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PENTACHLOROPHENOL - AN ENVIRONMENTAL POLLUTANT. MICROCALORIMETRIC INVESTIGATIONS OF AN ECOLOGICAL MODEL SYSTEM*

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SUMMARY

The influence of pentachlorophenol (PCP) on components of an ecological system was determined calorimetrically. After exposure to PCP at concentrations up to 0.4 g/l there was a significant stimulation to as great as 400 % of the rate of heat production in litter, soil and earthworms as a typical animal for this biotope. Even with the highest concentrations used no inhibitory effects could be detected in the present investigation.

INTRODUCTION

Pentachlorophenol (PCP) is one of the most intensively used pesticides in the world. It is applied in high quantities as fungicide, herbicide, insecticide, molluscicide and defoliant (Table 1). Thus, it can be assumed to be an ubiquious pollutant of any soil. Although its endangering potential has been well-known for many years, PCP is still used as an effective wood-protecting agent.

In many studies of its biological and biochemical activities (refs. 1,2) PCP has been established as a powerful uncoupler of oxidative phosphorylation and thus of the main energy producing step in metabolism (ref. 3). Severe influences on other enzymatic systems and on cell organelles also have been documented (refs. 4,5). The various actions of PCP on biological systems are listed in Table 2 .

Chemically pure PCP seems less poisoning than the technical product with its many impurities (ref. 6). Some of these contaminants, for example HCDD (hexachlorodibenzo-p-dioxine) and OCDD (octachlorodibenzo-p-dioxine), are highly toxic agents and thus responsible for fatal accidents initially ascribed to PCP. This fact has to be considered when laboratory results with pure PCP are related to ecological systems which have been treated with technical PCP.

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TABLE 1

Application of pentachlorophenol (PCP) in industry and agriculture

Pesticide	algicide
	bacteriocide
	fungicide
	herbicide (e.g. in flooded rice fields)
	insecticide
	molluscicide (e.g. against Schistosomia)
Utilization	wood protection
	crob protection in agriculture
	impregnation of textiles
	additive in synthetic materials
	in emulsion paints
	in glues

Until now only a few calorimetric papers have appeared on ecological and complex biological systems (refs. 7-9). Only one calorimetric publication has dealt with PCP, but exclusively in bacterial systems (ref. 10). As calorimetry is the only quantitative and unspecific method to monitor aerobic and anoxic metabolism (ref. 11), the present paper gives a calorimetric investigation of PCP as a representative of chlorinated phenols acting on an ecological soil system. This system comprised forest soil, forest litter of varying degree of rotting as well as some smaller animals (beetles, centipedes, earthworms, isopods) living in this biotope. These measurements were complemented by determinations of oxygen consumption and uptake of radioactively labelled PCP.

TABLE 2

Action of pentachlorophenol (PCP)

-	powerful decoupler of oxidative phosphorylation stimulation of respiration (at lower concentrations) stimulation of glycolysis (at lower concentrations) increase in heat production rate increased basal metabolism complete blocking of inorganic phosphore uptake
-	inhibition of respiration and glycolysis at higher concentrations
-	membrane made permeable to protons changes in membrane fluidity
-	induction of mitochondrial swelling stimulation of ATPase activity in mitochondria influence on microsomal enzyme systems
-	interruption of electron transfer from flavine to cytochrome P-450

MATERIAL AND METHODS

Ecological System

Two different forest systems were chosen which have been thoroughly investigated by other groups with respect to the action of PCP. System A (near Karlsruhe, West Germany) consisted of a mixed tree population including beech, birch, elm, lime-tree, maple, oak, poplar and willow, system B (near Frankfurt/Main, West Germany) of mainly beech trees. Leaves were found in the two litter layers "L" and "F" studied in these investigations. "L" is fresh material from the last autumn, while "F" contains older more rotten material. The third and lowest layer occupies the upper 2 cm of the actual soil.

A number of small animals were gathered in these ecotopes and used in calorimetric experiments: earthworms (*Eisenia foetida*), isopods (*Oniscus asellus*, woodlouse; *Armadillidium vulgare*, pill bug; *Lithobius forficatus*, *Polydesmus complanatus*) and carabid beetles (*Nebria brevicollis*). But only *E.foetida*, *O.asellus* and *A.vulgare* were used for systematic investigations of PCP because of their numerous occurrence. The animals were kept in plastic boxes with litter at 6 ^{o}C in the dark. Before and after each experiment the animals were weighed to the nearest 0.1 mg; all figures in this paper are given as wet weight. After the experiments the animals were immediately killed by deep freezing and stored at 4 ^{o}C in a refrigerator.

