Thermochimica Acta, 193 (1991) 125–134
Elsevier Science Publishers B.V., Amsterdam

Calorimetric investigations of pollution by xenobiotics

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(Received 19 December 1990)

Abstract

Parts of a forest ecosystem (decayed matter, soil and small animals) were investigated calorimetrically for the influence of a typical organic pollutant. Pentachlorophenol (PCP), a ubiquitously applied pesticide, was chosen as a representative man-made xenobiotic. At all concentrations used, PCP produced a pronounced stimulation of the energy metabolism by up to 350%.

INTRODUCTION

Living in an environment increasingly burdened with man-made chemicals, waste materials and radiation, scientists have been asked to develop methods to detect such poisonous substances and to study their unwanted influences on nature. These investigations have to be carried out on different levels: on isolated microorganisms or mixed microbial populations, being the initial agents of breakdown in nature, on insects as members of the food chain, and on complete ecosystems. The methods should be simple, cheap and unspecific in order to detect a large variety of xenobiotics. Modern (micro)calorimetry has been shown to be a suitable, promising tool for such experiments [1,2]. In addition to being as sensitive as polarographic or manometric techniques, direct calorimetry is the only method capable of monitoring aerobic as well as anoxic metabolic processes [3].

This paper is intended as a supplement to the review article by U. Reh (this issue: Thermochimica Acta, 193 (1991) 107–124): we intend to focus on organochemical compounds among the xenobiotics and, among them, on pentachlorophenol (PCP). PCP was, and in some countries still is, used intensively against algae, bacteria, fungi, herbs, insects and molluscs for wood and crop protection, and also as an impregnant for textiles and as an additive in synthetic materials, emulsion paints and glues. Because of the huge amounts of PCP produced worldwide, it can be assumed to be a ubiquitous, long-term contaminant of our environment. In addition, biotransformation of several highly chlorinated substances to less chlorinated ones proceeds via PCP [4].

The various biological and clinical effects of PCP are published in the literature: it is a powerful uncoupler of oxidative phosphorylation [5,6], stimulating respiration at lower concentrations and inhibiting it at higher ones, thus modifying the most important metabolic step for energy production in living systems [7]; it blocks the uptake of inorganic phosphorus into the cell [8] and influences other enzymatic systems and some cell organelles [9,10]. Pure PCP seems to be less toxic than the contaminants found in commercial PCP [8]. Moreover, its metabolite tetrachlorohydroquinone was recently shown to induce DNA single-strand breaks in mammalian cells [11].

Until now, defined microbial systems have been tested calorimetrically for antimicrobial drugs [12], for organic xenobiotics such as *m*-cresol [13] or phenol [14] and its derivatives [15–17], for heavy metals such as mercury [17–19] and cadmium [18], and for waste-water pollution [20,21]. Further calorimetric experiments were dedicated to the influence of cadmium as a representative of heavy metals on snails under various conditions [22]. Mixed and undefined microbial populations were studied for the degradation of a carbon source and the effects of chemicals in soil specimens [18,23–27]. Complex ecological and biological systems have been dealt with in only a few calorimetric studies [28–30]. The chapter by U. Reh (this issue: Thermochimica Acta, 193 (1991) 107–124) gives a review of this field. In the present contribution, PCP was chosen as a representative of chlorinated phenols from the group of organochemical xenobiotics, and forest soil was chosen as an example of a complex biological system. The system consisted of the soil itself, leaves ("decayed layers") of different ages and varying degrees of decomposition, and some smaller animals. Only one other calorimetric publication on PCP, dealing exclusively with bacterial metabolism, was found in the literature [31].

MATERIAL AND METHODS

Ecological system

A forest system that had already been studied by other groups with respect to the action of PCP was used for our calorimetric experiments. A mixed tree population of beech, birch, elm, lime, maple, oak, poplar and willow is typical for this system. Two decayed layers, "L" (fresh material from the previous autumn) and "F" (older, more rotten material), and a third 2 cm layer of underlying soil were investigated.

