STUDIES ON THE RESIDUAL PROPERTIES OF THE ORGANOPHOSPHORUS INSECTICIDE ACEPHATE (*ORTHENE* ^{R}) IN DOUGLAS-FIR NEEDLES, FOREST LITTER, AND WATER

by

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Studies on the residual properties of the organophosphorus insecticide acephate (Monitor^{R}) in Douglas-fir needles, forest litter, and water

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ABSTRACT

Acephate (O,S-dimethyl acetylphosphoramidothioate) (*Orthene* ^{R}) was applied by fixed-wing aircraft to a Douglas-fir forest, in the interior of British Columbia, at the rate of 1.12 kg/ha. Residues in Douglas-fir needles were greatest 3 to 27 h after application; they were greater in needles from upper than from lower, less exposed limbs. In needles from dominant and co-dominant trees they declined 50% in 4 - 5 days; in needles from intermediate trees in about 7 days. After 45 days, they had declined to less than 1% of the highest concentrations; after 60 days, they could no longer be detected.

The residues in forest litter varied with exposure: they were greatest (1.16 $\mu g/cm^2$) 10 to 15 m away from any tree canopy, and least (0.62 $\mu g/cm^2$) under dense cover. But they persisted longer (30 days) in litter under dense cover and shade than in litter from the open (10 days). The effect of tree canopy on the amount of chemical reaching the litter was confirmed by bioassay with adult worker ants, and by measuring the residues on clean glass surfaces, exposed at various sites shortly before spraying and retrieved 3 h after.

The concentrations of methamidophos (O,S-dimethyl phosphoramidothioate) (*Monitor* ^{R}) were low; they began to decrease within 3 days. They were last detected in needles after 45 days, in open and densely covered litter after 10 and 30 days, respectively.

Acephate on glass surfaces was readily decomposed when exposed to ultraviolet radiation of 253.7 nm at room temperature; after 25 h only

16.6% of the added acephate were recovered. Methamidophos was not detected in the photodecomposition of acephate.

Acephate was resistant to hydrolysis at low pH (4.0 to 6.9) but not at pH 8.2. Some conversion to methamidophos occurred in the hydrolysis of acephate. The maximum conversion was 4.5% of the added acephate, observed in samples incubated at 20° C and pH 8.2 for 20 days.

Acephate was somewhat persistent in natural waters incubated under simulated natural conditions: 78 to 83% were recovered from pond water after 42 days, and 45% from creek water after 50 days. The rate of degradation increased greatly when underlying sediments were incubated with water samples; but acephate was much more stable in water *plus* sediment samples which had been autoclaved prior to treatment and incubation. The greatest conversion of acephate to methamidophos in any natural water sample, with or without sediment, was 1.3% of the added acephate, observed in pond water incubated at 9° C for 42 days.

No residues of acephate or its metabolite, methamidophos, could be detected at any time from glass wool plugs used to stopper flasks containing natural waters for incubation. Some acephate residues moved from water into underlying sediments.

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INTRODUCTION

The Douglas-fir tussock moth, *Orgyia pseudotsugata*, (Lepidoptera: Lymantriidae) is a very serious defoliator of Douglas-fir (*Pseudotsuga menziesii*) and true firs (*Abies* spp.) in western North America. The early-instar larvae feed on the underside of new needles and the late-instar larvae feed on mature needles. Douglas-fir is the preferred host, but occasionally grand fir (*Abies grandis*), white fir (*A. concolor*), alpine fir (*A. lasiocarpa*), and several species of spruce (*Picea* spp.) are attacked by this pest. Infested trees can be partially or completely defoliated in one season, resulting in retarded growth. Tree mortality often occurs after two or more years of partial defoliation. Outbreaks of Douglas-fir tussock moths tend to develop explosively and cause enormous damage before they collapse from natural control mechanisms such as virus attack or severe winter. This collapse usually takes three years to develop (Johnson and Ross 1967).

The first recorded outbreaks of Douglas-fir tussock moth in North America were at Chase, British Columbia, in 1916 and at Jarbridge, Nevada in 1927. From 1927 to 1930 an outbreak in north-eastern Washington killed more than 700,000 m^3 (300 x 10^6 board feet) of Douglas-fir and grand fir trees (Johnson and Ross 1967). An outbreak that began in 1970 in Washington and Oregon reached epidemic proportion in 1971. By 1973, nearly 405,000 ha of fir timber had been defoliated, with damage estimated at $58.9 million (Graham *et al.* 1975).

In the past, infestations of Douglas-fir tussock moth were successfully controlled by applying DDT. Emergency use of DDT was permitted in 1974 to control the outbreak, which had spread also to Idaho, and western Montana. More than 172,000 ha were sprayed in that year (Graham *et al.* 1975). Despite the success of this program, the use of DDT remained a controversial matter owing to the adverse effects of DDT on the environment (Harwood 1975). High priority was then given to the search for alternative, environmentally less damaging and persistent chemicals for controlling the tussock moth in order to minimize the need for large-scale use of DDT in the future.

Several insecticides with less environmental impact than DDT had been introduced for controlling the tussock moth. Acephate (*Orthene* ^{R}) (O, S-dimethyl acetylphosphoramidothioate) and *Dimilin* ^{R} (1-(4-chlorophenyl)-3-(2,6-difluoro(benzoyl) urea) were the most promising; both had proved highly effective in field experiments. Laboratory studies by Robertson and Lyon (1973) showed that the LD_{90} of acephate when topically applied to 4th-instar larvae was 139.1 µg/g, compared to the LD_{90} of 5.52 µg/g for DDT. However, the results of field application of acephate in 1974 against the tussock moth in the interior of British Columbia showed that the application of acephate at 1.12 kg/ha reduced the population of tussock moth comparably with DDT at 0.84 kg/ha and provided excellent foliage protection with less apparent environmental impact (Neisess *et al.* 1976). As a result of this study, acephate was used in 1976 on an operational scale to control the tussock moth infestations in

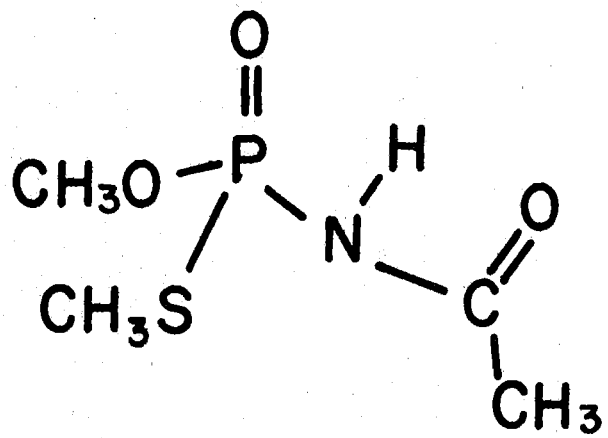
the forest of interior British Columbia.

Acephate is the N-acetylation derivative of methamidophos (O,S-dimethyl phosphoramidothioate) (Fig. 1). Methamidophos was discovered independently by Lorenz at Bayer in 1964 and by Magee at Chevron Chemical Company in 1967 (Magee 1974). The product was marketed under the trade name Tamaron ^{R} in Europe by Farbenfabriken Bayer and under the trade name Monitor ^{R} in the United States by Chevron Chemical Company and Chemagro in 1970. Methamidophos is an excellent systemic broad-spectrum insecticide and acaricide, but it is extremely toxic to higher animals (Oral LD_{50}, rat: 20 mg/kg). The discovery by Chevron Chemical Company that N-acetylation of methamidophos greatly reduced the mammalian toxicity led to the development of acephate as a new insecticide in 1968 which was then marketed by Chevron Chemical Company under the trade name Orthene ^{R} in 1972 (Magee 1974). In contrast to methamidophos, acephate is of low acute toxicity to higher animals (Oral LD_{50}, rat: 900 mg/kg; LC_{50}, 96 h, black bass: 1,725 ppm; gold fish: 9,500 ppm; blue gill: 2,050 ppm) (Spencer 1973; Chevron Chemical Company 1976, personal communication).

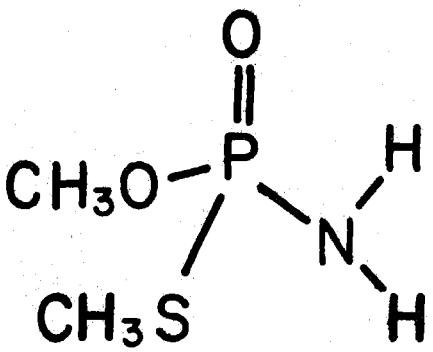
It is generally accepted that organophosphate insecticides are toxic to animals because of their ability, or that of their metabolites, to inhibit acetylcholinesterase (O'Brien 1967). Phosphoramidate and phosphorothionate insecticides in general are poor inhibitors of acetylcholinesterase *in vitro*. To exert their toxic action they have to

Figure 1

Planar structures of acephate and methamidophos.



ACEPHATE (ORTHENE®) (O,S-DIMETHYL-ACETYLPHOSPHORAMIDOTHIOATE)

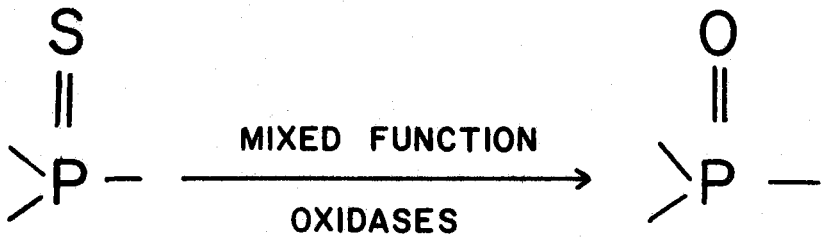


METHAMIDOPHOS (MONITOR®, TAMARON®) (O,S-DIMETHYLPHOSPHORAMIDOTHIOATE)

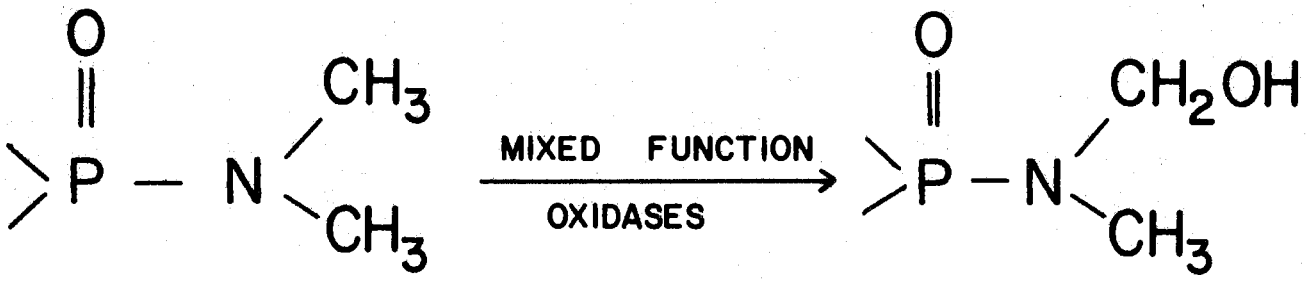
be activated *in vivo* by the mixed function oxidases in insects and higher animals. The activation products are the hydroxy alkyl derivatives of phosphoramidate and the oxygen (P=O) analogue of phophorothionate (Fig.2) (O'Brien 1960). Acephate is the N-acetylation derivative of methamidophos which is a phosphoramidate. Its *in vivo* activation mechanism in insects may therefore differ from that in higher animals as indicated by its low toxicity to higher animals. However, very little was known about the exact mode of action of acephate in both insects and higher animals. Rojakovick and March (1972) studied *in vitro* and *in vivo* inhibition of house fly acetylcholinesterase by acephate. They demonstrated that although acephate is a poor *in vitro* inhibitor of acetylcholinesterase ($I_{50} > 10^{-2}$ M), it effectively inhibits the enzyme *in vivo*. A 60-70% decrease in enzyme activity was observed 24 h after topical application of acephate at the LD_{90} level to the ventral abdomen tips of female house flies. They suggested that the toxicity of acephate is due to its stability *in vivo* and also to a gradual inhibition of acetylcholinesterase, possibly accompanied by irreversible phosphorylation and aging of the enzyme. This hypothesis was substantiated by Suksayretrup and Plapp (1977). They found that acephate is nearly as toxic to resistant strains of house flies as to susceptible strains, because acephate is a poor substrate for microsomal oxidases and transferases dependent on glutathione. These are enzymes responsible for the detoxification of organophosphate insecticides in resistant insects. The bioassay data of these workers indicated slow knockdown

Figure 2

Activation of phosphorothionate and phosphoramidate by mixed-function oxidases in both insect and higher animal.



PHOSPHOROTHIONATE ACTIVATION



PHOSPHORAMIDATE ACTIVATION

rates for flies exposed to lethal doses of acephate. Therefore, they speculated that acephate may have to be metabolically activated *in vivo* to become toxic. Similarly, the strikingly low toxicity of acephate to fish correlates very well with its low anti-acetylcholinesterase activity. Klaverkamp *et al.* (1975) reported that the LC_{50} for acephate to rainbow trout at 24 h and 15°C, was 975 ppm in contrast to 4.2 ppm for fenitrothion. Furthermore, the concentration which produced 50% acetylcholinesterase inhibition (I_{50}) was 1.4×10^{-1} M for acephate, compared to 7.4×10^{-2} M and 1.7×10^{-6} M for fenitrothion and fenitrooxon respectively. They speculated, therefore, that the high toxicity of fenitrothion to rainbow trout may have been due to the *in vivo* oxidative desulfuration of fenitrothion to fenitrooxon. On the other hand, the low toxicity of acephate to fish may be due to its inability to penetrate into the body either through the gills or the skin because of the high water solubility and hydrophilic nature of acephate.

Acephate has been tested for controlling insect pests in both agriculture and forest pest management. It is an effective systemic insecticide for many lepidopterous and coleopterous insect pests. Burbutis *et al.* (1972) reported that the application of acephate at 0.84 kg/ha reduced the population of green peach aphid, *Myzus persicae* (Sulzer), on sweet peppers by at least 95%. Similar results were obtained by Cruz (1974). Acephate applied at 15 g per tree gave 100% control for 4 weeks of palm aphid, *Cerataphis nucifera* (Reinert and Woodiel 1974). Other

agricultural insect pests successfully controlled by acephate include the leaf hopper, *Empoasca fabae* (Harris); tomato pinworm, *Keiferia lycopersicella* (Walsingham); potato tuber moth, *Phthorimaea operculella* (Zell); leafroller, *Platynota flavedana*; and scale, *Cerococcus deklei* (Cruz 1975, Poe and Everett 1974, Foot 1974, Bobb 1972, Reinert 1976).

Acephate was also investigated for stored-product pest control and preliminary results seemed promising. Watters (1977) reported that treatment with acephate at 32 ppm in stored wheat provided 90-100% mortality of rusty grain beetle and red flour beetle for 270-277 days.