Chemicals

Pentachlorophenol (PCP) was purchased from SIGMA/Deisenhofen (no. P1045, purity > 98 %) and dissolved in toluene. Before application it was diluted in a 10 % alcohol solution to final concentrations of 0.1, 0.2, 0.4 and 0.8 g PCP/l at a pH of 5.5. At 0.8 g PCP/l the remaining toluene concentration was 3 %, for the other dilutions correspondingly less. Radioactively labelled PCP-UL-^{14}C (purity > 98 %, 10.6 mCi/mmol in a concentration of 1 mCi/ml) was obtained from SIGMA/Deisenhofen, too. It was used in the same concentrations as the unlabelled one.

Animals were bathed in the solutions for different times or put in glass flasks with a bottom layer of filter paper thoroughly wetted with PCP solution. After this treatment the animals were dried with soft tissue paper. Litter and soil were treated with PCP by adding appropriate amounts of solution. Solutions without PCP served as controls.

Instrumentation

All experimemts were performed at 25 ^{o}C in an isoperibolic twin batch calorimeter of the Calvet type (MS 70, SETARAM/Lyon) with 4 vessels of 100 ml each and a sensitivity of approximately 60 mV/W. Specimens (litter, soil, ani-

mals) were placed in separate plastic containers of 45 ml with gauze covers (Reichelt/Heidelberg, no. 61167) which were transferred to the metallic calorimeter vessels after thermal equilibration. The volume of these vessels was large enough to avoid any significant drop in oxygen concentration during the experiments. The calorimetric output was registered as heat production rate versus time by a multichannel recorder (BD5 + BA5, Kipp&Zonen/ Delft). This direct calorimetric investigation was complemented by manometric and polarographic experiments (indirect calorimetry) which will be published elsewhere.

The uptake of radioactively labelled PCP was determined in a Beckman Scintillation Counter LS 1800 (Beckman Instruments/München).

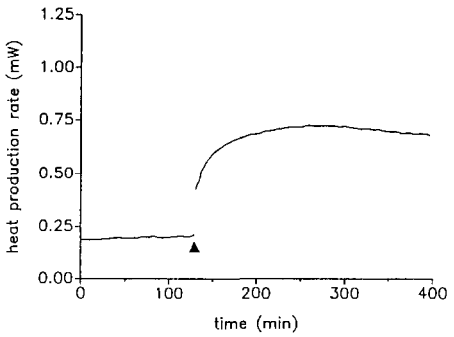

Fig. 1. Stimulation of the heat production rate of 1.29 g ww of litter of layer "F" after incorporation of 0.125 mg PCP/g ww (indicated by the triangle).

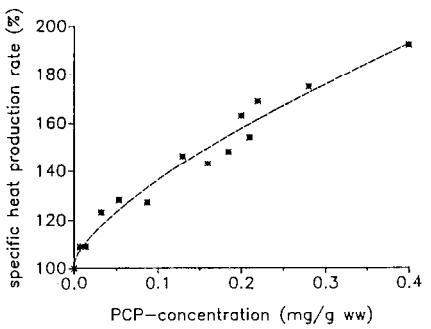

Fig. 2. Relative stimulation of the weight specific heat production rate of litter of layer "F" with increasing PCP concentrations.

RESULTS AND DISCUSSION

Litter, soil and animals readily took up PCP within a few minutes. The most effective application of PCP was obtained by bathing the animals in the solution. Creeping around over PCP-soaked paper produced an inhomogeneous contact due to periods of rest or intensive movement which caused a diminished or increased uptake of the toxic substance. The PCP uptake rate in earthworms (*E.foetida*) bathed in 0.4 g PCP/l solution corresponded well with that of snails (*Australorbis glabratus*) exposed for longer times to 2 ppm PCP (ref. 12). PCP uptakes in the order of 0.5 - 2 µg/g ww by the other animals used in

these experiments (isopods, beetles) were similar to those found in animals of a model ruderal ecosystem after three weeks (ref. 13).

Metabolic stimulation due to lower PCP concentrations occurs within a few minutes as measured by the respiration rate in yeast cultures (unpublished results, see also ref. 6). A time delay of at least 30 minutes after the addition of PCP occurs in calorimetric experiments because of the thermal inertia of the calorimeter. Adding equal amounts of solution without PCP as a control to litter and soil or bathing animals in such a solution induced no changes in the heat output level.

Fig. 1 shows the heat production rate of litter from the layer "F" before (left) and after addition of PCP (right of the triangle). In both cases steady states of heat flow were established. Within the chosen range of PCP concentration a strong stimulation was observed in the heat production rate of litter (Fig. 2) as well as soil (not shown) increasing with the amount of applied PCP. Even the highest PCP concentration of 0.4 mg PCP/mg w.w. never resulted in an inhibition of the heat production rate.

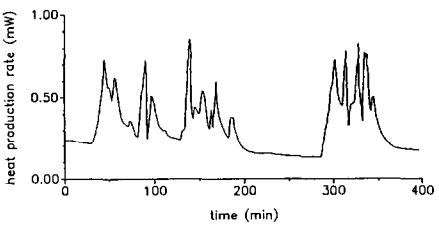

Fig. 3. Heat production rate of an earthworm (*E.foetida*) of 101 mg ww showing distinct peaks due to locomotory activities.

