DDT METABOLISM AND MOVEMENT IN DECIDUOUS FOREST SOIL MICROARTHROPODS

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ABSTRACT

DDT METABOLISM AND MOVEMENT
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By

George Edwin Klee

Much work has been done on DDT movement and metabolism in economically important insects and in aquatic ecosystems. However, suprisingly little has been discovered about metabolism by soil and litter arthropods, although these animals may have very prolonged and concentrated exposure to residues which accumulate in their habitats. Recent work at Michigan State University has indicated that soil-inhabiting Collembola and Acarina may play significant roles in DDT degradation.

Encouraging laboratory evidence in experiments with these two groups resulted in further work at Michigan State to set up field testing of the hypothesis that soil and litter arthropods may degrade DDT and possibly also its metabolites. In this way they could serve as a biological clean-up of persistent pesticides under natural conditions. DDT was introduced directly into an invertebrate food chain by feeding large numbers of a resistant species of Collembola, Folsomia candida, brewer's yeast food spiked with 100,000 parts per million DDT in laboratory culture. They were then fed uncontaminated yeast for 48 hours to flush out residues in the gut and released into fenced plots in a beech-maple forest in Central Lower Michigan over a two year period. The Collembola acted as "carriers" of the two major isomers of DDT and DDT, its major metabolite in arthropods. Three releases using o,p-DDT as the starting com-

pound were made, two using p,p'-DDT and one using p,p'-DDE. By using this new technique of introducing the chemical, levels of the insecticide were low enough to prevent mortality to members of the food chain, yet highly enough concentrated in the animals so that gas-liquid chromatographic analyses were possible. At selected time intervals the arthropod fauna was randomly sampled from subplots and all macroarthropods were removed for a companion study on macroarthropod DDT degradation. Over 800 microarthropod litter samples were processed by Berleze-Tullgren funnel extraction during the two years and over 1200 separate analyses were made on the gas-liquid chromatograph.

Of the various groups of microarthropods collected, the Collembola and the oribatid mites were found to show the most consistent pathways of DDT metabolism and movement. Each of the three compounds introduced reacted differently in the microarthropods analyzed, although the final detectable metabolite in each case was p,p'-DDE. O,p-DDT was found to give three detectable metabolites. A dehydrochlorination type of reaction resulted in o,p-DDE, which was rarely detected in microarthropods. A reductive dechlorination resulted in o,p-DDD, more commonly found in samples taken soon after releases. The most important metabolic pathway appeared to be the *in vivo* isomerization of o,p-DDT to p,p'-DDT, which was then dehydrochlorinated to form p,p'-DDE. When p,p'-DDT was introduced, almost all metabolism was to p,p'-DDE and it occurred at a faster rate than that of the o,p-DDT releases. There was some apparent conversion to p,p'-DDD, also, early in the sampling period, and this indicated some microbial breakdown. When p,p'-DDE was introduced, no

other detectable metabolites were found. Within two weeks after introduction, no further p,p'-DDE was found in Collembola, but oribatid mites maintained a slowly decreasing low level throughout its four week sampling period.

In all of the releases, microarthropods showed only traces or barely measurable levels of p,p'-DDE by the end of the sampling periods. The results of this work indicate that both major isomers and the major metabolite of DDT are degraded, or at least notaccumulated, by forest litter and soil microarthropods. The time required at the very low levels used here may be as short as four to five weeks. Apparent metabolic pathways of this degradation in both macro- and microarthropods under field conditions are also shown.

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George Edwin Klee

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INTRODUCTION

Probably no other pesticide is better known or more despised than DDT in this post-Silent Spring era of increasing public concern about all aspects of the quality of the global environment. Many organochlorine compounds which were developed after DDT was introduced have proved to be at least as persistent and have shown higher vertebrate toxicity. Apparently because it was the first synthetic organic insecticide to be widely used (and publicized), resistance to its continued use has continued to increase.

The persistence of DDT in the field was a great advantage for its use as an insecticide, but this persistence later proved to be of more concern than the acute toxicity of the compound. A large number of studies have been done by workers with a variety of equipment and expertise on biological magnification of DDT and its fat-soluable metabolites in food chains. A typical example and one that has been widely publicized is the work of Woodwell et al. (1967) on a marine estuary food chain on Long Island in New York. Some research indicated that DDT and/or its metabolites might remain in treated areas for a decade or even longer. Some scientists began referring to the "half-life" of DDT, as if it were as stable as some long-lived radioactive isotopes. However, the breakdown of DDT is largely a chemical process, not the strictly physical one involved in the disintegration of isotopes. Under the right conditions, DDT metabolism can be speeded up, as people working on insect resistance problems have discovered.

Resistance of some insects to the effects of DDT was noticed soon after the beginning of its widespread use following World War II. However, no good explanation for this resistance was discovered until 1950, when Sternberg et al. (1950a) found that resistant house flies metabolized DDT to the much less toxic DDE by means of a dehydrochlorination reaction. Little of either compound was lost by excretion and DDE appeared to be stored in the body in some way. A detailed follow-up of this work showed that both resistant larvae and adults were able to degrade DDT to DDE, and that DDE formed in the digestive tract was accumulated in the cuticle hypodermal layer and not excreted or metabolized further (Sternberg and Kearns, 1950b). The same workers (1952) later studied the metabolism of DDT in three other resistant insects: The differential grasshopper, Melanopus differentialis; the Mexican bean beetle, Epilachna varivestis; and the red-banded leaf roller, Argyrotaenia velutiana. Three different types of responses were described: (1) The grasshopper excreted large amounts of DDT virtually unchanged, (2) the beetle broke down some DDT to DDE and subsequently to an unknown metabolite, and (3) the lepidopteran degraded large amounts of DDT to DDE and then excreted both. Haskins and Witt (1958) who examined 30 species to get some idea of the range of types of DDT metabolism, found essentially the same three types of response and gave two examples of each: (1) Little degradation of DDT with most of it recovered unaltered (silkworm, mourning-cloak moth larvae), (2) absorbed DDT converted to unknown metabolites (Indian meal moth, soft-shelled tick), and (3) most of the absorbed DDT converted to DDE (tent caterpillars and resistant house flies).

Later workers found additional species, mainly in the latter two categories. Blum et al. (1959) reported that the boll weevil, Anthonomus grandis, broke DDT down to unknown metabolites but definitely not DDE. Agosin et al. (1964) investigated the breakdown in a hemipteran, Triatoma infestus, and found DDT was degraded to DDE and possibly some other metabolites. Kimura and Brown (1964) found a similar situation in the yellow fever mosquito, Aedes aegypti, with the breakdown primarily from DDT to DDE. Atallah et al. (1966) found that the ladybird beetle, Coleomogilla maculata, depends largely on the dehydrochlorination of DDT to DDE and it excretes both in the feces. DDE is the only metabolite here. Vinson and Brazzel (1966) reported the breakdown of DDT to DDE and DDA by tobacco bud worm larvae, Heliothis virescens. A good summary of all economically important arthropod species reported resistant to DDT up to that time was made by Brown (1968). He also reported resistant species for the other insecticide classes.

This synopsis by no means gives all of the investigations made on degradation of DDT by arthropods. Much additional work has been done, particularly on house flies and mosquitoes. It is intended, rather, to illustrate the variety of orders that have some resistant forms in them. Suprisingly, very little has been done on soil and litter species, although these may have very prolonged and intense exposure to residues which accumulate in their habitats. Most of the work that has been done with these groups has taken the form of mortality studies and population changes in soil as a result of pesticide treatments. Satchell (1955) studied the effects of benzene hexachloride, DDT and parathion on soil

animals. Stegeman (1964) analyzed the effects of varying amounts of carbaryl (Sevin) on populations of forest soil mites and Collembola. In a series of studies, Edwards (1965, '66, '67a&b, '68) tested the effects of DDT, several cyclodienes and several organophosphorus insecticides on soil animals. The converse of the above work--the possible effect of soil fauna on insecticides--has rarely even been considered until the last several years. Microbial breakdown of DDT has been reported by Chacko et al. (1967) and Guenzi and Beard (1967). Early work on the biology of Collembola in the soil zoology section of the Department of Entomology at Michigan State University revealed a high tolerance to DDT in Folsomia candida. High resistance to DDT by the same species was also reported by Scopes and Lichtenstein (1967) in work attempting to use the animals to bioassay contaminated soil. Drosophila were killed in the same experiments, but they were unable to determine an LD_{50} for the Collembola at any level of DDT tested.

