

highly depleted O. Regardless of the nuclear process, there must have been very little mixing of the materials ejected at different times (4, 14). These materials did not exchange isotopically with more abundant normal material, either before the formation of the solar nebula, in the solar nebula, or in the meteorite parent body. The Hg data indicate that only a low-temperature (< 100°C) history is acceptable for much of this matter. Suggestions for proton types of processes have been made by Heymann and Dziczkaniec (13) and by Turkevich (15). In our model these reactions would occur in a presolar nebula source.

Finally, the Hg results may be considered, to some extent, to be consistent with the proposal by Clayton (16) that anomalous Xe or its progenitor was trapped in circumstellar grains associated with explosive stars. We go beyond this proposal in requiring that the star eject material periodically.

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- The time intervals between pulses,  $\Delta t$ , are determined by the fractional  $^{202}\text{Pb}$  decay needed to give the observed excess of  $^{202}\text{Hg}$  in each step. Thus,  $\Delta t = (\ln 2/T) \ln (A_1/A_2)$  where the half-life,  $T$ , for  $^{202}\text{Pb}$  is  $\sim 3 \times 10^5$  years and  $A_1/A_2 = 5.1/22.8, 1.3/5.1, 0.44/1.3, \text{ and } 0.20/0.44$  for the fractional anomalies ( $A_1, A_2, \dots$ ) we observe.
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3 SEPTEMBER 1976

## Binding of [ $^{14}\text{C}$ ]Parathion in Soil: A Reassessment of Pesticide Persistence

**Abstract.** A steady decrease of extractable [ $^{14}\text{C}$ ]parathion residues in soils over a 1-month incubation period was accompanied by an increase of unextractable, bound  $^{14}\text{C}$ -labeled residues, resulting finally in total recoveries of extracted plus bound residues of 80 to 87 percent of the applied radiocarbon. Soils containing bound residues were nontoxic to fruit flies. Binding of  $^{14}\text{C}$ -labeled residues was related to the activity of soil microorganisms; soil sterilization resulted in a reduction of binding by 58 to 84 percent. Under flooded (anaerobic) conditions, the binding of compounds labeled with  $^{14}\text{C}$  doubled, and parathion was reduced to aminoparathion. Reinoculation of sterilized flooded soil fully reinstated the binding capacity. [ $^{14}\text{C}$ ]Aminoparathion was preferentially bound to soil, since its binding within 2 hours was 30 times greater than that of [ $^{14}\text{C}$ ]parathion. Because of the existence of formerly "unseen," unextractable residues, the concept of "persistent" and "nonpersistent" pesticide residues might have to be reconsidered.

Difficult problems have emerged regarding the ultimate fate of synthetic chemicals for agricultural pest control. Before 1962 both analytical methodology and knowledge about the metabolism of many pesticides were limited, so that analyses for these compounds were primarily for the applied "parent" compound. In addition, only easily extracted residues were detected. Accordingly, typical depletion curves for several chlorinated hydrocarbon insecticides in soils were published by this laboratory, showing the "persistence," "disappearance," or "loss" of the pesticides (1, 2). The relatively short persistence of parathion residues under both laboratory and field conditions was described a few years later (3). When the fate of pesticide residues was described, the vague terms "disappearance" or "volatilization" were widely used, but they do not include the formation of unextractable bound residues. The introduction of radiolabeled pesticides for experimental use made it possible to obtain a "balance" and to account for the fate of the applied radiocarbon. The use of combustion or strong hydrolytic techniques with the extracted soils revealed that unextracted or bound residues could be released and detected, as for example, with the herbicide propanil (4) and the insecticide dyfonate (5). The problem of the bound residues, however, is complicated since present methods for their release (combustion, hydrolysis) also result in the destruction of their identity.

Earlier we investigated the persistence of the organophosphorus insecticide, parathion, in a loam soil (3); we now report a study conducted with the same soil and an additional sandy soil, using parathion (*O,O*-diethyl *O-p*-nitrophenyl phosphorothioate) labeled with  $^{14}\text{C}$  either in the aromatic ring or in the ethoxy group. In this way, we intended to quantitatively ascertain the fate of

[ $^{14}\text{C}$ ]parathion in an attempt to clarify some questions pertaining to the mechanism of its binding to soils.

