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Monitoring toxicity, DNA damage, and somatic mutations in tobacco plants growing in soil heavily polluted with polychlorinated biphenyls

Tomáš Gichner [a,*], Petra Lovecká [b], Lucie Kochánková [c], Martina Macková [b], Kateřina Demnerová [b]

[a] *Institute of Experimental Botany, Academy of Sciences of Czech Republic, Na Karlovce 1a, 160 00 Prague 6, Czech Republic*
[b] *Faculty of Food and Biochemical Technology, Institute of Chemical Technology in Prague, 166028 Prague 6, Czech Republic*
[c] *Faculty of Environmental Chemistry, Institute of Chemical Technology, 166 28 Prague 6, Czech Republic*

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Abstract

Heterozygous tobacco (*Nicotiana tabacum* var. *xanthi*) plants were cultivated in soil from a dump site highly polluted with polychlorinated biphenyls (PCBs) at Lhenice in South Bohemia, Czech Republic. The total amount of PCBs in the polluted soil, measured by gas chromatography varied from 165 to 265 mg kg^{-1} of soil. In tobacco plants cultivated for 8 weeks in the polluted soil the amount of PCB in the leaves varied from 11 to 28 and in the roots from 104 to 308 mg kg^{-1} dry mass. The average leaf area of tobacco plants growing in the PCB-polluted soil was significantly reduced and the DNA damage in leaf nuclei, measured by the comet assay, was slightly but significantly increased compared with controls. The tobacco plants with increased DNA damage showed reduced growth and had distorted leaves. No increase in the frequency of somatic mutations was detected in tobacco plants growing in the PCB-polluted soil.

Keywords: Comet assay; Single-cell gel electrophoresis; Gas chromatography; *Nicotiana tabacum*

1. Introduction

Plants are exposed to various types of environmental agent, either deliberately as in the case of agricultural pesticides and plant-growth regulators, or accidentally as compounds present in polluted air, soil or water.

Polychlorinated biphenyls (PCBs) have been used as coolants and lubricants in transformers, capacitors, and other electrical equipment because they are highly resistant to high temperatures, and are good insulators. They belong to the group of persistent environmental pollutants, which can be found (at least in low quantities) in almost every compartment of terrestrial and aquatic ecosystems. The mechanisms by which PCBs exert their adverse effects on organisms are not fully understood and data on their genotoxic properties are still controversial [1,2].

In the work presented here, tobacco plants (*Nicotiana tabacum* var. *xanthi*) were cultivated for 2–8 weeks in soil from a dump site polluted with PCBs (locality Lhenice in South Bohemia, Czech Republic) and in

Abbreviations: PCBs, polychlorinated biphenyls; TM, tail moment

* Corresponding author. Tel.: +420 224 310 109; fax: +420 224 310 113.
E-mail address: gichner@ueb.cas.cz (T. Gichner).

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control garden soil. We have (1) assessed the toxic effects of the soil samples by measuring the tobacco leaf area, (2) evaluated induced DNA damage in leaf nuclei by the comet assay, and (3) scored the frequency of somatic mutations in leaves. In order to evaluate the possible adverse effects of poor nutrient values of the PCB-polluted soil, the tobacco plants were watered in parallel pots with distilled water or with 50% Hoagland's solution. The amount of PCBs in the soil and in the tobacco leaves and roots was determined by gas chromatography with electron-capture detection.

2. Material and methods

2.1. Chemicals and media

Ethyl methanesulphonate (EMS, CAS No. 62-50-0), maleic hydrazide (MH, CAS No. 123-33-1) and reagents for electrophoresis were purchased from Sigma Chemical Co., St. Louis, MO, normal and low melting-point agarose from Roth, Karlsruhe, Germany. Other reagents for the determination of the content of PCBs in the soil and in the tobacco plants were from Fluka, Germany and PCB standards were from Dr. Ehrenstorfer Co., Germany.

