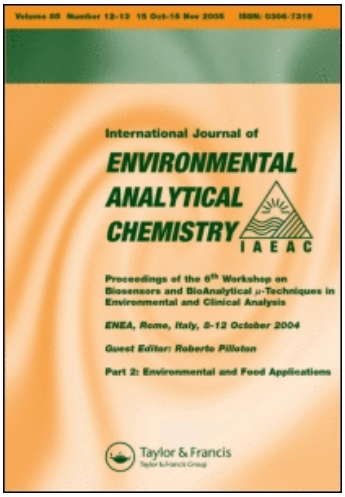

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Methods of Extraction and Analysis of PCBs from Earthworms

J. Tarradellas[a]; P. Diercxsens[a]; M. B. Bouche[b]
[a] Institut du Génie de l'Environnement, Lausanne, Switzerland [b] Laboratoire de Zooécologie du Sol, C.N.R.S., Centre Louis Emberger, Montpellier, France

To cite this Article Tarradellas, J. , Diercxsens, P. and Bouche, M. B.(1982) 'Methods of Extraction and Analysis of PCBs from Earthworms', International Journal of Environmental Analytical Chemistry, 13: 1, 55 — 67
To link to this Article: DOI: 10.1080/03067318208071583
URL: http://dx.doi.org/10.1080/03067318208071583

Intern. J. Environ. Anal. Chem., 1982, Vol. 13, pp. 55–67
0306-7319/82/1301–0055 $06.50/0

Printed in Great Britain

Methods of Extraction and Analysis of PCBs from Earthwormst

J. TARRADELLAS and P. DIERCXSENS
Institut du Génie de l'Environnement, EPFL—Ecublens, 1015 Lausanne, Switzerland

and

M. B. BOUCHE
Laboratoire de Zooécologie du Sol, C.N.R.S., Centre Louis Emberger, B.P. 5051, 34033 Montpellier, France

(*Received April 15, 1982*)

Earthworms can constitute an excellent indicator of soil pollution by micropollutants and PCBs in particular. The foresteps of an analysis of this organochlorine in earthworms must be considered seriously if reliable results are to be obtained.

In the first place the ecological class of earthworms (1) to be analysed must be chosen taking in account the pedological level in which they live and feed.

Secondly one must be able to distinguish the earthworms' contamination from the contamination of the earth present in their intestinal tube (30% of the total weight). Separation of this earth by dissection of earthworms is criticised because of subsequent PCBs losses. Two methods of emptying the intestinal tube are suggested: the first by ingestion of filter paper and the second by consumption of pure silicon powder. Application conditions of these methods are presented. The first, easier to use, is unfortunately not convenient to all earthworm species. The second is more universal.

The analysis of PCBs from earthworms and intestinal earth are realised by an acid digestion followed by *n*-hexan extraction, sulfuric acid and Florisil column cleanup and glass-capillary-gas-chromatography with ECD detection (split mode, methyl-phenyl-silicone SE 54).

This study has shown that the earth chosen by earthworms for feeding has different PCBs concentrations than the average earth. Earthworms are also better punctual indicators of the average PCBs contamination of soil than a soil sample.

KEY WORDS: PCB/Aroclor, earthworms, soil pollution, sewage sludges, compost.

INTRODUCTION

A good example of illustrating the state of contamination of a terrestrial ecosystem is the earthworm. That is:

†Presented at the 12th Annual Symposium on the Analytical Chemistry of Pollutants, Amsterdam, April 1982.

—they represent the first animal biomass of dry land.[1-3] This represents an analytical advantage, in so far as it is relatively easy to obtain a great quantity of them;
—they remain always in the ground;
—one can find them everywhere;
—they occupy an intermediate place between the microorganisms and the superior animals in the trophic relations;[4]
—they ingest enormous quantities of earth: in 1 hectare of land, up to 300 metric tons would be ingested;[1]
—the assimilation membranes of the worms are comparable with those of the major part of the other animals, including those of superior animals, but contrary to the assimilation membranes of the plants. This gives them a remarkable bio-sampler value;

Earthworms represent, therefore, a good example in the study of the micropollutants fluxes.[5-8] Sewage sludges and compost have been used more and more over the last few years in many countries, like Switzerland, as agricultural soil fertilizers. Several studies have showed that sewage sludges and composts contain PCB levels that are likely to contaminate the agricultural ecosystems over which they are spread.[10-15] The global study of this contamination cannot be performed without the knowledge of the PCB concentration in the earthworms. That is:

—earthworms constitute an integration of pollutant rejection during the time;
—earthworms constitute the food of a great number of mammals and birds;
—an eventual toxicity on the worms would in due time induce a decrease in soil fertility.