Preliminary studies were carried out with samples of a sandy soil collected periodically from a Wisconsin cranberry bog where parathion is used extensively, and the soil is periodically flooded and drained. In the laboratory, soil samples were treated with parathion labeled with  $^{14}\text{C}$  in the ring ([*phenyl*- $^{14}\text{C}$ ]parathion) at a concentration of 1 to 2 parts per million (ppm) and incubated under moist conditions at 23°C in the dark. After 14 days the soil was extracted three times by a routine method with a mixture of benzene, methanol, and acetone (1 : 1 : 1). A portion was then examined for the presence of unextractable or bound  $^{14}\text{C}$ -labeled residues by burning it to  $^{14}\text{CO}_2$  in a Packard model 305 Tri-Carb sample oxidizer (6). Up to 60 percent of the originally applied radiocarbon (in the form of [ $^{14}\text{C}$ ]parathion) was recovered in the form of bound residues. In an attempt to release the bound residues, samples of these soils were further extracted by using various solvent mixtures that ranged in polarity from benzene to water. The total  $^{14}\text{C}$  recovered by six additional extractions amounted to only 2.4 percent of the applied radiocarbon, while 56.5 percent still remained in the soil in unextractable bound residues. Autoclaving and subsequent extraction of this soil with a mixture of benzene, methanol, and acetone (1 : 1 : 1) and then with ethyl acetate resulted in an additional release of only 1.2 percent of the applied radiocarbon.

After these preliminary findings, detailed experiments were carried out with a sandy soil from a cranberry bog [composition in percentages: organic matter, 1.3; sand, 98; silt, 2; and clay, 0 (pH 7.2)] and Plano silt loam soil [composition in percentages: organic matter 4.7; sand, 5; silt, 71; and clay, 24 (pH 6.0)].

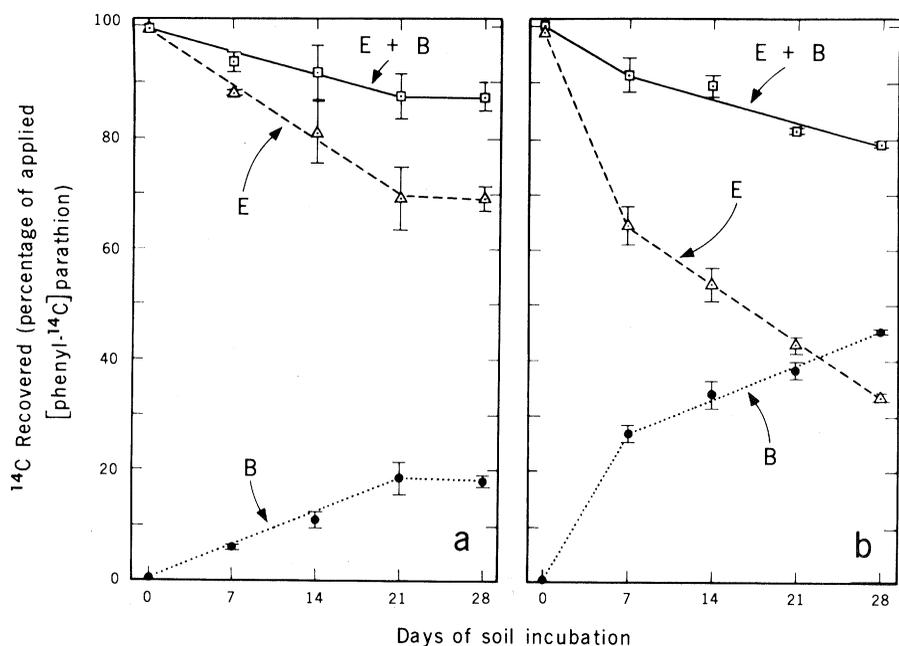


Fig. 1. Binding and extractability of  $[phenyl-^{14}C]$ parathion in two soils during a 28-day incubation period at 27°C (applied dose, 1 ppm). The amounts of parathion as determined by GLC in the loam soil extract were nearly identical to its radiocarbon content. (a) Soil from cranberry bog; (b) loam soil. Curve B, bound parathion; curve E, extracted parathion; and curve E + B, the total.

