March 1986

ANTICOAGULANT TRANSLOCATION AND PLANT RESIDUE STUDIES IN CROPS

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ABSTRACT: Three types of assessment techniques explore the possible environmental hazards of two anticoagulant compounds currently used for rodent control. In the first, rheological methodologies were used to assess the ability of pelletized baits to withstand precipitation. From these data, objective information was developed to assist agricultural producers to select a proper bait for a specific climatic period. Bioanalytical evaluations of chlorophacinone indicated that the compound decomposes when exposed to ultraviolet light into four nontoxic elements. Hence, if translocation were to occur, the elements—-not the parent compound—-would be the likely candidates. Finally, radioactive (^{14}C) braomdiolone was tested for translocatability. From the preliminary data developed to the date of this report, little, if any, translocation occurs.

INTRODUCTION

Two score and two years ago, a financial emergency brought forth onto this continent a new vertebrate pest management specialist, conceived in deliberation, and dedicated to the proposition that all rodents were created equal.

Now we are engaged in a great civil war, testing whether that notion or any notion so conceived and so dedicated can long endure.

And so, under this notion, a new researcher was thrust: a researcher with the idea that a great deal of intelligence can be invested in ignorance if the need for illusion is deep. And ignorance was found to be rampant: first, with the researcher's own lack of knowledge; second, with the lack of any extensive environmental hazard assessment data relevant to the State of Washington. When first confused by this omission, the researcher soon found a nitch into which he could comfortably fit.

Preliminary field assessments indicated a profound need for encompassing vertebrate pest management programs; those that relied not only on habitat management and toxicants, but the use of these toxicants throughout the year as well. From these investigations, two programs were developed: the first to explore the effect of habitat manipulation on rodents; the second to test the efficacy of different chemical and biological controls; finally, to assess chemical hazards in plant ecosystems.

Several types of data were found that fit nicely into the program: vegetation manipulation work, scatter-and-count technologies, toxicology, and hazard assessment. The latter, however, was found to focus on secondary-species poisoning. Little data were found on the physical properties and biological effects of these chemicals.

A complete rodent control program, particularly in Washington's agriculture, means some form of population control must be available throughout the year. In two types of agriculture, no-till grain production and apple production, rodent control throughout the year is severely limited by the grower's inability to destroy the rodent's habitat and to use toxicants during the growing season. Habitats may be modified through the elimination or reduction of vegetation; but since many of the rodents spend much of the summer under the ground—to escape high temperatures and low moisture—-these measures can be ineffective, particularly when population levels are high. Out of fear of crop contamination, none of the currently registered rodenticides can be used for Washington's crops during the growing season. Thus, during the spring and late summer, when rodents have been found to reemerge, the grower does not have any practical means of controlling an expanding population. For a rodenticide to be registered for crop use, five things need to be known about a specific chemical or group of chemicals: The first, structure and mode of action. Second, the effects on nontarget species. Third, the effect on secondary species. Fourth, the effect of environmental factors on the compound. And, finally, crop contamination.

With one group of rodenticides—-anticoagulants—-a series of research programs have been undertaken by the manufacturers and, in some cases, federal agencies. These programs are currently working at answering the second and third questions—-nontarget and secondary-species poisoning problems. The research reported here, as elsewhere, focused on the fourth and fifth questions about environmental factors and crop contamination.

Three studies or two chemicals were undertaken to answer one question. Do anticoagulant rodenticides, namely chlorophacinone and bromadiolone, translocate through actively growing plant systems?

The study was broken down into three phases: physical assessment, bioanalytical evaluation, and translocation. In the first series of tests, a simple procedure was devised to ascertain the degree at which a commercially prepared rodenticide bait might withstand precipitation regimes.

WEATHERABILITY PROFILE

Rheological tests were selected as the most efficient means of establishing the physical integrity of rodenticide pellets after being subjected to known amounts of precipitation. Rheological tests, because they are mechanical measurements of certain physical factors, provide a precision for developing data. In rheological tests, the basic measurement in hardness determination involves the low deformation relationship. Cohesiveness may be measured as a rate at which the material disintegrates under mechanical action. The smaller the deformation under a given load, the lower the cohesiveness (Pomeranz and Meloan 1978).