EXPERIMENTAL

Choice of earthworms samples

With the view to study the relation between the PCB content of earthworms and those from the soils, it is necessary to choose the samples in conjunction with the ground levels in which they live and eat. The ecological classification proposed by Bouché,[1] distinguishes three classes:

1) The epigeic worms

—form about 1% of the earthworm's biomass in fruitful grounds;
—live and feed on the surface;
—are small in size;
—e.g.: *Lumbricus castaneus.*

2) The anecic and epianecic worms

—form about 80% of the earthworm's biomass in fruitful grounds;
—live deep down but feed on the surface;
—are of medium and large size;
—e.g.: *Nicodrilus longus, Lumbricus terrestris L.*

3) The endogenic worms:
—form about 20% of the earthworm's biomass in fruitful grounds;
—live and feed deep down;
—are of small and medium size;
—e.g.: *Allolobophora icterica* and *chlorotica.*

From our previous experience it has proved preferable to study principally the anecics and epianecics owing to their abundance, their relative easy manipulation and the fact that they feed principally on superficial ground levels where PCBs are concentrated.[16]

Up to 30% of the fresh body weight in earthworms sampled in their natural environment is earth in gut. In certain cases it will be sufficient to analyse the worm as a whole (i.e.: worm+gut earth). This is the case when one wishes to know the PCB quantity transmitted to gulls by earthworms, which constitute their principal nourishment.[17] Meanwhile, in many cases, it is important to know the worm's PCB concentration without his gut content. This is necessary for the study of the toxicity of PCBs towards earthworms, for investigations of PCB fluxes between the soil and the earthworms, but also when one wishes to know the PCB quantity transmitted to certain animals which, like the mole, empty the gut content of the earthworm before eating.[18]

This important problem is infrequently discussed in literature. The present paper discusses the methods used in the laboratory in the separation of the earthworm and its gut content in view to analyse PCBs.

Methods used in cleaning the digestive tract

Three methods were tested and are briefly discussed below.

1) *The dissection*

The earthworms are dissected one after the other with small ophthalmologic scissors. By means of pins one can carefully open the length of the worm. The gut content is eliminated by rinsing them abundantly with distilled water which has been previously washed with hexane for residue analysis and then boiled.

2) *Emptying by filter paper*

The method is cited by Yadav.[5] Worms of a relative large size are placed

in two's or three's in Petri dishes after having been rinsed. Filter paper, torn to shreds and humidified, is placed in the dishes. All is carefully conserved at a temperature of 13°C, a humidity level of 100% and in total darkness. The worms consume the filter paper proven by the nibbled edge.

3) *Emptying by fine silica powder*

This method is based on the works of Ferrière, Fayolle and Bouché.[19] The earthworms are placed in cutaneous and intestinal contact with an artificial and totally synthetic medium, called "Artisol". The "Artisol" is made up of two elements:

—a framework of glass marbles each of which has a 2 cm diameter;
—a matrix composed of two products: amorphous hydrated silica powder and distilled water.

The worms penetrate into the "Artisol", placed in glass flasks, owing to the controlled porousness of the glass marble framework. The glass flasks are conserved at a temperature of 13°C and in total darkness. Like in the case of filter paper, the worms excrete their gut content by eating the silica matrix.

Analytical methods

Each sample, accurately weighed, is composed of 10 to 20 g of earthworms, washed with distilled water. It is digested by approximately 80 mls of acid solution (i.e.: 2 vols 70% perchloric acid in 3 vols glacial acetic acid),[20] for 24 hours over a steam bath. After cooling and doubling the volume by distilled and hexane washed water, the sample is transferred to a separatory funnel and extracted with 3 vols of 20 ml each of *n*-hexane for residue analysis. The hexane extract is concentrated to approximately 5 ml; this volume of concentrated hexane extract is accurately determined. Approximately 2 ml of concentrated hexane extract is transferred into a centrifuge tube and 1–2 ml of concentrated sulfuric acid is added for the first cleanup.[21] The centrifuge tube is shaken for 1 min and then centrifuged at 4,000 r.p.m. for 10 min. After centrifugation an aliquot of 1.5 ml of the hexane layer is treated with two drops of metallic mercury to remove sulfur.[22] Black precipitate formed and mercury are separated and remaining hexane extract finally cleaned on Florisil column chromatography.[23]

In view to determine the quantity of PCBs excreted in silica of "Artisol" medium by earthworms, marbles are first rinsed three times in 60 ml of *n*-hexane for residue analysis. This hexane is mixed with methylene chloride (85:15) and this solvent mixture is used to extract the silica and the

earthworms washing water[21] (see above). The phases are separated and the same cleanup procedure as above is employed.