This ecotope was typically populated by animals such as earthworms (*Eisenia foetida*), isopods (*Oniscus asellus* (wood lice), *Armadillidium vulgare* (pill bugs), *Lithobius forficatus* and *Polydesmus complanatus*) and carabid beetles (*Nebria brevicollis*). All were investigated calorimetrically, but only *E. foetida*, *O. asellus* and *A. vulgare* were investigated systematically for

PCP actions because of their frequent occurrence. The storage, handling and preparation of the animals are described elsewhere [32].

Chemicals

Pentachlorophenol (Sigma, Deisenhofen, no. P1045, purity > 98%) and radioactively labelled PCP–UL $-^{14}C$ (purity > 98%, 10.6 mCi $mmol^{-1}$, concentration 1 mCi ml^{-1}, Sigma, Deisenhofen) were dissolved in toluene and diluted in a 10% alcohol solution to final concentrations of 0.1, 0.2, 0.4 and 0.8 gPCP l^{-1} before application. PCP was applied by bathing the animals for different times in solutions of appropriate concentration. A second technique was to let them creep around in glass flasks, the bottoms of which contained a layer of filter paper saturated with PCP solution. After the desired exposure time, one animal of the group was separated, quickly frozen and prepared for uptake measurements as given in ref. 32. The samples of decayed matter and soil also received appropriate amounts of PCP solution.

Instrumentation

An isoperibolic twin-batch calorimeter of the Calvet type (MS 70, Setaram, Lyon) with four vessels of 100 ml capacity and a sensitivity of approximately 60 mV W^{-1} was used throughout the experiments. Animals, soil and decayed matter in 45 ml plastic containers (Reichelt, Heidelberg, no. 61167) were equilibrated to 25 °C and then transferred to the metallic calorimeter vessels. If both metallic vessels and plastic containers are open to the air, no significant drop in oxygen concentration is recorded during the experiments. The calorimeter was connected to a multichannel recorder (BD5 + BA5, Kipp and Zonen, Delft). The uptake of radioactively labelled PCP was determined in a Beckman Scintillation Counter LS 1800 (Beckman Instruments, Munich).

RESULTS

Soil and decayed matter

Specimens were treated with PCP by bathing, wetting, or by adding smaller amounts of PCP solution to the decayed matter and soil. Care was taken in the latter cases that all liquid was absorbed in the leaves and that no "free" solution remained on the bottom or walls of the vessel. Likewise, the animals were carefully washed and dried after bathing in PCP solution. Similar treatment with the pure solvent showed no significant influence either on the animals or on decayed matter and soil.

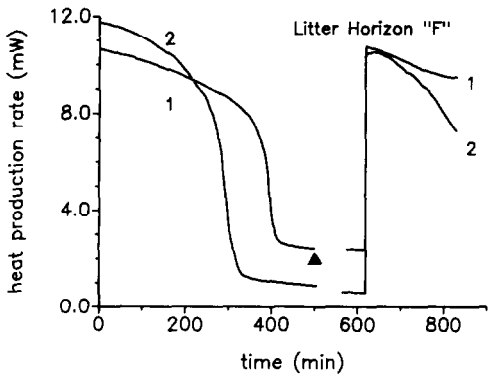

Fig. 1. Heat production rate plotted versus time for decayed matter (layer "F") in hermetically sealed plastic containers (curve 1, 6.56 g ww; curve 2, 8.18 g ww). The triangle indicates the moment when both containers were aerated intensively outside the calorimeter. Container 1 remained open; container 2 was closed again.

As PCP mainly acts on the energy production during oxidative phosphorylation, a sufficient supply of oxygen had to be assured during the experiment. Its concentration may become critical in larger, densely packed specimens of decayed matter from layer "F" with high metabolic activity. Figure 1 shows the results of two decayed matter samples measured in hermetically sealed plastic containers. After a short period of high intensity, heat production rates drop to the low level of anoxic fermentation. After the re-opening of the container and aeration, the heat output returns close to the initial level and remains there as long as the container remains open (compare curves 1 and 2 of Fig. 1).

