RED PINE RELEASE AND RESIDUE PERSISTENCE AFTER HEXAZINONE SPOTGUN TREATMENT IN NORTHERN ONTARIO

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ABSTRACT

Effects of spotgun treatment with hexazinone [3-cyclo-hexyl-6-(dimethylamino]-1-methyl-1,3,5 triazine-2,4-(1H,3H)-dione] (Velpar-L) in a red pine [Pinus resinosa (Ait.)] plantation in northern Ontario (Great Lakes/St. Lawrence Forest Region) were examined. Residue persistence and lateral movement in the sandy loam soil were monitored for one year: while weed control and conifer release were observed for three years after treatment. Grid-pattern spot applications of Velpar-L, at 2 mL (480 mg ai)/spot resulted in a dose for the entire treatment area of approximately 1.64 kg ai/ha. This dosage was effective in controlling aspen (Populus tremuloides Michx.), white birch (Betula papyrifera Marsh.), and pin cherry (Prunus pensylvanica L.), and enhancing the growth of red pine. One year after treatment, residues of hexazinone and its metabolites A and B from single spot applications (equivalent to 140.25 kg ai/ha within a 18.5 by 18.5 cm surface area), were found at 0.78, 0.17 and 0.25 ppm or 1.14, 0.24 and 0.36 kg/ha, respectively, in the 0 to 15 cm soil layers, and at 0.22, 0.05 and 0.07 ppm or 0.45, 0.11 and 0.15 kg/ ha, respectively, in the 15 to 30 cm soil layers. Evidence was found of hexazinone leaching to, and degrading in, the 15-30 cm soil layer. Hexazinone residues in the soil column were equivalent to 0.81% (0 to 15 cm layer) and 0.33% (15 to 30 cm) of the initial amount applied per spot at surface level. No trace of hexazinone was detected in soil samples at distances of 60 to 90 cm from the treated spots. Symptoms of phytotoxicity observed in adjacent deciduous plants were probably caused by absorption through lateral adventitious roots. Residue levels in the soil sampling stations one year after spotgun treatment were below the concentrations that would cause damage to red pine, suggesting that regeneration would not be adversely affected after such a time.

Additional index words. Velpar, leaching, conifer (competition) release, weeds

INTRODUCTION

Weed competition is a major problem in pine plantations in northern Ontario. Determining the appropriate herbicide treatment for conifer release is necessary to manage these areas effectively. Furthermore, studies on efficacy and environmental fate are requisite to the registration of new herbicides before they can be made available to forest managers.

In early attempts to control forest weeds, gridball pellets of hexazinone were found to be an effective means of conifer release, and the selectivity was enhanced if the granules were kept away from pine roots (18, 24, 27). Shipman (21) advanced the idea of a chemical "pill" or pelleted herbicide to be applied in strips or in a grid pattern. He found that effective brush control was obtained at lower than normal application rates and conifer tolerance was enhanced with this technique. However, in subsequent trials using this method, Drouin[3] found extensive damage to crop trees caused by movement of hexazinone with rain water.

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Hexazinone is a relatively new herbicide that is effective on a wide range of weeds with little or no adverse effect on recently field-planted red pine seedlings (5, 13, 14). Three formulations of hexazinone [90% wettable powder, 5 and 10% granular (Pronone), and 25% water miscible liquid] are available commercially. The herbicide can act through both foliar and root absorption, with the latter process apparently being the more effective (19).

A conifer release project planned for a local red pine plantation by the Ontario Ministry of Natural Resources (OMNR) was used as an opportunity to evaluate a modified version of the hexazinone grid-pattern treatment. To reduce the risk of damage to the crop species, the liquid formulation of hexazinone[4] was chosen over the afore-mentioned pellet formulation and application was conducted using a spotgun. The specific objectives of the study were: 1) to monitor the persistence, lateral movement and degradation of hexazinone from spotgun treatments of Velpar-L in a sandy loam soil; 2) to measure the phytotoxicity of the spotgun treatment on aspen and red pine; and 3) to measure the growth response of red pine to competition release.