A Perkin-Elmer Sigma 2 electron capture gas chromatograph (^{63}Ni detector) coupled to a Perkin-Elmer Sigma 10B data station is used to quantify the PCB present in each sample. A glass capillary column (length: 30 m, i.d.: 0.25 mm) coated with SE 54 is used in split mode. Other conditions are: carrier gas: N_2, injector temp.: 260°C, detector temp.: 325°C, oven temp.: 205°C up to 245°C.

The samples were quantified by individual comparison of computed area of 45 peaks with standards realized by a mixture in equal quantities of Monsanto Aroclors 1242, 1254 and 1260 at different concentrations. Calculations are made by a Hewlett-Packard 9845 B desk computer.

Criticism of the dissection and filter paper method

The PCB concentration balance-sheet in the dissected worms with regard to the entire worms (Table I) shows clearly a PCB loss during the dissection and the rinsing of the intestinal earth. The PCB losses are caused, on the one hand, by bleeding and, on the other hand, by the unavoidable loss of smaller organs. This dissection work is moreover very painstaking, particularly when the worms are of small size. The results depends highly upon the operator's ability.

TABLE I

PCB balance-sheet in % for three dissected lots of earthworms (different operators)
(earthworms for this experience were not from Chardonne but from Worben, Canton of Bern CH.)

	PCB balance sheet in %		
Lot	A	B	C
Entire worms (worm + earth gut)	100	100	100
Dissect worms	50	41.5	38
Earthgut	24	46	32
Rinsing water	10	10	6
Loss	16	2.5	24

The filter paper cleaning technique attends with the disadvantage to be not available to small worms: they do not ingest the filter paper.

Study of the PCB elimination by earthworms in an artificial medium "Artisol"

1) *Experience description*

—About 800 anecic and apianecic earthworms were sampled in a vineyard of the village of Chardone (Canton of Vaud, Switzerland) where 150 tons per hectare of sewage sludges and compost, with an estimated PCB concentration of 0.9 ppm fresh weight, were spread in 1978.

—These worms were immediately washed and sorted to obtain the most uniform lots as possible. Part of them were distributed amongst 14 glass flasks of 1 litre each, filled with the "Artisol" medium. Each flask contains 700 g of marbles (i.e.: about 50 marbles), 50 g of amorphous hydrated silica ("Levilite", registered mark of Rhône–Poulenc) and 110 ml of distilled water. In each flask the earthworm quantity varies between 35 and 38 and their weight between 10 and 17 g. The other part was used to determine the weight of the gut content.

—Before further analysis, the marbles and the silica had been demonstrated to be free of detectable organochlorine pollutant.

—The flasks, containing the artificial medium and the worms, had been placed in a sink filled with constantly flowing fresh water and carefully isolated from light. The water temperature varied between 13 and 14.5°C.

—The contents were analysed one after the other at different periods of time, the last one being after 21 days. The silica had been changed on the 4th, 8th and 15th day.

—On the moment of the analysis, the worms were carefully separated from the matrix using a grip and rinsed with distilled water. The water and the silica were recuperated separately from the marbles until the moment of the PCB extraction. The worms were immediately weighed and placed in the acid solution where they were digested.

2) *Weight determination of the gut content*

Three large quantities of earthworms were dissected with view to determine the weight of earth contained in the digestive tract. The results are on Table II.

Contrary to the above findings about concentration balance of PCBs, there is, in our opinion, little information lost in weight balance.

