FORMATION AND BIORELEASE OF BOUND RESIDUES OF PESTICIDES IN TWO BRAZILIAN SOILS [1]

II. [14C] - PARATHION

MARA MERCEDES DE ANDRÉA[2] and FREDERICO MAXIMILIANO WIENDL[3]

ABSTRACT - The use of radiolabelled pesticide molecules in researches proved that some of the applied pesticides remain unextractable and become bound into the soil and other matrixes. Since the available methods to identify the nature of bound residues from parathion application were little effective, this study investigated the persistence and biorelease of ^{14}C - parathion bound residues from soil to bean plants, and as $^{14}CO_2$, by using biometer flasks. Bound residues formation was studied in two soils immediately, and 3 months after the pesticide application. Results proved that not only the pesticide, but also the soil characteristics and the aging of parathion into the soils determined the amounts and biorelease of bound parathion residues. These residues were weaker bound immediately after the parathion treatment than after an aging period. The rhizosphere did not have more influence on the biorelease than the microflora only, and most of the bound residues remained as still bound and inactivated into the soil.

Index terms: biorelease of ^{14}C - parathion, bean plants, $^{14}CO_2$, biometer flasks.

FORMAÇÃO E BIOLIBERAÇÃO DE RESÍDUOS-LIGADOS DE PESTICIDAS EM DOIS SOLOS BRASILEIROS

II. [14C] - PARATHION

RESUMO - O uso de moléculas radiomarcadas de pesticidas em pesquisa provou que uma parte dos pesticidas aplicados permanece não extraível e ligada ao solo e outras matrizes. Como os métodos disponíveis para identificação da natureza dos "resíduos-ligados" a partir da aplicação de parathion foram pouco eficientes, este estudo investigou a persistência e bioliberação de resíduos-ligados de ^{14}C - parathion do solo para plântulas de feijão e como $^{14}CO_2$, através de frascos biométricos. A formação de resíduos-ligados foi estudada em dois solos, imediatamente e 3 meses após a aplicação do pesticida. Os resultados provaram que não somente o pesticida, mas também as características dos solos e o tempo de interação do parathion nos solos determinaram as quantidades e a bioliberação dos resíduos-ligados. Estes resíduos foram mais fracamente ligados imediatamente após o tratamento com parathion do que após o tempo maior de interação. A rizosfera não teve influência maior na bioliberação do que a microflora apenas, e a maior parte dos resíduos-ligados permaneceu como ainda-ligados e inativados nos solos.

Termos para indexação: bioliberação de ^{14}C - parathion, frascos biométricos, rizosfera, microflora.

INTRODUCTION

The use of radiotracers allowed the detection of non extractable and firmly soil bound residues from pesticide treatments. Although the organophosphate pesticides are considered low persistent, they are reported as one of the chemical classes which forms the highest amounts of these bound residues in soils (Roberts, 1984; Calderbank, 1989). Reported bound residues recovery after soil treatment with parathion were from 17% (Katan et al., 1976) to 72% (Lichtenstein et al., 1983), according to soil type and treatment conditions.

In theory, the binding process would reduce the toxic effects of the applied compounds; but, as the release of bound residues was proved, the permanent inactivation was not assured. Since the biorelease of parathion soil bound residues by earthworms and

plants was detected by Fuhremann & Lichtenstein (1978), the significance of the bound residues was directed on its toxicity and bioavailability into the environment (Helling & Krivonak, 1978; Ebing, 1987; Khan, 1991).

In spite of the desirable identification of the bound residues nature to know about their toxic potential, the available methods were not effective for parathion. High temperature distillation (HTD) failed because of the thermal labillity of the compounds (Helling et al., 1986). The supercritical fluid extraction (SFE) with methanol could recover only small amounts of the total parathion bound residues (Capriel et al., 1986). Thus, the possible effects of parathion bound residues has to be studied through the environment where it is bioreleased.