The smooth, unstructured curves that are observed for decayed matter of layer "L" are similar to those seen in Fig. 2 for the heat production rate of 5.8 g of forest soil. At the triangle on the figure, 1 ml of 0.2 g l^{-1} PCP

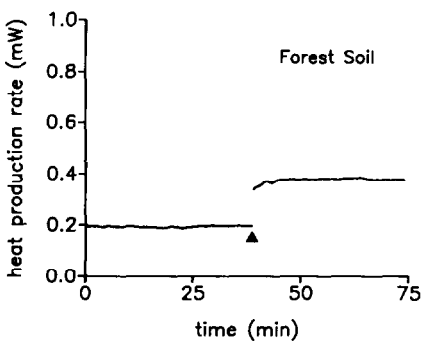

Fig. 2. Heat production rate of 5.8 g ww forest soil as a function of time. At the triangle, 1 ml of 0.2 g l^{-1} PCP solution was added, giving a final concentration of 34.5 μg PCP g^{-1} ww of soil.

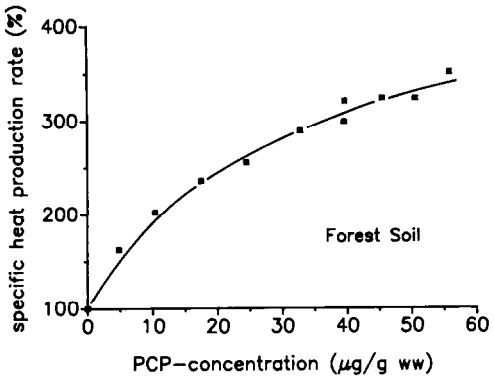

Fig. 3. Metabolic stimulation of forest soil by PCP: relative weight specific heat production rate of forest soil as a function of the final concentration of PCP.

solution is added, leading to a significant stimulation of the metabolic rate. Addition of the same amount of solvent without PCP has no influence. Figure 3 shows the weight specific heat production rates of soil as a function of the final PCP concentration. It is clear from the slope that with the concentrations used in these experiments, there is always a stimulation of heat production and no inhibition of metabolism. Decayed matter "L" is less sensitive to PCP stimulation (Fig. 4): within the investigated concentration range, no change towards a maximum and no subsequent inhibition could be detected. Decayed matter experienced approximately 250% stimulation, less than observed in soil.

Animals

The reproducible treatment of soil or decayed forest material is readily performed by addition of the corresponding amount of PCP in solution.

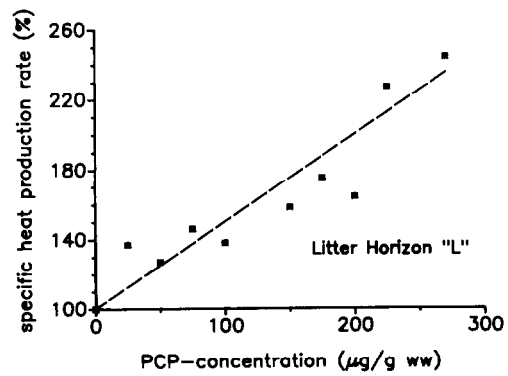

Fig. 4. Metabolic stimulation of decayed matter "L" by PCP: weight specific heat production rate as a function of the final concentration of PCP.

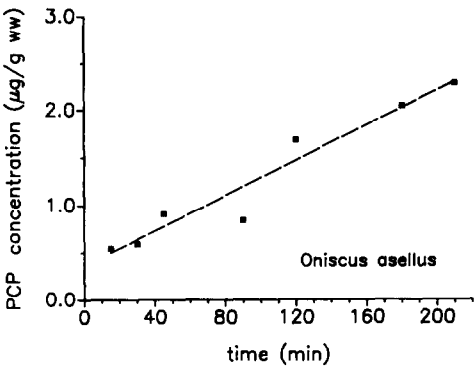

Fig. 5. Uptake of radioactively labelled PCP by wood lice (*O. asellus*) as a function of the time of creeping on PCP wetted paper (see text).