Acephate was also effective in controlling many forest insect pests in addition to Douglas-fir tussock moth. Goyer (1973) reported that LD_{50} and LD_{90} of acephate to first-stage gypsy moth larvae, *Porthetria dispar* (L.), reared on an artificial diet containing acephate, were 15-25 ppm and 25-30 ppm respectively, indicating that acephate is toxic to gypsy moth larvae at low dosages. Consequently, acephate seemed to be a likely candidate for field testing. Brewer (1973) demonstrated that foliar spray with acephate at 1 g/l until dripping, reduced the population of elm leaf beetle larvae, *Pyrrhalta luteola* (Muller). The mean number of elm leaf beetle larvae per 46-cm Siberian elm branch was 1.72 two weeks after acephate treatment compared to 7.89 in the untreated control. Similar success was indicated in the control of Japanese beetles, *Popillia japonica* (Newman) by Lawrence *et al.* (1973). Japanese beetles were

allowed to feed for 48 h on soybean leaves collected from the field after spraying a 3 m x 3 m soybean plot with acephate at 1 g/l until runoff. The mortalities (48 h) were 100%, 96% and 93% for Japanese beetles fed on soybean leaves collected 1-2, 3-4 and 6-7 days after acephate treatment. Chemical control of the forest defoliating spruce budworm, *Choristoneura fumiferana* (Clemen) has been investigated intensively. Preliminary results indicated that acephate is very effective (Armstrong and Nigam 1975, Nigam and Hopewell 1973). Hopewell (1975) reported that acephate at 0.3 kg/ha produced a 90% reduction in a spruce budworm population and a 70% reduction in defoliation when applied as a simulated aerial spray.

Recently, the possibility of using acephate in combination with pathogens to control spruce budworm and white-marked tussock moth, *Orgyia leucostigmata* (J. E. Smith) has been investigated and the results are encouraging. Morris (1975) found that applications of *Bacillus thuringiensis* Berliner produced about 45% mortality in third and fourth-instar larvae of spruce budworm. Combination with a dosage of acephate causing 5-25% mortality, resulted in low budworm survival. The mortality of pathogen *plus* acephate treatment showed additive effect, and all survivors eventually died before pupation. This alternative approach provided successful insect pest control with less insecticide, thus minimizing adverse effects on the environment.

The limited information available suggests that acephate is not persistent and has little apparent impact on the environment. At 0, 7

and 14 days after the last of 6 spray applications of acephate at 1.13 kg/ha, Tappan *et al.* (1974) found residues of 21.6, 16.5 and 15.7 ppm of acephate and 2.5, 1.6 and 1.3 ppm of methamidophos on green cigar-wrapper tobacco. No detectable residue of either compound was found on cured and fermented cigar-wrapper or flue-cured tobacco regardless of the formulation or time interval from last application to harvest. Similar residual properties were demonstrated in the foliage of greenhouse tomato and pine seedlings. Lindquist and Krueger (1975) detected 252.4 ppm of acephate in greenhouse tomato leaves immediately after spraying with a formulation of 0.12% acephate. The residue level decreased to 130.9 ppm after 7 days and 56.8 ppm after 21 days. Werner (1974a) determined acephate residues by bioassay with weevils in loblolly pine seedlings after soaking the seeds in aqueous solution of acephate (10.4 mg/ml). He reported that 16.2% of the initial residue level were found 1 week after treatment. Only 3.1% were left after 10 weeks and no residues were detected after 11 weeks.

Buckner and MacLeod (1975) monitored the populations of forest-inhabiting small birds, small mammals, amphibia and honey bees during the application of acephate at two rates (0.56 and 1.4 kg/ha). Population data and field observations indicated that these amounts affected only the honey bees. Bee mortalities were observed in the treated plots up to 14.5 h after acephate application. Increase in hive entrance activity

and reduction in pollen collection occurred in the hives of the treated plots up to 4-5 days after acephate application. However, examination of the colonies 3 months later showed no differences in colony strength or honey production between the treated and untreated hives. Focht and Joseph (1974) observed that over a 50-day period after three repeated applications of either acephate or its metabolite, methamidophos at the rate of 20 ppm to soil, the population levels of actinomycetes, bacteria, and fungi were not affected.

The initial success of acephate in controlling forest pests with little apparent environmental impact suggests the possibility of its increasing use in forest pest management. However, little is known about its persistence and metabolism in the forest ecosystem following large-scale aerial application. Sundaram and Hopewell (1976) studied its persistence in spruce trees in a nursery after a simulated aerial spray. They reported that immediately following the application of 0.28 kg/ha concentrations in the needles averaged 55.15 ppm, decreased to 2.92 ppm within 5 days, and were non-detectable after 32 days. Only traces were found of the toxic metabolite, methamidophos. The possibility of significant conversion of acephate to methamidophos, itself an effective insecticide and acaricide, is of great importance since methamidophos is very toxic to higher animals.

The low fish toxicity of acephate compared with carbaryl and fenitrothion, makes it preferable for use against forest pests near

bodies of water. However, nothing was known about its persistence and behaviour in water. Oloffs *et al.* (1972, 1973) demonstrated that organochlorine insecticides and polychlorinated biphenyls in waters tend to escape into the atmosphere by evaporation and co-distillation. They may thus contaminate the environment away from the point of release. For a better understanding of the behaviour and persistence of acephate in a forest ecosystem and in natural waters, further research is needed.

The objectives of this study were: (1) to investigate the persistence of acephate and its conversion to methamidophos in Douglas-fir needles from different classes of trees, and in forest litter under different shade conditions, following an aerial application to control the Douglas-fir tussock moth; (2) to determine the effects of ultraviolet radiation on the degradation of acephate under laboratory conditions; (3) to determine the effects of pH and temperature on hydrolysis of acephate under laboratory conditions; and (4) to study its behaviour in two natural waters, with or without bottom sediments, under simulated natural conditions in the laboratory.

MATERIALS AND METHODS

I. Field Studies.

1. Sampling Plots. The plots were in a tussock-moth-infested Douglas-fir forest in the interior of British Columbia on the eastern slopes of the North Thompson River (50°, 51.5' N.; 120°, 12' W.; 850 m elevation). The unsprayed control plots were about 2 km to the south and 1 km away from the edge of the sprayed area. Acephate was applied by aerial spray at 1.12 kg/ha. *Orthene* R 75 S soluble powder (US Pat. No. 3,716,600), containing 75% of acephate and 25% inert ingredients by weight, was diluted with an appropriate amount of water for application. Spraying was done at 0700 h, 2 June, 1976. Samples were taken 1 day prior to spraying and 3 h, 27 h, 51 h, 3 days, 10, 20, 30, 45, and 60 days thereafter.

2. Weather. The weather after spraying was cool with occasional light showers throughout the district. The closest weather station, 11.5 km away at Heffley Creek, reported 0.5 mm of rain 2 days after spraying and nothing more until 7 days after spraying when 4.8 mm fell. A total of 31 mm was recorded at Heffley Creek in June and 33 mm in July. Maximum daily temperatures ranged from 13° - 19° C for 4 days after spraying, then increased to 23° - 24° C for 3 days, and cooled to 13° - 20° C for the next 6 days. Mean daily temperatures for June and July were 12.5° and 15.6° C respectively.

3. Trees. In each spray and control area, 30 Douglas-fir trees were selected and marked with surveyor's tape. Ten were *dominant* trees, with fully developed crowns, ample growing space and exposure to sunlight; 10 were *Co-dominant* trees, with fairly well developed crowns, but were partially shaded by adjacent, larger trees; and 10 were *Intermediate* trees with poorly developed crowns, and heavily shaded by surrounding, larger trees.

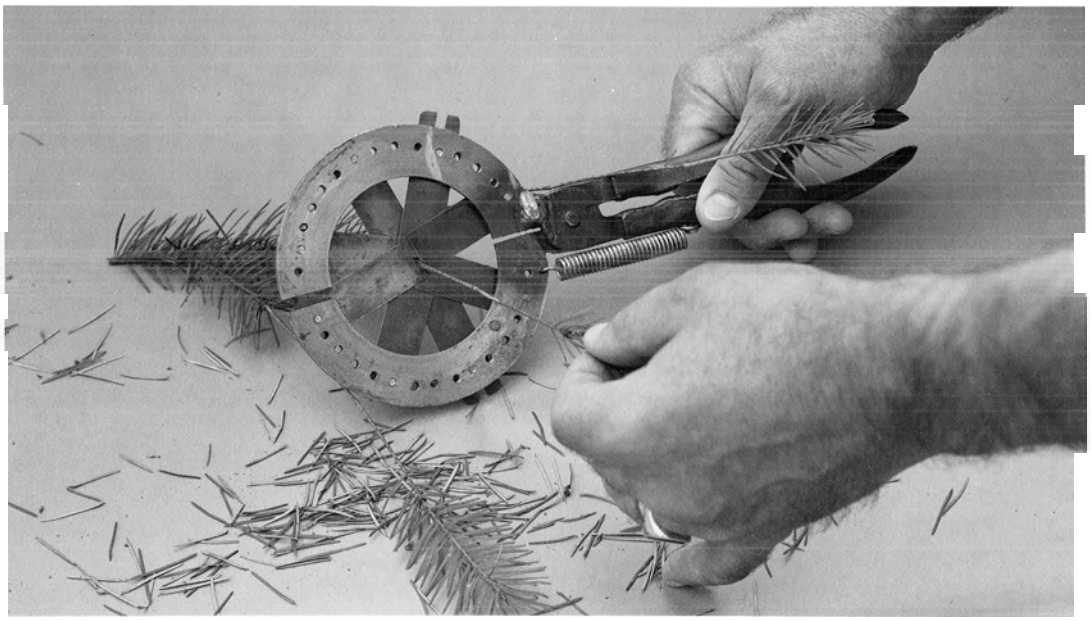
4. Forest Litter. The forest floor, from which litter samples were obtained, was classified as: *Open*, fully exposed, 10 to 15 m away from the nearest tree canopy; *Semi-open*, partially shaded by a light tree canopy above the site, and *Dense*, heavily covered by tree canopies, having no direct exposure to sunlight. A plot of 2 x 2 m was marked at each of these 3 sampling sites and cleared of large objects such as branches and rocks, prior to spraying.

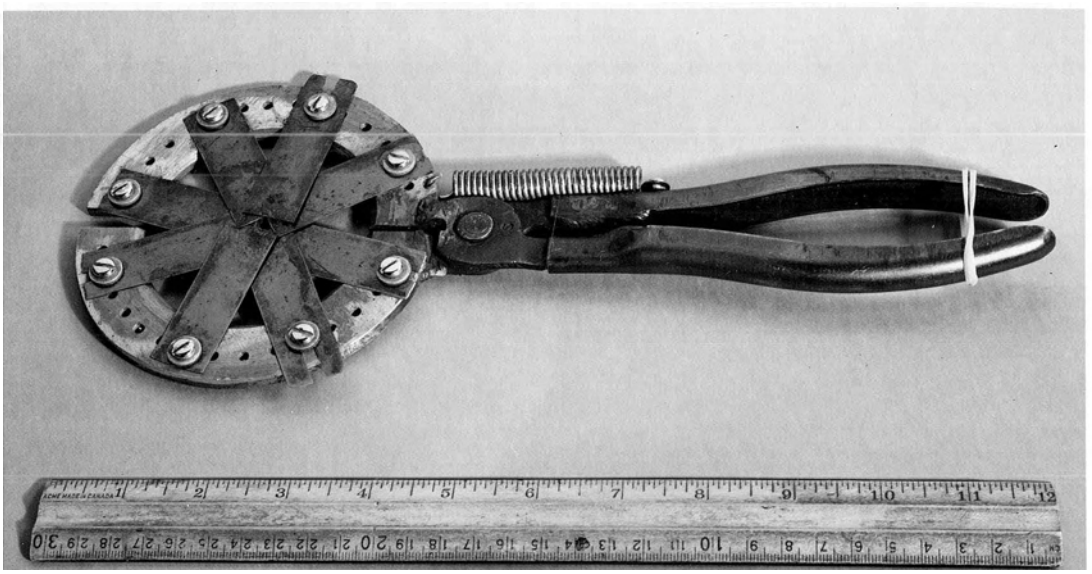
5. Sampling Needles. At each sampling, one branch was taken from the upper, middle, and lower one-third of every dominant tree; the upper and lower one-half of every co-dominant tree; and one branch from every intermediate tree. The needles were then stripped with *Noble Needle Nippers* (Fig. 3), wrapped in heavy-duty aluminium foil, and frozen on dry ice immediately in the field for transport to the laboratory, where they were held at -20° C until analysis.

6. Sampling Litter. Litter samples of 30.5 x 30.5 cm, 2.5 cm deep, were taken from the open, semi-open, and dense plots with a

Figure 3

Noble Needle Nippers: stripping Douglas-fir needles (above), and arrangement of adjustable and exchangeable steel blades (below).





sharpened spade, placed on heavy-duty aluminium foil, rolled up and wrapped, and further handled as described for needles.

7. Sampling with Glass Surfaces. Lids and bottoms of clean 15-cm glass Petri dishes were placed near the 3 marked plots. In the spray area, 2 dishes were used in the open plot, and 3 dishes each in the semi-open and densely covered plots; 1 dish per plot was used in the unsprayed control area. Three hours after spraying, the dishes were closed, wrapped in aluminium foil, packed in dry ice and transported to the laboratory. The surface deposits were analyzed upon arrival.

8. Bioassay. A total of 4,187 adult worker ants (*Formica integroides* Emery) were collected from a nearby colony 12 h prior to spraying and placed into 12 dissecting dishes with the inside walls coated with Fluon (polytetrafluoroethylene). Next morning, 1 h prior to spraying, they were distributed in the litter sampling plots of the spray area as follows: 3 dishes each in the open (1,099 ants), semi-open (879 ants), and dense (845 ants), plus 1 dish in each of the corresponding plots of the unsprayed control area (419, 566, and 379 ants, respectively). They were placed immediately adjacent to the glass Petri dishes and the marked litter sampling plots, then collected 3 h after spraying and moved to a nearby temporary laboratory for mortality counting 12, 24, and 36 h after spraying.

From the mortality data, corrected for control mortalities, LT_{50}

values and their 95% confidence limits were determined according to the method of Swaroop (1966). LT_{50}'s were also computed by Fortran Probit analysis.

II. Laboratory Studies.

1. Ultraviolet Radiation Studies. Five µg of acephate in 0.5 ml of acetone were applied to a 5-cm glass Petri dish and the solvent was removed by evaporation at room temperature under a gentle nitrogen stream. The treated glass Petri dish was placed in a Chromatovue Model CC-2-UV Viewer and irradiated with either ultraviolet light (253.7 nm) or incandescent light at about 20° C for 25 hours. The control samples were shaded by a cardboard cover to shield them from the light. After irradiation, residues were removed immediately from the glass surfaces of the Petri dishes for analysis.

2. Hydrolysis Studies. Samples of glass-distilled water were buffered to pH 4.0, 5.0, 5.6, 6.0 (citrate plus sodium citrate, 0.2 M), 6.9 and 8.2 (Tris plus HCl, 0.2 M) according to Dawson *et al.* (1969). One-ml aliquots of an aqueous solution containing 1,000 µg/ml of acephate and 9-ml aliquots of the buffer solutions were added to 25-ml glass-stoppered Erlenmeyer flasks, mixed, and then held for 20 days at either 20° C or 30° C. At the end of incubation, one-ml aliquots of each sample were diluted with glass-distilled acetone for immediate analysis of acephate and methamidophos by GLC without further clean-up.