This biorelease was related mainly with microorganisms (Gerstl & Helling, 1985; Klein & Scheunert, 1985; Khan & Dupont, 1987; Golovleva et al., 1990), but also with chemical changes as consequence of environmental or agricultural changes (Gerstl & Helling, 1985; Calderbank, 1989). The current study reports laboratory experiments under standardized conditions which were used to provide information about the relative persistence and biorelease of parathion bound residues in two types of Brazilian soils.

MATERIALS AND METHODS

Insecticide

Parathion (O,O-diethyl O-4-nitrophenyl phosphoro-thioate) pure standard was purchased by Greyhound Chromatography and Allied Chemicals, Merseyside, England. The corresponding radiolabelled (ethyl [1-^{14}C]-parathion) was purchased by Amersham International, Amersham, England, with specific activity of 703 MBq/mmol (19 mCi/mmol) and 99% of radiochemical purity as determined by thin-layer chromatography (TLC).

Soils

Samples of the two soils used in the experiments were described elsewhere (Andréa & Wiendl, 1995). The main silty clay soil characteristics are: 10.9% of sand, 36.9% silt, 52.8% clay, 4.7% of organic matter and pH of 4.7. The sandy loam soil contains 61.0% of sand, 14.0% of silt, 25% of clay, 3.3% of organic matter and pH of 4.5.

Plant, soil treatment and incubation, and liquid scintillation counting (LSC)

It followed the same pattern of treatment and concentration used for [^{14}C]-lindane, and described in Andréa & Wiendl, 1995.

Thin-layer chromatography (TLC) and Radioscanner

As also previously described the hexane extracts were examined by TLC using hexane : acetone (4:1) as developing solvent, and the R_f of parathion was 0.34 under ultraviolet light (254 nm). All the extracts were also examined on TLC Scanner II LB 2723 (Berthold).

RESULTS AND DISCUSSION

Immediately after the application of 1.41 kBq/g of soil (or 0.038 µCi/g, Table 1) as ^{14}C-parathion, radiocarbon recoveries from the silty clay soil were about 84% as extractable and 15% as immediately bound residues. The reactivation of the extracted soil with fresh soil inoculum and nutrient solution did not change the amount of bound residues for the biotests. Parathion bound residues formed immediately after the application were also detected by others (Katan et al., 1976; Gerstl & Helling, 1985).

The investigation of the biorelease of these bound residues formed immediately after the ^{14}C-parathion treatment showed no biomineralization to ^{14}CO$_2$ independently of both the presence of a plant and its rhizosphere (Table 1). However, about 8% and 10% were recovered as re-extractable residues, respectively in the biotests with and without plant, thus indicating a weak binding of the initially formed bound residues. Nevertheless, the rhizosphere did not add any effect to the microflora action.

Very little was uptook (0.13%) and bound (0.05%) into the plant tissues (Table 1). But, high amounts of the initially formed bound residues remained as still bound, independently of the rhizosphere or microflora only (6.9%, Table 1). As Katan & Lichtenstein (1977) stated, once the parathion degradation products were formed, their binding is strong.

Results of the silty clay soil incubated for 3 months with ^{14}C-parathion are presented in Table 2. The amount of radiocarbon still present after the aging period (1.33 kBq/g, Table 2) was very close

to that applied (1.41 kBq/g, Table 1), which indicates that volatilization or degradation to volatile products did not occur. The amount of extractable radiocarbon (88%, Table 2) was also near to that extracted immediately after the treatment (84%, Table 1). It proved that either the microflora was not able to metabolize the residues, or the binding was due to

TABLE 1. Radiocarbon recovery from silty clay soil immediately after treatment with ^{14}C-parathion.