Probably the best single summary of all pesticide interactions in the soil habitat was published in the proceedings of an international symposium held at the Michigan State University Pesticide Center dedication in February 1970: "Pesticides in the Soil: Ecology, Degradation, and Movement." All the papers were invitational, and the resulting reviews of research done and in progress in a wide range of disciplines by international authorities will be a reference work for research done in soil biology for many years.

This work led to laboratory testing by the soil zoology group at Michigan State on Folsomia candida to determine the cause of its high DDT resistance. Cultures of the Collembolan were fed upon p,p'-DDT (10-20 ppm) slant cultures which were inoculated with Penicillium and Verticillium fungi. The fungal mycelium penetrated the surface of the agar and picked up DDT, while the Collembola fed on the fungi (and apparently also, to a limited extent, directly on the agar). The animals were subsequently macerated and analyzed for DDT and its metabolites. Within 24 hours after they began feeding, quantities of DDE were recovered from the extracts as contrasted with that recovered from the agar surface and subsurface. The percentage of DDE, of total DDT and its metabolites increased consistently during the sampling periods of from 12 to 69 days; up to 30% (Butcher et al., 1969).

This encouraging evidence resulted in further work by members of the soil zoology group to set up field testing of the hypothesis that other soil and litter arthropods may also degrade DDT and possibly also its primary metabolites. This work suggests that they may be of significance in biological clean-up under natural conditions in the environment.

MATERIALS AND METHODS

Introduction of DDT into a Forest Litter Food Chain

Much of the past work on the effects of insecticides on soil arthropods has simply utilized a direct application of the compound to the plots, with subsequent soil samples being taken to obtain arthropods for analysis of the insecticide under investigation. For this study, a more direct method of introducing DDT into an arthropod food chain was sought which would give a level low enough to avoid mortality to different members of the chain. However, it also had to be at a level high enough to allow the DDT and its metabolites to be detected in the various species by gas liquid chromatography (GLC) analysis.

We decided to introduce Folsomia candida as a "carrier" of DDT directly into a food chain by feeding it contaminated food. F. candida was chosen for several reasons: (1) It is very resistant to DDT and it has been fed as much as 100,000 ppm in its food with no noticeable effect on reproduction or lifespan, (2) it is parthenogenic, with a relatively short life cycle of 21 days, and each female can lay hundreds of eggs over a life span of up to six months under ideal conditions, and (3) it is very easily reared in large numbers in the laboratory. The selection of a forest litter food chain for introduction was made because we thought there would be a better chance of getting a forest area with no or very low background amounts of DDT. Also, it could be assumed that there would be more species and biomass in a forest litter food chain. This assured that we would recover enough insecticide to be able to quantify the DDT and metabolites present.

Since the laboratory medium for F. candida was powdered brewer's yeast, DDT was dissolved in an acetone solution and mixed with the yeast as a slurry. The acetone was then allowed to evaporate, leaving crystalline DDT spread throughout the powdered yeast. The contaminated yeast was then weighed and placed into 25 by 35 by 10 cm clear styrene plastic rearing containers. The substrate in the bottom of the boxes was a 2 to 6 cm thick layer of a 1:1 mixture of finely powdered decolorizing charcoal and plaster of Paris, kept close to saturation by periodic addition of distilled water. Collembola from similar sized rearing containers were weighed and introduced into those with the DDT contaminated yeast. The animals were allowed to feed for two days before field release.

Forty-eight hours before the carrier Collembola were to be released into the plots, they were again fed uncontaminated yeast to flush out DDT which had not actually been incorporated into the tissues of the animals. Immediately prior to release, small numbers of Collembola were taken from several of the rearing containers for later GLC analysis in order to approximate the actual amount of DDT in ppm in the carriers at the time of introduction into the field plots. During the week before release, the Collembola were also gradually cooled from the 75°F rearing temperature to about the temperature of the litter at the site and time of release.

Field Plots and Sampling Procedure

A beech-maple hardwood forest site was selected in an area in Central Michigan that showed relatively little recent agricultural disturbance. Analyses of soil samples from the woodlot showed only trace amounts of DDT and/or its metabolites. Two 5 by 5 m plots were used for each sampling period; one in which the carrier Collembola were introduced and one which served as a control. Each plot was surrounded by a 45 cm high window screen frame fence, clamped onto 5 by 10 cm timbers nailed to strips of galvanized steel driven into the soil. This enclosure served to keep movement of crawling animals into and out of the plots to a minimum. The plots were divided by string into one-half meter squares to form a grid, which facilitated systematic sampling after the Collembola were introduced (Fig. 1).

At dusk on the day of release, carrier Collembola were brought to the forest plots in insulated styrofoam containers and released. This was done in order to give them several hours of darkness and relatively humid, cool conditions for penetrating below the upper litter surface and acclimating somewhat to field conditions with a minimum of mortality. Samples were taken in both control and treated plots the following day, and then at increasing intervals thereafter. Sampling was stopped when little or no DDT or metabolites were found in the animals collected.

On each sampling day, eight .25 M^2 samples were taken in each plot. The locations were chosen randomly and mapped out for the entire sampling period before the releases were made. No samples were taken within .25 M of the plot wall or from .25 M wide walkways across the center of the