3. Studies with Two Natural Waters and Their Sediments.

Approximately 10 litres of water and 5 kg of sediment were taken from each a small pond and a creek. Both are located in a forested area in the Fraser Canyon (49°, 40'N,; 121°, 22'W.) draining into the salmon-bearing Fraser River. The samples, kept separately in glass containers on ice, were transferred to the laboratory immediately. The temperatures of the pond and the creek waters at the time of sampling were 9° C and 6° C respectively. Subsequent incubations in the laboratory were carried out at 9° C.

(a) Incubation of Water Samples. Ten 150-ml aliquots of creek water, and 12 150-ml aliquots of pond water were measured into individual 500-ml Erlenmeyer flasks. Then, 100 µl of an aqueous solution, containing 1,500 µg/ml of acephate, were added to each flask, giving a concentration of 1 ppm of acephate in each water sample. The flasks were then plugged with glass wool (Oloffs *et al.* 1972, 1973) and incubated at 9° C, creek water for 0, 7, 21, 34, and 50 days, and pond water for 0, 2, 7, 14, 21, and 42 days.

(b) Incubation of Water Samples with Sediments. Ten 100-g aliquots of creek sediment, and 12 of pond sediment, were placed into individual 500-ml Erlenmeyer flasks to give a layer of even thickness. Then, a 150-ml portion of the corresponding water was added slowly to each flask so that the layer of sediment was not disturbed. Each water sample was then treated with acephate, plugged with glass wool, and incubated as

described under (a).

At the end of each incubation period, the water was separated from the sediment by filtering through Whatman No. 1 filter paper. The glass wool plugs, water samples, and sediment samples (including the filter paper) were analyzed for acephate and methamidophos.

(c) Incubation after Autoclaving. Twelve samples of creek water, and 12 of creek water plus sediment, were placed into 24 500-ml Erlenmeyer flasks as described under (a) and (b). Six flasks of each set were then wrapped in aluminium foil and autoclaved for 45 min. After the 12 autoclaved samples had cooled, all 24 were treated with acephate as described under (a) to give a concentration of 1 ppm in the water, and incubated at 9° C for 0, 43, and 50 days.

III. Analytical Procedures.

1. Extraction and Clean-up.

(a) Needles, Litter, Glass Surfaces. Sub-samples of 10 g of needles, and 7.6 x 7.6 x 2.5-cm sub-samples of litter, were extracted in a Lourdes blender three times each with 100 ml pesticide-grade ethyl acetate; during the first extraction 5 g of anhydrous granular sodium sulfate were added. The extracts were filtered through glass wool and a layer of granular anhydrous sodium sulfate into 500-ml round-bottom flasks, then concentrated to approximately 10 ml in a flash evaporator at 38° C. After quantitative transfer with 100 ml acetonitrile into a 250-ml separatory funnel, each extract was partitioned three times with 25 ml

hexane to remove terpenoid interferences. The acetonitrile phase was then evaporated just to dryness, the residue dissolved in 5 ml ethyl ether and transferred to a 15-g silica gel column. The column was eluted with 100 ml ethyl ether, followed by 100 ml of 2% methanol in ethyl ether. These eluates were discarded. Acephate and methamidophos were then eluted from the column with 250 ml of 10% methanol in ethyl ether. The eluate was evaporated just to dryness and the residue dissolved in 5 ml acetone for GLC (gas-liquid chromatography) analysis.

Two sub-samples of each needle sample and 4 sub-samples of each litter sample were analyzed. The procedures described were adopted mainly from Leary (1971, 1974) and were similar to those reported by Sundaram and Hopewell (1976).

The residues were removed with 10 ml of acetone from the glass surfaces of the Petri dishes used in the field studies (see I, 7, p. 16) and the ultraviolet radiation studies (see II, 1, p.17). The acetone solutions were analyzed for acephate and methamidophos by GLC without further clean-up.

(b) Water. Acephate and methamidophos were extracted from water samples and simultaneously cleaned by a coconut charcoal column (McKinley 1977, personal communication). Coconut charcoal (Fisher Scientific Company) was sieved through a 60-mesh screen and the fines were discarded. The charcoal retained by the screen was washed three times with glass-distilled acetone and dried at 130° C for 48 h before

use. Chromatographic columns (50 x 1.0 cm i.d.) with Teflon stopcocks and 250-ml reservoirs were packed, in that order, with a glass wool plug, a 5-cm layer of Ottawa sand, 2 g of coconut charcoal, and a second 5-cm layer of Ottawa sand. Each column was connected to a 500-ml suction flask. The column was first washed with 500 ml of glass distilled water under aspiration to remove charcoal fines. The water sample containing acephate and methamidophos was then allowed to pass through the charcoal column without aspiration. The sample container was rinsed three times with 10 ml of glass-distilled water and the rinsings transferred to the charcoal column. After elution without suction was completed, residual water was removed from the column by aspiration for 30 min. The acephate and methamidophos were then eluted from the column with 50 ml of glass-distilled acetone, and the eluate concentrated to 5 ml for GLC analysis.

(c) Sediments. One hundred grammes of sediment were extracted three times with 100 ml of pesticide-grade acetonitrile by blending in a Lourdes homogenizer for 5 min each time; 100 g of anhydrous granular sodium sulfate were added at the beginning of the first extraction. After each extraction, the liquid phases were filtered through glass wool and a layer of granular anhydrous sodium sulfate into a 500-ml round-bottom flask. The filtrate was evaporated just to dryness in a flash evaporator at 38° C. The residues were dissolved in 4 ml of glass-distilled acetone then 50 ml of coagulating solution were added to the round-bottom flask. The coagulating solution was 1.5 g of ammonium chloride and 3 ml of 85% phosphoric acid in 1 litre of aqueous solution.

After 15 min, the sample was filtered through a sintered-glass filter funnel (medium) with 2.5 cm of Celite 545. The filter funnel and flask were rinsed twice with 25 ml of coagulating solution and three times with 25 ml of glass-distilled water. The acephate and methamidophos were extracted from the filtrates and simultaneously cleaned as described in Section III, 1, p. 20.

(d) Glass Wool. Glass wool plugs were extracted with 10 ml glass-distilled acetone. The extracts were concentrated to 2 ml under a gentle nitrogen stream for GLC analysis without further clean-up.

2. GLC Analysis. For this study, a Tracor MT 220 gas chromatograph was used, equipped with a flame photometric detector (526 nm phosphorus filter) and Teflon columns (61 x 0.32 cm o.d.) packed with 1% Carbowax 20M TPA on Chromosorb W "HP", 100/200 mesh. Operating parameters were: detector 165° C; injection port 185° C; column oven programmed from 135° C to 185° C at 30° C/min; gas flow rates for N_2, H_2, O_2, and air, respectively, 60, 180, 15, and 80 ml/min.

Clean extracts (1 ml = 2 g of needles; 11.5 cm^2 of litter; 30 ml of water; 20 g of sediments; 4 cm^2 of glass surfaces; or one half of the glass wool plug) were suitably diluted for GLC analysis if necessary. If 8 µl of undiluted extract gave no response, the results were reported as non-detectable (N.D.). The limits of quantification of acephate (methamidophos) were: 0.01 (0.005) ppm for needles; 0.004 (0.002) $\mu g/cm^2$ for litter; 0.002 (0.001) ppm for water; 0.004 (0.002) ppm for sediments;

0.001 (0.0005) µg/cm^2 for glass surfaces; and 0.075 (0.025) µg/glass wool plug. For the hydrolysis samples, the limit was 4 µg of methamidophos per sample (acephate not applicable). Detectable responses below these limits were reported as *Trace* and computed as one half of the appropriate quantification limit.

3. Recovery Studies. Recovery studies on needles, litter, water, and sediment were conducted on 4 replicate sub-samples from the unsprayed controls. Needle and litter samples were fortified with acephate and methamidophos at 1-ppm and 0.1-ppm levels of each. Recoveries ranged from 95.9% to 98.4% for acephate, and 92.2% to 96.9% for methamidophos at the 1-ppm level; from 90.0% to 98.0% for acephate, and 83.0% to 89.0% for methamidophos at the 0.1-ppm level. Water and sediment samples, also fortified with both insecticides at 1-ppm and 0.1-ppm levels, gave 86.1 to 93.1% recoveries.

4. Storage Stability. Residue-free needle and litter samples from the unsprayed control plots were fortified with acephate to give concentrations of either 1.0 or 0.1 ppm; additional samples were fortified at the same rates with both acephate and methamidophos. Sub-samples of these were analyzed shortly after fortification. The remains of the fortified samples were stored at -20° C, together with the field samples. Further sub-samples were analyzed periodically, until all the field samples had been analyzed, to determine any possible residue loss and/or conversion of acephate to methamidophos during storage.

RESULTS AND DISCUSSION

1. Storage Stability of Acephate. No residues, or equivalent GLC responses, were detected in any control or pre-spray samples. Analyses of needle and litter samples were completed after 5 and 9 months, respectively. During this storage period, no detectable decrease occurred in residue concentrations and there was no conversion of acephate to methamidophos.

Short-term laboratory experiments, carried out prior to the field work, had produced similar results, but it was important to monitor the storage stability until the analyses had been completed, since no hard data in this respect were available. It is evident that freezing the samples on dry ice immediately in the field successfully stabilized the acephate residues. Other contributing factors were the relatively small samples and the wrapping in aluminium foil, a good heat conductor, so that the samples were quickly frozen.

2. Deposition of Acephate at Ground Level after Aerial Application. Acephate deposits on the glass surfaces, collected 3 h after spraying, were 1.95, 1.59, and 0.55 $\mu g/cm^2$ at the open, semi-open, and densely covered plots, respectively. No methamidophos could be detected on the glass surfaces. The results of bioassay in the field with ants are shown in Table I. They reflect the differences in acephate deposition on the glass surfaces in the three sampling plots. LT_{50}'s and their 95% confidence limits ( ), calculated according to Swaroop (1966), for ants

Table I.

Mortality of ants (*Formica integroides*) 12, 24, and 36 h after exposure to acephate applied by aircraft to a Douglas-fir forest at 1.12 kg/ha (%: observed mortality in percent; %corr.: after correction for control mortality).

	Number of ants tested	12 hours		24 hours		36 hours	
		%	%corr.	%	%corr.	%	%corr.
Acephate:							
Open	1,099	54.3	54.3	98.0	98.0	100	----
Semi-open	879	54.8	54.8	90.4	90.3	98.3	98.1
Dense cover	845	5.2	5.2	21.0	20.0	42.7	36.9
Control:							
Open	419	0	----	1.0	----	10.7	----
Semi-open	566	0.2	----	1.1	----	6.5	----
Dense cover	379	0	----	1.8	----	10.3	----

exposed at the respective sites were: 11.6 h (11.16 to 11.85); 11.2 h (10.6 to 11.8); and 47.5 h (44.3 to 49.9).

Fortran-computed regression lines of the mortality data are shown in Figures 4 to 6. The Fortran-computed LT_{50} values for the open and semi-open locations were also 11.6 h and 11.2 h, respectively, but 48.2 h for the densely covered plot. The difference between acephate deposits in open and semi-open areas was small, not enough to affect the mortality amongst the ants. But densely covered area received so much less chemical that the time to kill 50% of the ants exposed at this site was more than four times longer.

3. Residues in Douglas-fir Needles. Residues found in the needles are shown in Tables II and III and Figures 7 to 12. As expected, the initial concentrations were highest at 9.2 ppm in needles from the upper crowns of dominant trees, i.e. that portion of the forest tree canopy which is most exposed to aerial spray. They were predictably lower in needles from the middle and lower crowns of dominant trees, which were comparable with those in needles from co-dominant trees. The lowest initial concentrations occurred in the needles of intermediate trees; they were about one-third of the maximum.

Rates of residue decay are shown in Table IV. Calculated regression lines for residue decay in Douglas-fir needles from different classes of trees and crown levels are shown in Figures 7 to 12. After the application of acephate, the loss in residues of acephate *plus* methamidophos

(Cont'd p. 39)

Figure 4

Regression line (Fortran probit analysis) calculated from mortality of 1,099 ants exposed to aerial spray in a open sampling plot: LT_{50} = 11.6 h.

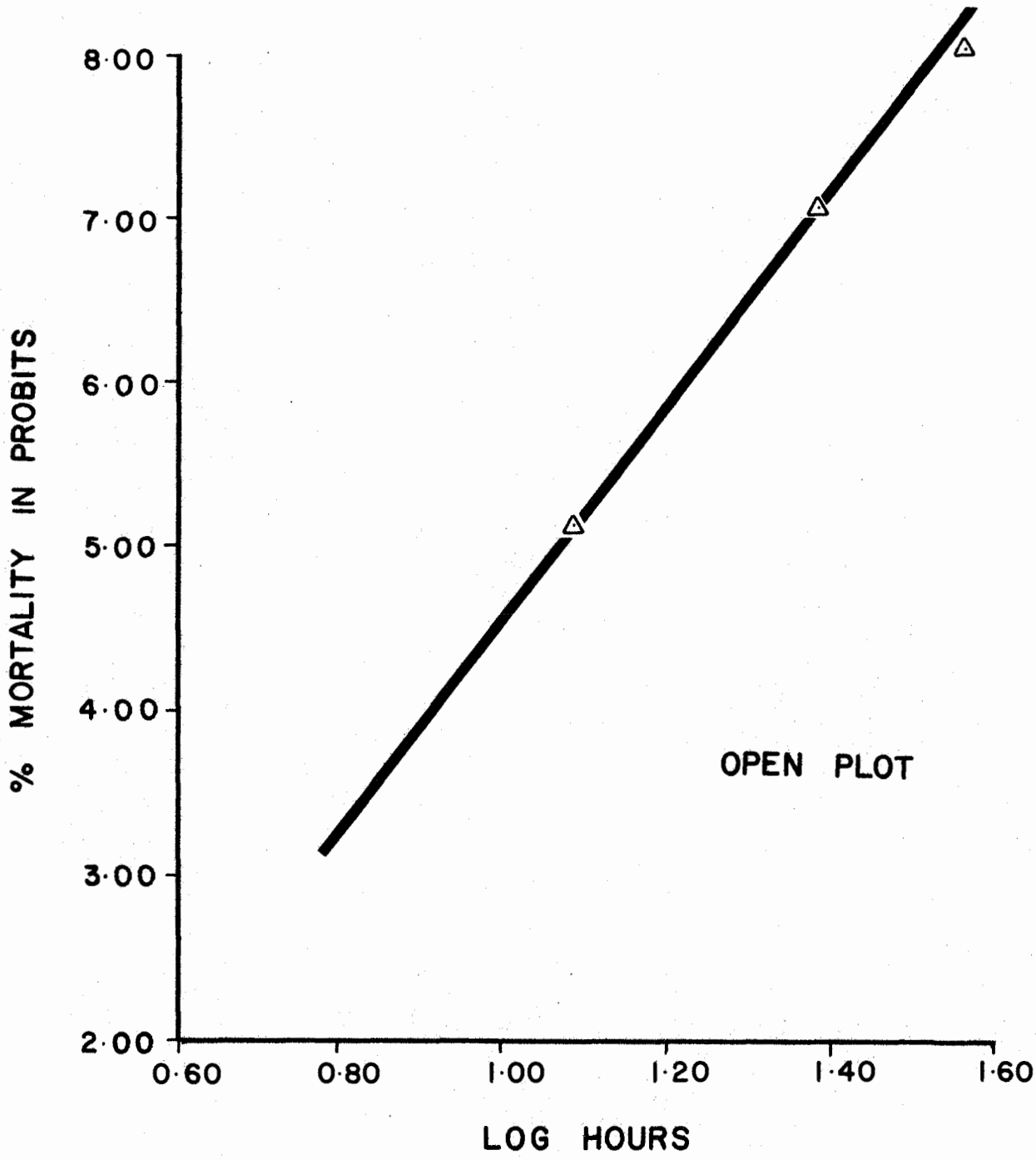

8·00
7·00
6·00
5·00
4·00
3·00
2·00
% MORTALITY IN PROBITS
0·60
0·80
1·00
1·20
1·40
1·60
LOG HOURS
OPEN PLOT

Figure 5

Regression line (Fortran probit analysis) calculated from mortality of 879 ants exposed to aerial spray in a semi-open sampling plot: LT_{50} = 11.2 h.