Acetone solutions of  $[phenyl-^{14}C]$ parathion (specific activity, 2  $\mu\text{C}/\text{mg}$ ) were mixed with the soils at a rate of 1 ppm, unless otherwise stated. Soil moisture was adjusted to 9 and 20 percent for sandy and loam soils, respectively. In some experiments parathion labeled with  $^{14}C$  in the ethoxy group ( $[ethoxy-^{14}C]$ parathion) (specific activity, 4.56  $\mu\text{C}/\text{mg}$ ) was also used. Portions of the treated soils (10 g each) were placed in cotton-plugged glass vials (82 by 20 mm) and incubated in the dark at 27°C, unless otherwise stated. Moisture levels of the soils were maintained by periodically weighing the vials and adding distilled water. One hour after treatment, 90 to 100 percent of the applied  $[^{14}C]$ parathion could be extracted. At the end of the incubation periods, the soil in each vial was extracted three times with 60 ml of a mixture of benzene, methanol, and acetone (1 : 1 : 1). The combined extracts were concentrated and adjusted to 100 ml with the same solvent mixture, and portions were used for liquid scintillation counting (LSC) (7). The extracted soil was divided into 1.4-g portions that were burned to  $^{14}CO_2$  for determination of the bound residues by LSC (6). In order to test the involvement of soil microorganisms in the binding of parathion residues, soil was sterilized either by autoclaving [1 hour at 121°C and 1 atm) on two successive days or by gamma irradiation (45,000 rads for 70 hours), which causes less alteration of the soil structure and composition. When sterile soils were

used, all procedures up to the extraction were carried out aseptically; the sterility of these soils was confirmed by incubating samples in yeast extract-dextrose medium. The sterilized soils were reinoculated either by mixing 0.5 g of nonsterile soil with 10 g of sterilized soil (sandy soil) or by mixing 0.5 ml of a water slurry of nonsterile soil with 10 g of irradiated soil (loam soil). All experiments were carried out with three replicates and later repeated at least once.

The extent of binding of  $[phenyl-^{14}C]$ parathion with time was determined by treating sandy and loam soils and incubating them for 1, 2, 3, and 4 weeks. After incubation the soils were extracted and analyzed as described (see Fig. 1 for results). With longer incubation times the amount of unextractable or bound residues increased, while the amounts of extractable residues decreased. During the 28 days of incubation the bound residues amounted to 17.55 and 45.75 percent of the applied radiocarbon in sandy and loam soils, respectively. The total  $^{14}C$  recovered (extracted plus bound) decreased somewhat, although 87.5 percent of the applied  $^{14}C$  was still recovered after 28 days from the sandy soil and 79.2 percent from the loam soil. This partial loss could have occurred from the formation of volatile degradation products.

The depletion curve of the extracted residues from a loam soil treated with  $[^{14}C]$ parathion (Fig. 1a, curve E) is similar to the curves described in 1964 for

parathion in the same soil under laboratory (3) and later under field conditions (8) (see Fig. 2). In all three studies the amount of parathion extracted from the loam soil after 24 to 28 days of incubation was 30 to 36 percent of the applied dose, which suggests that parathion is a relatively "nonpersistent" insecticide (Fig. 2). However, when the unidentified bound residues are taken into account (Fig. 1b), the total residues amount to 80 percent of the applied  $[^{14}C]$ parathion, although the nature of these bound residues is not known. Thus the depletion curve for total parathion residues in the soil indicates a much slower disappearance of residues, resembling depletion curves obtained by standard extraction methods, for the more persistent pesticides such as aldrin, for which 68 percent of the extractable residues (aldrin and dieldrin) were recovered after 1 month of incubation under laboratory conditions (9) and 53 percent under field conditions (Fig. 2). Thus it is necessary to consider bound residues in our deliberations about "persistent" and "nonpersistent" pesticides.

The extractable radiocarbon-containing residues were examined after the extracts were partitioned into benzene and water fractions. Analysis by LSC showed that  $^{14}C$ -labeled, water-soluble products comprised only 0.55 to 1.36 percent of the radiocarbon originally applied as  $[^{14}C]$ parathion. Analysis of the benzene fraction by gas-liquid chromatography (GLC) (10) indicated that 92 to 100 percent of the extractable radiocarbon was parathion. No degradation products could be detected.