MATERIALS AND METHODS

Site description. The study site was a 10 ha red pine plantation, located in Parkinson Township, in the Blind River District of northern Ontario. The red pine seedlings were planted in 1974. In 1983, the year of the proposed trial, their growth was being suppressed by clones of aspen, intermixed with a few white birch and pin cherry. These species were about 2 to 3 m high.

The plantation was situated on predominantly flat terrain, in soil composed of a well-drained sandy loam, with 4.5% organic carbon, a pH of 5 and a relatively low N, P, and K content. Red pine is well adapted to the conditions of the Great Lakes/St. Lawrence forest zone and is very productive the in sandy loam soils of this region (13, 14, 20).

Spotgun treatment. Five replicate plots of 10 m by 10 m were laid out (in association with randomly selected crop trees), within the proposed treatment area for future evaluation of weed-control and conifer release. A second set of five plots, located within the plantation but outside the treatment area, were to act as controls. Six quadrats (1.83 m by 1.83 m), near the perimeter of the treatment area, were designated as soil sampling stations to monitor residues from individual hexazinone spots.

Treatment was conducted on July 30, 1983, by an OMNR crew using spotguns[5] calibrated to dispense 2 mL (480 mg ai) of hexazinone per spot. The treatment procedure involved applying spots at the base of competing brush trees, midway between rows and columns of planted red pine seedlings. These spray locations represent the safest distance from each set of four surrounding crop seedlings within the plantation's grid-pattern. Spots were only applied when competing aspen were present. Based the volume of hexazinone used, dosage over the entire treated area was calculated to be 1.64 kg/ha. To accommodate the soil residue experiment, a single 2 mL spot of hexazinone (480 mg ai) was sprayed in the centre of each designated soil sampling station. An effort was made not to treat the immediate vicinity of these stations to prevent the possible interference with residues from adjacent spot applications.

Efficacy and Conifer Release Measurement. Twenty healthy red pine and twenty aspen from each of the 10 plots (five treated and five control) were

4. Velpar-L provided by Dupont Canada Ltd., Mississauga, Ont.
5. Model 20 ml Autodrencher; Sanex Inc., Mississaugi, Ontario.

selected to evaluate efficacy and conifer release. Aside from the criterion of uniform size, selection of sample trees was unbiased. Due to the spotty distribution of both pin cherry and white birch in these plots, their responses to treatment was observed but not measured.

To evaluate hexazinone efficacy, each of the designated aspen was assessed for the degree of defoliation and stem die back on a scale of 0 - 100%, [according to the Expert Committee on Weeds guideline (13, 14)]. Conifer response to release from competition was determined by measuring the basal (ground-level) diameter and height of each sample red pine (4, 15). Data was analyzed using the Tukey test (22).

Measurement of Hexazinone Persistence in Soils. To study hexazinone persistence in soils, 6 treated spots and the soil immediately surrounding them were sampled 365 days after the treatment. Two strata of the soil column (surface dimensions of 18.5 by 18.5 cm) were excavated at the center of each station, one from the 0 to 15 cm depth and the second from the 15 to 30 cm depth. Soil samples from each depth and each station were stored separately and immediately transported to the Forest Pest Management Institute in Sault Ste. Marie

Core samples taken from two depths (0 to 15 cm and 15 to 30 cm levels) were extracted with a soil auger (5 cm diameter) within a radial surface distance of 10 to 50 cm of the treated spot to study the lateral movements of hexazinone residues The process of selecting the extraction sites within this area was random. Samples were taken at each sampling station prior to treatment, immediately following treatment, and 7 and 365 days after treatment. As a check to confirm that no significant amount of residue had leached outside this perimeter, an additional set of soil cores were collected at a radial distance of between 60 and 90 cm from the treated spot.

Pre-analysis and soil processing. Soil samples were stored at -14°C immediately upon their arrival at the FPMI analytical laboratory.