Methods

Ten commercially available products were tested in this study: Field Mouse Plus, Maki, ORCO Rabbit Bait, Parapel (large and small), Ramik Brown and Green, Rozol, Volid, and Z.P. Rodent Bait AG.

Dry samples of materials were tested and then placed in a mist chamber on perforated plastic trays and subjected to six different amounts of simulated precipitation (0.4 mm, 0.8 mm, 1.6 mm, 3.2 mm, 6.4 mm, and 12.7 mm). Precipitation was simulated by using a TN-4W Tee Jet spray tip placed 61 cm above the table surface. Precipitation was measured with an E.C. Geiger 1.75 Amp mist-a-matic precipitation gauge and regulators. Trays were withdrawn from the chamber at the end of each simulated precipitation treatment. Twenty pellets were randomly selected every 5 minutes from each tray after the trays were removed from the mist chamber. Each pellet was individually tested with a Magness-Taylor (1925) apparatus to identify the point at which the material crumbled.

A simple analysis of variance was conducted between samples, tests and categories of test material.

Results

The candidate materials were grouped into two categories: those which quickly decomposed and those which slowly decomposed under different amounts of precipitation (Figures 1 and 2). Significant differences ($P < .001$) were found between categories but not within categories. Neither were any significant differences found between standard deviations for any of the individual materials tested. The weighted means, plotted in Figures 1 and 2, graphically depict the differences between the two categories.

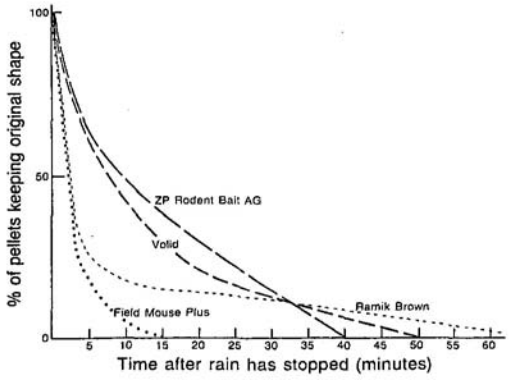

Figure 1. Rate at which four pelletized rodent baits fell apart after being subjected to rain.

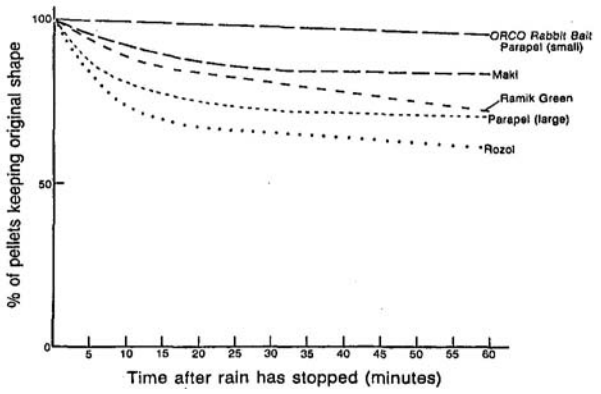

Figure 2. Rate at which six pelletized rodent baits fell apart after being subjected to rain.

No correlation could be found between pellet size (length and diameter) and structural integrity. The pressure at which a product when dry crumbled ranged from 189 to 700 g/cm^2. No predictions about a product's ability to withstand moisture over time based on its visible characteristics or initial test are possible (Askham 1985).

BIOANALYSIS

Bioanalysis studies have taken two forms: first, in the degradation analysis of chlorophacinone; second, in high-pressure liquid chromatographic (HPLC) analysis of plant materials subjected to chlorophacinone under field conditions.