Amount still present	1.41 kBq/g (or 0.038 µCi/g)
	%
Extracted	84.00 ± 2.64
Bound	15.33 ± 2.11
Present after reactivation	15.99 ± 2.19

Biotest	With plant	Without plant
^{14}CO$_2$	0	0
Released and extracted	8.17 ± 1.45	9.83 ± 3.00
Uptaken by plant	0.13 ± 0.13	—
Bound in the plant	0.05 ± 0.01	—
Still bound in soil	6.90 ± 1.43	6.90 ± 0.63

TABLE 2. Radiocarbon recovery from silty clay soil three months after treatment with ^{14}C-parathion.

Amount still present	1.33 kBq/g (or 0.036 µCi/g)
	%
Extracted	88.30 ± 4.17
Bound	11.48 ± 1.67
Present after reactivation	11.20 ± 2.18

Biotest	With plant	Without plant
^{14}CO$_2$	0.37 ± 0.13	0.39 ± 0.08
Released and extracted	1.33 ± 0.53	1.74 ± 0.68
Uptaken by plant	0.05 ± 0.02	—
Bound in the plant	0.03 ± 0.02	—
Still bound in soil	10.88 ± 0.99	8.50 ± 0.62

chemical process, as suggested by Katan et al. (1976). Values of bound and present after reactivation (both about 11%, Table 2) were only slightly smaller than that detected immediately after the parathion application (about 15%, Table 1).

The biotests released little ^{14}CO$_2$ (about 0.4% in both biotests), indicating that the rhizosphere did not contribute for higher effect than did the microflora only (Table 2). The amount of re-extractable residues were small (1.3% or 1.7%, Table 2) as compared with the re-extractable recovered immediately after the application (8.2% or 9.8%, Table 1). These values represented only between 12% and 15% of the amount to be released. Therefore, after the residence time of parathion in this soil, the bound residues became less susceptible to the microflora and/or rhizosphere actions.

Very little was absorbed by the plants (0.05%) and became bound into the plant tissues (0.03%, Table 2). The recalcitrance for bioreleasing of the bound residues after the aging time was evident by the still bound residue values detected after the biotests with and without plant (respectively, 11% and 8.5%, Table 2). These values are very close to that detected before the biotests (11%). Racke & Lichtenstein (1985) also detected large amounts of still bound residues after some assays to release them.

In the sandy loam soil, the amount of extractable radiocarbon recovered immediately after the ^{14}C-parathion treatment was very high (about 92%, Table 3), and only 9.6% remained unextractable or bound in the soil (Table 3). The amount present after the reactivation changed very little and 8.7% were present for the biotests (Table 3).

After the biotests, very little ^{14}CO$_2$ was detected (0.11%), only in the biotest without plant (Table 3). The highest recovered values were again as released and re-extractable residues, and values were near in the two biotests (5.2% and 4.8%, Table 3). Thus, as occurred for the silty clay soil, the amount of released and re-extracted residues represented more than 50% of the immediately formed bound residues present for the biotests.

Again very little was uptook (0.04%) and bound into the plant (0.07%), and the highest amounts remained as still bound in the soil after the action of the microflora or rhizosphere (about 3%, Table 3).

However, the aging of the ^{14}C-parathion in this

soil caused 40% of decrease of the applied amount (1.59 kBq/g, Table 3) related to the amount still present after the 3 months period (0.96 kBq/g, Table 4). Thus, in this soil, some of the pesticide residues were volatilized and/or metabolized to volatile products, as found by Katan et al. (1976) which detected volatilization losses from 12% to 21% of the parathion applied to different soils, after 28 days incubation period.

The highest recovery was as extractable residues (65%), but about 40% became bound and remained as present after the reactivation (Table 4). So that, the aging time was responsible for the higher formation of bound residues, as pointed by Khan & Dupont (1987). However, it did not occur with the silty clay soil indicating that the soil characteristics influenced more for the bound residues production with time than the pesticide itself.