Results

1) *PCB released by earthworms in "Artisol"*

Figure 1 shows the PCB distribution between the entire earthworms and the silica during the three weeks of the experiment. We established during

TABLE II
Earth content in digestive tract of earthworms

Lot	A	B	C
Weight worm + earth (g)	124.60	111.19	131.08
Weight dissected worms (g)	104.89	90.97	112.03
Weight earth (g)	19.71	20.22	19.05
% Earth	15.8	18.2	14.5

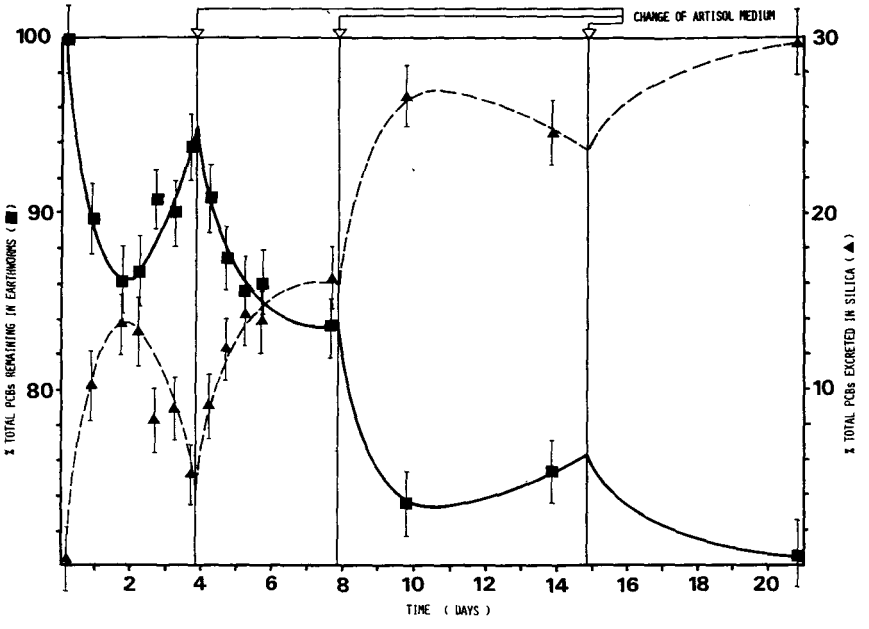

FIGURE 1 Distribution of PCB between earthworms and silica as a function of time.

the first two days a rapid loss of PCB. This loss is due to the release of the digestive tract's content. An equivalent weight of silica matrix replaces this content, proven by almost identical worm weight at the moment of sampling and analysis at all times during the experiment.

PCB reabsorption is observed during the following two days. That is, in our opinion, due to:

—either ingestion of silica contaminated by excreta;
—or a cutaneous absorption due to the contact with dirty silica.

This phenomenon is broken when, on the 4th day, the dirty silica matrix is replaced by clean product. From that time forth, the loss of PCB continues until the reabsorption phenomenon manifests itself again, but in reduced manner, less PCB being lost in the new silica. A new loss of PCB is observed after the second and the third silica change.

2) *The three periods of PCB losses from earthworms*

If we eliminate the samples resulting from PCB intake due to dirty silica, we obtain Figure 2.

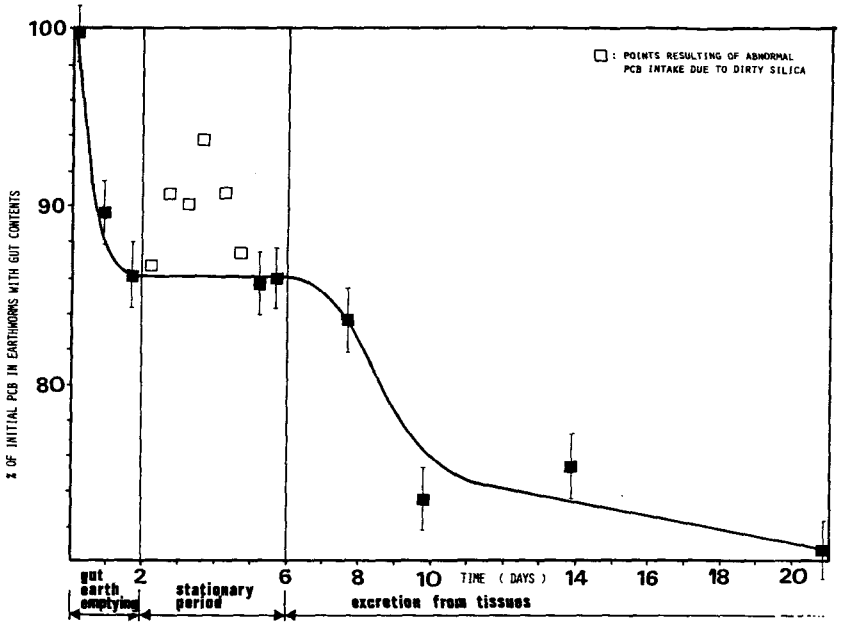

FIGURE 2 Periods of PCB losses from earthworms as a function of residence time in "Artisol".