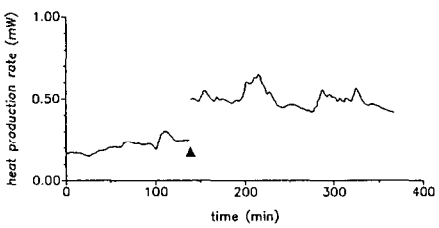

Fig. 4. Stimulation of the heat production rate of an earthworm (*E.foetida*) of 365 mg ww after the uptake of 31.8 µg PCP/g ww (triangle).

The situation is more complicated with animals. Due to locomotory activity their heat production rates showed strong fluctuations and only short steady states of basal metabolism. The following experiments concentrate on the earthworm (*Eisenia foetida*). It has no protecting cuticle so that the PCP uptake through the surface is facilitated and therefore more reproducible than in the two isopods (*Oniscus asellus* and *Armadillidium vulgare*). Intensive investigation of the isopods will be published elsewhere. A typical graph of heat production rate versus time for a single earthworm (*E.foetida*) of 101 mg wet weight is given in Fig. 3. Periods of active locomotion exchanged with periods of rest at a nearly constant level of activity. Heat-production-curves

were not corrected for the thermal inertia of the calorimeter, otherwise the peaks would be even more pronounced (ref. 14). Due to this scatter it is more difficult to determine the exact percentage of stimulation by PCP in animals rather than in soil or litter, although the effect was seen in all cases.

Fig. 4 presents - in comparison with Fig. 1 for litter - the heat production rate for a single earthworm (*E.foetida*) without (left) and with PCP. The shift in the heat flow level is clearly seen, but until now it cannot be established if the strong fluctuations in the curves have changed significantly. Obviously, PCP treatment produced no additional restlessness of the animals as shown by the calorimetric curves. Thus, the increased heat production rate seems to result only from an elevated basal metabolism. Fig. 5 shows the PCP uptake of earthworms after bathing in a ^{14}C-labelled PCP solution as a function of time and Fig. 6 the relative stimulation of the heat flux as a function of PCP uptake. Again there is a strong increase by more than a factor 4 in the applied PCP range without any indication of saturation or transition to inhibition.

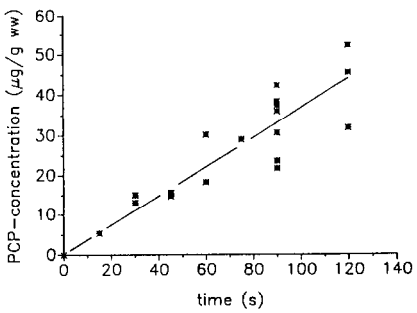

Fig. 5. PCP uptake by earthworms (*E.foetida*) as a function of time after exposure to a solution of 0.4 g PCP/l.

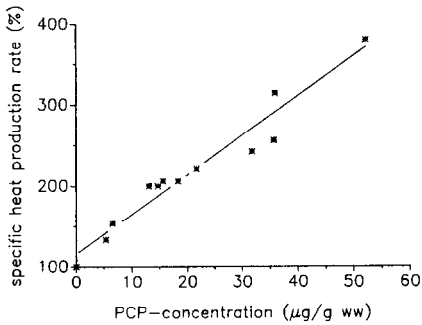

Fig. 6. Stimulation of the weight specific heat production rates of earthworms (*E.foetida*) as a function of the concentration of PCP taken up during bathing.

The present calorimetric results are in good agreement with data from the literature on respiration after the application of PCP (refs. 12,15). This is supposed to be a powerful uncoupler of oxidative phosphorylation (refs. 3,6), cells consuming energy without producing the corresponding energy equivalent in form of ATP necessary for cellular processes. Thus the metabolic rate increases in order to cover the energy demand of the organism: oxygen consump-

tion and heat production rate increase proportional to the amount of PCP applied.

Rao and coworkers (ref. 15) observed in the grass shrimp, *Palaemonetes pugio*, also an initial strong increase of oxygen consumption which was followed by a steep decrease after 4 h and the subsequent death after 8 h. But their experiments were performerd at a much higher PCP concentration of 20 ppm which corresponded to the concentration used in Egyptian irrigation systems to control molluscan schistosoma vectors (ref. 16). At such concentrations of PCP a complete interruption of oxidative phosphorylation occurs in snail tissue, while anaerobic processes remain intact. The snail may thus survive for a limited time period using glycolytic reactions (refs. 12,17). Highest concentrations of PCP in water without lethal effects range from 0.16 to 1.2 ppm for some small crustaceae and to 0.2 ppm for snails (ref. 2).

In summary it can be concluded that microcalorimetry may readily be applied to monitor toxic effects in ecological (model) systems both on the level of microbes and that of small animals. Compared with other techniques calorimetry is a fast and unspecific tool for recording aerobic as well as anaerobic metabolism (ref. 11).

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