PRODUCTS OF METABOLIC PATHWAYS OF PCBSs IN BACTERIA AND PLANTS – COMPARISON OF THEIR TOXICITY AND GENOTOXICITY

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ABSTRACT

Some different systems for the measurement of ecotoxicity have been used for the estimation of the toxicity of intermediates of bacterial and plant PCB metabolism in comparison with primary polychlorinated biphenyls. Luminescent bacteria (*Vibrio fischeri)* and mammalian cells (keratinocytes) showed similar reactions response to the different toxicants examined. Chlorobenzoic acids (intermediates of bacterial PCB metabolism) exhibited the lowest toxicity. Products of plant metabolism (hydroxychlorobiphenyls) were the most toxic compounds; their toxicity exceeded that of initial individual monochlorobiphenyls. Other method (Bioscreen and germination of seeds) following growth in presence of the toxicants, showed a different response of the selected model organisms based on their abilities to degrade PCBs. Ames test with *Salmonella typhimurium* was used for genotoxicity measurements. Chlorobenzoic acids exhibited lower toxicity then initial PCB, but in some cases significant genotoxicity.

1. INTRODUCTION

Microbial and plant species may possess enzymes capable of metabolizing certain environmentally - persistent xenobiotics that contaminate soil and water. It has been shown that both bacteria and plants metabolize different organic molecules including highly persistent polyaromatic hydrocarbons and polychlorinated biphenyl (1,2). Bacterial metabolism of PCBs is well-known and it has been described in detail (1). Generally, plant metabolism has been studied to a lesser extent than that in bacteria or mammals and little information is available. Little is known about the intermediates in plants, their toxicity and the effect that such compounds may have to animals and other organisms. In plants, organic compounds are transformed into less phytotoxic ones, then conjugated with sugars, amino acids, etc. and deposited in vacuoles or lignified parts of the cell wall. Unfortunately this fact does not mean that metabolites or products have less toxicity towards other living systems. Bacterial metabolism of PCBs is well-known and it has been described in detail (1,3,4). Generally, plant metabolism has been studied to a lesser extent than that in bacteria or mammals and little information is available.

Different systems for ecotoxicity measurement were evaluated during last 15 years. Many of them are based on measurement of viability of different organisms and their ability to survive in presence of different toxicants. In our experiments we studied metabolism of PCBs in bacteria and plants (3). These organisms are mainly involved in transformation of toxic compounds in nature and they are responsible for further fate of those xenobiotics and their intermediates in the environment. We use bacterial strains and several plant species that are able to transform PCBs. As a model, aseptic plant cells cultivated *in vitro* in liquid media containing PCBs and normal plants cultivated in contaminated soil were also used as well. The decrease of PCB congeners after biotransformation is detected by gas chromatography with EC detection. Toxicity of identified bacterial and plant products was studied using plant cells, microbial and mammalian cell system (5).

2. MATERIALS AND METHODS

2.1 Polychlorinated biphenyls

Individual congeners of monochlorinated PCBs were used as models for toxicity studies. Bacterial and plant products and intermediates of PCB metabolism were chosen according to previous results (see Table 1) (6).

TABLE 1: Compounds used for the toxicity measurements

PCB 1		2 -chlorobiphenyl
PCB 2		3 - chlorobiphenyl
PCB 3		4 - chlorobiphenyl
Hydroxychloroderivatives		(RPM)
RPM 3		3 – chlor - 4 - hydroxybiphenyl
RPM 4		4 - chlor - 4 - hydroxybiphenyl
RPM 6		2 -chlor - 5 - hydroxybiphenyl
Chlorobenzoic acids		
3cl a		3 - chlorobenzoic acid
2,3cl a		2,3 – chlorobenzoic acid
2,5cl a		2,5 - chlorobenzoic acid

2.2 Toxicity assay using luminescent bacteria

A working suspension of luminescent bacteria was prepared by reconstitution a vial of lyophilized cells of *Vibrio fischeri,* using 0,5 ml of 2% NaCl aqueous solution at 2-5°C. The bacterial suspension was added to 0,5 ml dilution series of toxicant (chlorobenzoic acids, congeners of PCB, hydroxychlorobiphenyls) in 2% NaCl. Luminescence was measured after 15 minutes incubation .