In the first phase of the study, chlorophacinone dissolved in acetone was applied to four TLC plates coated with silica gel G and developed in ethylacetate. The bands of chlorophacinone were identified with UV light and marked on each plate. After 48 hours, the intensity of quenching was noted and fluorescence had decreased but not totally disappeared. The material collected from the plate was heterogeneous and seemed to be different from the parent material (chlorophacinone). It was further

purified by TLC to separate the individual components. The plates were again observed under UV light in which nonpolar fractions and quenching spots were noted. These are referred to as fractions A, B, C, and D. The major product, fraction D, produced approximately 70 to 80% of the yields. A pair of infrared spectral analysis of the pure chlorophacinone and all the fractions was then conducted in chloroform and Beckman Aculab 1. It appears from the spectrum that all the fractions were totally different from the chlorophacinone and also appeared to be different from each other (Figure 3). A photon magnetic resin sprectra of chlorophacinone and the major fraction D were then recorded. Because fractions A, B, and C were not present in any significant quantity to evaluate, attention was directed to fraction D. In this, the mass spectrum analysis showed two absorption bands for carbonyl. These two absorption bands were different from that of the parent compound and of the other three degradation products. From the nature of the absorption, it may be argued that both the carbonyls are of the same nature, just split into two distinct units. From this, it was derived that there's a formation of 1,3-indandione in which two carbonyls with the same nature were the formation of 1,3-indandione with two carbonyls. A proton magnetic resonance spectrum of this fraction also suggests the formation of 1,3-indandione.

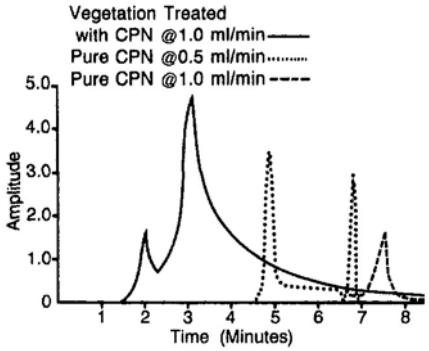

Figure 3. High pressure liquid chromatographic analysis of chlorophacinone at 1.0 ml/tnin (A) and 0.5 ml/min (B) flow rates and bluegrass vegetation (Poa sp.) control and chlorophacinone treated samples (C)* in 100% methanol in a 254 x 4.6 mm column, Si 60, 10 m mobile phases with UV detection at 286 nm. All vegetation samples tested produced identical results.

Once the ground work had been established, plant materials field-treated with chlorophacinone pelletized rodenticides, were assessed with HPLC. The fields were treated at 10 and 20 pounds per acre, respectively. From these studies, it was found that no residues of the compound could be detected in the plant material harvested from these plots. This led the researchers to believe that either the application rates used were such slow intensity that they could not be detected or that the environmental conditions to which they were subjected had affected the material in some manner (Askham et al. 1984, 1985).

TRANSLOCATION STUDIES

An analysis of chemical structures and physical characteristics for both chlorophacinone (CPN) and bromadiolone (BDN) indicated a low translocation potential. Both compounds are only slightly soluble in water, are thus less likely to be transported through soils and plant tissue membranes. In addition, both compounds are made up of large molecular weight structures (CPN = 374.82, BDN = 527.4) which generally precludes their passage through root membranes. Additional data, however, were felt necessary before either of these compounds could be released for use during the growing season for food crops.

Earlier studies tested alfalfa (Medicago sp.) grown in soil treated with ^{14}C labeled CPN. The researchers concluded that the alfalfa "does not absorb either the active ingredient, nor the degenerated products" (Lipha, ND, p. 108). They did, however, record low levels of radioactivity in the aerial fractions. This, however, only accounted for 0.4% of the total radioactivity of the soil. When soil columns were tested, 82% of the radioactivity was distributed in the top sixth of the column. Only 14% was recorded in the second sixth, and 4% in the remaining four sixths (Lipha, ND).