The biotests showed that the reactivated microflora of this soil was little able to metabolize the aged bound residues to $^{14}CO_2$ (Table 4). The released and re-extractable residue amounts were small (between 4% and 5%), representing only about 12% of the radiocarbon amount present for the biotests (38%, Table 4). But this release was of the same order of magnitude of that in the sandy clay soil. Hsu & Bartha (1976) proposed a two step

TABLE 3. Radiocarbon recovery from sandy loam soil immediately after treatment with ^{14}C-parathion.

Amount still present	1.59 kBq/g (or 0.043 µCi/g)	
	%	
Extracted	91.72 ± 5.02	
Bound	9.60 ± 4.99	
Present after reactivation	8.73 ± 5.90	
Biotest	With plant	Without plant
$^{14}CO_2$	0	0.11 ± 0.03
Released and extracted	5.16 ± 1.78	4.82 ± 3.98
Uptaken by plant	0.04 ± 0.03	—
Bound in the plant	0.07 ± 0.04	—
Still bound in soil	2.98 ± 1.03	3.37 ± 2.51

TABLE 4. Radiocarbon recovery from sandy loam soil three months after treatment with ^{14}C-parathion.

Amount still present	0.96 kBq/g (or 0.026 µCi/g)	
	%	
Extracted	65.09 ± 2.67	
Bound	40.03 ± 3.62	
Present after reactivation	38.15 ± 3.92	
Biotest	With plant	Without plant
$^{14}CO_2$	0.91 ± 0.35	0.99 ± 0.07
Released and extracted	4.30 ± 0.80	5.05 ± 1.14
Uptaken by plant	0.15 ± 0.11	- - -
Bound in the plant	0.12 ± 0.04	- - -
Still bound in soil	28.44 ± 4.18	29.81 ± 1.56

bounding mechanism for the herbicide propanil which may have happen for parathion: firstly the biological activity releases the weakly bound residues, and then, a chemical bounding into the organic matter is involved.

Therefore, the aging of applied ^{14}C-parathion applied favoured the high formation of bound residues which remained still bound after the biotests in both soils (Tables 2 and 4), although the highest values were detected in the sandy loam soil (Table 4). Katan & Lichtenstein (1977) proved that once the bound degradation products were produced, they were not released even in the presence of microorganisms.

The degradation of ^{14}C-parathion to apolar compounds in the silty clay and sandy loam soils are presented in Tables 5 and 6. In the silty clay soil (Table 5), all the radioactivity in the hexane soil extracts was identified solely as from parathion (R_f 0.34). However, immediately after the application, some metabolites were detected in the plant extract (R_f 0-0.10 and 0.60-0.70). After the 3 months aging period, the little amount of bound residues which could be bioreleased and extracted was not parathion (R_f 0.60-0.70). On the other hand, just after the ^{14}C-parathion application in the sandy loam soil (Table 6), some apolar metabolites were detected in the soil after the rhizosphere and microflora actions,

TABLE 5. Chromatographic profile of ^{14}C-parathion from the silty clay soil.

R_f	0 - 0.10	0.10 - 0.27	0.34	0.42 - 0.53	0.60-0.70
			(^{14}C-parathion)		
		Immediately after the treatment			
Extracted	-	-	xxx	-	-
Released and extracted					
- with plant	-	-	xxx	-	-
- without plant	-	-	xxx	-	-
Uptaken by plant	x	-	-	-	x
		Three months after the treatment			
Extracted	-	-	xxx	-	-
Released and extracted					
- with plant	-	-	-	-	xx
- without plant	-	-	-	-	-

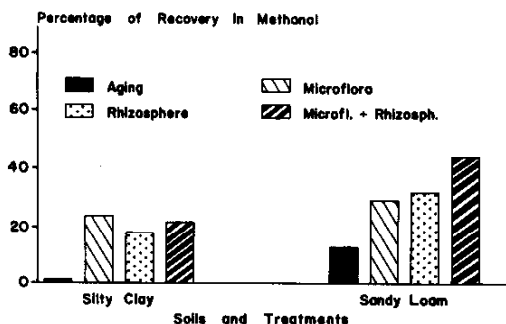- no radioactivity; x some radioactivity; xx evident radioactivity; xxx maximum radioactivity.