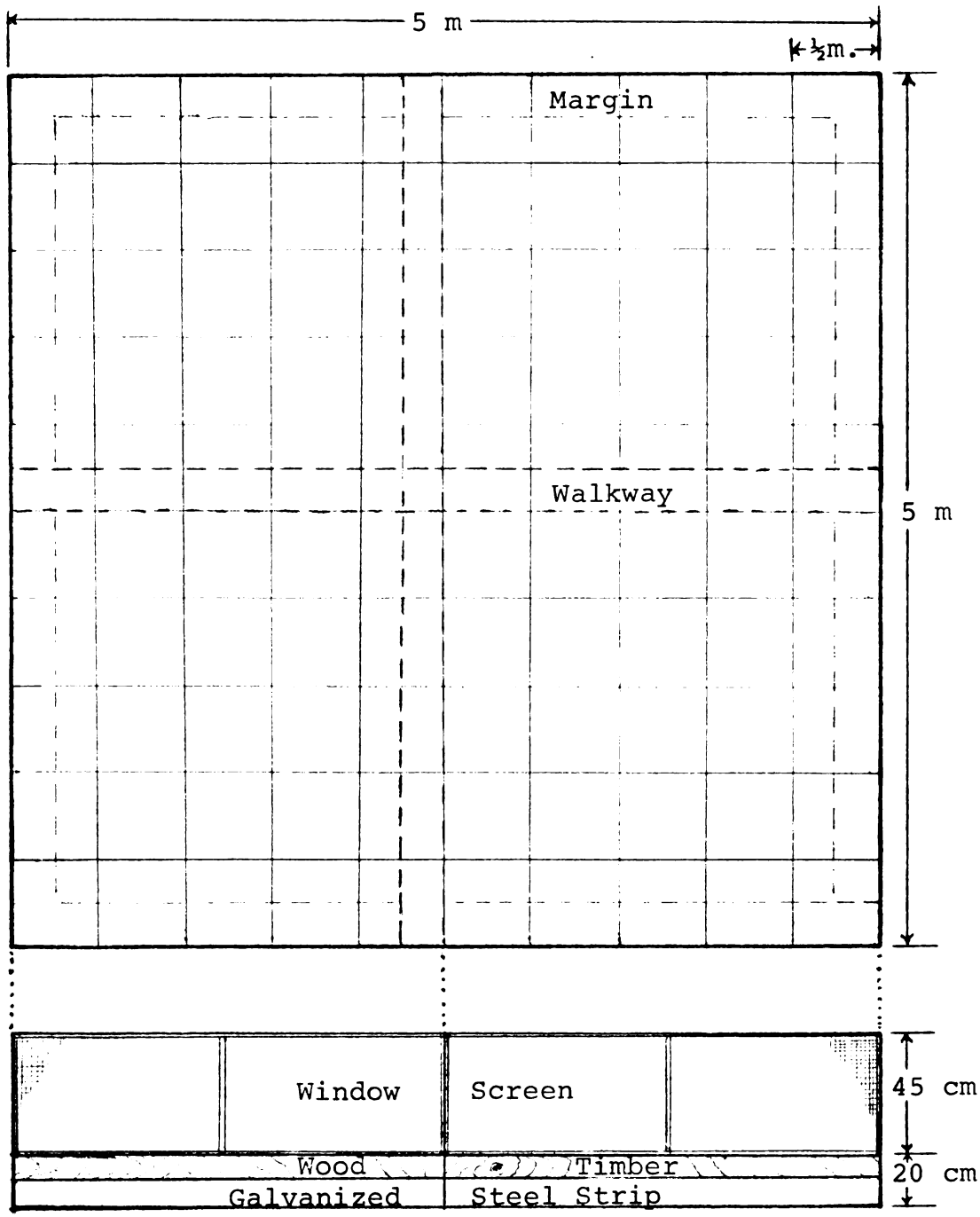


Fig. 1. PLOT LAYOUT FROM ABOVE, AND LATERAL VIEW OF ONE SIDEWALL

plots. Samples near the center of the plots were reached by placing a board along the walkway area to minimize compaction. Each of the eight samples consisted of litter and humus layer to the depth of the mineral soil layer (the top of the A_1 horizon). A steel frame was pushed in around each sample, with the aid of a knife to cut the leaves and other organic matter. The sample was then scraped off and placed in a plastic bag which was subsequently sealed. Bags containing the samples were then placed in styrofoam insulated chests and brought back to the laboratory for immediate extraction of both micro- and macroarthropods.

Six separate releases were made on different plots in the woodlot. These were run in succession during the summer field seasons of 1969 and 1970. For the purpose of comparisons, both major isomers and a metabolite were used in the releases; o,p-DDT in three of them, p,p'-DDT in two, and p,p'-DDE in one. Fifteen to 20 grams of Collembola, containing 1000 to 5000 ppm of each compound, were released each time; this was comparable to an application rate of four to seven grams/acre.

Extraction

All macroarthropods were hand sorted by shaking the samples into a large white sheet of paper. The large moving animals were collected with forceps. Gross identifications were made as the animals were picked out. The samples of litter were quickly placed back in the plastic bags before they could dry out. They were then placed in Berlese-Tullgren funnels for microarthropod extraction. The electric light source of heat was gradually turned up during the extraction period of four days. Control

samples were collected directly into alcohol, but those from treated plots were collected in 5 cm diameter glass jars with a moist charcoal-plaster of Paris base, in order to keep the specimens alive until they could be identified and weighed. Distilled water was added daily to maintain moisture levels, and any large specimens were immediately taken out of the jars, preserved and labeled in order to cut down on predation in the jars themselves. (Most had already been removed by the hand sorting process, but occasionally a few were missed.) All live material was kept in 34°F coolers to keep both the animals from freezing and their metabolism as inactive as possible before sorting.

Taxonomic Identifications

Because of the large numbers of species involved and the small amounts of biomass, the project was divided into two areas; macroarthropods and microarthropods. The macroarthropod portion of the study, involving such groups as the spiders, centipedes, millipedes and other large animals was done by a colleague, Dr. Gary Manley, and therefore, no detailed references will be made concerning these groups. A few macroarthropods were collected at fairly high levels in the samples, however, so they will be noted in passing. Among the microarthropods, the Collembola and the oribatid mites were selected for detailed work because they showed the most insecticide residues and because of ready access to determined synoptic collections from the area and usable keys. Identifications for the Collembola were based on Snider (1967) and those for the oribatid mites on Balogh (1963). Confirmations of the oribatid

identifications were made much easier by comparison with a large identified synoptic collection from Michigan determined by Dr. E. Piffl of the University of Vienna, Austria. This, combined with a course given at MSU by Professor Piffl on oribatid taxonomy, were invaluable in making determinations.

Identified animals were either chilled in a freezer or anesthetized with carbon dioxide so that they could be weighed in a Cahn electrobalance which was accurate to .002 mg. Numerous, very active samples had to be anesthetized with an ether cone to quiet them enough for identification and weighing. Any taxonomic groups of samples that weighed less than .1 or .2 mg were lumped with related groups for weighing, because the inherent error in analysis became too large in samples below this weight. As it turned out, most taxa were lumped into general categories to get large enough samples for GLC analysis.

Analytical Techniques

After the samples were weighed, they were placed in glass vials with .5 ml of glass distilled hexane, capped, and forzen for storage before analysis. They were maintained at -20 to -30°F. Previous to analysis, each sample was macerated with a glass rod and a few crystals of anhydrous sodium sulfate were added to pick up any water present, since water in samples can affect sensitivity of the GLC detector and give false peaks. A Hamilton 10 microliter syringe was used to inject two microliter portions of the extracts into a Beckman GC-4 gas-liquid chromatograph equipped with a discharge electron capture detector.

Because the samples were run over a two year period, several columns were used, with slightly different packings. Most were packed with 11% DC 200, 3% QF-1, 60/80 GCQ, and temperature was usually 225-230°C. Peak heights of both isomers of DDT, DDE and DDD were measured and converted to ppm based on live weights of the samples. Standards were run daily and repeated if the machine was operated for more than six hours at a time.

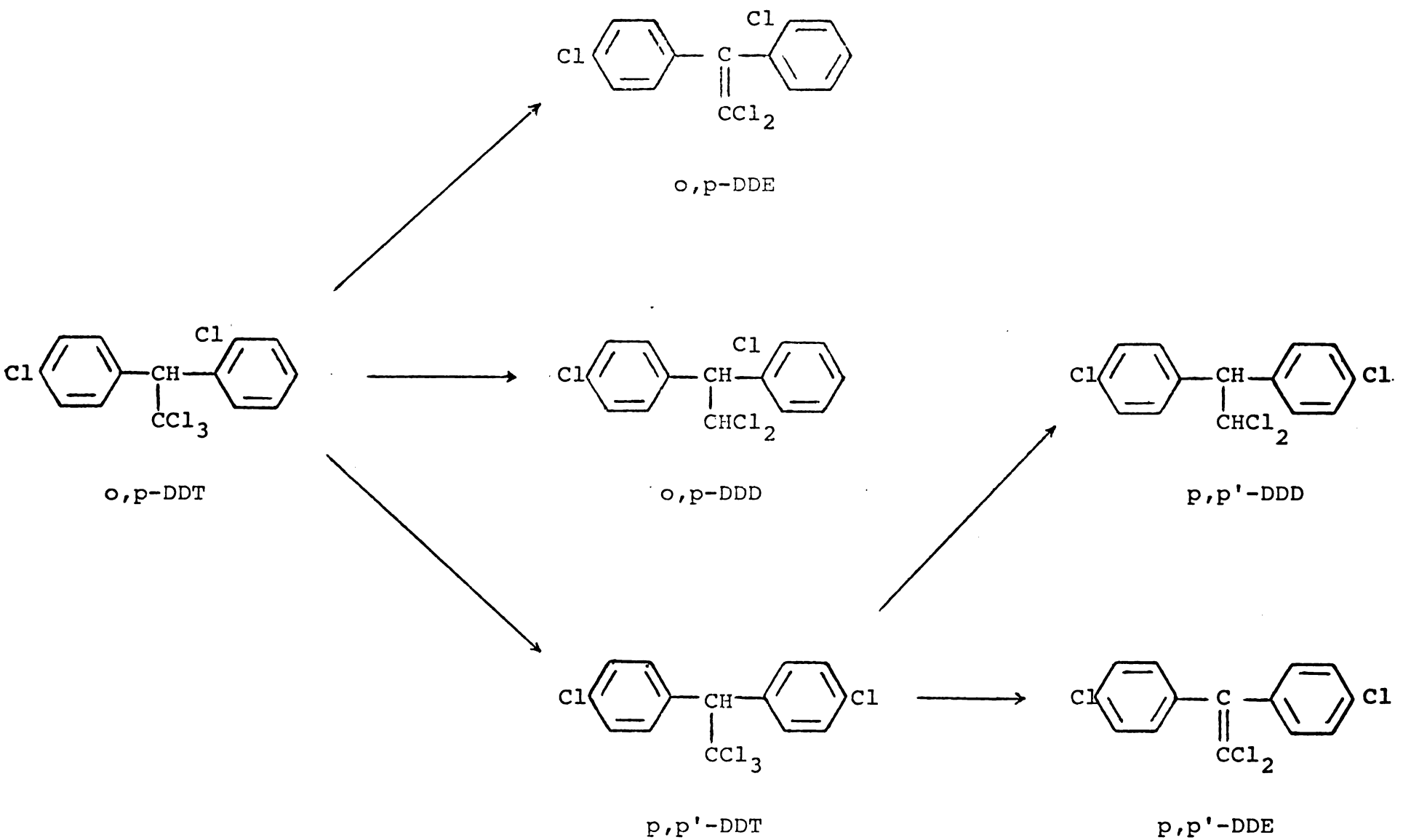
Before proceeding to the results of the analyses, a brief description of the known metabolic routes of DDT by arthropods and other soil fauna should be given, to aid in visualizing the compounds and reactions involved. Menzel and Abou-Donia (1970) list 14 different DDT type compounds which they have discovered in work with chickens. Menzie (1969) also lists 14 compounds in his review of known metabolism of almost every substance that has been used as a pesticide. Many of these, such as DDA, a major metabolite in vertebrates (O'Brien, 1967), have been found almost exclusively in vertebrate systems, which have a more complex array of detoxifying mechanisms. Also, some of them are more polar than DDT and its primary metabolites, and thus are not extracted very well by non-polar solvents such as the hexane which was used exclusively to extract DDT from sample material in this study. Thirdly, even if these compounds were present and could be extracted efficiently, most would be in such small quantities that they would be almost impossible to identify or quantify. All GLC work is based on comparing unknown peaks and retention times to those of standards of known concentration, and reliable standards of many minor metabolites are not generally available. There-