Poisoning the animals is more difficult. Brief bathing (a few minutes) in PCP solution results in strong locomotor activity and thus in increased PCP uptake. This corresponds well with the observed behaviour of isopods or beetles when bathed in PCP solution. Such bathing represents a severe disturbance of the animals. Creeping on PCP-contaminated wet papers is less stressful and less effective, as shown by the amount of PCP taken up with time. Bathing resulted in 15 μg PCP g^{-1} ww in 3 min, creeping in 0.6 μg^{-1} in 200 min (Fig. 5). However, due to periods of rest alternating with periods of motion, the uptake by creeping is less uniform and introduces a larger scatter. This can be seen in Fig. 5 for wood lice (*O. asellus*) and Fig. 6 for pill bugs (*A. vulgare*). Corresponding results are found for earthworms (*E. foetida*) bathed in PCP solution (Fig. 5 in ref. 32).

Figures 7 and 8 show the heat production rates of isopods and beetles before and after stimulation by PCP. The calorimetric traces are not as steady and smooth as those of decayed matter or soil (Fig. 2). Temporal fluctuations are due to locomotory activities of the animals and thus to an

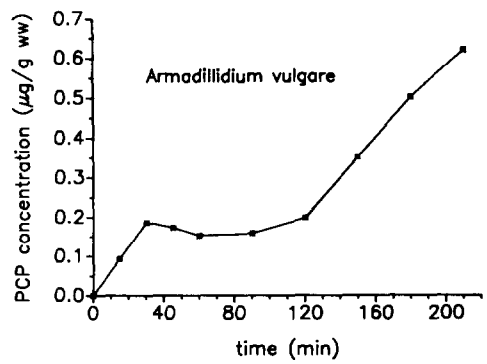

Fig. 6. Uptake of radioactively labelled PCP by pill bugs (*A. vulgare*).

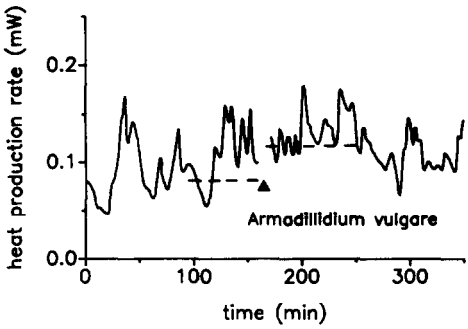

Fig. 7. Heat production rate of five pill bugs (*A. vulgare*, 161 mg ww) before and after stimulation by PCP (90 min creeping on paper wetted with 0.8 g l^{-1} PCP). The stimulation amounts to 135%.

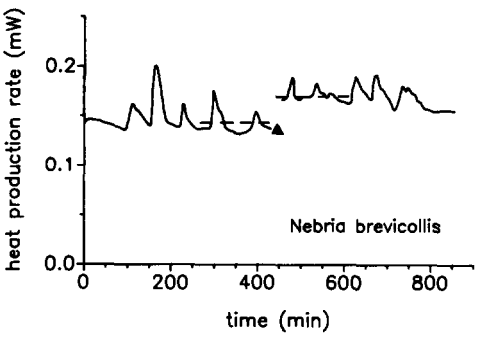

Fig. 8. Heat production rate of two carabid beetles. (*N. brevicollis*, 68 mg ww) before and after stimulation by PCP (2 min bathing in 0.8 g l^{-1} PCP). The stimulation amounts to 120%.

increased heat dissipation compared with their low resting metabolism. To obtain a larger signal, 5 pill bugs (*A. vulgare*) were placed in a plastic container together with some wet paper to prevent desiccation and to make it easy for them to walk around. Their mean weight specific heat production rate rose from 0.56 to 0.75 mW g^{-1} ww (increase of 135%) after 90 min of creeping around on wetted paper (Fig. 7). To facilitate the comparison between the two states of metabolism, the mean heat production rates are indicated by dashed lines in Figs. 7 and 8.