This figure shows the existance of three distinct periods of the PCB elimination by entire earthworms. During the first two days a rapid decrease of PCB happens due to the replacement of the gut content by silica. The second period continues for 4 days and shows a stationary PCB level in the entire worm. From the 6th day, the PCB quantity in the entire worm diminishes once again. We cannot attribute this fact to an intestinal excretion. The gut is at this moment already full of clean silica. This phenomenon appears to us to arise from a PCB loss from the earthworm's tissues, something similar was observed with DDT.[24]

3) *PCB concentrations in the earthworm's tissues*

With a view to calculate the PCB concentration in the earthworm's tissues alone, several earthworm lots were, on the sampling day, directly submitted to a very precise dissection. These lots served as control samples. After having balanced all the losses due to this method, we have established the concentration in the tissues of these samples.

The weight of the earthworm's tissues alone, after cleaning with "Artisol", is easily determined by deducing from the total weight the proportion corresponding to the intestinal earth, which is determined during the dissection of the first lot (as mentioned before, the weight of the silica matrix is equal to that of the original intestinal earth).

We have thus established the PCB concentrations in the earthworm's tissues during their time spent in the "Artisol" medium. It is a matter of course that we eliminated the samples which resulted from an abnormal PCB intake due to dirty silica.

This result is represented on Figure 3.

The examination of this result confirms the assumption previously made: the level of PCB in the earthworm's tissues remains constant during a period of approximately 6 days and decreases asymptotically afterwards.

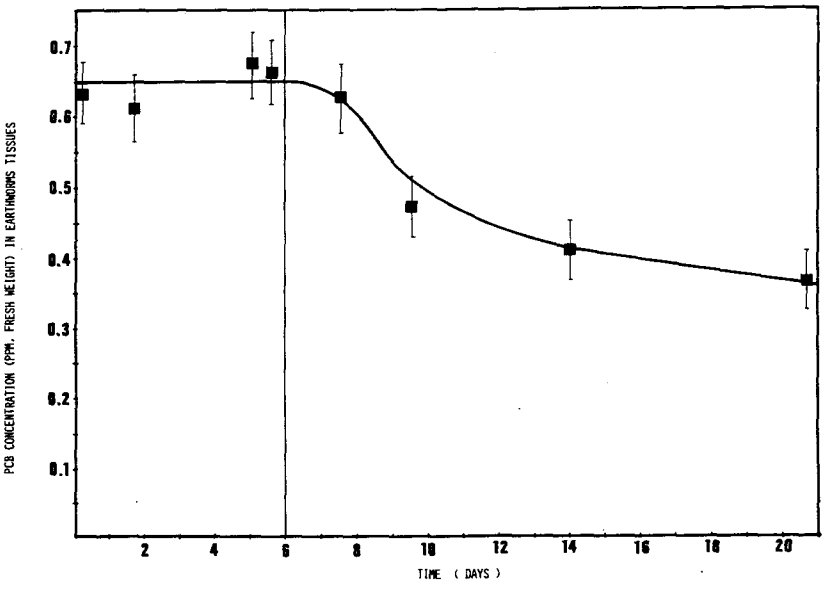

FIGURE 3 PCB concentrations in earthworms' tissues as a function of residence time in "Artisol".

Proposal for a procedure of preparation of earthworms for extraction and analysis of PCB

The results presented previously shows us that the most favourable moment for establishing the PCB concentration in earthworms, prepared for analysis by the "Artisol" cleaning technique, occurs after 48 hours spent in this medium.

At this moment the worms have excreted the PCB being in the original intestinal earth, but not yet those which are enclosed in the tissues. Moreover, at this moment, the absorption of the PCB present in the dirty silica did not manifest at all. We propose the following analysis procedure for the establishing of the PCB concentration in the earthworm's tissues:

—divide the total amount of earthworms sampled into two homologous parts;
—dissect the first part as a view to determine the weight balance-sheet;
—allow the earthworms of the second part to remain in the "Artisol" for 48 hours and analyse the PCB in the entire worms according to the operating methods described earlier;
—the PCB concentration in the tissues alone is obtained using the balance between emptied worms and gut content found above.