The possible role of microorganisms in the binding of  $[^{14}C]$ parathion to soil was tested by subjecting samples of sandy and loam soils to various treatments that might affect microbial activity. After 14 days of incubation, the extractable and bound residues were determined. Basically, the binding of radiocarbon was studied with sterilized soils, with and without reinoculation, and with soils under anaerobic conditions. Results with sandy soil (Table 1) show that binding of radiocarbon in sterilized soil was reduced by 80 to 84 percent. In nonsterile sandy soil at 6°C, in which only low microbial activity would be expected, binding was also reduced by 88 percent. Reinoculation of the sterilized soil restored only part of the binding; apparently, under these conditions, microorganism populations were not fully reestablished during the 14-day incubation period. Binding with sterile loam soil was greater than with sterile sandy soil, yet sterilization of the loam soil by

autoclaving or by irradiation still reduced the binding by 58 to 60 percent as compared to that of nonsterile soil. Incubation at 6°C reduced binding by 91 percent in loam soil in comparison to incubation at 27°C.

To determine whether anaerobic microorganisms might be important in the binding process, loam soil was either flooded with water, by pipetting distilled water onto the treated samples until it was 15 mm above the soil surface, or was held under nitrogen during the incubation period. Flooding the soil had the pronounced effect of increasing binding of radiocarbon by 92 percent as compared to the binding in a moist, non-flooded soil (Table 1). In soil sterilized by irradiation and then flooded, the binding of <sup>14</sup>C was reduced by 77 percent as compared to binding in a nonsterile flooded soil. Reinoculation of the sterile soil and subsequent flooding completely restored its binding ability, while reinoculation of the nonflooded soil produced only a partial restoration. Thus it is possible that the reported rapid disappearance of parathion from submerged soils (11) is due to binding of this pesticide through the activity of anaerobic microorganisms that rendered it unextractable. When the binding of [<sup>14</sup>C]parathion was determined in loam soil held under nitrogen, binding was significantly increased by 19.5 percent (Table 1) in comparison to a similar test of the soil with air. After 14 days of incubation pH values of the flooded and the nonflooded soils were 5.74 and 6.13, respectively.

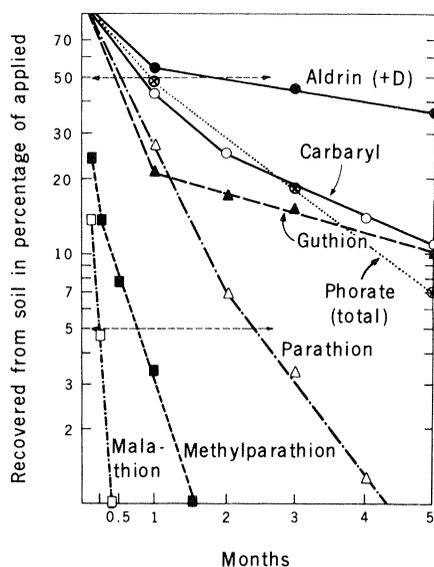


Fig. 2. Depletion curves depicting extractable residues of various insecticides applied at 6 kg/hectare (concentration, 3.1 ppm) to loam soils under field conditions.

The solvent extracts of the flooded soils were also partitioned into benzene and water fractions and were subsequently analyzed. The water fraction contained 3.4 percent of the originally applied radiocarbon. Analysis of the benzene phases by GLC showed that 100 percent of the radiocarbon in extracts from nonflooded soil was present as parathion, but only 48 percent in extracts from flooded soil. Thus the extensive binding in the flooded soil was also accompanied by the increased degradation of parathion. Benzene-extraction phases from flooded soil also contained amino-

parathion as shown by thin-layer chromatography (TLC) and GLC (10), but the amount of this metabolite was too small to be accurately quantitated.

When loam soil was treated with [*phenyl*-<sup>14</sup>C]parathion at a concentration of 1 ppm and incubated for 2 weeks at 27°C, 34.8 percent of the applied radiocarbon was bound to the soil, while at a concentration of 10 ppm only 14.5 percent was bound. Therefore, increasing the pesticide concentration in the soil resulted in relatively less binding, but the absolute amount of the bound residues was 4.2 times greater at the higher concentration. This indicates that at the lower concentration only a portion of the binding sites in the soil were saturated. Similar results have been reported for concentration-dependent binding of propanil (3',4'-dichloropropionanilide) (4) and 3,4-dichloroaniline (DCA) (12) in soil.