A similar situation is shown in Fig. 8 for 2 carabid beetles (*N. brevicollis*), but the calorimetric traces are less intensively structured. In this case, the mean weight specific heat production rate increased from 2.05 to 2.48 mW g^{-1} (120%) after 2 min of bathing in a PCP solution. As with isopods, the beetles remained fully active for many hours after PCP application.

DISCUSSION

Figures 1 and 2 show examples of heat production rates of decayed matter and soil. It becomes clear that the heat production is much higher in

the decayed matter than in the soil because of the smaller amount of organic matter and microbial contamination in the latter. Decayed matter of layer "F" has a mean weight specific heat production rate of 0.98 ± 0.09 mW g^{-1} ww, decayed matter "L" has 1.11 ± 0.28 mW g^{-1} ww and soil has 0.022 ± 0.006 mW g^{-1} ww. The order of the rates of stimulation caused by a given concentration of PCP is the reverse: highest in the soil samples, lowest in decayed matter "L". Concentrations that stimulated heat production in decayed matter caused inhibitory effects in animals and pure microbial cultures.

Because of the necessary thermal equilibration of the calorimeter, it was not possible in these experiments to show the immediate effect of PCP on the specimens. However, it is known from calorimetric and polarographic experiments with microbial cultures (unpublished results) and from the literature [8,17] that the response to PCP occurs within 1–2 min. Moreover, it has been observed that PCP is readily excreted by animals and not accumulated in the food chain [33]. Continuous microbial cultures poisoned ballistically with PCP showed a decreasing effect of PCP proportional to the wash-out from the culture [17].

In general, it was found, on varying levels, that low concentrations of PCP lead to the expected uncoupling effect with an increased metabolic rate and heat production. Higher concentrations produce a strong metabolic inhibition or lethal damage to the animals [15–17,34,35]. The stimulation of heat production rates in forest soil by PCP (Fig. 3) may be extrapolated to a maximum of 350% at approximately 70 μgPCP g^{-1} soil (which is in agreement with results for pure cultures of the yeast *Saccharomyces cerevisiae*, to be published elsewhere). Moreover, this concentration corresponds well with the results of Weppen and Schuller [17] for microbial cultures. Itoh et al. [15] found stimulation of mammalian cell cultures in the same range, while synchronous algae populations were more sensitive to PCP [16]. Grass shrimps (*Palaemonetes pugio*) showed a markedly increased respiration at 20 μgPCP g^{-1} ww for some hours, followed by death, while respiration in isolated crab tissue was reduced to 50% by 1.3 mg g^{-1} [34]. Values of toxic concentrations for aquatic organisms, invertebrates and vertebrates are presented in ref. 6.

Uptake rates of radioactively labelled PCP in isopods creeping on wetted paper ranged from 3 to 10 ng g^{-1} min^{-1} and were thus at least one order of magnitude smaller than the uptake rates found for animals bathed in PCP solution [32 and unpublished results] or for continuous exposure of snails to PCP [36].

The present experiments show that microcalorimetry is a suitable method for monitoring xenobiotic effects in biotopes. The problem of inhomogeneities occurring in smaller samples is avoided. In larger vessels with volumes up to 100 ml, even ill-defined, complex material can be investigated. Furthermore, the response of such samples on application of pollutants are

immediate and the overall effects occur within a few hours; thus, a rapid method is necessary, a requirement which is also met by microcalorimetry.