fore, this work is restricted to the six DDT type compounds that are relatively fat soluble and which were found most frequently in the members of the forest microarthropod food chain under study.

For comparative purposes, separate releases of Collembola which had been fed o,p-DDT, p,p'-DDT and p,p'-DDE were made in the test plots. The three compounds each reacted differently in the microarthropod food chain, but the relationships between them can be seen in an overall schematic diagram of the major routes of DDT metabolism (Fig. 2). The diagram is shown here to illustrate the structure of the compounds which will subsequently be mentioned in the data section. The chemical reaction and the relative importance of the different conversion pathways will be summarized in the discussion following the presentation of results.

During the six collection periods, which extended over a two year period, 816 Berlese-Tullgren funnel samples were extracted. With this large number of samples to sort, it was very difficult to make many specific determinations. Also, many of the samples had only .1 to .5 mg of Collembola of all types and perhaps 1 mg of oribatid mites, so they were left in general categories unless there were very large numbers of one type. The sensitivity of the gas-liquid chromatograph used is about .01 part per million, or .01 microgram per gram for chlorinated hydrocarbons when it is running at top efficiency. Samples below .1 or .2 mg necessarily would need very high levels of insecticide for detection, and inherent errors in analysis would probably exceed the concentration. Samples weighing less than this were not analyzed. Finally, even when enough of a lower taxon was extracted and run separately, few differences were shown. It was seldom possible to get more than one or two samples

Fig. 2. MAJOR REPORTED ROUTES OF DDT METABOLISM IN TERRESTIAL ECOSYSTEMS



of the taxon out of the eight sub-samples taken per sample day. Therefore, for graphic representations the general taxa "Collembola" and "Oribatei" will be used, with mention of more specific collections and analysis runs made in the respective discussion sections. Even with this restriction, over 1200 analytical runs of samples and standards were made with the GLC. The machine was being run concurrently for the macroarthropod part of the study and several other research projects, and at times was reserved up to two months in advance, including weekends. This further limited the detail with which the material could be identified, as one could, at best, run only 25-30 DDT residue samples and standards per 12 hour day.

A few selected samples were run on a special column which could give baseline separation of all peaks of the six DDT type compounds under study. (The column was packed with 11% [OV-17 + QF1] on 80/100 gas chrom Q; temp. was 180°C [Henly, et al. 1966].) It was quantitatively less sensitive than the first column and took 60 to 70 minutes to process a complete sample; however, it did serve to give further confirmation of the actual metabolites produced. Samples were also run on thin layer plates in an attempt to get additional verification of the compounds detected. The thin layer method is about .001 times as sensitive to chlorinated hydrocarbons as electron capture gas-liquid chromatography. Apparently the samples were too small, because nothing was detected except the standards. Verification of unknowns by two different qualitative methods or at least two different columns is quite important, as is illustrated in residue work by Frazier, et al. (1970).

All graphs show average values for the ppm and the percent DDE to DDT found in the different releases, as will be evident from the figures. The time axis is given as days from release and the day refers to the day the sample was taken from the field in order to maintain some continuity with the macroarthropod study results. However, it should be remembered that all microarthropod samples were placed on Berlese-Tullgren funnels for four days after the initial collection date and thus some metabolism could have continued before the animals were stored in the refrigerator. Some allowance should be made for this time lag to give the true stopping point of metabolism. This is an unfortunate but practically unavoidable problem when working with microarthropods that cannot be sorted immediately.

RESULTS AND DISCUSSION

General Survey of Arthropods Collected

The Collembola collected included a number of species from different litter layers. The most numerous soil forms identified were Folsomia candida and Tullbergia granulata. Some common lower litter forms were Hypogastrura armata and Neanura muscorum. Two other species, infrequent, but very characteristic upper litter layer forms were Orchesella hexafasiata and Tomocerus flavescens.

The oribatid mites were by far the most numerous litter arthropod group, as has been reported before by numerous workers, including Lawrence (1953) and Haarløv (1959). Huge numbers of oribatids were collected (over 1000 per sample on many days) and certainly not all were identified, but some of the more numerous Oribatei Inferiores were included. Among these were the genera Phthiracarus, Camisia and Protoribatritia. In the Oribatei Superiores, Pycnonoticae subdivision, Gymnodamaeus, Epidaemaeus, Eremulus and members of the Oppiidae were found. In the Poronoticae subdivision of the Superiores group, Lepidozetes, Achipteria, Ceratozetes, Galumna, Oribatula, Scheloribates, Peloribates and Protoribates were encountered frequently. Other mites were much less common, and although numerous samples of several predaceous and scavanger mite groups were run, a trace of either DDT or one of its metabolites were seldom found.

Other taxa, such as small millipedes, centipedes, pseudoscorpions and staphylinid beetles that somehow had escaped the hand-sorting process were also weighed, prepared and analyzed as they were picked up in the samples. These were macroarthropods and not within the scope of this project, but will be mentioned in the discussion of different compound breakdown pathways.

p,p'-DDT Metabolism

P,p'-DDT was metabolized very rapidly to p,p'-DDE and p,p'-DDD in most microarthropods analyzed. In one of the releases (Figure 3), no DDT was detected by the eighth day after introduction, and in the other p,p'-DDT release (Figure 4), all DDT was gone by the sixteenth day, and most had disappeared by the eighth. Metabolism apparently started very rapidly, as there were high concentrations of DDD and DDE in Collembola in both releases on the first day after introduction.

P,p'-DDE was one of the first metabolites identified in arthropod metabolism of p,p'-DDT (Sternberg & Kearns, 1952). It was found that an enzyme produced by DDT resistant insects, DDT-dehydrochlorinase, was responsible for this breakdown (Lipke & Kearns, 1958). Butcher et al. (1969) has also shown this ability in Folsomia candida under laboratory conditions. It has been found, by using highly sensitive enzyme tests, that the culture of F. candida used for releases also produces significant amounts of DDT-dehydrochlorinase (Mr. L. Besaw, personal communication). The DDE was found in the samples on the first day of p,p' releases. It was about 1/10 as concentrated as the p,p'-DDT found, but it was definitely present.