The toxicity to insects of bound residues labeled with <sup>14</sup>C derived from [*phenyl*-<sup>14</sup>C]parathion was compared to that of equivalent amounts of parathion freshly added to soil by exposing fruit flies (*Drosophila melanogaster* Meigen) to these soils (13). Mortalities of the insects after 24 hours of exposure to sandy soil containing 0.43 ppm of bound residues and sandy soil containing 0.43 ppm of freshly added parathion were 0 and 87 percent, respectively; mortalities after similar exposure to loam soils containing 3.3 ppm of bound residues or 3.3 ppm of freshly added parathion were 5 and 96 percent, respectively. No mortality was observed with exposure to soil free of insecticides. Therefore, soil-bound resi-

Table 1. Effect of temperature, soil sterilization, and anaerobic conditions on the binding of [*phenyl*-<sup>14</sup>C]parathion to sandy and loam soils during a 2-week incubation period.

Soil treatment	Incubation temperature (°C)	<sup>14</sup> C recovered (in percentage of [ <i>phenyl</i> - <sup>14</sup> C]parathion applied)*					
		Bound†		Extractable		Total (bound + extractable)	
		Sandy	Loam	Sandy	Loam	Sandy	Loam
None (control)	27	11.1 ± 1.8	34.8 ± 1.2	80.7 ± 5.9	51.8 ± 1.4	91.7 ± 5.4	86.6 ± 1.6
None	6	1.3 ± 0.5‡	3.2 ± 0.2‡	96.3 ± 2.1	97.8 ± 3.7	97.6 ± 1.7	101.0 ± 3.6
Autoclaved	27	2.2 ± 0.1‡	14.7 ± 1.1‡	94.7 ± 1.1	76.1 ± 4.9	96.9 ± 1.2	90.8 ± 3.8
Autoclaved and reinoculated	27	4.2 ± 0.7‡§		93.8 ± 1.2		98.0 ± 1.3	
Irradiated	27	1.8 ± 0.1‡	14.0 ± 1.4‡	93.5 ± 5.6	74.8 ± 3.0	95.3 ± 6.0	88.8 ± 5.0
Irradiated and reinoculated	27	5.8 ± 0.6‡§	17.1 ± 0.4‡	89.2 ± 1.0	69.8 ± 1.3	95.0 ± 1.4	86.9 ± 0.9
Flooded	27		66.7 ± 2.1‡		28.3 ± 2.9		95.0 ± 4.1
Irradiated and flooded	27		15.6 ± 2.7‡		74.1 ± 0.7		89.7 ± 1.9
Irradiated, reinoculated, and flooded	27		67.2 ± 1.8‡§		18.0 ± 1.6		85.2 ± 0.2
None, air	27		36.4 ± 0.4		51.4 ± 4.8		87.8 ± 4.4
None, N <sub>2</sub>	27		43.5 ± 0.5		55.0 ± 1.7		98.5 ± 1.2

\*[*phenyl*-<sup>14</sup>C]Parathion had been applied to soil at 1 ppm (specific activity, 2 μc/mg). †Carbon-14 determined by combustion of previously extracted soils. ‡Significantly different (at the 0.1 percent level) from the control of the respective soil at 27°C. §Significantly different (at the 0.1 percent level) from the respective sterilized soil that was not reinoculated. ||Soils incubated with either air or nitrogen. Differences between two treatments are significant at the 1 percent level.

dues derived from parathion either lost the original toxicity of the parent compound or are present as other biologically inactive compounds.

In order to specify the nature of the bound residues, the rate of binding of [ethoxy-<sup>14</sup>C]parathion was compared to that of [phenyl-<sup>14</sup>C]parathion. Results with [ethoxy-<sup>14</sup>C]parathion were very similar to those shown in Table 1 for [phenyl-<sup>14</sup>C]parathion. Bound residues expressed in percentage of applied [ethoxy-<sup>14</sup>C]parathion amounted to 11.0 percent in the sandy soil, 33.1 percent in the loam soil, and 67.2 percent in the flooded loam soil. It appears, therefore, that bound residues contain both the aromatic and ethoxy moieties of the original parathion molecules.