Slightly different results were observed when ^{14}C BDN was placed on four different soils. In clay soils, approximately 95% of the ^{14}C was found in the upper 2.54 cm of the column. In a silt loam, 91.7% was retained in the same region. As the porosity of the soil increased, less was retained in the upper regions. In loamy sands, 54% was retained in the upper 2.54 cm. Lechates produced 1.3%. In sand, only 26.4% was retained in the upper 2.54 cm while 66.9% was collected in the lechate (Lipha 1980).

The question remains, however, whether or not these chemicals move through the soil into the plants and are translocated through the plant system.

This research tests the ability of three plants--apple (Malus sp.), wheat (Triticum sp.), and clover (Trifolium sp.)--to translocate Carbon 14 labeled bromadiolone from a growing medium through their systems. Further, it tests the translocation of bromadiolone when applied directly to a wheat plant.

MATERIALS AND METHODS

Three sample vials of one milligram (mg) ^{14}C labeled (specific activity: 20 m Ci/m Mole) pure (99.7%) BDN (C_{30} H_{23} BrO_4) (Figure 1) of molecular weight 527.4 were received for testing in June 1984. The contents or two vials (1 mg each) were dissolved in 10-ml ethanol and 10-ml water to form parent solutions. One-half of one parent solution (10 ml) was further dissolved in 1300 ml water (the treatment solution) for application on apple (Malus sp.) seedlings. The remainder was dissolved in 1200 ml of water for application to wheat (Triticum sp.) seedlings. The second parent solution was equally divided and dissolved into two equal 1200-ml water solutions in order to treat clover (Trifolium sp.) seedlings as well as perform hydroponic studies with wheat.

Prior to the application of the C BDN, each of the three plant species were established in a clay-loam soil obtained from production orchards in Wenatchee, Washington.

Twenty-five bare root apple seedlings (1-m high) were each planted in 17.75-cm diameter by 40.65-cm-high fiber pots containing 7.0 kg of soil and allowed to grow until fully leafed. Twenty-four pots of clover, each containing 10 plants, in 20.30 cm diameter by 19.00-cm-high plastic containers containing 3.3 kg of soil, were grown from seed to a height of 15 cm. The same number of wheat plants were similarly established and allowed to grow to 30 cm. In addition, 24 15-cm tall wheat plants were placed in aerated 250-ml neoprene containers containing 200 ml of Hoaglands 1 N. solution.

One hundred ml of test solution (10 ml. 95% aqueous ethanol and 90 ml distilled water), with a specific activity of 310,625 dpm (2028.46yg ^{14}C bromadiolone) was applied to the soil surface of the 13 apple seedlings. Similarly, 100 ml of test solution, with a specific activity of 336,510 dpm (2199.39µg ^{14}C bromadiolone) was applied to the soil surfaces of 12 growing wheat and clover plants. An additional 100 ml of full strength Hoagland solution was used to fill the growing chambers of 12 wheat plants growing in hydroponic solutions. Finally, 1µl of pure ^{14}C BDN was placed at the juncture of the flag leaf (last leaf produced before seed-head formation) and stem of 12 semimature (30 cm) wheat plants growing in soil.

On days 4, 8, and 16, one-third of the leafy portion of each plant (apples) or group of plants (clover and wheat) was harvested, weighed, and oven dried. At the end of the experiment, the apple seedling stems and roots were divided into three sections (top, middle, and bottom thirds), harvested, and dried.

The hydroponically treated wheat samples were harvested in total (stems and roots) on the same days. No roots were obtainable from wheat samples grown in soil.

All vegetation samples (stems, leaves, seed heads, and roots) were hand-ground and burned in a Packard Tri-Carb Sample Oxidizer (88.2% efficiency). Oxidized samples were collected in 20 ml scintillation vials containing 8 ml Carbo-Sorb II and 12 ml Permafluor V Carbon Dioxide Cocktail.

The soils from each pot were also divided into three sections (top, middle, and bottom), and 5-cm cubes extracted from each layer for evaluation. Soil samples were dried, subsampled, and weighed. Each subsample was placed in a 200-ml vial containing 100 ml water, agitated for 24 hours, and allowed to precipitate for another 24 hours. One ml of aqueous solution was drawn from each subsample and placed in a 20-ml scintillation vial with Phase Combining System (PCS) liquid. All subsamples were processed through a Packard Tri-Carb Liquid Scintillation Spectrometer.