P,p'-DDD was also found early in the sampling period, but only through the fifth day after release. After the DDT was gone, DDD also disappeared. This corresponds with the concurrent disappearance of DDT and DDD found by Manley in macroarthropods (1971). Previous work has shown that DDD is a metabolite of a variety of soil organisms (Ko and

Fig 3. P,P'-DDE AS % P,P'-DDT FOUND IN COLLEMBOLA AND ORIBATEI(First p,p' Release)

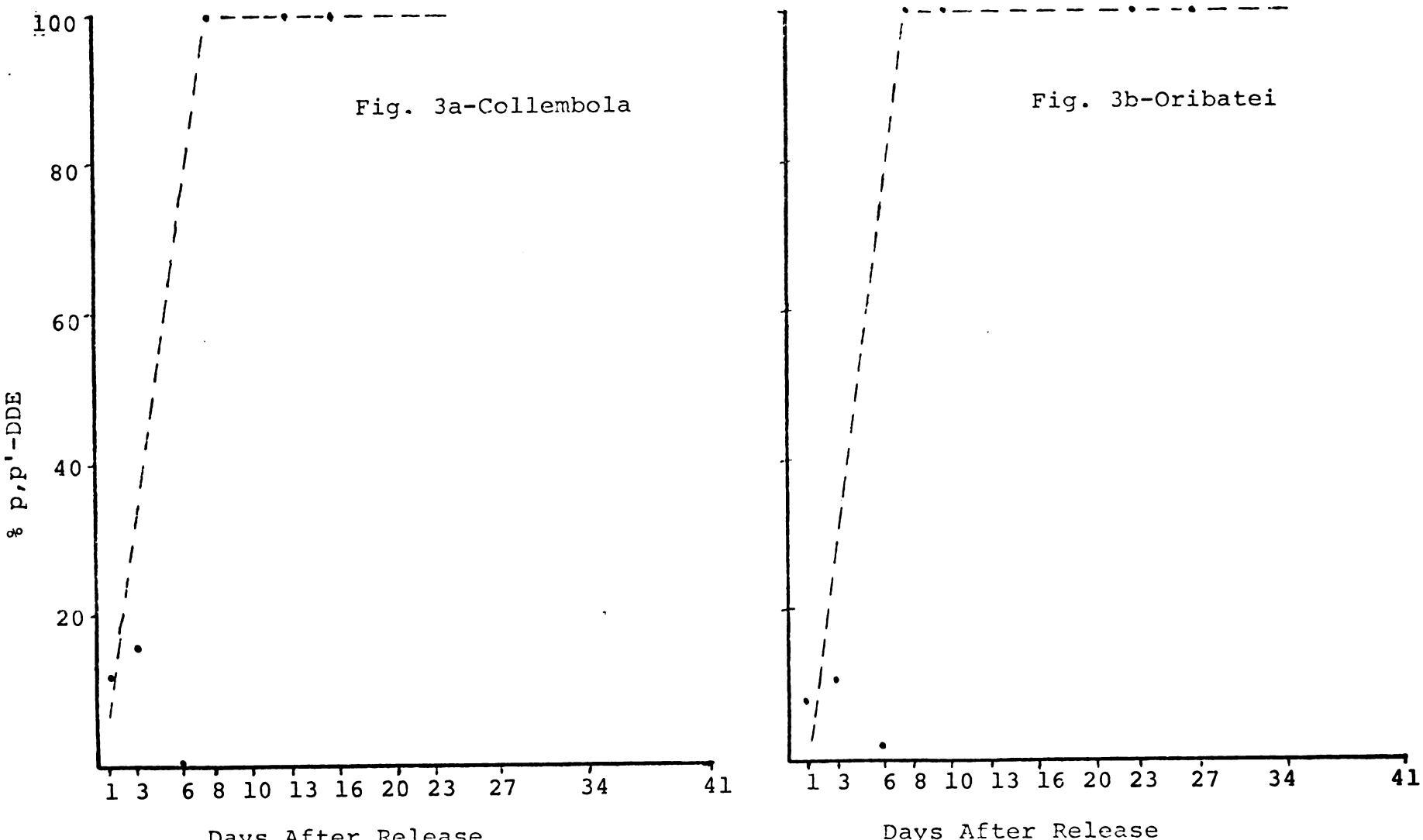
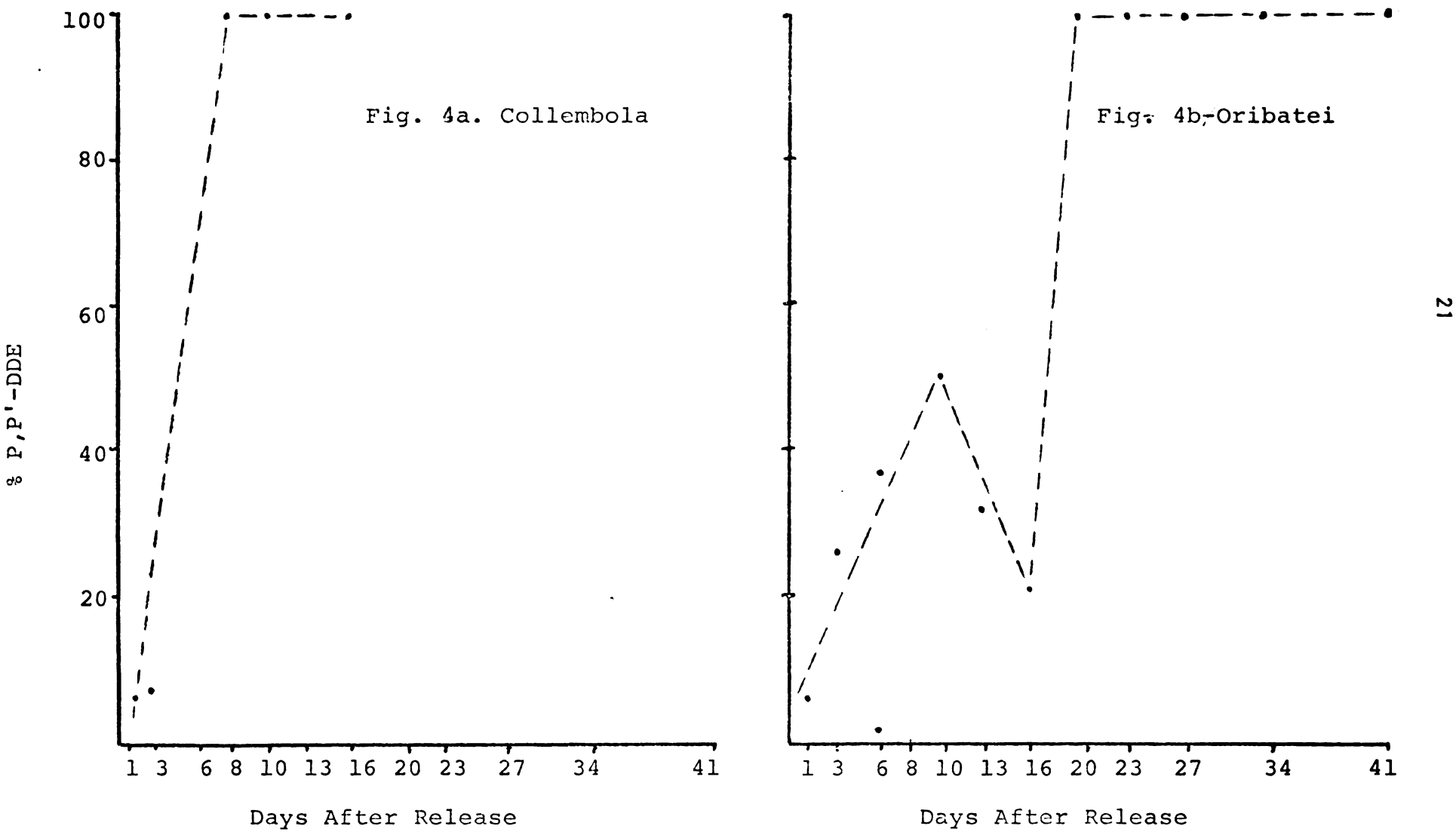


Fig. 4. P,P'-DDE AS % P,P'-DDT FOUND IN COLLEMBOLA AND ORIBATEI (Second p,p' Release)



Lockwood, 1968). Guenzi et al. (1967) showed that DDT is slowly converted to DDD in the soil under anaerobic conditions. These data appear to support a generalization that gut flora may be aiding in DDT breakdown as long as there is DDT present.