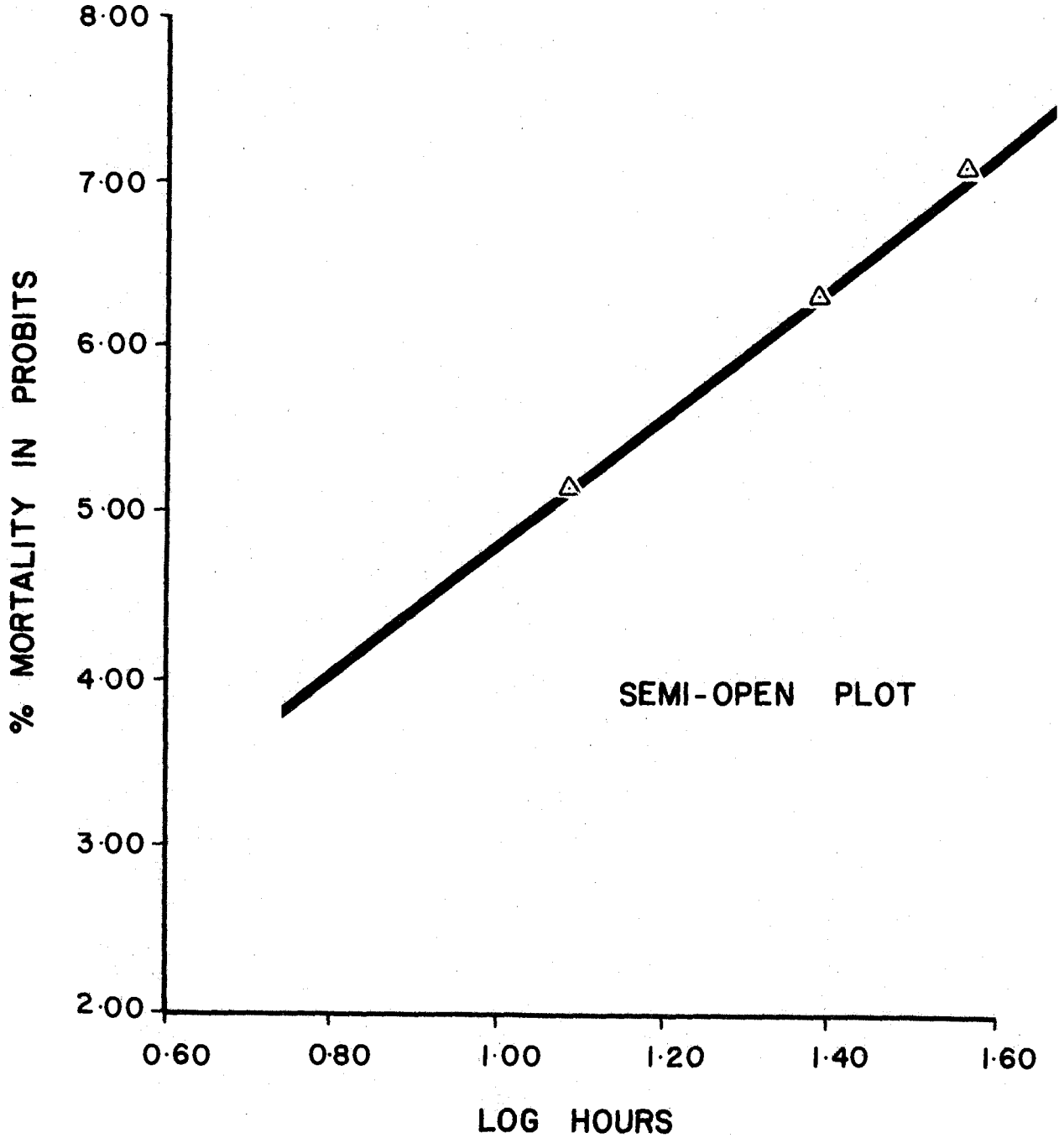

8·00
7·00
6·00
5·00
4·00
3·00
2·00
% MORTALITY IN PROBITS
SEMI-OPEN PLOT
0·60
0·80
1·00
1·20
1·40
1·60
LOG HOURS

Figure 6

Regression line (Fortran probit analysis) calculated from mortality of 845 ants exposed to aerial spray in a densely covered sampling plot: LT_{50} = 48.2 h.

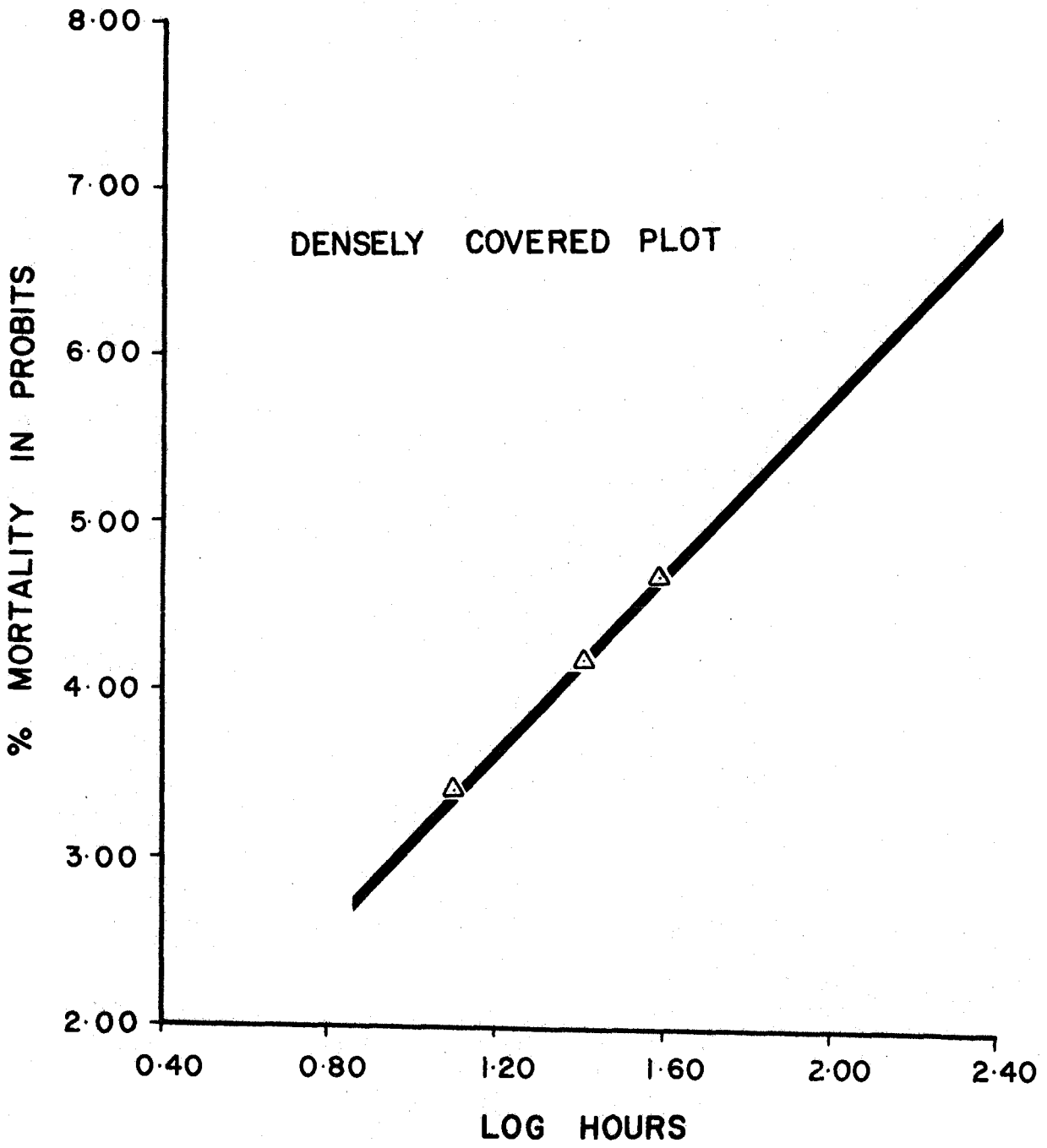

DENSELY COVERED PLOT
% MORTALITY IN PROBITS
8·00
7·00
6·00
5·00
4·00
3·00
2·00
0·40
0·80
1·20
1·60
2·00
2·40
LOG HOURS

Table II.

Residues in needles of dominant Douglas-fir trees (U = upper, M = middle, L = lower crown).

Time after Spraying		Crown	Acephate			Methamidophos		
Hours	Days	Level	ppm ±	SD	%[a/]	ppm ±	SD	%[a/]
3		U	9.08	0.07	98.9	0.093	0.02	1.1
		M	7.33	0.04	98.5	0.115	0	1.5
		L	6.21	0.34	98.1	0.118	0.016	1.9
27	1	U	9.18	0.40	98.8	0.11	0.01	1.2
		M	6.83	0	98.3	0.113	0.003	1.7
		L	5.71	0.10	98.4	0.084	0.004	1.6
51	2	U	5.63	0.05	98.3	0.11	0.02	1.7
		M	4.58	0.29	97.9	0.101	0.01	2.1
		L	4.14	0.30	97.0	0.129	0.003	3.0
72	3	U	4.70	0.30	98.9	0.051	0.006	1.1
		M	3.39	0.09	98.0	0.067	0.004	2.0
		L	3.22	0.17	97.0	0.103	0.02	3.0
240	10	U	0.550	0.007	94.7	0.031	0.001	5.3
		M	0.364	0.006	92.4	0.033	0.004	7.6
		L	0.692	0.08	94.5	0.040	0.001	5.5
480	20	U	0.163	0.006	91.1	0.016	0.004	8.9
		M	0.121	0.008	91.0	0.013	0.0006	9.0
		L	0.127	0.006	86.4	0.02	0.01	13.6
720	30	U	0.092	0.002	87.6	0.013	0.002	12.4
		M	0.068	0.003	85.0	0.011	0.001	15.0
		L	0.079	0.02	86.8	0.012	0.008	13.2
1,080	45	U	0.04	0.007	85.1	0.008	0.0003	14.9
		M	0.022	0.005	84.6	0.006	0.001	15.4
		L	0.018	0.003	75.0	0.006	0	25.0
1,440	60	U	N.D.[b/]			N. D.		
		M	N.D.			N. D.		
		L	N.D.			N. D.		

a/ Percent of acephate *plus* methamidophos.

b/ N. D. = non-detectable: acephate <<< 0.01 ppm;
methamidophos <<< 0.005 ppm.

TABLE III.

Residues in needles of co-dominant and intermediate Douglas-fir trees (CU = co-dominant, upper; CL = co-dominant, lower crown; IN = intermediate).

Time after Spraying		Crown	Acephate			Methamidophos		
Hours	Days	Level	ppm ±	SD	%[a]	ppm ±	SD	%[a]
3		CU	7.79	0.23	98.2	0.138	0.006	1.8
		CL	6.14	0.05	97.2	0.18	0.02	2.8
		IN	3.28	0.25	96.8	0.111	0.015	3.2
27	1	CU	7.64	0.22	98.8	0.098	0.01	1.2
		CL	6.45	0.06	98.6	0.087	0.005	1.4
		IN	3.53	0.16	97.8	0.082	0.001	2.2
51	2	CU	4.54	0.23	97.6	0.112	0.004	2.4
		CL	3.90	0.02	95.6	0.124	0.001	4.4
		IN	3.62	0.121	96.8	0.121	0.004	3.2
72	3	CU	3.76	0.09	98.2	0.072	0.002	1.8
		CL	3.30	0.01	97.6	0.079	0.001	2.4
		IN	2.31	0.35	96.7	0.073	0.008	3.3
240	10	CU	0.733	0.18	94.6	0.042	0.003	5.4
		CL	1.14	0.07	91.9	0.096	0.01	8.1
		IN	1.03	0	91.2	0.1	0.02	8.8
480	20	CU	0.135	0.002	91.8	0.012	0.0005	8.2
		CL	0.141	0.004	89.8	0.016	0.008	10.2
		IN	0.148	0.01	89.2	0.018	0.007	10.8
720	30	CU	0.109	0.01	88.6	0.014	0.0005	11.4
		CL	0.076	0.006	86.4	0.012	0.007	13.6
		IN	0.128	0.02	88.9	0.016	0.005	11.1
1,080	45	CU	0.023	0.003	79.3	0.006	0.0007	20.7
		CL	0.027	0.004	79.4	0.007	0.001	20.6
		IN	0.023	0.003	88.5	0.005	0	11.5
1,440	60	CU	N. D.[b]			N. D.		
		CL	N. D.			N. D.		
		IN	N. D.			N. D.		

a/ Percent of acephate *plus* methamidophos.

b/ N. D. = non-detectable: acephate <<< 0.01 ppm; methamidophos <<< 0.005 ppm.

Figure 7

Residues of acephate *plus* methamidophos in Douglas-fir needles from the upper crowns of dominant trees, computed linear regression lines, and *half-life* of residues computed from Phase 1.