RESULTS

Leaf material from apples treated with radioactive BDN contained only 63 dpm and 86 dpm after 4 and 8 days, respectively (Table 1). Sixteen days after application, this level increased slightly to 112 dpm. Assuming that no BDN degradation has occurred and the radioactivity is only associated with BDN, approximately 0.8, 1.0, and 1.3 parts per billion (ppb) of the BDN was translocated into this region of the plant. The radioactivity in the stems was highest in the basal sections (610 dpm, 7.3 ppb). The levels of radioactivity in the roots was highest in the middle section and lowest in the bottom section. The roots contained the highest levels of radioactivity in the plant, but was still only equivalent to 187 ppb BDN. Preliminary analysis of the soil indicate that most of the radioactivity was retained in the top third of the profile.

In clover leaves and stem portions, a higher concentration of radioactivity was found than in the apple samples (Table 2). Radioactivity levels increased from 10,000 dpm after 4 days to 16,000 dpm at 8 days but then decreased at 16 days to 8,000 dpm. Like apple, the roots contained the highest levels of radioactivity (560 ppb). Most radioactivity in the soil was retained in the top one-third of the soil profile similar to apples.

Increased levels of radioactivity over a 16-day period were found in the leaves and stems of plants grown in a clay loam soil (Table 3). After 16 days, the calculated level of BDN, assuming no degradation, was, however, only 353 ppb. Root samples could not be assayed because their fiberous structure precluded them from being separated from the soil. Radioactivity levels in the soil profile were still highest in the top section, but the overall levels were much lower than in the other experiments.

When ^{14}C BDN was applied to the base of the flag leaf of wheat plants, *very* little of the radioactivity was translocated to the seed head (Table 4). The majority of the material remained at the site of application.

Table 1. Radioactivity found in the leaves, stems, roots of apple (Malus sp.) and soil 4, 8 and 16 days after the application of 2028.46µg of ^{14}C bromadiolone dissolved in 10 ml 95% aqueous ethanol and 90 ml distilled water to the growing medium. 57.14% recovery of total ^{14}C applied.

	Days after application					
	4 days		8 days		16 days	
Location	DPM/g*	µg/g	DPM/g*	µg/g	DPM/g*	µg/g
Leaves	70.51	8.4537×10^{-4}	96.14	1.1422×10^{-3}	126.64	1.4939×10^{-3}
Stems						
Top					170.92	1.6476×10^{-3}
Middle					438.82	5.2599×10^{-3}
Bottom					680.04	8.1487×10^{-3}
Roots						
Top					8,717.37	1.0444×10^{-1}
Middle					17,434.73	2.0817×10^{-1}
Bottom					4,207.24	5.0429×10^{-2}
Soil†						
Top					51,449.28	6.1668×10^{-1}
Middle					167.08	2.0026×10^{-3}
Bottom					358.55	4.2977×10^{-3}

* Oven dry weight.

** NOTE: $\left(\frac{DPM}{g}\right)\left(\frac{1\mu M \text{ chemical}}{4.4 \times 10^{7} \text{ DPM}}\right)\left(\frac{527.4\mu g}{\mu M}\right) = \frac{\mu g \text{ chemical}}{g \text{ test material}}$

$$\left(\frac{DPM}{g}\right)\left(\frac{527.4\mu g}{4.4 \times 10^{7} \text{ DPM}}\right) = .000011986 \frac{\mu g}{g} = 1.1986 \times 10^{-5} \frac{\mu g}{g}$$

† This represents preliminary data.

Table 2. Radioactivity found in the leaves, stems, and soil of clover (Trifolium sp.) 4, 8 and 16 days after the application of 2199.3µg of ^{14}C bromadiolone dissolved in 10 ml 95% aqueous ethanol and 90 ml distilled water to the growing medium. 13.96% recovery of total ^{14}C applied.