Prior to analysis, the frozen soil samples were taken to an insulated drying room that was equipped with a dehumidifier, metal shelves and styrofoam insulation board to maintain a constant room temperature (72°C) and complete darkness and placed on separate disposable cardboard trays (35 cm x 50 cm) with aluminum foil linings. After thawing and drying overnight, large soil clumps were broken into fine pieces and air drying was continued. One day was normally required to reduce the moisture content to approximately 5% and 2 to 4 days to 1% in sandy loam soils. Air dried soils were pulverized with a heavy duty Waring blender (No. S-61643-50) equipped with a 4 L container, and sieved with a 2 mm mesh brass sieve with cover and pan (Tyler No. 10) (7).

Extraction and cleanup. Holt's (9) method was modified and used for extraction and cleanup of hexazinone and its metabolites (A and B)[6] from soil samples. The method is briefly summarized as follows. An aliquot of 25 gm processed air dried soil was weighed in a 150 mL Nalgene bottle (Nalge 2107), mixed with 15 mL of distilled water, capped tight and shaken horizontally on an Eberbach reciprocating shaker at 280 excursions per minute for 15 min. Acetone (60 mL) was then added and the mixture was shaken for another 15 min. The sample was then centrifuged at 350 x g (1150 rpm, rotor radius 23.8 cm) for 10 min. The extracts were filtered through a Millipore Filter Apparatus (47 mm) with Mitex membrane filter (5 m, Millipore LSWP 04700) under reduced pressure. Soils were extracted twice more with 75 mL of an acetone-water solution (80:20 v/v). They were shaken for 2 min and centrifuged for 10 min, as that described above. The extracts were filtered and combined, and the acetone was flash-evaporated in a va-

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6. A = 3-(4-hydroxycyclohexyl)-6-(dimethylamino)-1-methyl-1,3,5,-triazine-2,4(1H,3H)-dione
   B = 3-cyclohexyl-6-(methylamino)-1,3,5-triazine-2,4(1H,3H)-dione

cuum rotary evaporator at 60°C. The remaining aqueous solution was washed and extracted three times with 50 mL of n-hexane and 75 mL of chloroform, respectively. Chloroform extracts were combined, passed through anhydrous sodium sulfate, and flash-evaporated to dryness.

The residues were redissolved in 50 mL of acetonitrile and washed twice with 50 mL of hexane. The acetonitrile phase was combined and flash-evaporated to dryness. The residues were finally dissolved in 10 mL of ethyl acetate and filtered with a Millex SR (0.5 µm) filter unit (Millipore SLSR 025NB) before gas chromatographic (GC) analysis. If a sample extract contained more than twice the concentration of that in the mix-standards after preliminary GC analysis, the sample extract was diluted to near the concentration of the mix-standards and was re-analyzed.

Gas chromatography. Sample extracts in ethyl acetate were analyzed alternately with mix-standard solution containing 2.5, 10 and 5.0 ppm of hexazinone, metabolite-A and metabolite-B, respectively, on a Varian VISTA 6000 GC (Varian Canada Inc.) equipped with a Thermionic Specific Detector (TSD) and a Varian data system (DS402). The specific gas chromatographic conditions were as follows:

- chromatographic column:	60 cm glass, 2 mm id, packed with 10% SP2250 DA on 100/120 Supelcoport, and with acid-treated glasswool plugs.	
- temperatures:	injector	- 260°C
	detector	- 300°C
	column initial	- 250°C (2.5 min)
	column ramp rate	- 10°C/min
	column final	- 280°C (3.5 min).
- gas flow rates:	N2 (UHP grade)	- 3 mL/min
	H2 (pre-purified)	- 5 mL/min
	air (zero gas grade)	- 175 mL/min.

Retention times under these GC conditions were 2.6, 5.5 and 3.5 min for hexazinone, metabolite A and metabolite B, respectively. Peak heights were used for the calculation of residue concentration. When a sample injected showed more than 5 ppm of hexazinone, the sample was diluted to near 2.5 ppm with ethyl acetate and re-analyzed. The average of two peak heights, obtained from a mix-standard solution analyzed immediately prior to and after sample analysis, was used proportionally in calculating the residue concentration in the sample. When the difference between the average value and either one of the standard peaks was greater than 10% of the average value, the sample analysis was rejected. Alternate analysis of the standard solution and the sample was repeated sequentially at least 3-4 times until the standard peak height stabilized.