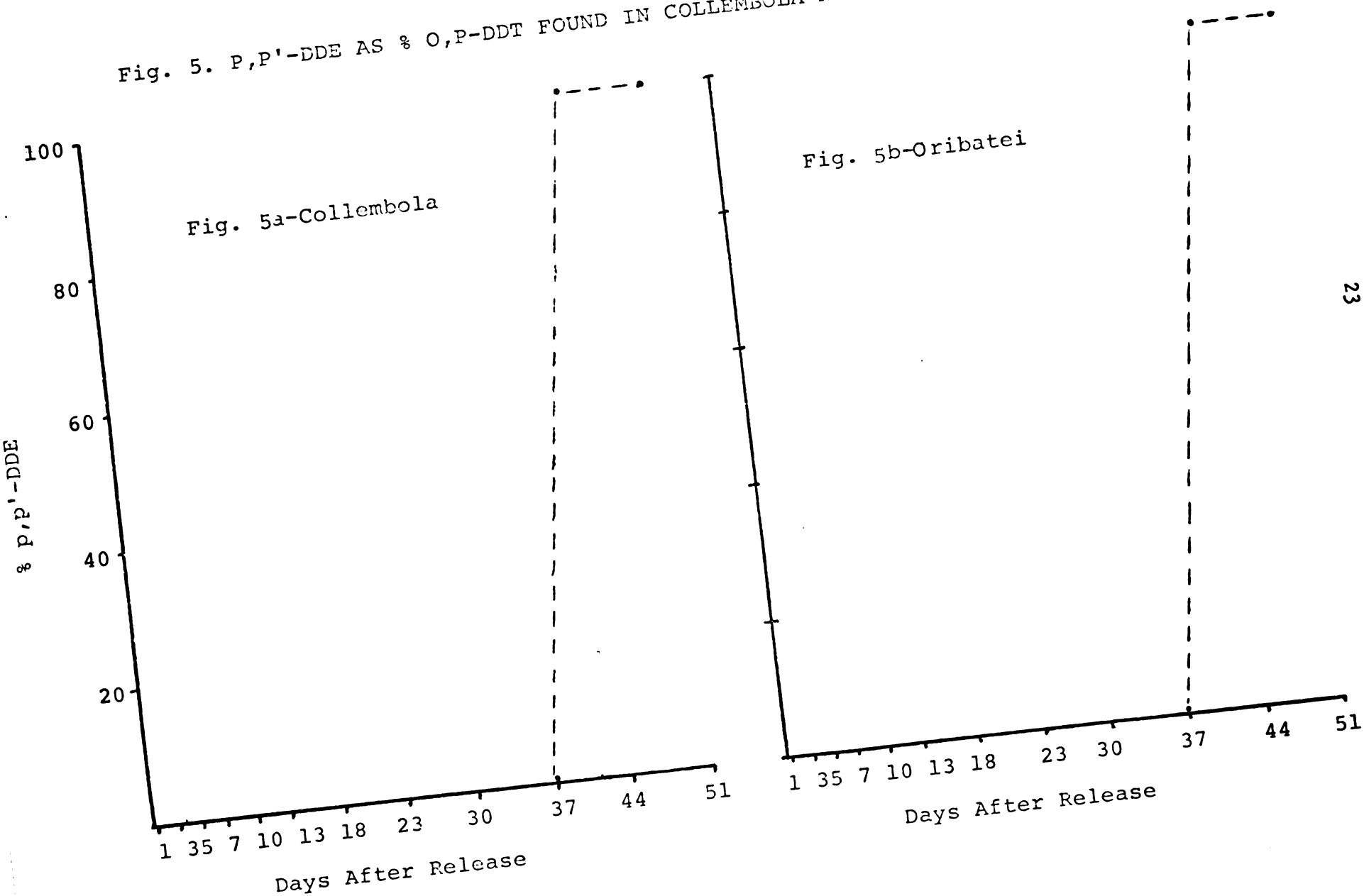
A few staphylinid beetles also showed measurable concentrations of DDE. In fact, that is all they ever showed in the p,p'-DDT releases, even on the first sampling day. No DDT or DDD was detected in them; suggesting that they apparently can break DDT down very rapidly.

o,p-DDT Metabolism

The conversion of o,p-DDT is shown in Figure 5. It can be seen that breakdown is not nearly as rapid with o,p-DDT although the same compound, p,p'-DDE, is the final result. Traces of o,p-DDD also were found in the first two samples, showing another possible example of microbial degradation; but it was not as concentrated as was the p,p'-DDD.

From the eighteenth to the thirtieth day after release, only traces of DDT were found in any animals tested, but during the following three weeks, the only compound detected was p,p'-DDE. This was found in very large quantities on day 44 in Collembola (467 ppm), oribatid mites, and juliform millipedes. By day 51, only traces of DDE were found. The same thing happened in another release of o,p-DDT, with nothing detected during the middle of a sample period and a buildup of DDE at the end, on the eighteenth and twenty-fifth days. The lapse in both release periods and the sudden appearance of DDE may be due to a general dilution of the o,p-DDT as time passed; and then it was gradually picked up again

Fig. 5. P,P'-DDE AS % O,P-DDT FOUND IN COLLEMBOLA AND ORIBATEI(o,p Releases)



by animals eating contaminated fungi and/or feces. Also, o,p-DDT apparently is isomerized to p,p'-DDT and then converted to p,p'-DDE, and this *in vivo* isomerization may take time. O'Brien (1967) states that *in vivo* isomerization of DDT is rare, and this one would be especially difficult, moving the chlorine two positions on the aromatic ring. However, Dr. J. Bedford (personal communication) has stated that this also occurred often in aquatic ecosystems, so it is not unknown.

p,p'-DDE Introduction

The introducing of p,p'-DDE (probably the major arthropod metabolite of DDT) was done for comparative purposes and to test predator-prey relationships in the macroarthropod food chain. It also was done to see if DDE might be broken down or eliminated from the food chain. This metabolite, although almost non-toxic in arthropods, may be responsible for upsetting avian calcium metabolism (Cade, et al., 1971) and thus helping to create the "thin eggshell" problem. The polychlorinated byphenyls (PCB's) may also be involved, however (Risebrough et al. 1968). The results are shown in Figure 6. It is evident that the results are rather inconclusive, but there is a gradual drop in all animals in which DDE was detected as time passed. The oribatids show the most complete record, with small amounts of DDE shown even on the twenty-eighth day. The juliform millipedes show high but erratic concentrations. Note that the Collembola show no DDE whatever after the fifth day, even though they were collected and analyzed for the next three sample days (see circles at baseline in Fig. 6). The Collembola data might show more if specimens