Other results

The analysis we described before with a view to establish the preparation procedure of the earthworms by the "Artisol" method, allows us to observe the following points:

1) Figure 4 shows a typical chromatogram obtained after preparation of earthworms in the "Artisol" medium and afer 2 days spent in it.

These earthworms have lived in a field where a mixture of compost and sewage sludges of 0.9 ppm (fresh weight) had been spread 4 years before the sampling moment. The PCB content of the compost was estimated by means of an analysis made in 1980 on the compost produced in the same treatment plant. With the method proposed above we can calculate that the average PCB concentration in the tissues of these earthworms is 0.65 $\pm$0.05 ppm (fresh weight).

We did not observe on the chromatograms obtained significant differences in the relative concentrations of the individual PCB isomers found in the worms immediately analysed after sampling, in those having remained in "Artisol" medium and in the earthworm's intestinal earth. The cleaning by "Artisol" seems then not to change the balance between the different isomers of PCB.

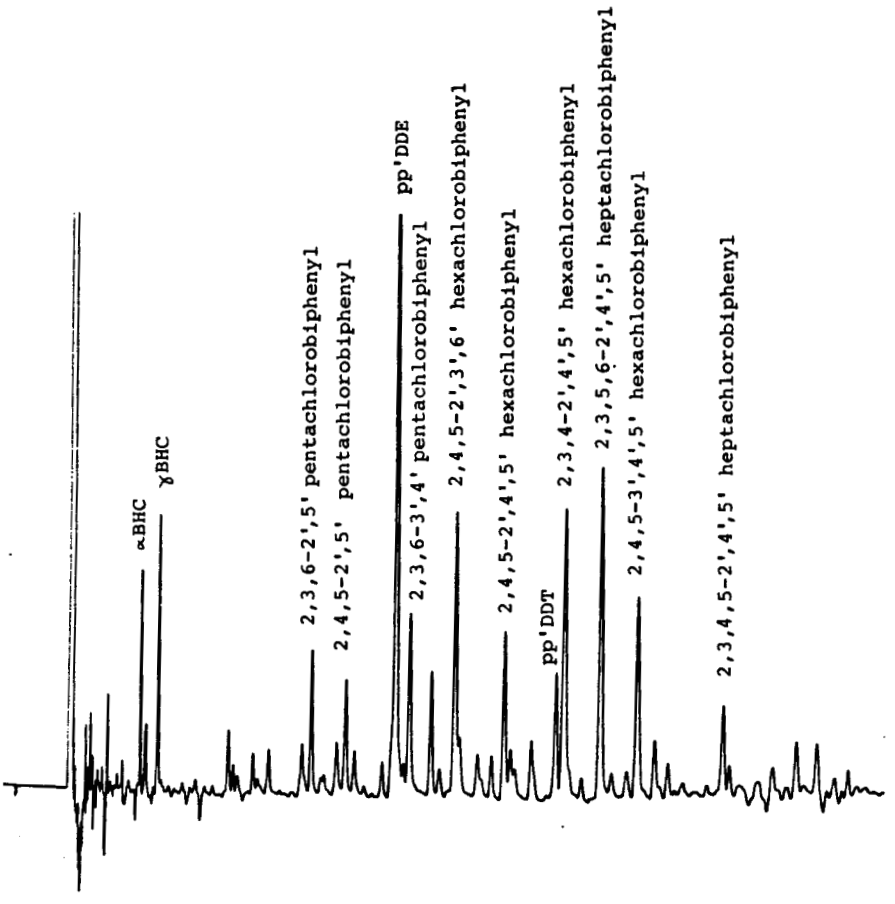

FIGURE 4 Chromatogram of PCBs from earthworm tissues after cleanup of gut earth by a residence time of 2 days in "Artisol".

2) The PCB concentration in the earthworm's intestinal earth differs appreciably from the mean concentration found in the ground level where the worms feed. This fact is illustrated on Table III.

The difference of concentration between intestinal earth and soil samples seems to indicate that the worms make a choice of earth they ingest in the ground level in which they feed. We may validly propose this hypothesis owing to the fact that the soil of the vineyard where those results were established was complately free from litter, which could constitute an addition of pollutants other than that of the earth.

