die Notwendigkeit Synergistische Modelle mit der Berücksichtigung der Möglichkeit des Auftretens neuer Eigenschaften und unerwarteter Folgen wird heute offenbar anerkannt; Streit besteht aber nach wie vor um die Frage, wieweit Schlußfolgerungen daraus für Risikoabschätzung und Risikovorbeugung zu berücksichtigen sind. In diesem Abschnitt gibt es (p. 11) eine bemerkenswerte Zweigliederung von Nichtwissen: solches das auf noch nicht geprüften, konkreten Hypothesen beruht und sich z.B. durch einen Auftrag zu einem For-