Analysis of soil characteristics. The air-dry mass of soil samples was measured for bulk density calculations. Soil analyses for N, P, K, pH, cation exchange capacity (CEC), organic content (OC) and moisture (oven-dry basis) were based on the mass of air-dry samples and were carried out according to a standard procedure (2). In reporting the residue levels, the sample bulk densities were used to calculate residue values from ppm (µg/g) concentrations to kg/ha. Residue values were reported as both µg/g and kg/ha (i.e. kg/ha = ppm (µg/g) x D (cm) x B (g/mL)/10); where:

- the sample bulk density B (g/mL) = w (g) / [A (cm 2) x D (cm)]
- the mass of the whole core is w (g)
- the soil sample is collected from a defined area A (cm^2)
- the soil sample is collected from a defined depth D (cm)

RESULTS AND DISCUSSION

Efficacy and Conifer Release. Results indicate that a substantial level of aspen control was achieved by spotgun treatment with hexazinone (Table 1). Control of pin cherry and white birch was observed to be somewhat more variable. This species-specific tolerance may be either a genetically controlled characteristic or a reflection of the difference in capacity of the various root systems to absorb and translocate the herbicide once it has leached into the root zone. This latter process appears to be very effective in aspen.

The success of the spotgun treatment in controlling aspen, even at some distance from the points of application, suggest that the tap roots and adventitious roots of these off-target trees were somehow able to pick up sufficient concentrations of hexazinone to cause mortality. Because aspen are a clonal species and individual shoots are often interconnected by underground suckers, it is also possible that some phytotoxic effects from directly treated shoots may get transferred laterally through the sucker network. No attempt was made to expose the roots and study this question.

Although some resprouting of aspen near the treated spots was evident by the third year (Table 1), the substantial reduction in species density and competition was reflected in the vigorous growth of the crop species (Table 2). Significant increases in both height and basal diameter of red pine in the treated area demonstrate that spotgun treatment with hexazinone was effective for conifer release.

Table 1. Phytotoxic effects on aspen in a red pine plantation, three years after hexazinone spotgun treatment.

Treatment	Number of plants	Mean height	Mean damage	Mean defol.	Mean stem dieback	Resprouting
		(m)	(%)	(%)	(%)	(%)
Treated	100	2.35 [a]	82.5	78.3	88.3	15
Control	100	3.34	0	0	0	0

[a]Significant at P = 0.05; (Tukey test)

Table 2. Growth response of red pine, 3 yrs. after hexazinone spotgun treatment.

Treatment	Number of trees	Height	Increase	Basal diameter	Increase
		(m)	(%)	(cm)	(%)
Treated	100	2.76 [a]	131	6.05 [b]	150
Control	100	2.05	100	4.02	100

[a]Significant at P = 0.05
[b]Significant at P = 0.01; (Tukey test)

Reduction in crop yields due to weed competition, and subsequent amelioration of the situation through the use of herbicides has not been suffi-

ciently documented for forestry situations (16, 25). In this study the hexazinone spotgun treatment elicited a classic weed control response. The elimination or suppression of the competing weed species probably acted to divert limited resources such as nutrients, water and sunlight to the crop trees (8, 10, 11, 27), in a manner similar to that observed under agricultural conditions. This type of conifer release data may prove useful to forest managers in i) predicting the productivity of plantations; ii) estimating crop losses and cost-benefit ratios; and iii) offsetting environmental concerns about the use of herbicides.

Persistence and Lateral Mobility of Hexazinone in Soils. Characteristics of the soils at the treatment sites are summarized in Table 3. The nutrient content of this soil was low, and the moisture content varied from 3 to 26% for fresh field samples and was consistent for air-dried samples (1% in the top 15 cm and 0.7% in the bottom 15 cm samples). Soil bulk density averaged 0.98 for the top 15 cm and 1.38 for the bottom layers.