REFERENCES

1 I. Lamprecht, Application of calorimetry to different biological field and comparison with other methods, Boll. Soc. Nat. Napoli, 92 (1983) 515–542.
2 E. Gnaiger, Pharmacological application of animal calorimetry, Thermochim. Acta, 49 (1981) 75–85.
3 E. Gnaiger, Direct calorimetry in ecological energetics. Long term monitoring of aquatic animals, in E. Marti, H.R. Oswald and H.G. Wiedemann (Eds.), Angewandte chemische Thermodynamik and Thermoanalytik, Birkhäuser, Basel, 1979, pp. 155–165.
4 G. Renner and C. Hopfer, Metabolic studies on pentachlorophenol (PCP) in rats, Xenobiotica, 20 (1990) 573–582.
5 K.R. Rao (Ed.), Pentachlorophenol — Chemistry, Pharmacology, and Environmental Toxicology, Plenum Press, New York, 1979.
6 K.R. Rao, Umweltchemikalie Pentachlorphenol, Forschungsbericht 10604007, UBA-FB 83-009, Schmidt-Verlag, Berlin, 1987.
7 W.G. Hanstein, Uncoupling of oxydative phosphorylation, Biochim. Biophys. Acta, 456 (1976) 129–148.
8 M. Kunde and C. Böhme, Zur Toxikologie des Pentachlorphenols: Eine Übersicht, Bundesgesundhbl. Nr. 19/20 vom 22. September 1978, pp. 302–310.
9 F.R. Fox and K.R. Rao, Effects of sodium pentachlorophenate and 2,4-dinitropehnol on hepatopancreatic enzymes in the blue crab, *Callinectes sapidus*, in K.R. Rao (Ed.), Pentachlorophenol — Chemistry, Pharmacology, and Environmental Toxicology, Plenum Press, New York, 1979, pp. 265–275.
10 M. Löwer, Untersuchung zur normalen Pentachlorphenolkonzentration in menschlichen Organen, in K.R. Rao, Umweltchemikalie Pentachlorphenol. Forschungsbericht 10604007, UBA-FB 83-009, Schmidt-Verlag, Berlin, 1987, pp. 163–184.
11 W. Ehrlich, The effect of pentachlorophenol and its metabolite tetrachlorohydrquinone on cell growth and the induction of DNA damage in Chinese hamster overay cells, Mutat. Res., 244 (1990) 299–302.
12 A.E. Beezer and B.Z. Chowdry, Microcalorimetric investigations of drugs, in A.E. Beezer (Ed.), Biological Mircocalorimetry, Academic Press, London, 1980, pp. 195–246.
13 J. Hartung, Zur Einschätzung der biologischen Wirkung nachteiliger Stoffe auf *E. coli* mit der Flow-Mikrokalorimetrie, Zbl. Bakl. Mikrobiol. Hyg. B-Umwe, 183 (1986) 58–66.
14 A.E. Beezer, W.H. Hunter and D.E. Storey, The measurement of phenol coefficients by flow microcalorimetry, J. Pharm. Pharmacol. 33 (1981) 65–68.
15 E. Itoh, H. Sakihama, K. Toyama and K. Matsui, Effects of 2,4-dinitrophenol and other metabolic inhibitors on the thermograms of Ehrlich ascites carcinoma cells registered with a microcalorimeter, Cancer Res., 44 (1984) 1985–1990.
16 J.-E. Tillberg, A. Kylin and I. Sundberg, Microcalorimetric measurements of the heat evolution and its sensitivity to dinitrophenol during different stages of synchronous cultures of Scenedesmus, Plant Physiol., 48 (1978) 779–782.
17 P. Weppen and D. Schuller, Microcalorimetric studies of the mode of action of environmental chemicals on continuous microbial cultures, Thermochim. Acta, 72 (1984) 95–102.
18 T. Kawabata, H. Yamano and K. Takahashi, An attempt to characterize calorimetrically the inhibitory effect of foreign substances on microbial degradation of glucose in soil, Agric. Biol. Chem., 47 (6) (1983) 1281–1288.
19 P. Ohlmeyer, C. Dilgerakis and D. Mühlen, Wirkung von Quecksilbersalz auf Hefen, Z. Naturforsch., 28c (1973) 628.