TABLE 6. Chromatographic profile of ^{14}C-parathion from the sandy loam soil.

R_f	0 - 0.10	0.10 - 0.27	0.34	0.42 - 0.53	0.60-0.70
			(^{14}C-parathion)		
		Immediately after the treatment			
Extracted	-	-	xxx	-	-
Released and extracted					
- with plant	-	-	xxx	x	-
- without plant	-	-	xx	xx	xx
Uptook by plant	-	-	-	-	-
		Three months after the treatment			
Extracted	-	-	xxx	xx	x
Released and extracted					
- with plant	-	-	-	-	-
- without plant	-	-	x	x	-

- no radioactivity; x some radioactivity; xx evident radioactivity; xxx maximum radioactivity.

FIG. 1. Degradation of [^{14}C]-parathion to water soluble compounds in soil extracts.

(R_f 0.42-0.53), and another metabolite was also detected as microflora action (R_f 0.60-0.70). Three months after, besides parathion, the same two metabolites were detected in the soil extract before the rizosphere and microflora actions, and only one of these metabolites (R_f 0.42-0.53) was bioreleased by the microflora.

Results accounting for the water solubles remained in the methanol phase after the extraction with hexane are shown in Fig. 1. The aging of parathion in the silty clay soil was responsible for less than 1% of degradation to polar compounds. But, the pesticide was degraded to water soluble compounds in about 23% and 18%, respectively by the actions of the microflora and rhizosphere, thus proving that microflora was the main responsible for degradation to polar compounds. But the highest radiocarbon recoveries were transferred to the hexane phase, and the radioactivity was mostly identified as from parathion itself (Table 5). The same trend was observed in the sandy loam soil (Fig. 1), although the aging period determined formation of 12% of polar compounds and the amounts detected by the actions of the microflora only or rhizosphere were 28% and 31%, respectively. In this soil, the amounts of ^{14}C released and extracted were relatively high (Table 4), and the degradation to polar compounds, as found by Katan & Lichtenstein (1977), reached about 44%. The radiocarbon which remained in the hexane phase was distributed among different R_f (Table 6).

Therefore, in the sandy loam soil both the aging and the rhizosphere contributed for the degradation of parathion to polar compounds. But, in the silty clay soil, the action of the microflora was the main cause. The sandy loam soil characteristics also determined that some parathion had been lost to the atmosphere during the aging time, and the amounts of still bound residues detected after the 3 months period were higher than in the silty clay soil.

CONCLUSIONS

Results here presented demonstrated that the aging of parathion was the main cause of the parathion bound residues which were inactivated into the soils, because neither the microflora nor the

rhizosphere released most of them. From the environmental point of view, although bound residues of parathion had been formed in large amounts, they did not pose potential hazard because mostly was inactivated. But the bound residues bioreleased must be taken into account for the concept of persistence of pesticides.

REFERENCES

ANDRÉA, M.M.; WIENDL, F.M. Formation and biorelease of bound residues of pesticides in two Brazilian soils. I. [¹⁴C]-lindane. **Pesquisa Agropecuária Brasileira**, Brasília, v. 30, n. xxxx, p. xxxx, 1995.

CALDERBANK, A. The occurrence and significance of bound pesticide residue in soil. **Review of Environmental Contamination and Toxicology**, v.108, p.71-103, 1989.

CAPRIEL, P.; HAISCH, A.; KHAN, S.U. Supercritical methanol: an efficaceous technique for the extraction of bound pesticide residues from the soil and plant samples. **Journal of Agriculture and Food Chemistry**, v.34, p.70-73, 1986.