2.3 Toxicity assay using the Bioscreen test

This test allows evaluation of the effect of various concentrations of toxicants on bacterial viability and growth in comparison with the controls cultivated without toxicants. Values of OD_{400nm} and the time dependence of growth are monitored in parallel samples incubated with or without toxicants for 2 days. The effect of three different concentrations of toxicants (PCB, chlorobenzoic acids and hydroxychlorobiphenyls) at three concentrations (10mg/l, 20mg/l and 40mg/l) was followed using three different bacterial strains (*Pseudomonas* sp. R9 - isolated from the soil contaminated with polyaromatic hydrocarbons, *Pseudomonas* sp. P2 - isolated from the soil contaminated with PCB and *E. coli* from the Collection of the Department of Biochemistry and Microbiology). Minimal medium containing glycerol (5g/l) and toxicants as described above was used for the incubation.

2.4 Toxicity assay using germination of seeds

This method is based on measuring of the germinating root lenght of *Lactuca sativa* in presence of toxicants. The temperature of incubation is 22°C without light for 4 days and referent medium consists of 18,5 g/l $CaCl.2H_2O$, 2,3 g/l KCl, 49,3 g/l $MgSO_4.7H_2O$, 25,9 g/l $NaHCO_3$ (2,5 ml for 1000ml H_2O). Results are expressed as coefficient of inhibition **I** (%). The sample is interpreted to be toxic, when **I** – value is greater than 30 %.

2.5 Measurement of genotoxicity using Ames test

The Ames test is used world-wide as an initial screen to determine the mutagenic potential of new chemicals and drugs. First of all the toxicity of the substances to *Salmonella* strains was tested. The Ames test has a range of specific modifications and enables detection of a wide variety of mutagens. The detection system using *Salmonella typhimurium* His^- differs in mutations within histidine operon. compared with the original strain. Mutations in the histidine operon are induced by mutagens leading to reversion to protothrophy, e.g. the ability to synthesize histidine. To enhance the sensitivity of indicator strains differing in mutation type with the tested substance, markers were added. Finally, the results were compared using the parameter Rt/Rc, where. Rt represents the total number of revertants of a particular concentration of a tested substance, where Rc is a total number of spontaneous revertants on control plates.

3. Results and discussion

3.1 Measurement of the toxicity

The main products of bacterial degradation of PCB are chlorobenzoic acids, which can be further degraded by other bacteria present in contaminated environment.

Analysis of the products of bacterial and plant metabolism has shown that plant products (hydroxychlorobiphenyls) of the first phase of PCB transformation are similar to those detected in mammalian cells. While bacteria degrade PCB to chlorobenzoic acids, plants are generally unable to open the ring and degrade the chemical structure of biphenyl .[6]

PCBs were produced as mixtures of different congeners varying in their degree of chlorination. The simplest PCBs are monochlorobiphenyls which are usually not present in commercial mixtures, but they can be formed in the natural environment by microbial dechlorination or degradation of more highly chlorinated congeners. We used monochlorobiphenyls and their products identified in bacterial and/or plant cells as models for the toxicity measurements of compounds which can exist naturally in contaminated soils and increase the toxicity of polluted areas. As was previously described bacterial and plant intermediates of PCBs are structurally different. Toxicity of bacterial (chlorobenzoic acids) and plant intermediates (hydroxychlorobiphenyls) of PCB transformation compared to the toxicity of initial monochlorobiphenyls measured using luminescent bacteria is shown in Figure1.

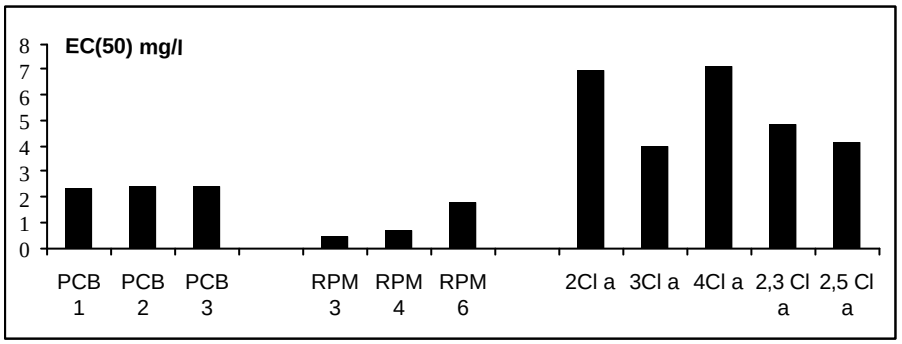

FIGURE 1: Toxicity of monochlorobiphenyls and products of bacterial and plant metabolism measured by luminescent bacteria *Vibrio fischeri*

From the results it can be concluded that hydroxychlorobiphenyls as primary products of PCB metabolism in plants are the most toxic (i.e. the lowest LD50), comparing to all three groups of tested compounds. Hydroxychlorobiphenyls are more soluble in water than the original PCB and thus are more available and toxic for living organisms. A similar response was previously obtained with mammalian cells (keratinocytes), which also showed the highest sensitivity to hydroxychlorobiphenyls. Comparing both systems for the ecotoxicity measurement luminescent bacteria are susceptible to lower concentrations of tested compounds.