One possible explanation of the role of microorganisms in the production of bound residues in soil is that they degraded parathion to products that are more readily bound to soil. Since binding was enhanced under microbial anaerobic conditions, aminoparathion, which is formed via reduction of parathion and contains both aromatic and ethoxy moieties, is a likely compound. [Aminoparathion was a degradation product of parathion in submerged soils (11), in lake sediments (14), in a simulated cranberry bog (15), in loam soil (3), and, in the present study, in flooded soil.] This possibility was investigated by comparing the binding of parathion and aminoparathion in soil during brief incubation periods. Aminoparathion labeled with <sup>14</sup>C in the ring was prepared from [phenyl-<sup>14</sup>C]parathion by reduction with nascent hydrogen produced from HCl and zinc. The resulting [<sup>14</sup>C]aminoparathion was isolated and purified by TLC (10); loam soil was then treated with 1 ppm of the purified product. After only 2 hours of incubation, 49 percent of the radiocarbon applied as aminoparathion labeled in the ring was bound to the soil while only 1.6 percent of [phenyl-<sup>14</sup>C]parathion was bound. Thus, the amount of aminoparathion residues bound to soil in 2 hours was 30 times greater than that of parathion residues bound during the same time and also greater than the amount bound to parathion-treated soil after a 4-week incubation period. Since binding of aminoparathion occurred within 2 hours, this process apparently does not involve significant microbial activity. It has been reported (3) that most of the non-radiolabeled aminoparathion added to loam soil "had disappeared" 1 day after its application. The binding of aminoparathion, shown in the present study, may explain the earlier inability to recover this compound even a short time after

its application to soil. The rapid binding of another aromatic amine, 4-chloroaniline, has also been reported; about 50 percent of the originally applied compound was bound to soil in 1 hour (12). It appears, therefore, that microorganisms reduce the insecticide to compounds that are easily and rapidly bound to soil, thus making them nonextractable by standard methods. Other possibilities concerning the role of microorganisms in the production of bound parathion residues are (i) splitting the parathion molecule into two compounds that are highly and equally bound, (ii) the binding of parathion to cells of living microorganisms, and (iii) binding of the pesticide to soil by means of some microbial metabolic products. Our results suggest that the formation of bound residues of parathion in soil involves two processes: (i) a microbial process leading to the "conditioning" of parathion for binding, by one or more of the above-mentioned mechanisms, and (ii) a physicochemical process finally leading to the strong retention of the conditioned product by the soil. Hsu and Bartha (16), who studied the binding mechanisms of chloroanilines, suggested a covalent binding of the nitrogen atom of these compounds to the carbon of carbonyl groups or to quinoidal rings of humic substances in the soil. A two-step binding process was also proposed by Hsu and Bartha (12) to explain the binding of propanil in soil, that is, a microbial activity leading to the liberation of easily bound chloroanilines (such as DCA) and a subsequent chemical binding of these compounds to the organic matter of soil. Chisaka and Kearny reported that propanil was highly bound in a natural but not in a sterile soil (17), while the degradation product DCA (12, 17) was highly bound in both natural and sterile soils. The basically similar binding phenomena between two different pesticides, such as the herbicide propanil and the insecticide parathion, may be related to the existence of an amino group on the aromatic ring in the degradation products of both pesticides. Environmental factors and agricultural practices may affect parathion binding in soil through their effect on each of the two proposed processes involved in production of bound residues. Changes in the persistence of parathion resulting from soil treatments that affect microorganism populations or those caused by environmental factors may be attributed not only to the effect of these treatments on the biodegradation of parathion but also to their effect on its binding.

Smaller but significant amounts of bound residues were found in sterile

soils (Table 1). This could have occurred from the production of easily bound compounds through chemical degradation in soil or through adsorption of intact parathion to soil (18).

Binding of parathion residues to soil results in a pronounced reduction of its insecticidal activity. This is a beneficial environmental consequence of the binding—at least for a short term. However, unless we know under which circumstances and in what forms the bound residues can be released or reactivated, or interact with other compounds in the environment, "loss" of toxicity should not be regarded as permanent.

Our results regarding the relatively rapid disappearance of the extractable parathion residues in soil confirm other reports. However, because of the concomitant formation of bound residues, as a consequence of its rapid degradation, the classification of this highly toxic pesticide as "nonpersistent" is arbitrary until the fate of its bound residues is revealed. This may also be true for other "nonpersistent" pesticides.

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