2.2. PCB-polluted soil

PCB-contaminated soil was taken from a protected dump site in Lhenice, South Bohemia, Czech Republic. The soil was originally collected from places with high concentrations of PCBs, mostly from factories using PCBs for the production of incombustible materials, e.g. dyes, transformers or tar. The PCBs for these factories (mostly the commercial PCB mixture Delor 103) were produced by Chemko Strážské until 1984. The area of the dump site is about 500 m^2 and contains approximately 250 t of contaminated soil. The PCB content varied between 5 and 400 mg kg^{-1} soil. The PCB content in the control soil and in control plants was so low that it could not be detected.

2.3. Tobacco growth, treatment conditions, measurement of the leaf area and the frequency of somatic mutations

Seeds of the double heterozygous *N. tabacum* var. *xanthi* ($a_1^+/a_1; a_2^+/a_2$) plants [3] were germinated under sterile conditions in plastic vented containers that contained 50 ml of solid growth medium. At the stage of three to four true leaves, the plants were transferred to plastic pots with the test soil. Ten plants in pots *per* variant were cultivated in a cultivation room with artificial light with an 18-h photoperiod at 22–26 °C.

The leaf area of one leaf per plant, thus 10 leaves per variant, was measured by a planimeter and expressed in square centimetre. The measurements started after 2 weeks with the first newly formed leaves after planting the seedlings into the polluted soil. After 3, 4, 6 and 8 weeks the area of the subsequent leaves was measured.

Three main types of mutagenic event were scored on the greenish-yellow leaves of the heterozygous tobacco plants cultivated on the test soil: (1) dark green, (2) yellow and (3) green/yellow twin sectors [3]. The somatic mutations were scored under a stereomicroscope.

2.4. DNA-damage studies

The preparation of agarose microscope slides with isolated nuclei, the DNA unwinding, and the conditions of electrophoresis and ethidium bromide staining in the comet assay were as previously described [4,5]. For each slide, 25 randomly chosen nuclei were analyzed using a fluorescence microscope with an excitation filter of BP 546/10 nm and a barrier filter of 590 nm. A computerized image-analysis system (Komet Version 3.1, Kinetic Imaging Ltd., Liverpool, UK) was employed. The tail moment parameter (integrated value of tail DNA density multiplied by the migration distance/100) was used as a measure of the DNA damage. For DNA-damage studies, three leaves were taken from different plants of each treatment variant, and from each leaf two comet slides were prepared. In total, 150 nuclei were analyzed *per* variant.

2.5. Determination of the content of PCB in the soil and in tobacco plants

Aliquots (1 g) of soil samples that had been air-dried overnight and sieved (1-mm mesh), and aliquots (5 g) of harvested and air-dried tobacco leaves and roots, homogenized in liquid nitrogen, were extracted with hexane for 4 h. The extracts were concentrated to 1 ml by a nitrogen flow, purified on a Florisil column, and diluted with hexane to the same volume as was used for the extraction. These extracts were analyzed using a Hewlett-Packard 5890 gas chromatograph with an electron-capture detector and a fused silica capillary column (30 m, 0.2 mm inner diameter) coated with 0.25 μm immobilised phase SE-54 with nitrogen as carrier gas (flow rate 1 ml min^{-1}). The temperature program was as follows: 50 °C for 1 min, followed by an increase at a rate of 25 °C min^{-1} until the temperature was 280 °C and then maintained at this temperature. The injection volume was 2 μl. Results were calculated from the residual amounts of congener peaks present in each sample. For the evaluation of the experiments, the US EPA method 8089/8081 for expressing the total content of PCBs as a sum of recommended indicator congeners (PCB 77, 101, 118, 138, 153, 180) was applied [6,7].

2.6. Statistical analysis

Data were analyzed using the statistical and graphical functions of SigmaPlot 8.0 and SigmaStat 3.0 (SPSS Inc., Chicago, IL, USA). The median tail moment values were used in a one-way analysis of variance test. If a significant F-value of $P < 0.05$ was obtained, a Dunnett's multiple comparison versus the con-