Table 3. Soil analysis of air-dried samples collected from treatment sites.

	Sample No. 1		Sample No. 2	
	(0-15 cm)	(15-30 cm)	(0-15 cm)	(15-30 cm)
pH	4.99	5.18	4.77	4.82
%N	0.067	0.035	0.157	0.055
%P	0.0007	0.0004	0.0009	0.0011
%K	0.0045	0.0018	0.0080	0.0016
Cation Exchange Capacity (CEC)	6.15	2.50	11.77	5.54
% Organic Carbon (OC)	2.53	1.33	6.32	1.66
% Moisture Content (MC)	0.95	0.74	1.07	0.69

Herbicide residue levels were converted to application rates to allow direct comparison of the rate of persistence and dissipation in soil profiles.

Analysis of soil samples for monitoring the lateral movement of hexazinone from the spot, detected no residues in lateral and adjacent core samples in either top or bottom layers. (The limit of detection was 0.03, 0.06 and 0.06 ppm (µg/g) for hexazinone and its metabolites A and B, respectively.)

Soil samples measuring persistence, taken one year after hexazinone spotgun treatment, contained <1% of the initial applied dosage (Table 4). The small amount persisting (0.78 µg/g) was below the phytotoxic level for conifer seedlings planted on these sites (20, 23). Differential phytotoxicity to conifer species by hexazinone treatment has been noted, with red pine being more tolerant than jack pine in light textured soils (3, 13, 14, 20).

Feng and Campbell (6), working with two forestry soil types, demonstrated considerable breakdown of hexazinone and noted that the rate of

dissipation was faster in sandy loams than in clay loams. Similar conclusions were drawn by Holt (9) and Rhodes (17) with agricultural soils.

Table 4. Hexazinone residue levels one year after spotgun treatment in two soil profiles collected from treated spots[a].

Soil Profile	Soil residue level[b]		Residue remaining
cm	µg/g	kg/ha	%
0-15	0.78 ± 0.34	1.14 ± 0.49	0.81 ± 0.36
15-30	0.22 ± 0.13	0.45 ± 0.25	0.30 ± 0.19

[a]Initially applied hexazinone: 480 mg spot or a dosage equivalent to 140.25 kg/ha within a 18.5 x 18.5 cm area.
[b]Average value of 6 samples.

The content of metabolites A and B isolated from soils treated with hexazinone, after one year, was only 0.3% of the initial amount of chemical applied: the concentrations in the 15 to 30 cm layer were less than half those in the 0 to 15 cm zone (Table 5). Because the concentrations of metabolites were exceedingly low, it seems unlikely that they would cause any environmental concern even though Sung et al. (23) have suggested that metabolite B inhibits photosynthesis in loblolly pine (Pinus taeda L.).

Table 5. Metabolite A and B residue levels in two soil profiles collected from the treated spots one year after treatment with hexazinone[a].

Soil profile	Metabolite	Soil residue level[b]	
		µg/g	kg/ha
0-15 cm	A	0.17 ± 0.08	0.24 ± 0.12
15-30 cm	A	0.05 ± 0.02	0.11 ± 0.04
0-15 cm	B	0.25 ± 0.14	0.36 ± 0.20
15-30 cm	B	0.07 ± 0.03	0.15 ± 0.06

[a]Initially applied hexazinone: 480 mg or 140.25 kg/ha within a 18.5 x 18.5 cm treated area.
[b]Average value of 6 samples.

It is not possible to elucidate the mechanism of formation of these 2 metabolites without further study, but according to Holt (9) microbes are largely responsible for major degradation of the metabolites in soils. Miller and Bace (12) found a rapid transformation of hexazinone in aquatic environments treated with pelleted hexazinone, but only trace amounts of metabolite A were detected. Only metabolite A was formed in blueberry (Vaccinium spp. L.) and goldenrod (Solidago fistulosa Mill) as reported by Baron and Monaco (1). Whatever the route of degradation of hexazinone, no deleterious effects of any metabolite were noticed on red pine.