Phase 1 (3 - 240 h): $y = 8.738 - 0.036\ x$

$r = -0.939$; significant at $p = 0.05$

half-life: 113.6 h (= 4.7 days)

Phase 2 (240 - 1,080 h): $y = 0.592 - 0.0006\ x$

$r = -0.858$

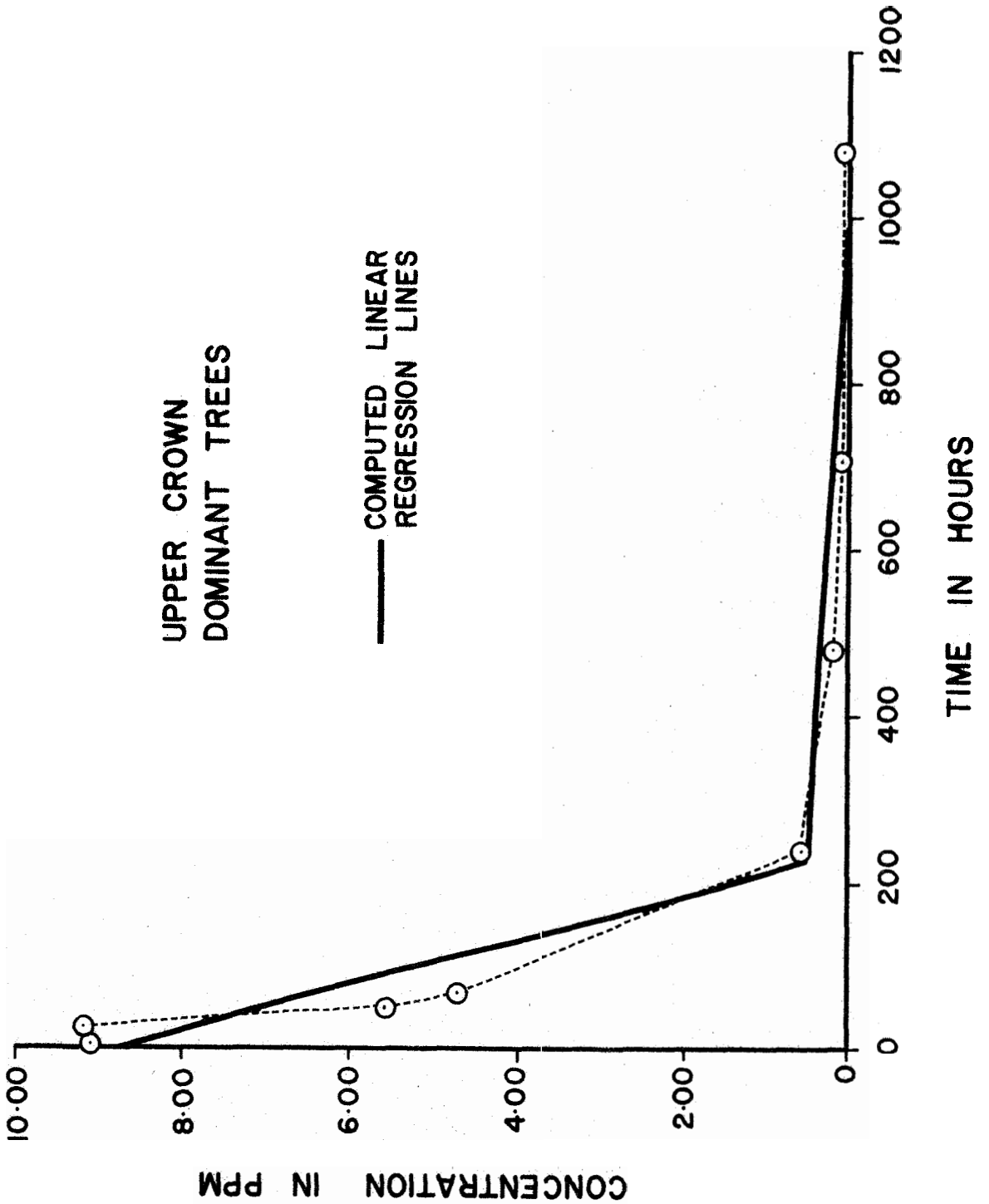

UPPER CROWN
DOMINANT TREES
COMPUTED LINEAR
REGRESSION LINES
CONCENTRATION IN PPM
10·00
8·00
6·00
4·00
2·00
0
0
200
400
600
800
1000
1200
TIME IN HOURS

Figure 8

Residues of acephate *plus* methamidophos in Douglas-fir needles from the middle crowns of dominant trees, computed linear regression lines, and *half-life* of residues computed from Phase 1.

Phase 1 (3-240 h): $y = 6.836 - 0.029\ x$

$r = -0.942$; significant at $p = 0.05$

half-life = 108.7 h (= 4.5 days)

Phase 2 (240 - 1,080 h): $y = 0.413 - 0.0004\ x$

$r = -0.880$

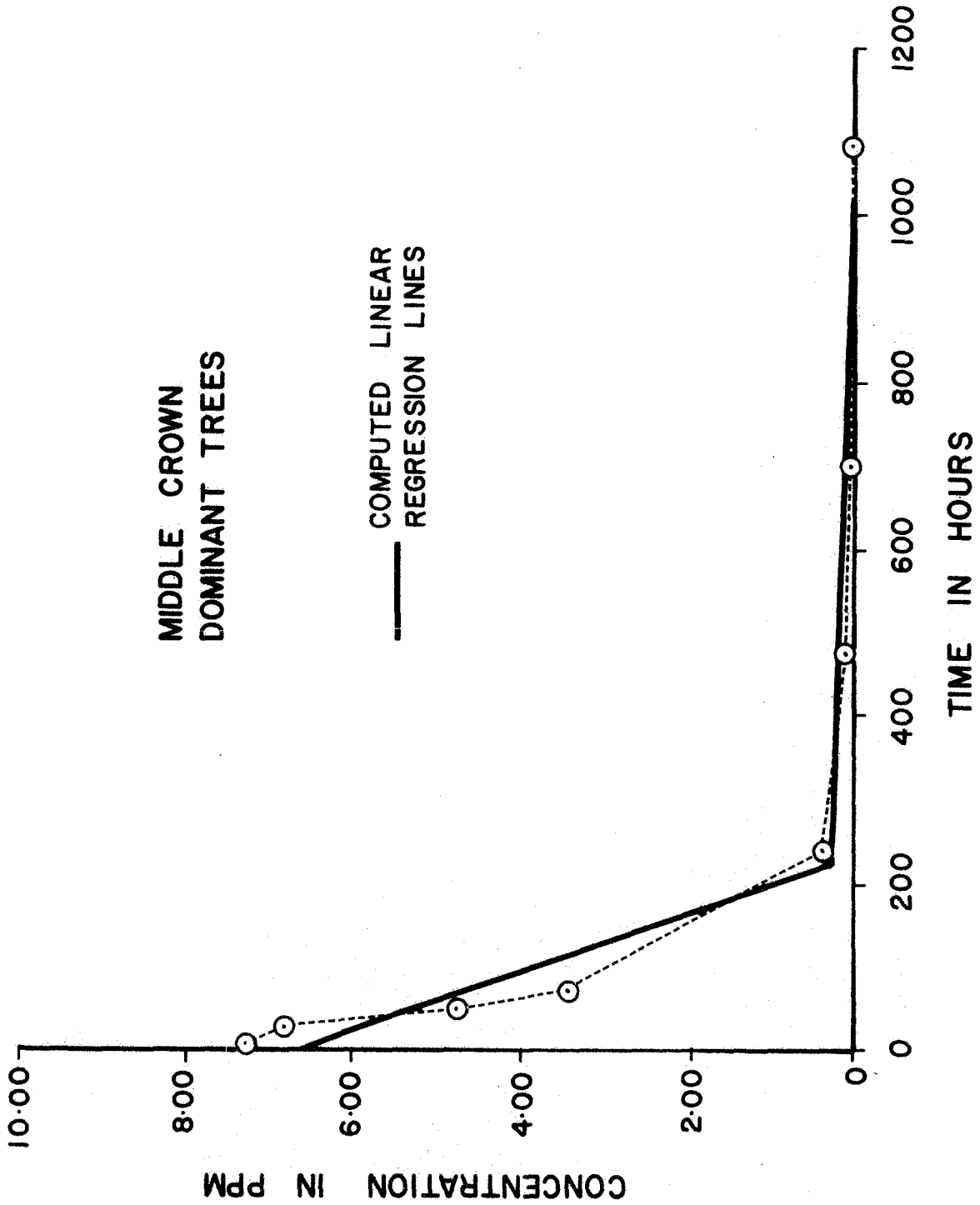

MIDDLE CROWN
DOMINANT TREES
COMPUTED LINEAR
REGRESSION LINES
CONCENTRATION IN PPM
10·00
8·00
6·00
4·00
2·00
0
0
200
400
600
800
1000
1200
TIME IN HOURS

Figure 9

Residues of acephate *plus* methamidophos in Douglas-fir needles from the lower crowns of dominant trees, computed linear regression lines, and *half-life* of residues computed from Phase 1.

Phase 1 (3-240 h): $y = 5.872 - 0.023\ x$

$r = -0.956$; significant at $p = 0.05$

half-life = 119.4 h (= 5 days)

Phase 2 (240 - 1,080 h): $y = 0.720 - 0.0007\ x$

$r = -0.823$

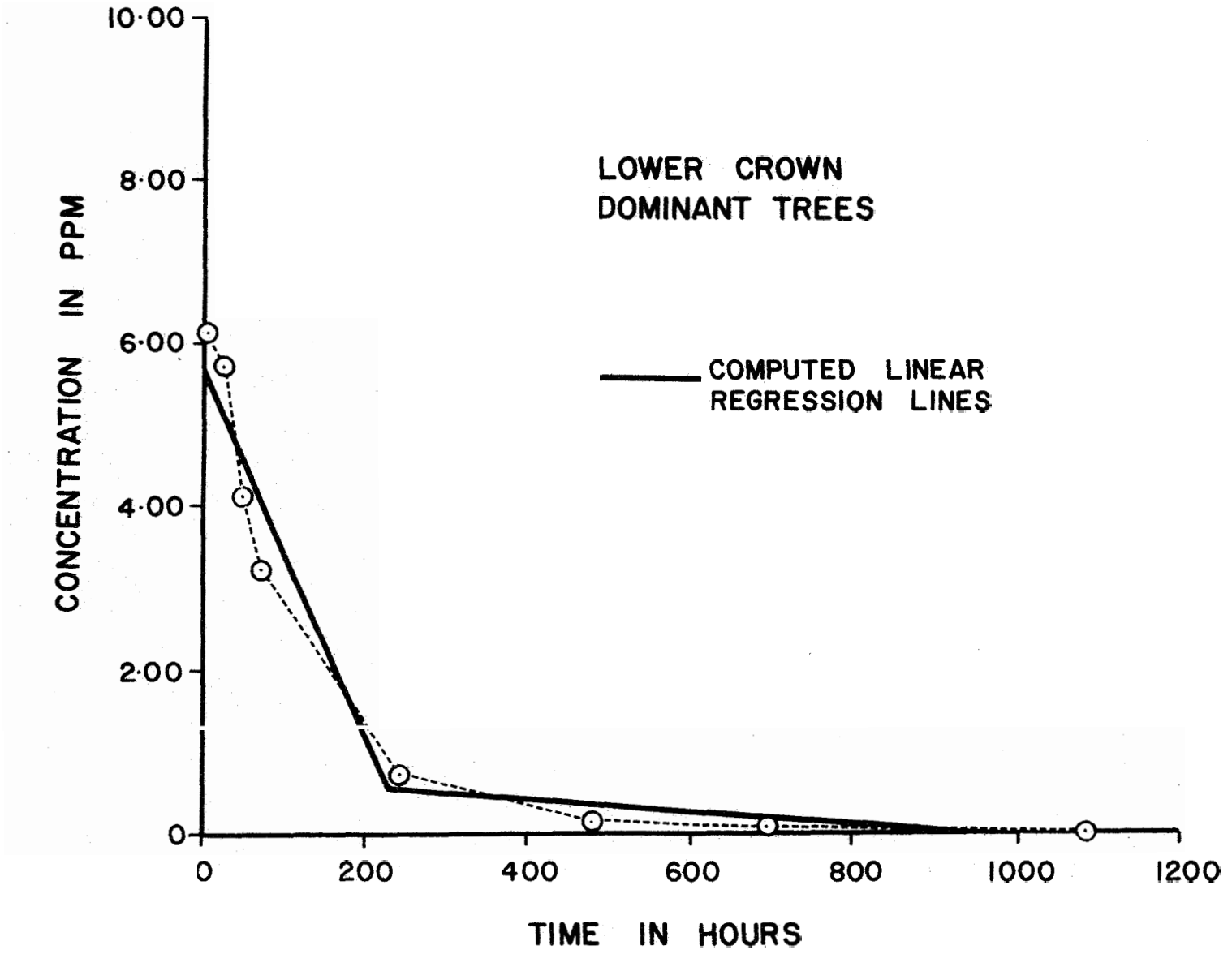

10·00
8·00
6·00
4·00
2·00
0
CONCENTRATION IN PPM
LOWER CROWN
DOMINANT TREES
COMPUTED LINEAR
REGRESSION LINES
0
200
400
600
800
1000
1200
TIME IN HOURS

Figure 10

Residues of acephate *plus* methamidophos in Douglas-fir needles from the upper crowns of co-dominant trees, computed linear regression lines, and *half-life* of residues computed from Phase 1.

Phase 1 (3 - 240 h): $y = 7.277 - 0.029 x$

$r = -0.920$; significant at $p = 0.05$

half-life = 113.6 h (= 4.7 days)

Phase 2 (240 - 1,080 h): $y = 0.759 - 0.0008 x$

$r = -0.818$

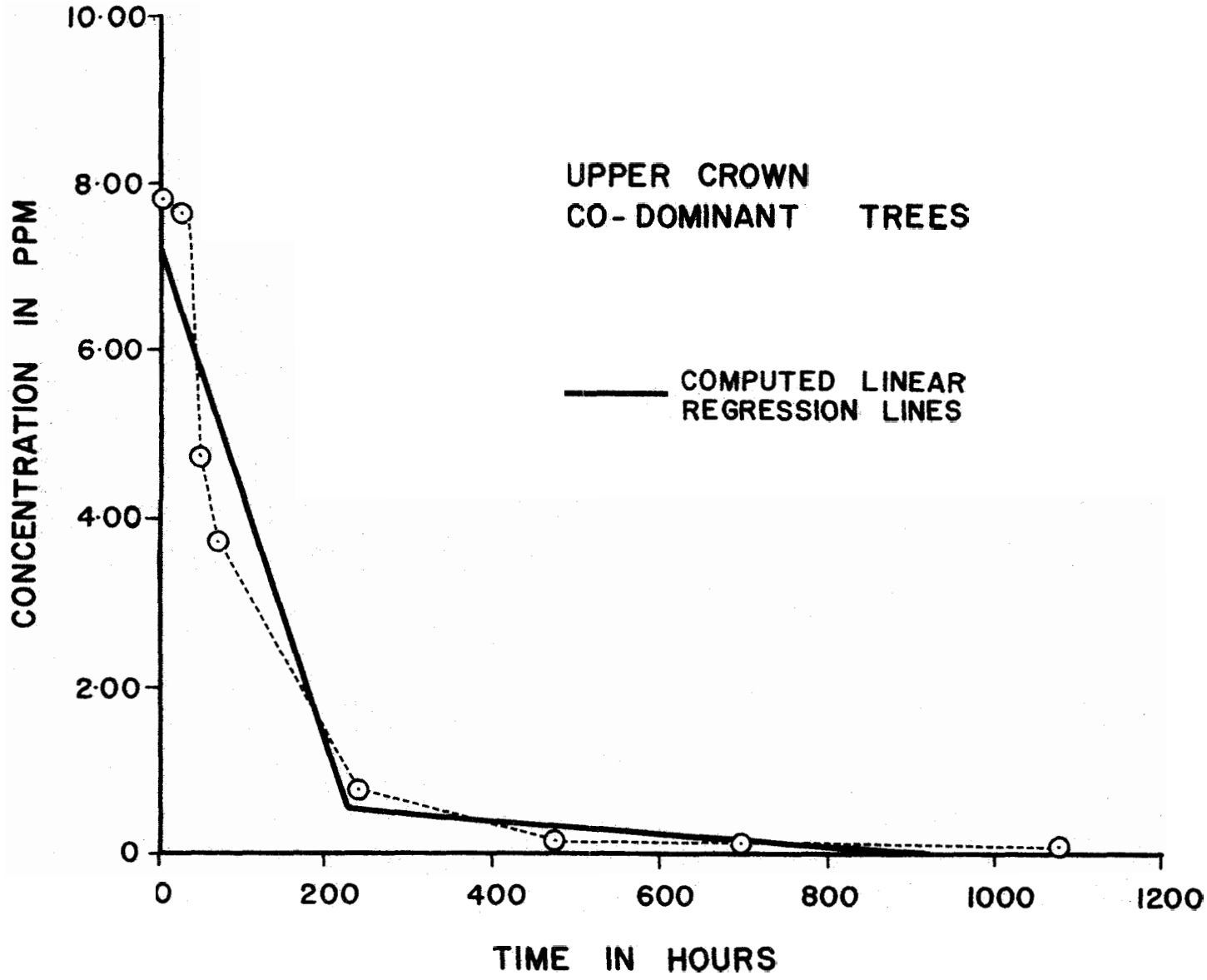

10·00
8·00
6·00
4·00
2·00
0
CONCENTRATION IN PPM
0
200
400
600
800
1000
1200
TIME IN HOURS
UPPER CROWN
CO-DOMINANT TREES
COMPUTED LINEAR
REGRESSION LINES

Figure 11

Residues of acephate *plus* methamidophos in Douglas-fir needles from the lower crowns of co-dominant trees, computed linear regression lines, and *half-life* of residues computed from Phase 1.