EBING, W.K. Fate of pesticides in soil. In: GREENHALG, R.; ROBERTS, T.R. (Ed.) **Pesticide Science and Biotechnology**. 6ᵗʰ IUPAC Congress. Oxford Blackwell Scientific Publication , 1987, p. 411-416.

FUHREMANN, T.W.; LICHTENSTEIN, E.P. Release of soil bound methyl [¹⁴C] - parathion residues and their uptake by earthworms and oat plants. **Journal of Agriculture and Food Chemistry**, v.26, p.605-610, 1978.

GERSTL, Z.; HELLING, C.S. Fate of bound methyl parathion residues in soils as affected by agronomic practices. **Soil Biology and Biochemistry**, v.17, p.667-673, 1985.

GOLOVLEVA, L. A.; AHARONSON, N.; GREENHALG, R.; SETHUNATHAN, N.; VONK, T.W. (Eds.) The role and limitations of microorganisms in the conversion of xenobiotics.

Pure and Applied Chemistry, v.62, p.351-364, 1990.

HELLING, C. S.; KRIVONAK, A. E. Biological characteristics of bound dinitroaniline herbicides in soil. **Journal of Agriculture and Food Chemistry**, v. 26, p. 1164-1171, 1978.

HELLING, C.S.; GERSTL, Z.; KLOSKOWSKI, R. Stability of aged bound residues of methyl parathion in soil. In: INTERNATIONAL ATOMIC ENERGY AGENCY **Quantification, nature and bioavailabilty of bound ¹⁴C - pesticide residues in soil, plants and food**. Vienna, 1986. p. 137-153.

HSU, T.-S.; BARTHA, R.J. Hydrolizable and non hydrolyzable 3,4-dichloroaniline - humus-complexes and their respective rates of biodegradation. **Journal of Agriculture and Food Chemistry**, v.24, p.118-122, 1976.

KATAN, J.; FUHREMANN, T.W.; LICHTENSTEIN, E.P. Binding of [¹⁴C] parathion in soil: a reassessment of pesticide persistence. **Science**, v.193, p.891-894, 1976.

KATAN, J.; LICHTENSTEIN, E.P. Mechanisms of production of soil-bound residues of [¹⁴C] parathion by microorganisms. **Journal of Agriculture and Food Chemistry**, v.25, p.1404-1408, 1977.

KHAN, S.U. Bound (non extractable) pesticide residues in soil and plants. In: INTERNATIONAL ATOMIC ENERGY AGENCY. **Pesticide Transformation Products. Fate and Significance in the Environment ACS** Washington: American Chemical Society, 1991. p. 108-121. (Symposium Series, 459).

KHAN, S.U.; DUPONT, S. Bound pesticide residues and their bioavailability In: GREENHALG, R.; ROBERTS, T.R. (Ed.) **Pesticide Science and Biotechnology**. 6ᵗʰ IUPAC Congress. Oxford: Blackwell Scientific Publication, 1987. p. 417-420.

KLEIN, W.; SCHEUNERT, I. **Appraisal of tests to predict the environmental behaviour of chemicals**. New York: SCOPE, John Wiley & Sons Ltd. Publisher, 1985. p. 195-211.

LICHTENSTEIN, E.P.; LIANG, T.T.; KOEPPE, M.K. Effects of soil mixing and flooding on the fate and metabolism of ¹⁴C - fonofos and ¹⁴C - parathion in open and closed agricultural microcosm. **Journal of Economic Entomology**, v.76, p.223-238, 1983.

RACKE, K.D.; LICHTENSTEIN, E.P. Effects of soil microorganisms on the release of bound ¹⁴C residues from soils previously treated with [¹⁴C] - parathion. **Journal of Agriculture and Food Chemistry**, v.33, p.938-943, 1985.

ROBERTS, T.R. Non extractable pesticide residues in soil and plants. **Pure Applied Chemistry**, v.56, p.945-956, 1984.