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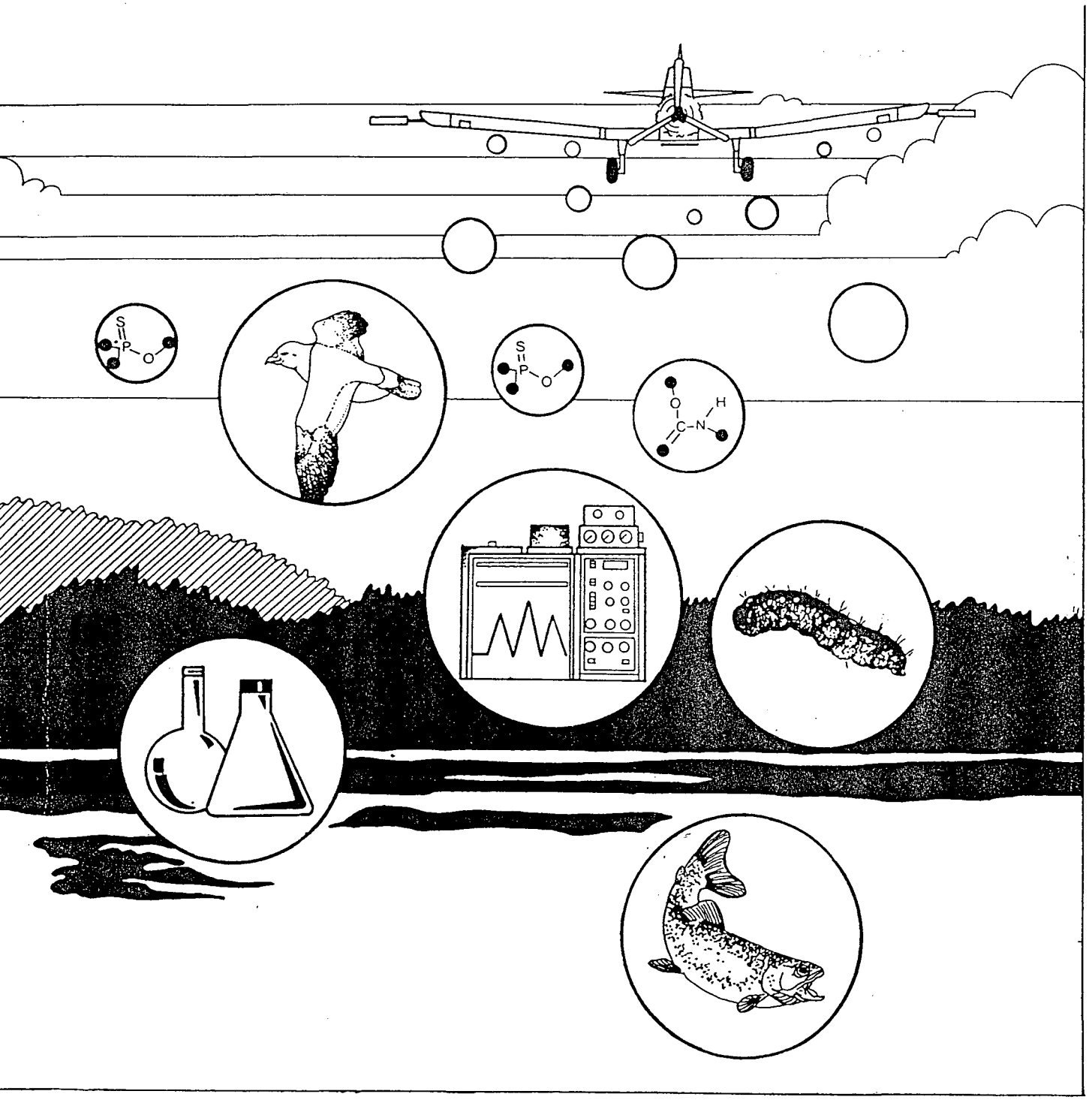

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