Phase 1 (3 - 240 h): $y = 5.970 - 0.021 x$

$r = -0.906$; significant at $p = 0.05$

half-life = 127.1 h (= 5.3 days)

Phase 2 (240 - 1,080 h): $y = 1.168 - 0.001 x$

$r = -0.782$

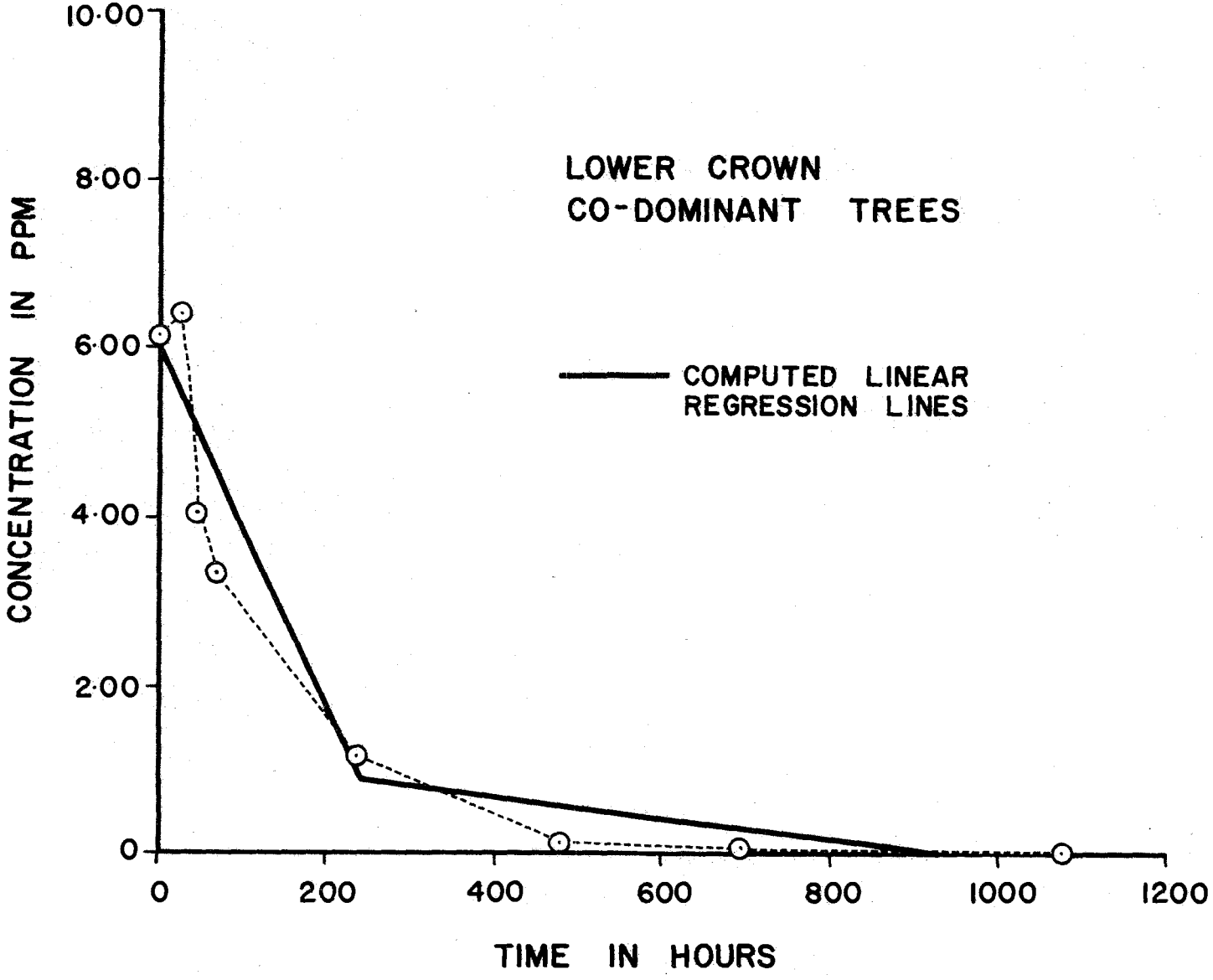

10·00
8·00
6·00
4·00
2·00
0
CONCENTRATION IN PPM
LOWER CROWN
CO-DOMINANT TREES
COMPUTED LINEAR
REGRESSION LINES
0
200
400
600
800
1000
1200
TIME IN HOURS

Figure 12

Residues of acephate *plus* methamidophos in Douglas-fir needles from intermediate trees, computed linear regression lines, and *half-life* of residues computed from Phase 1.

Phase 1 (3 - 240 h): $y = 3.698 - 0.011 x$

$r = -0.918$; significant at $p = 0.05$

half-life: 169.7 h (= 7 days)

Phase 2 (240 - 1,080 h): $y = 1.09 - 0.011 x$

$r = -0.80$

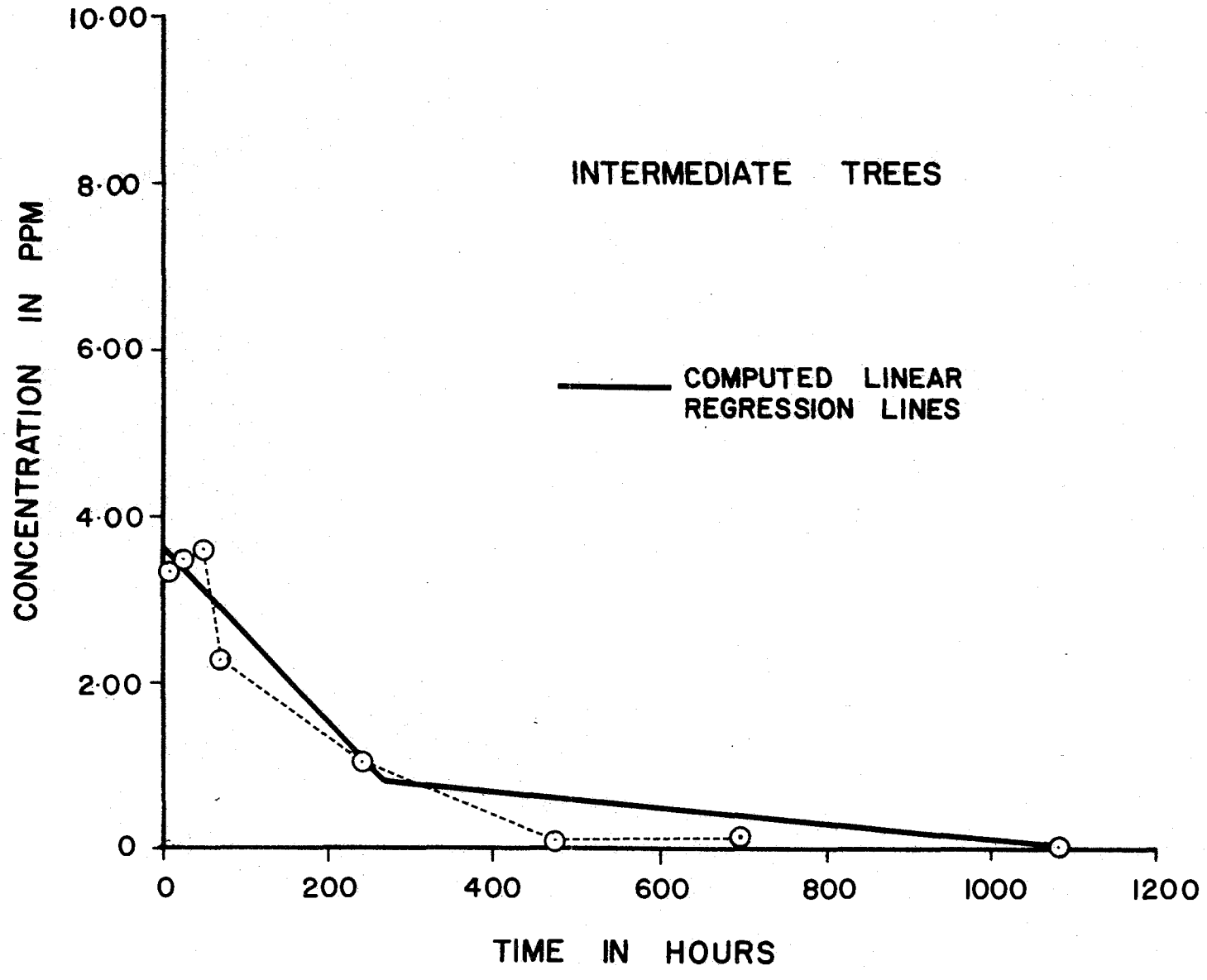

10·00
8·00
6·00
4·00
2·00
0
CONCENTRATION IN PPM
INTERMEDIATE TREES
COMPUTED LINEAR
REGRESSION LINES
0
200
400
600
800
1000
1200
TIME IN HOURS

TABLE IV.

Decay of residues in needles. Acephate *plus* methamidophos in percent of highest concentrations shown in Tables II and III.

Time after Spraying		Dominant			Co-dominant		Intermediate
Hours	Days	U	M	L	U	L	
3		99	100	100	100	97	91
27	1	100	93	92	98	100	97
51	2	62	63	68	59	62	100
72	3	51	46	53	48	52	64
240	10	6.3	5.3	12	9.8	19	30
480	20	1.9	1.8	2.3	1.9	2.4	4.4
720	30	1.1	1.1	1.4	1.5	1.3	3.9
1,080	45	0.51	0.37	0.39	0.37	0.52	0.75
1,440	60	--	--	--	--	--	--

appeared to be biphasic. During the first phase, there was a rapid decline in residue concentrations of acephate *plus* methamidophos from 3 to 240 h. The linear correlation between residue concentrations and time was significant at $p = 0.05$. During the second phase, residues decreased slowly. But the correlation between residue concentrations and time was not significant at $p = 0.05$.

In all needle samples, less than 1% of the highest concentration was present after 45 days. No residues could be detected after 60 days. However, the concentrations declined more slowly in intermediate than in dominant and co-dominant trees (Table IV). The *half-life*, or time after which 50% of the initial residues were left, was between 4 and 5 days in dominant and co-dominant trees, but 7 days in intermediate trees (Figures 7 to 12). The slower rate of residue decrease in intermediate trees remained apparent for 30 days (Table IV). This may have been due to lower metabolic activity of the suppressed intermediate trees, or to lack of direct exposure to sunlight and thus to reduced photo-decomposition. In dominant and co-dominant trees, the residues declined more slowly in needles from lower, less exposed branches than they did in the upper branches of the same trees (Table IV), which suggests that sunlight may have been an important factor.

These results for Douglas-fir are different than those reported by Sundaram and Hopewell (1976) for spruce trees, *Picea Glauca* (Moench). They reported initial concentrations of 55.15 ppm following an application

of only 0.28 kg/ha. By comparison, the initial residue concentrations found in this study following an application of 1.12 kg/ha seem low, even in view of the fact that the deposits found on fully exposed glass surfaces from the open area were only 17.6% of the theoretical value of 11.07 $\mu g/cm^2$, which is based on the assumption that 100% of 1.12 kg/ha were to reach ground level in the open area.

Sundaram and Hopewell (1976) also reported a *half-life* of less than 1 day, a decrease to 2.92 ppm (= 5.3% of initial) within 5 days, non-detectability after 32 days, and only traces of methamidophos. They did not, however, define traces. The differences between initial concentrations found in this study and those reported by Sundaram and Hopewell (1976) are possibly due to the different application methods. The other discrepancies suggest that geographic location, climate, and species studied, may significantly affect the persistence of residues of acephate.

4. Residues in Forest Litter. Residues found in litter from the three sampling plots are shown in Table V, and Figure 13. The data are given in $\mu g/cm^2$ rather than in ppm. The samples collected from the different plots, as well as the sub-samples from each sample varied greatly so that the weights of sub-samples (7.6 x 7.6 x 2.5 cm) for residue analyses differed from 21.4 to 239.1 g. Thus there is a lack of a constant weight base for comparison in ppm. Moreover, residue data in $\mu g/cm^2$ give a clearer indication of the actual distribution of residues on the ground.

TABLE V.

Residues in forest litter (O = open, S = semi-open, D = dense cover).