Toxicity of the same compounds was tested using an independent method – namely Bioscreen measurement. The Bioscreen test showed a different response of the selected model organisms. *Pseudomonas sp.* R9, which is not able to degrade PCBs, exhibited the strongest response to individual congeners of polychlorinated biphenyls, while hydroxychlorobiphenyls and chlorobenzoic acids were less toxic. The opposite effect was followed with *Pseudomonas sp.* P2 which is able to degrade PCBs and thus it was not susceptible to PCBs but to products of PCB degradation - chlorobenzoic acids. *E coli* exhibited a similar response to all three groups of tested compounds (Figure. 2).

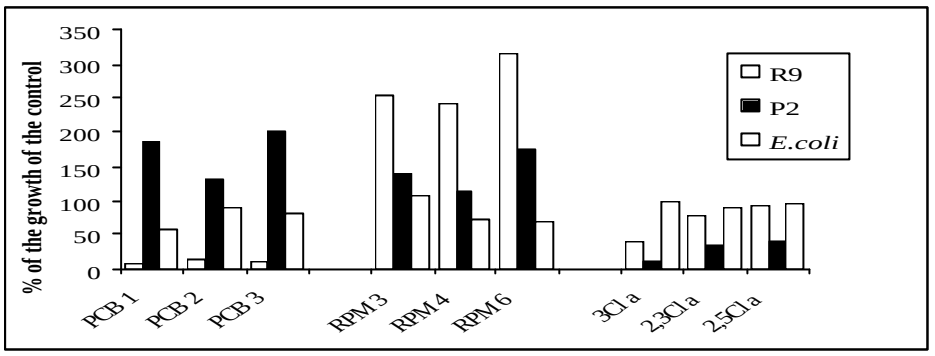

FIGURE 2: Evaluation of specific growth rates of three bacterial strains incubated with PCBs, chlorobenzoic acids and hydroxychlorobiphenyls at a concentration of 10 mg/l

Results of measuring with seeds of *Lactuca sativa* are shown in Table 2. Monochlorbiphenyls are not toxic for plant system, but chlorobenzoic acids and hydroxychlorobiphenyls are more toxic then PCB. It is probably due to their better solubility in water.

TABLE 2: Toxicity of products of bacterial and plant metabolism measured by seeds of *Lactuca sativa*

I(%)	PCB 1	PCB 2	PCB3	RPM 1	RPM 3	RPM 4	3 Cl a	2,3Cla	2,5Cl a
3 mg/l	19,1	16,5	7,8	4,7	2,0	15,7	55,5	44,5	53,7
50mg/l	25,8	27,3	7,5	86,9	81,8	93,6	79,1	93,7	99,6

3.1 Measurement of genotoxicity by Ames test

Due to the toxicity of the tested substances for our tested model microorganisms it was necessary to establish an appropriate concentration, which did not kill the bacterium itself. The lowest concentration showing any mutagenic effect was chosen and a concentration gradient was prepared. Genotoxic effect was proved in case of chlorobenzoic acids. Original PCBs and hydroxychlorobiphenyls showed less genotoxicity than chlorobenzoic acids, only with TA 100 cells weak genotoxic effect was measured.

4. CONCLUSION

In our study we showed different behaviour of organisms to the presence of pollutants, namely of some PCBs and their bacterial products. These data show the phenomena which could appear with low soluble contaminants transformed by any living system, unfortunately can not be generalised to properties of all contaminants polluting the environment. In each case basic data documenting behavior of pollutants and their products should be evaluated using proper system for ecotoxicity analysis and compared with analytical results determining chemical origin and concentrations of pollutants.

Acknowledgement : The work was supported by the grant of the Czech Ministry of Education No. LN 00B030 and the 5FW EU grant No. QLK3-CT-2001-00101

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