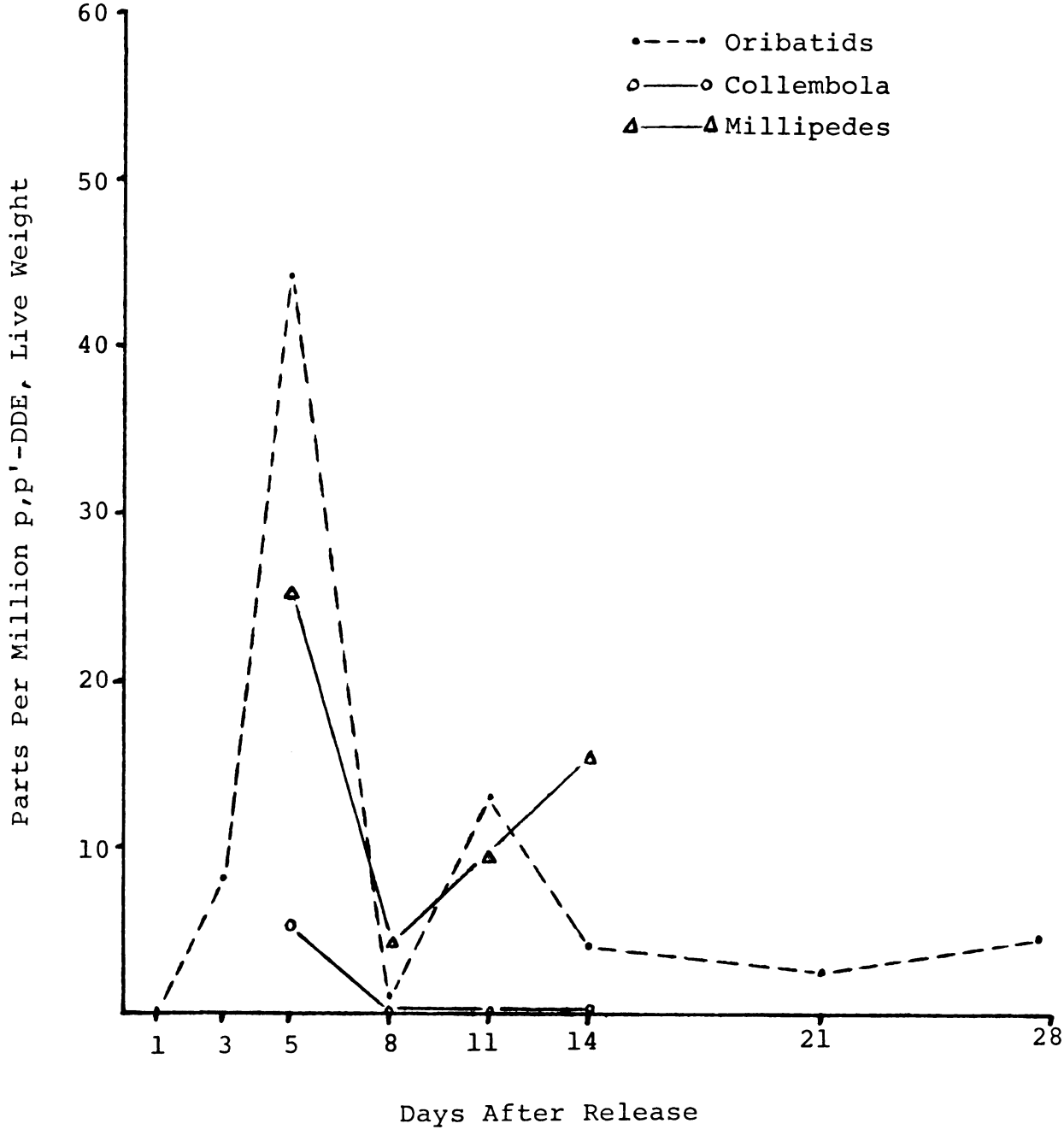


Fig. 6. MOVEMENT OF P,P'-DDE AFTER INTRODUCTION

had been available for the first two sample days; but unfortunately few were collected and none were analyzed. In any event, p,p'-DDE doesn't build up very much in microarthropods, as Manley reported in macroarthropod predators.

Discussion

Manley (1971) found ten major groups of cryptozoan macrofauna in the forest plots under study. These included Araneae, Chilopoda, Coleoptera, Diplopoda, Diptera, Hymenoptera, Lepidoptera larvae, Orthoptera, Pulmonata, and Oligochaeta. The first three taxa comprised the majority of predators in the forest litter food chain, with Araneae being the most important. They formed the most diverse fauna in terms of niches filled and predatory activities among the cryptozoan fauna of the forest floor.

The two predominant microarthropod groups in the food chain, the Collembola and oribatid mites, have generally been considered to feed almost exclusively on vegetable matter of various kinds (Lawrence, 1953, Michael, 1884, Engelmann, 1966, 1968). Christensen (1964) gave a range of reports of Collembola feeding habits and noted a few exceptions to this general rule, however. Members of the genus Friesia were reported as feeding on rotifers, Protura and tardigrades in the Pyrenees in Southern France, while MacNamara (1924) reported that Isotoma grandiceps is both cannibalistic and carnivorous on other species of Collembola. In addition, this same author reported Anurida maritima (in the same family as Friesia) as feeding on decaying animal matter on beaches between tidal marks,

while an Isotomid species was cited as having been found feeding on human cadavers in over half of 150 exhumed bodies. More recently, work done by Gilmore (1970) showed that eight out of ten Collembola species tested would eat significant numbers of nematodes. Two species, Entomobryoides dissimilis and Sinella caeca, were selected for more detailed predation and nutritional work and the following findings were made. (1) Eight out of ten species of nematodes tested (several of them economically important), were eaten by the Collembola; (2) Collembola grew faster on a diet of pure nematodes than on brewer's yeast; (3) As many as 2400 nematodes a day were eaten daily by all animals involved in the tests. Work on E. purpurascens, a closely related species, at Michigan State University supports the premise that E. dissimilis is an aggresive predator. E. purpurascens is cannibalistic in culture, particularly when there is a food shortage (Mrs. R. Snider, pers. comm.).

The above cited information indicates that Collembola may be more omnivorous than had previously been thought. The feeding on non-vegetable matter should still probably be regarded as an opportunistic type of feeding behavior in Collembola until more field observations are made under natural conditions. As for the oribatids, their feeding habits are less well known than the Collembola. What is known has been reviewed by Butcher, et al. (1971). Oribatids have also been observed eating plant feeding nematodes (Rockett & Woodring, 1966), although not as many were consumed daily per animal as was described for some Collembola.

Work on the formation of DDE has already been discussed in detail, but research on the formation of DDD as a metabolite has been more recent

and merits additional comment, since a coherent pattern seems to be emerging. First, metabolism to DDD is actually not a complete detoxification. DDD is 50-60% as toxic to insects as DDT itself (Ludvik, 1953), while DDE is almost non-toxic to invertebrates. Metabolism of DDT to DDD was demonstrated more than ten years after metabolism of DDT to DDE by Kallman and Andrews (1963) who first noticed it in yeast. Bridges et al. (1963) showed that 80% of the total DDT present in farm pond rainbow trout twelve months after application was DDD. The level was about 50% in other aquatic animals tested. Allison et al. (1963) in further work on the same pond found that when several types of microorganisms found in the pond were exposed to C^{-14} labeled DDT, DDD was the only metabolite. They suggested that the DDD found in the fish might be produced by microflora prior to uptake by the fish. Barker et al. (1965) demonstrated that the bacterium Proteus vulgaris isolated from mouse intestine was able to metabolize DDT to DDD. DDE was not converted to DDD, however. Datta et al. (1964) showed a similar conversion to DDD in the liver of the rat, in the absence of organisms. No DDD was found in the fat and kidneys of the same animals. Mendel and Walton (1966) in further work on the rat found that the intestinal flora, rather than the liver, may be primarily responsible for conversion of p,p'-DDT to p,p'-DDD. Coliform bacteria converted DDT rapidly under anaerobic conditions. Aerobacter aerogenes cultures showed 55% of total DDT compounds present as DDD after 24 hours, while Escherichia coli cultures yielded 30% DDD. Further work on A. aerogenes by Wedemeyer (1966) showed a 70% conversion to DDD in 24 hours under anaerobic con-

ditions. The use of selected metabolic inhibitors indicated that reduced Fe(II) cytochrome oxidase was responsible for DDT dechlorination. In another publication, Wedemeyer (1967) showed a seven-step metabolic pathway by which A. aerogenes degraded DDT to DDD and then on to dichlorobenzophenone. Plimmer et al. (1968) did a very elegant study on the mechanism of conversion of DDT to DDD by A. aerogenes. They demonstrated by the use of deuterated DDT that the conversion of p,p'-DDT proceeds by direct reductive dechlorination to p,p'-DDD without the formation of an unsaturated intermediate, p,p'-DDE. Braunberg and Beck (1968) showed that other microbes in the intestinal flora of the rat gut also can produce DDD. Eight out of nine types collected converted p,p'-DDT to DDD (6 to 90%) after 20 hours incubation. Ko and Lockwood (1968) showed that several soil bacteria and Actinomycetes rapidly converted DDT to DDD under waterlogged conditions. In a striking comparison of the different processes involved in DDE and DDD formation, French and Jeffries (1969) showed that o,p-DDT was broken down to p,p'-DDE in living avian tissue, but in the anaerobic conditions existing after death, o,p-DDT was metabolized to o,p-DDD.

Manley (1971) found formation of o,p-DDD from o,p-DDT and p,p'-DDD from p,p'-DDT in almost all groups of macroarthropods which he sampled. It was found as soon as sampling began, but could be detected only as long as DDT itself was present in the system. When DDT could no longer be detected, DDD was also missing. There was a direct relationship to the amount of DDT present in animals, also. Animals with a high level

of DDT contained a high level of DDD. In both o,p- and p,p'-DDT introductions, DDD concentrations dropped off at about the same rate as DDT in time. The highest DDD levels were never as great as those for DDT, but were found on the first sampling days. This is just the opposite of DDE, which gradually built up over the first few sampling days. Manley suggests that DDD is a metabolic product of gut micro-flora of macroarthropod predators and is produced only as long as DDT itself is present. His observation that DDD was probably formed by the gut micro-flora in proportion to the amount of DDT present lends support to the microbial formation of DDD hypothesis.