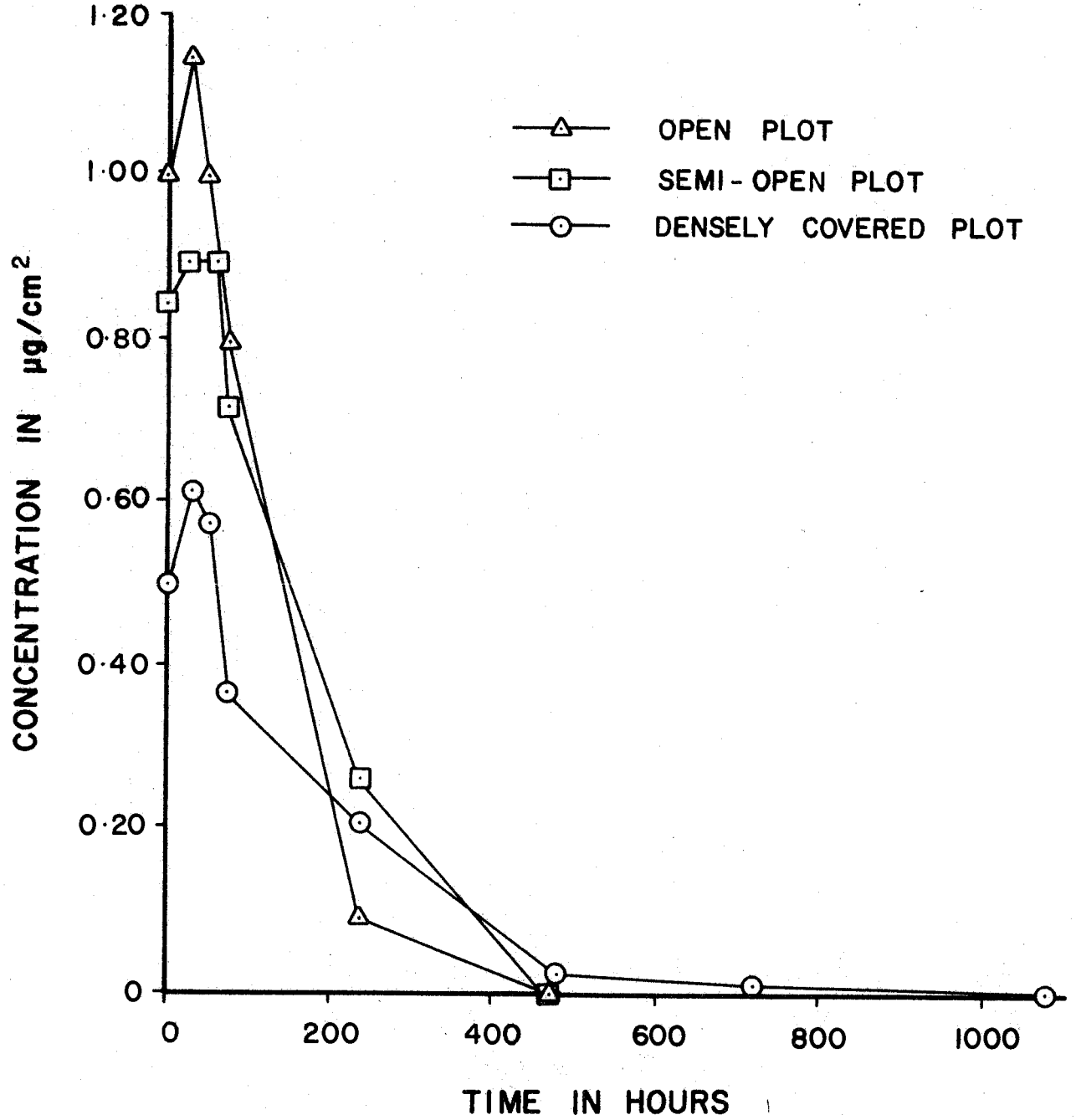
Time after Spraying Hours	Days	Forest Floor	Acephate µg/cm^2	± SD	%[a/]	Methamidophos µg/cm^2	± SD	%[a/]
3	-	O	0.886	0.114	87.0	0.133	0.012	13.0
		S	0.810	0.147	95.1	0.041	0.003	4.9
		D	0.438	0.043	88.2	0.059	0.002	11.8
27	1	O	1.102	0.133	92.8	0.054	0.003	7.2
		S	0.838	0.068	95.6	0.039	0.001	4.4
		D	0.581	0.051	94.3	0.036	0.004	5.7
51	2	O	0.943	0.100	91.6	0.087	0.012	8.4
		S	0.855	0.097	95.6	0.039	0.006	4.4
		D	0.503	0.079	88.5	0.065	0.008	11.5
72	3	O	0.700	0.100	88.7	0.089	0.005	11.3
		S	0.700	0.100	92.7	0.018	0.001	7.3
		D	0.326	0.036	89.2	0.040	0.008	10.8
240	10	O	0.079	0.01	89.7	0.009	0.001	10.3
		S	0.244	0.019	95.5	0.012	0.003	4.5
		D	0.173	0.021	85.8	0.029	0.003	14.2
480	20	O	N. D.[b/]			N. D.		
		S	N. D.			N. D.		
		D	0.016	0.005	69.0	0.007	0.001	31.0
720	30	O	N. D.			N. D.		
		S	N. D.			N. D.		
		D	0.012	0.003	70.9	0.005	0.0007	29.1
1,080	45	O	N. D.			N. D.		
		S	N. D.			N. D.		
		D	N. D.			N. D.		

a/ Percent of acephate *plus* methamidophos.

b/ N. D. = non-detectable: acephate <<< 0.004 µg/cm^2
methamidophos <<< 0.002 µg/cm^2.

Figure 13

Residues of acephate *plus* methamidophos in forest litter from the open, semi-open, and densely covered sampling plot.


1·20
1·00
0·80
0·60
0·40
0·20
0
CONCENTRATION IN µg/cm²
OPEN PLOT
SEMI-OPEN PLOT
DENSELY COVERED PLOT
0
200
400
600
800
1000
TIME IN HOURS

The initial concentrations in open and semi-open areas were little different but considerably higher than those in the densely covered area. These results corroborate those from deposits on glass surfaces and from bioassay with ants in the field.

Although residues in litter from open and semi-open plots were higher initially, they persisted only for 10 days. But residues in litter from the densely covered plot, lower initially, persisted for 30 days (Table V). As with the decay of residues in Douglas-fir needles from trees of different dominance and from branches at different crown levels, the decay of acephate residues in litter also suggests that sunlight may have been an important factor. Rates of residue decay are shown in Table VI. No decrease in residues of acephate plus methamidophos occurred in any of the litter samples up to 51 h after the aerial spray, but they rapidly decreased thereafter (Table VI, and Figure 13). This may have been due to the cool, cloudy weather shortly after spraying.

Fenitrothion (O, O-dimethyl O-(4-nitro-m-tolyl) phosphorothioate), a broad-spectrum organophosphorus insecticide, has been used intensively for the control of spruce budworm in eastern Canada since 1969. In comparison with acephate, it appears that fenitrothion is much more persistent than acephate. Yule and Duffy (1972) studied the fate and persistence of fenitrothion in forest soil and the foliage of balsam fir (*Abies balsamea* (L.) Mill) and mixed spruce (*Picea* spp.) following an aerial spray of fenitrothion at 0.28 kg/ha. They reported that concen-

TABLE VI.

Decay of residues in forest litter. Acephate *plus* methamidophos, in percent of highest concentrations shown in Table V.

Time after Spraying		Forest Floor		
Hours	Days	Open	Semi-open	Dense Cover
3		88	95	81
27	1	100	98	100
51	2	89	100	92
72	3	68	80	59
240	10	7.6	29	33
480	20	--	--	3.7
720	30	--	--	2.8
1,080	45	--	--	--

trations of residue in forest soil were 0.02-0.03 ppm up to 32 days after spraying; and decreased to less than 0.01 ppm after 167 days. Also, 2.25 ppm and 2.5 ppm of fenitrothion residues were found in the foliage of balsam fir and spruce, respectively, 1 day after application. The residues in foliage decreased by about 50% within 4 days. However, 0.28 ppm in balsam fir and 0.25 ppm in spruce were still detected after 336 days. In contrast, the application of acephate in this study was at the rate of 1.12 kg/ha, which is 4 times higher than that of fenitrothion in the study by Yule and Duffy (1972). However, acephate residues in forest litter persisted only for 10 days in the open and semi-open areas; and 30 days in the densely covered area (Table V). Also, in all Douglas-fir needle samples, less than 1% of the highest residue concentration was present after 45 days. No residues could be detected after 60 days (Table IV). Furthermore, in the study by Sundaram and Hopewell (1976), the initial concentration of acephate in spruce foliage was 55.15 ppm following a simulated aerial application of 0.28 kg/ha. The residue concentration decreased to 2.92 ppm (= 5.3% of initial) within 5 days, non-detectability after 32 days. It is apparent that acephate is non-persistent compared with fenitrothion.

5. Conversion of Acephate to Methamidophos. Some conversion of acephate to methamidophos occurred in all needle and litter samples. The percentage of methamidophos in the total residue (acephate *plus* methamidophos) increased from 1.1% to 28% in Douglas-fir needles up to 45 days

after acephate application. However, the actual concentrations of methamidophos declined rapidly, beginning 2 to 3 days after the application of acephate. They became non-detectable by the time acephate could no longer be detected.

The concentrations of methamidophos residues found in both Douglas-fir needles and litters were low compared to initial acephate concentrations. Therefore, the primary degradation process of acephate could be through the rupture of P—N, P—O, or P—S bonds rather than the N—C bond. The low concentrations of methamidophos residues found in this study are in agreement with those reported by Sundaram and Hopewell (1976) but differ from Werner's findings (1974 b). Werner reported that ^{14}C-acephate was absorbed rapidly from nutrient solution by 130-day old loblolly pine seedlings within 1 h after treatment. Acephate was converted to methamidophos and accumulated in various parts of the plant. Methamidophos residues which accounted for 42.6% of the total radioactivity, were found in the needles 6 h after treatment. The significant conversion of acephate to methamidophos observed by Werner may be due to enzymes in the young roots of pine seedlings. For instance, Oloffs (1970) demonstrated that aldrin was converted to its epoxide, dieldrin by enzymes from roots of young pea seedlings.

6. Photodecomposition of Acephate. Acephate on clean glass surfaces was readily decomposed when exposed to UV radiation (253.7 nm) at room temperature for 25 hours (Table VII). The recovery of acephate

TABLE VII.

Decomposition of acephate on glass surfaces (5 µg/20 cm^2) exposed to 253.7-nm ultraviolet (UV-open) and incandescent (I-open) light for 25 h at 20° C. Control samples were shaded by cardboard paper (UV-shaded; I-shaded).

Sample	Acephate recovered [a/] microgrammes $\bar{X}$ ± S. D.[b/]	percent
UV-open	0.83	16.6
UV-shaded	3.22	64.4
I-open	3.48	69.6
I-shaded	3.29	65.8

a/ No methamidophos detected; detection limit: 0.0005 µg/cm^2.

b/ N = 4

after exposure to UV radiation was 16.6%. On the other hand, 69.6% were recovered after 25 hours in samples exposed to white light at room temperature, comparable to the 65.8% recovery for samples without white light exposure and the 64.4% recovery for samples without UV-exposure (Table VII). It is evident that white light has no apparent effect on the photodecomposition of acephate, the loss in the samples not being subjected to UV radiation may only be due to the volatilization of acephate from the glass Petri dishes.

Methamidophos was not detected in any sample in this study. It suggests that the primary photodecomposition process of acephate could be through the rupture of P-N, P-O, and P-S bonds rather than the N-C bond of the molecule.

The effect of UV radiation on the phototransformation and photodecomposition of organic pesticides has been well documented. The classic example is the photoisomerization of isodrin; photoisodrin was obtained after photolysis with a 2537-nm lamp for 7 days in a carbon dioxide atmosphere (Bird *et al.* 1961). Other cyclodiene insecticides also undergo photoisomerization. Benson *et al.* (1971) reported that sunlight or short-wave UV light produced photo-cis-chlordane from cis-chlordane through hydrogen migration and carbon-carbon bond formation. But does the photodecomposition and phototransformation of pesticides, as demonstrated under laboratory conditions, really take place in the environment? Are light-energized decomposition and transformation

of pesticides environmentally important? Generally, it has been believed that all of the sun's emitted radiation in the UV region below 285 nm is absorbed by the layer of ozone in the earth's atmosphere. Therefore, it should be doubtful that photodecomposition and phototransformation of pesticides by short-wave UV radiation really takes place in the environment.

Nevertheless, phototransformation products of pesticides have been detected in environmental samples. Wilson and Oloffs (1973a, 1973b) found 0.1533 ppm of photo-cis-chlordane in soil samples taken 3 months after the application of HCS-3260 High-Purity Chlordane at 11.2 kg/ha, and about 0.06 ppm in alfalfa 2 months after application to the soil. Also, it has been reported that the solar flux between 200 and 285 nm reaching the earth's surface is in the order of 10^{16} photons/cm^2/month (Barker 1968) because the absorption co-efficient of ozone drops off sharply at 220 nm. Thus pesticides may indeed undergo photodecomposition and phototransformation in sunlight. The rates of decline of acephate residues in Douglas-fir needles and forest litter found in this study also suggested that sunlight may well be an important factor in the disappearance of acephate residues. They always declined more rapidly in needles and litter fully exposed to sunlight than in those from shaded areas.

7. Hydrolysis of Acephate under Laboratory Conditions. The results of hydrolytic degradation of acephate under laboratory conditions at 20° C

and 30° C are given in Tables VIII and IX. Acephate was quite resistant to hydrolysis between pH 4 and 6.9, regardless of temperature. More than 80% of the added acephate were still recovered from samples in this pH range, and there were no statistically significant ($P = 0.0$) differences between corresponding 20°-C and 30°-C results up to pH 6.9. Within the same temperature series, however, acephate recoveries declined inversely with pH.

At pH 8.2, hydrolysis of acephate was strongly affected by temperature. While 78% were left when incubated at 20° C, only 18% could be recovered from those samples incubated at 30° C (Tables VIII and IX).

These results indicated that the susceptibility of acephate to hydrolysis at elevated pH is comparable to that of most organophosphorus insecticides. For example, Gomaa and Faust (1972) reported that the half-life of parathion at 20° C was 3,670 h at pH 5.0, but only 523 h at pH 9.0.

Some conversion of acephate to methamidophos occurred during hydrolysis. In samples with pH values between 4.0 and 6.0, trace amounts of methamidophos, i.e., less than 4 µg, were found. At pH 6.9 and 8.2, i.e., near physiological pH, quantifiable amounts of methamidophos were detected. The highest conversion was 4.5% of the added acephate, observed in samples incubated at pH 8.2. This pH-dependent conversion of acephate to methamidophos was not significantly affected by temperature (Tables VIII and IX). This indicates that methamidophos is not the

TABLE VIII.

The effect of pH on the hydrolysis of 1,000 µg acephate in 10 ml of buffered water at 20° C for 20 days.

pH	Acephate recovered (µg) $\bar{X} \pm$ S. D.	Methamidophos recovered $\bar{X} \pm$ S. D.	in % of Acephate added Acephate	Methamidophos
4.0	977.5 20.8	Trace[b/]	97.8	0.2
5.0	926.5 15.5	Trace	92.7	0.2
5.6	881.9 17.5	Trace	88.2	0.2
6.0	860.6 16.1	Trace	86.1	0.2
6.9	841.5 15.5	10.0 0.4	84.2	1.0
8.2	778.8 8.5	44.6 0.5	77.9	4.5

a/ N = 4

b/ Trace = less than 4 µg and considered as 2 µg for computation.

TABLE IX.

The effect of pH on the hydrolysis of 1,000 µg acephate in 10 ml of buffered water at 30° C for 20 days.

pH	Acephate recovered (µg) $\bar{X}$ ± S. D.		Methamidophos recovered $\bar{X}$ ± S. D.		in % of Acephate added Acephate	Methamidophos
4.0	955.4	21.0	4.9	0.2	95.5	0.5
5.0	902.3	18.8	Trace[b/]		90.2	0.2
5.6	837.5	15.0	Trace		83.8	0.2
6.0	830.0	14.1	Trace		83.0	0.2
6.9	824.5	6.9	16.7	1.9	82.5	1.7
8.2	177.8	13.0	43.4	2.9	17.8	4.3

a/ N = 4

b/ Trace = less than 4 µg and considered as 2 µg for computation.

primary degradation product of acephate hydrolysis, and that the primary hydrolytic pathway of acephate could be through the rupture of some bond other than the N—C bond of the molecule.

8. The Fate of Acephate in Natural Water. Acephate was more persistent in the pond than in the creek water. Pond water still contained about 80% of the amount added after incubation for 42 days at 9° C in the laboratory (Table X). Creek water, incubated at the same temperature, contained 58% and 45% after, respectively, 34 and 50 days (Table XI). Considering that the pH of the pond water was 7.5 to 8.0, while that of the creek water was 7.0 to 7.2 (Tables X and XI), and considering acephate's susceptibility to elevated pH (Tables VIII and IX), these results may appear to be contradictory at first sight. However, under the conditions of this experiment, namely incubation at 9° C - *not* at 20° C or 30° C - and in water samples with pH values approaching 8 - *not* exceeding 8 - acephate probably is not subject to nucleophilic attack by hydroxyl ions, and its disappearance from the creek water must have been owing to other factors than elevated pH.