3) It is noticeable, on Table III, that the relative dispersion of the soil results, for a piece of ground as small as (1,000 m^2) that where did our

TABLE III

Differences in the PCB concentrations in the earthworm's tissues, in the intestinal earth and the earth in which they feed.

	Results (ppm fresh weight)	Divergence to the mean in %
Mean concentration of PCB in the tissues	0.65 ± 0.05	8.5
Mean concentration of PCB in the intestinal earth	0.44 ± 0.05	12.0
Mean concentration of PCB in 3 soil samples	0.08 ± 0.05	60.0

samples, is much higher than that of the earthworms sampled at the same place. This seems to indicate that earthworms would be better punctual indicators of the average PCB contamination of soil than soil samples.

DISCUSSION

The authors have only tested the conditions of the above proposed method for PCB extraction and analysis in tissues of worms on anecics and epianecics. It would be necessary to make a similar study to establish the conditions of this method for other soil pollutants and for other classes of earthworms.

Acknowledgements

Some preliminary studies for this research were made by D. de Weck, N. Borsinger and B. Rosset during the 9th Postgraduate Course in Environmental Engineering of Swiss Federal Institute of Technology of Lausanne (EPFL).

The authors wish to thank M. Olivier Neyroud, electrical engineer and vineyardist at Chardonne (canton of Vaud, Switzerland) whose vineyard not only have interesting earthworms but also, and principally, produces one of the finest Lavaux wines.

References

1. M. B. Bouché, Lombriciens de France, écologie et systematique (I.N.R.A. Publ. 72, Paris) 671 (1972).
2. A. Stöckli, *Landw. Jahrb. Schweiz.* **72,** 699 (1959).
3. E. Nowak, *Pol. Ecol. Stud.* **2,** 195 (1976).
4. M. B. Bouché, In: *La vie dans les sols* (Gauthier Villars, Paris) 187 (1971).
5. D. V. Yadav, M. K. K. Pillai and H. C. Argawal, *Bull. Environm. Contam. Toxicol.* **16,** 541 (1976).

6. B. N. K. Davis and R. B. Harrison, *Nature* **211,** 1424 (1966).
7. G. A. Wheatley and J. A. Hardman, *J. Sci. Food Agr.* **19,** 219 (1968).
8. B. N. K. Davis and M. C. French, *Soil Biol. Biochem.* **1,** 45 (1969).
9. A.R.P.E.A., Bulletin, numéro spécial boues d'épuration dans l'agriculture, Hauterive, Ne, Suisse, **76,** 95 (1976).
10. A. K. Bergh and R. S. Peoples, *Sci. Total. Environ.* **8,** 197 (1977).
11. C. Schweizer and J. Tarradellas, *Chimia* **34,** 509 (1980).
12. D. Pal, J. B. Weber and M. R. Overcash, *Residue Review* **74,** 45 (1980).
13. A. E. McIntyre, J. N. Lester and R. Perry, *Environ. Pollut. Ser. B,* **2,** 309 (1981).
14. Environment Canada, Background to the regulation of polychlorinated biphenyls in Canada, *Techn. Rep.* **76,** 61 (1976).
15. M. Suzuki, *Arch. Environ. Contam. Toxicol.* **5,** 343 (1977).
16. P. Moza, L. Kilzer, I. Weisberger and W. Klein, *Bull. Environ. Contam. Toxicol.* **16,** 454 (1976).
17. G. Cuendet, Etude du comportement alimentaire de la mouette rieuse, Ph.D., Conservation de la Faune, Lausanne, Suisse, (1979).
18. B. Keck, Biologie et écologie de la taupe, internal report, Ecole vétérinaire de Lyon, France (1981).
19. G. Ferriere, L. Fayolle and M. B. Bouché, *Pedobiologia* **22,** 196 (1981).
20. R. L. Stanley and H. T. Lefavoure, *J. Ass. Offic. Anal. Chem.* **48,** 666 (1965).
21. P. G. Murphy, *J. Ass. Offic. Anal. Chem.* **55,** 1360 (1972).
22. C. F. Rodriguez, W. A. McMahon and R. E. Thomas, Technical Report Data—EPA 600/2-80-029, (1980).
23. P. A. Mills, B. A. Bong, L. R. Camps and J. A. Burke, *Journal of the AOAC* **55,** 39 (1972).
24. C. A. Edwards, A. E. Whiting and K. A. Jeffs, Pesticides and earthworms, *Rept.* Rothamsted Expt. Station, 1970, 193 (1971).