20 F. Tiefenbrunner, Mikrokalorimetrische Untersuchungen im Vorfluter, Zbl. Bakl. Mikrobiol. Hyg. B-Umwe, 161 (1976) 519–526.
21 B. Redl and F. Tiefenbrunner, Determination of hydrolytic activities in waste water systems by microcalorimetry, Water Res., 15 (1) (1981) 87–90.
22 G.B. Joachimsohn, I.H.D. Lamprecht and B. Schaarschmidt, Cadmium induced changes of the metabolic activity of the freshwater snail *Planorbis corneus* estimated by direct calorimetry, J. Therm. Anal., 35 (1989) 659–669.
23 T. Kimura and K. Takahashi, Calorimetric studies of soil microbes: Quantitative relation between heat evolution during microbial degradation of glucose and changes in microbial activity in soil, J. Gen. Microbiol., 131 (1985) 3083–3089.
24 K. Ljungholm, B. Noren and I. Wadsö, Microcalorimetric observations of microbial activity in normal and acidified soils, Oikos, 33 (1979) 24–30.
25 K. Ljungholm, B. Noren, R. Skoeld and I. Wadsö, Use of microcalorimetry for the characterization of microbial activity in soil, Oikos, 33 (1979) 15–23.
26 K. Ljungholm, B. Noren and G. Odham, Microcalorimetric and gas chromatographic studies of microbial activity in water leached, acid leached and restored soils, Oikos, 34 (1980) 98–102.
27 H. Yamano and K. Takahashi, Temperature effect on the activity of soil microbes measured from heat evolution during the degradation of several carbon sources, Agric. Biol. Chem., 47 (1983) 1493–1499.
28 H. Bolouri, I. Lamprecht and B. Schaarschmidt, Adiabatic calorimetric investigations on complex biological systems, in W. Hemminger (Ed.), Thermal Analysis, Vol. II, Birkhäuser, Basel, 1980, pp. 577–582.
29 D. Coenen-Staß, B. Schaarschmidt and I. Lamprecht, Temperature distribution and calorimetric determination of heat production in the nest of the wood ant, *Formica polyctena* (Hymenoptera, Formicidae), Ecology, 61 (1980) 238–244.
30 I. Bachem, I. Lamprecht and B. Schaarschmidt, Energetical investigation on the ecological system: ant hill, in W. Hemminger (Ed.), Thermal Analysis, Vol. II, Birkhäuser, Basel, 1980, pp. 571–575.
31 P. Weppen and D. Schuller, Microcalorimetric studies of the mode of action of environmental chemicals on continuous microbial cultures, Thermochim. Acta, 72 (1984) 95–102.
32 I. Lamprecht, Ch. Motzkus, B. Schaarschmidt and D. Coenen-Staß, Pentachlorophenol – an environmental pollutant. Microcalorimetric investigations of an ecological model system, Thermochim. Acta, 172 (1990) 87–94.
33 H. Gruttke, U. Kielhorn, W. Kratz, G. Weigmann and A. Haque, Verteilung von ^{14}C-Natriumpentachlorphenol in einem geschlossenen Modellökosystem unter besonderer Berücksichtigung der Detritusnahrungskette, Verh. Ges. Ökologie, 15 (1987) 351–357.
34 A.C. Cantelmo, P.J. Conklin, F.R. Fox and K.R. Rao, Effects of sodiumpentachlorophenate and 2,4-dinitrophenol on respiration in crustaceans, in K.R. Rao (Ed.), Pentachlorophenol — Chemistry, Pharmacology, and Environmental Toxicology, Plenum Press, New York, 1979, pp. 251–263.
35 A. Bevenue and H. Beckman, Pentachlorophenol: A discussion of its properties and its occurrence as a residue in human and animal tissues, in F.A. Gunther (Ed.), Residue Reviews, Vol. 19, Springer-Verlag, Berlin, 1967, pp. 83–134.
36 E.C. Weinbach and M.O. Nolan, The effect of pentachlorophenol on the metabolism of the snail *Australorbis glabratus*, Exp. Parasit., 5 (1956) 276–284.