Results presented in Tables XII and XIII indicate that one major factor was microbial break-down of acephate. Autoclaving the creek water prior to incubation with acephate increased the recovery, after 50 days of incubation, from 45% in non-autoclaved water to 76% in autoclaved water (Table XII). In samples containing both creek water and sediment, autoclaving more than doubled the recovery, namely from about

TABLE X.

The fate of 1 ppm acephate in 150-ml pond water samples held in the laboratory for 42 days at 9° C [a/].

Time, Days	Acephate recovered (ppm)	Methamidophos recovered (ppm)	in % of Acephate added Acephate	Methamidophos
0	0.902	N. D. [b/]	90.2	0
0	0.903	N. D.	90.3	0
2	0.967	0.003	96.7	0.3
2	0.940	0.008	94.0	0.8
7	0.867	0.005	86.7	0.5
7	0.887	0.004	88.7	0.4
14	0.867	0.007	86.7	0.7
14	0.873	0.007	87.3	0.7
21	0.853	0.005	85.3	0.5
21	0.847	0.006	84.7	0,6
42	0.780	0.010	78.0	1.0
42	0.833	0.013	83.3	1.3

a/ pH was 7.5 at day 0 and had changed to 8.0 after 42 days.

b/ N.D. = non-detectable and the detection limit for methamidophos in this study was 0.001 ppm.

TABLE XI.

The fate of 1 ppm acephate in 150-ml creek water samples held in the laboratory for 50 days at 9° C [a/].

Time, Day	Acephate recovered (ppm)	Methamidophos recovered (ppm)	in % of Acephate added Acephate	Methamidophos
0	0.955	N. D. [b/]	95.5	0
0	0.898	N. D.	89.8	0
7	0.809	0.004	80.9	0.4
7	0.743	0.003	74.3	0.3
21	0.887	0.003	88.7	0.3
21	0.873	0.004	87.3	0.4
34	0.598	0.003	59.8	0.3
34	0.570	0.003	57.0	0.3
50	0.450	0.004	45.0	0.4
50	0.455	0.003	45.5	0.3

a/ pH was 7.0 at day 0 and had changed to 7.2 after 50 days.

b/ N. D. = non-detectable and the detection limit for methamidophos in this study was 0.001 ppm.

TABLE XII.

Effect of autoclaving on fate of 1 ppm acephate in 150-ml creek water samples incubated in the laboratory for 50 days at 9° C [a/].

Time, Days	Acephate recovered (ppm)	Methamidophos recovered (ppm)	in % of Acephate added Acephate	Methamidophos
		Not Autoclaved		
0	0.955	N. D. [b/]	95.5	0
0	0.898	N. D.	89.8	0
34	0.598	0.003	59.8	0.3
34	0.570	0.003	57.0	0.3
50	0.450	0.004	45.0	0.4
50	0.455	0.003	45.5	0.3
		Autoclaved		
0	0.955	N. D.	95.5	0
0	0.898	N. D.	89.8	0
43	0.750	0.004	75.0	0.4
43	0.765	0.004	76.5	0.4
50	0.768	0.005	76.8	0.5
50	0.760	0.005	76.0	0.5

a/ pH was 7.0 at day 0 and had changed to 7.2 after 50 days in samples not autoclaved.
pH was 7.0 at day 0 and remained unchanged in autoclaved samples.

b/ N. D. = non-detectable and the detection limit for methamidophos was 0.001 ppm.

TABLE XIII.

Effect of autoclaving on fate of 1 ppm acephate in 150-ml creek water samples in the presence of bottom sediments, incubated in the laboratory for 50 days at 9° C [a/].

Time, Days		Acephate recovered (ppm)		Methamidophos recovered (ppm)		in % Acephate added			
						Acephate		Methamidophos	
		Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment
0	Not Autoclaved	0.955	N. D. [b/]	N. D.	N. D.	95.5	0	0	0
0		0.898	N. D.	N. D.	N. D.	89.8	0	0	0
34		0.365	0.031	0.005	0.002	36.5	2.1	0.5	0.1
34		0.347	0.032	0.004	0.002	34.7	2.1	0.4	0.1
50		0.259	0.037	0.003	Trace [c/]	25.9	2.5	0.3	0.07
50		0.251	0.032	0.004	Trace	25.1	2.1	0.4	0.07
0	Autoclaved	0.955	N. D.	N. D.	N. D.	95.5	0	0	0
0		0.898	N. D.	N. D.	N. D.	89.8	0	0	0
43		0.540	0.146	0.004	0.003	54.0	9.7	0.4	0.2
43		0.520	0.155	0.004	0.002	52.0	10.3	0.4	0.1
50		0.568	0.138	0.006	0.004	56.8	9.2	0.6	0.3
50		0.532	0.153	0.005	0.003	53.2	10.2	0.5	0.2

a/ pH was 7.1 at day 0 and had changed to 7.8 after 50 days in samples not autoclaved. pH was 7.1 at day 0 and had changed to 7.3 after 50 days in autoclaved samples.

b/ N. D. = non-detectable and the detection limits were 0.002 ppm for acephate and 0.001 ppm for methamidophos.

c/ Trace = less than 0.002 ppm and considered as 0.001 ppm for computation.

27% to 65% (Table XIII).

Incubation of acephate-treated pond and creek water samples in the presence of their respective sediments also affected the fate of acephate in the water. It hastened its disappearance. After 42 days, only about 20% could be recovered from the pond water/sediment combination; and 28% from the creek water/sediment combination after 50 days (Tables XIV and XV). The presence of sediments also reversed the order of acephate's rates of decline. In pond water without sediment, acephate was considerably more persistent than in creek water (Tables X and XI); but in the presence of sediments, the opposite was found (Tables XIV and XV).

It was also found that in the presence of sediments about 20% of the acephate, applied to the water, had moved to the bottom sediments after 2 (pond) and 7 days (creek). Interestingly, the acephate concentrations in the sediments remained constant for at least 21 days. But after 42 days, they had decreased to approximately 5% in pond sediments, while in creek sediments they had decreased to slightly over 2% after 34 days, without further changing during the remaining 16 days (Tables XIV and XV).

The disappearance of acephate from creek water without sediments could be attributed mostly to microbial activity (Table XII). But other, non-biological forces, not eliminated by autoclaving and attributable to sediments, must have contributed to the break-down of acephate when water samples were incubated in the presence of sediments (Table XIII). Although autoclaving more than doubled acephate recovery after 50 days of

TABLE XIV.

The fate of 1 ppm acephate in 150-ml pond water samples in the presence of bottom sediments incubated in the laboratory for 42 days at 9° C [a].

Time, Days	Acephate recovered (ppm)		Methamidophos recovered (ppm)		in % Acephate added			
					Acephate		Methamidophos	
	Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment
0	0.902	N. D. [b]	N. D.	N. D.	90.2	0	0	0
0	0.903	N. D.	N. D.	N. D.	90.3	0	0	0
2	0.563	0.292	0.006	0.005	56.3	19.5	0.6	0.3
2	0.587	0.310	0.008	0.004	58.7	20.7	0.8	0.3
7	0.429	0.320	0.010	0.006	42.9	21.3	1.0	0.4
7	0.400	0.273	0.013	0.006	40.0	18.2	1.3	0.4
14	0.378	0.336	0.007	0.006	37.8	22.4	0.7	0.4
14	0.397	0.308	0.009	0.006	39.7	20.5	0.9	0.4
21	0.313	0.324	0.004	0.004	31.3	21.6	0.4	0.3
21	0.337	0.263	0.005	0.004	33.7	17.5	0.5	0.3
42	0.178	0.086	0.002	Trace [b]	17.8	5.7	0.2	0.07
42	0.150	0.058	0.002	Trace	15.0	3.9	0.2	0.07

a/ pH was 7.5 at day 0 and had changed to 7.9 after 42 days.

b/ N. D. = non-detectable and the detection limits were 0.002 ppm for acephate and 0.001 ppm for methamidophos.

c/ Trace = less than 0.002 ppm and considered as 0.001 ppm for computation.

TABLE XV.

The fate of 1 ppm acephate in 150-ml creek water samples in the presence of bottom sediments, incubated in the laboratory for 50 days at 9° C [a].

Time, Days	Acephate recovered (ppm)		Methamidophos recovered (ppm)		in % Acephate added: Acephate		Methamidophos	
	Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment
0	0.955	N. D. [b]	N. D.	N. D.	95.5	0	0	0
0	0.898	N. D.	N. D.	N. D.	89.8	0	0	0
7	0.360	0.288	0.006	0.006	36.0	19.2	0.6	0.4
7	0.380	0.286	0.006	0.006	38.0	19.1	0.6	0.4
21	0.271	0.243	0.005	0.005	27.1	16.2	0.5	0.3
21	0.315	0.200	0.005	0.006	31.5	13.3	0.5	0.4
34	0.365	0.031	0.005	0.002	36.5	2.1	0.5	0.1
34	0.347	0.032	0.004	0.002	34.7	2.1	0.4	0.1
50	0.259	0.037	0.003	Trace [c]	25.9	2.5	0.3	0.07
50	0.251	0.032	0.004	Trace	25.1	2.1	0.4	0.07

a/ pH was 7.1 at day 0 and had changed to 7.8 after 50 days.

b/ N. D. = non-detectable and the detection limits were 0.002 ppm for acephate and 0.001 ppm for methamidophos.

c/ Trace = less than 0.002 ppm and considered as 0.001 ppm for computation.

incubation, 25% to 30% remained unaccounted for. It is well possible that interactions of acephate with colloidal particles, containing highly reactive surfaces, mediated the chemical inactivation of that fraction of acephate. Details of colloid-mediated reactions of pesticides in soils are still incompletely understood (Oloffs 1975). But it is very possible that electron withdrawal from acephate's phosphorus atom by an electrophilic group of a colloid particle can initiate hydrolysis. This mechanism of rendering the acephate molecule subject to hydrolysis at pH < 8, would be comparable with the initial step in the reaction of organophosphorus insecticides with cholinesterases. Soil colloids are known to contain electrophilic groups; and organophosphorus insecticides are known to be inactivated by clay minerals with reactive surfaces (Oloffs 1975).

It is likely that microbial activity in sediments would be reflected, at least to some extent, by that in the corresponding waters, taken at the same time and locations. Therefore, it is also likely that the reversal of acephate persistence described earlier (Tables X and XI *versus* Tables XIV and XV) was probably caused by a higher "concentration" in pond sediment of non-biological forces catalyzing acephate inactivation.

Neither acephate nor methamidophos were found on any of the glass wool plugs used to stopper the flasks during incubation, indicating that movement of acephate out of water bodies, followed by transport through the atmosphere and contamination of other environs, far removed from points of release, is not likely to occur with this insecticide. This

contrasts the behaviour of several chlorinated hydrocarbon compounds, including PCB's, studied by Oloffs *et al.* (1972, 1973) under similar conditions with three natural waters and their sediments, obtained from two rivers and the Pacific Ocean. They found that DDT, PCB's, α- and γ-chlordane rapidly moved from the water to the water-air interface and from there into the atmosphere if the waters, treated with any of these compounds, were incubated without the sediments. Presence of the corresponding sediments, however, prevented any detectable movement into the atmosphere and caused complete movement into the sediments. After 42 days, residues could only be found in the sediments, none in the waters, and no movement into the atmosphere during that period could be demonstrated.

Lindane, studied at the same time (Oloffs *et al.* 1972, 1973), behaved differently and in a manner more comparable to the behaviour of acephate described here. Lindane did not move into the atmosphere, regardless of sediments. In the case of ocean water *plus* sediment, almost as much was recovered from the water as from the sediment after 42 days of incubation.

Oloffs *et al.* (1972, 1973) attributed these differences to different water solubilities. The four materials which either moved into the atmosphere or, if present, into sediments, are highly water-insoluble and have strong affinities to clay minerals and organic matter in soils (Wilson and Oloffs 1973 b). Lindane's water solubility, on the other hand, is approximately 40 times higher than the initial concentrations used by

Oloffs *et al.* (1972, 1973), who concluded that the behaviour of chemicals in aquatic systems will depend on their water solubilities. If present in water at concentrations exceeding their solubility, chemicals will tend to behave as they found for DDT, PCB's, α- and γ-chlordane. If present at concentrations below their solubilities, they will tend to remain dissolved in the water and not move into the atmosphere. The results obtained with acephate, predictable on account of its water solubility of 65%, have confirmed this concept: acephate could not be shown to escape into the atmosphere and more remained in solution than partitioned into sediments (Tables XIV and XV).

Small quantities of methamidophos were found in all samples throughout the incubation periods. This conversion of acephate to its much more toxic metabolite, however, remained quantitatively insignificant (Tables X to XV), so that introduction of acephate into aquatic systems is not likely to pose environmental hazards in this respect.

CONCLUSIONS

This study on the residual properties of acephate in Douglas-fir needles and forest litter following an aerial application of acephate at 1.12 kg/ha, and on the fate of acephate in natural waters with or without their sediments under simulated natural conditions has demonstrated the following points:

1. The initial concentrations of acephate residue in Douglas-fir needles were higher in trees or parts of trees which were most exposed to aerial spray. The highest initial concentration was 9.2 ppm in needles from the upper crowns of dominant trees.
2. The decay of acephate residues in Douglas-fir needles showed a significant correlation with time ($p = 0.05$) up to 10 days after aerial application. In all needle samples, less than 1% of the highest concentration was found after 45 days. No residues could be detected after 60 days. The *half-life*, or time after which 50% of the initial residues were left, was about 4-5 days in dominant and co-dominant trees, but 7 days in intermediate trees.
3. The initial concentrations of acephate residues in forest litter from open and semi-open areas were higher than those in forest litter from a densely covered area. However, the difference between concentrations in open and semi-open areas was small.
4. Acephate residues in forest litter decayed considerably faster in open and semi-open areas than in a densely covered area. Acephate

residues persisted for 10 days in open and semi-open areas, but for 30 days in a densely covered area.

5. Some acephate was converted to methamidophos in all Douglas-fir needle and litter samples. Concentrations of methamidophos, relative to total residue (acephate + methamidophos) found at any one time, increased in needles up to 45 days and in litter from densely covered area up to 30 days. However, the actual concentrations of methamidophos declined rapidly and became non-detectable by the time acephate could no longer be detected.
6. White light had no apparent effect on acephate. However, acephate was readily decomposed when exposed to 253.7-nm ultraviolet radiation at room temperature. No methamidophos was detected in the photodecomposition of acephate.
7. Acephate was resistant to hydrolysis at acidic pH (4.0 to 6.9). It was hydrolyzed at pH 8.2 if held at 30° C, but at 20° C hydrolysis was minimal. Some acephate was converted to methamidophos in the hydrolysis. However, the primary hydrolytic pathway must be through the rupture of bonds other than the N—C bond of the molecule.
8. Persistence of acephate in natural waters varied: about 80% were recovered from pond water after 42 days and 45% from creek water after 50 days if incubated at 9° C.
9. Degradation in these waters, without sediments, was mostly by microorganisms. Break-down in the presence of sediments was much more

pronounced, but only a part of the sediment-mediated break-down could be prevented by sterilization.

10. No escape of residues from water into the atmosphere could be shown to occur. Some acephate moved from water into the sediment when samples were incubated in the laboratory under simulated natural conditions.

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