Manley's findings are supported by the results of the microarthropod part of the study. DDD was found in a number of different microarthropods during the first several sampling days in the case of both o,p- and p,p'-DDT releases at only very low concentrations; most at less than 1 ppm. Only a few individual samples of Collembola from the p,p'-releases, predominantly Folsomia candida, showed as high as 20 to 40 ppm.

These numerous described instances of DDD production by micro-organisms suggest a possibility that the presence of DDD in DDT residues indicates anaerobic microbial breakdown, while DDE indicates an enzymatic breakdown by animals themselves.

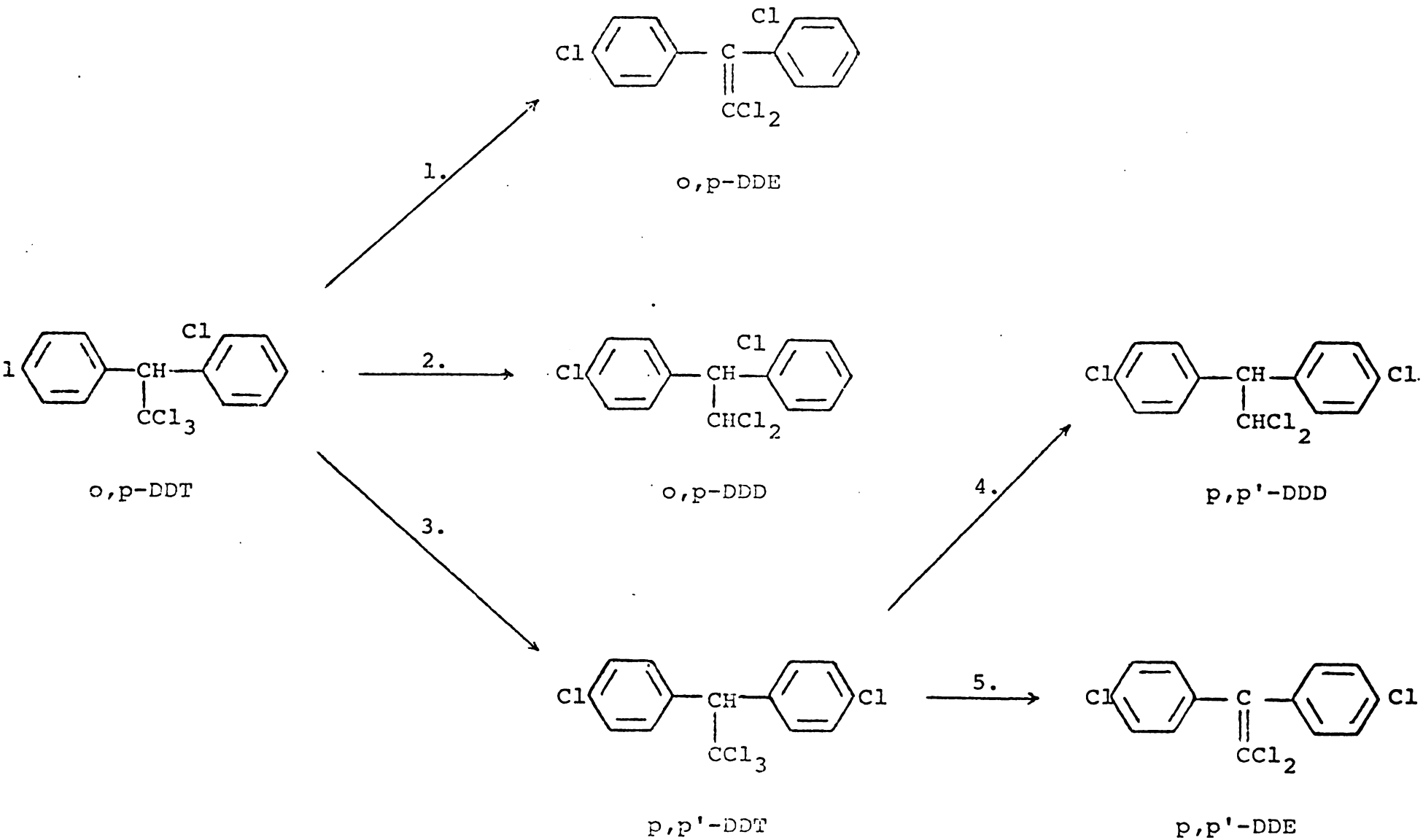
The following summary is a concise compilation of the pathways of movement and metabolism of both isomers of DDT in a deciduous forest soil and litter arthropod food web. It includes the microarthropod material presented here and also the macroarthropod data obtained by

Dr. Gary Manley, and is an attempt to integrate the results of the studies in order to make the results of the entire project more accessible.

Figure 7 shows the six major detectable compounds involved and the five reaction routes. O,p-DDT was found to give three detectable metabolites. A dehydrochlorination type of reaction (route 1) resulted in o,p-DDE, rarely detected in microarthropods here but found in several types of spiders (especially the Hahniidae), and to a lesser degree in larval Coleoptera in the families Cantharidae and Carabidae. It had disappeared in all taxa sampled by the end of the sampling periods. A reductive dechlorinationation resulted in o,p-DDD (route 2), found at low levels in almost all taxa sampled during the first two or three sampling days, but rapidly disappearing after about a week following releases. An in vivo isomerization of o,p-DDT to p,p'-DDT was the most important pathway used (route 3); probably over 90% of the detected metabolites came through this route. In most cases, p,p'-DDT was a short-lived intermediary, which was then broken down to p,p'-DDE via metabolic route 5. In fact, in some taxa, namely the spider family Thomasidae and the coleopteran family Carabidae (larvae), no p,p'-DDT was found at all. Conversion from o,p-DDT to p,p'-DDE was so rapid here that routes 3 and 5 seem to be combined.

P,p'-DDT was found to give two detectable metabolites, but the dehydrochlorination reaction resulting in p,p'-DDE (route 5 mentioned above) was by far the most important route. P,p'-DDD was formed in several macro- and microarthropod taxa, but was detected only as long as p,p'-DDT itself could be detected. Thus, this metabolic pathway (route 4) was used only for a few days after introduction of p,p'-DDT.

Figure 7. Major Routes of DDT Metabolism in a Forest Litter Arthropod Food Chain



Formation of p,p'-DDD from p,p'-DDT was more rapid than the corresponding reductive dechlorination to o,p-DDD in the o,p-DDT releases.

When p,p'-DDE was introduced, no other detectable metabolites were found. Within 2 weeks after introduction, no further DDE was found in Collembola, but oribatid mites maintained a slowly decreasing low level throughout the four week sampling period.

Two other taxa that should be mentioned because of the surprising lack of residues detected in them are the Acarina other than oribatids and the Pseudoscorpionida. Both of these taxa have many predatory species, yet almost no DDT residues of any type were detected in the numerous samples analyzed. This may only indicate the speed with which the residues move out ofthese groups, but this is not a complete answer; more work needs to be done on these groups.

CONCLUSIONS

Almost all micro- and macroarthropod forest litter taxa analyzed metabolized the two major isomers of DDT, o,p-DDT and p,p'-DDT, after they had been directly introduced into the food chain via resistant carrier Collembola. The final products detected in the introduction of each isomer was p,p'-DDE. The intermediate products of metabolism varied between the isomers introduced and between the arthropod groups analyzed.

Major results of the study were: (1) successful use of a new technique in terrestial food chain studies of pesticide movement; e. g., use of a carrier arthropod to introduce a pesticide directly into a food chain, thus allowing maximum recovery from other members of the chain without using high enough concentrations to cause direct contact mortality and disrupt the chain under study. This method also causes minimal disruption of the natural habitat; (2) an insight into the possible roles of various soil and litter arthropods in movement and degradation of DDT; (3) illustration of the detectable metabolic pathways of o,p-DDT, p,p'-DDT and p,p'-DDE for several taxa of forest arthropods; and (4) evidence that both isomers of DDT can be degraded by arthropods under natural conditions in forest soil and thus are biologically detoxified.

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