	Days after application					
	4 days		8 days		16 days	
			Calculated uptake			
Location	DPM/g*	µg/g	DPM/g*	µg/g	DPM/g*	µg/g
Leaves & Stems	11,144.83	1.3347×10^{-1}	18,027.30	2.1607×10^{-1}	9070.71	1.0872×10^{-2}
Roots					523,976.85	6.28
Soil†						
Top					17,450.21	2.0916×10^{-1}
Middle					1,218.29	1.4567×10^{-2}
Bottom					8.92	1.0691×10^{-4}

* Oven dry weight.

† This represents preliminary data.

Wheat plants grown in a radioactive BDN-treated Hoaglands solution produced the highest concentration levels (Table 5). Most of the radioactivity, however, was retained in the roots. The concentration of presumed BDN, based on calculated values, was 2.9 and 3.2 parts per million (ppm), respectively after 16 days.

Table 3. Radioactivity found in the leaves, stems, and soil of wheat (Triticum sp.) 4, 8 and 16 days after the application of 2199.3µg of ^{14}C bromadiolone dissolved in 10 ml 95% aqueous ethanol and 90 ml distilled water to the growing medium. 5.49% recovery of total ^{14}C applied.

	Days after application					
	4 days		8 days		16 days	
			Calculated uptake			
Location	DPM/g*	µg/g	DPM/g*	µg/g	DPM/g*	µg/g
Leaves & Stems	19,170.43	2.2978×10^{-1}	25,550.71	3.0625×10^{-1}	32,800.51	3.9315×10^{-1}
Soil						
Top					9,234.56	1.1068×10^{-1}
Middle					142.82	1.7119×10^{-3}
Bottom					282.33	3.3841×10^{-3}

* Oven dry weight.

Table 4. Radioactivity found in the stems, leaves, and roots of wheat (Triticum sp.) 16 days after being placed in 10 ml 95% aqueous ethanol, 100 ml full strength Hoaglands solution, and 90 ml distilled water with 1389.03µg labeled bromadiolone. 5.49% recovery of total ^{14}C applied to sample.

	Calculated uptake	
Location	DPM/g*	µg/g
Stems & Leaves	273,192.52	3.2745
Roots	300,403.90	3.6006

* Oven dry weight.

Table 5. Radioactivity found in the seed heads, flag leaf, and stem of wheat (Triticum sp.) 16 days after 15.99µg of ^{14}C bromadiolone was placed directly at the flag leaf shield and stem junction. 98.13% recovery of total ^{14}C application.

	Calculated uptake	
Location	DPM/g*	µg/g
Seed Heads (above treatment site)	1,389.29	1.8537×10^{-2}
Flag Leaf (at treatment site)	1,077,294.89	1.4374×10^{-1}
Stem (below treatment site)	23,825.00	3.1790×10^{-1}

* Oven dry weight.

DISCUSSION

The weatherability tests showed that not all rodenticide bait formulations are created equal. Some will withstand more moisture before they loose their physical integrity than others. This does not condemn any specific bait for agricultural use. Rather, it provides an indication as to which bait should be used for different moisture conditions.

The data from the chlorophacinone bioanalysis indicate that this material may not have any long-term hazard potential. If the compound decomposes into nontoxic elements when subjected to sunlight, it will only be effective for short periods of time–several hours to a few days.

In each of the soil related experiments, radioactive BDN did not readily move downward through the soil profile. Low solubility of BDN in water and the adsorption to soil particles appears to have caused a retention of the compound to the upper soil strata. These results are consistent with those reported earlier (Lipha 1980).

Apple and clover roots contained higher levels of radioactivity than their respective stems or leaves. Based on the molecular characteristics and size of the compound and insolubility in water, the BDN molecules were not expected to be transportable across plant tissue membranes. The actual amounts transported are small but vary between species. Also the low specific activity of the BDN required an application rate approximately 10,000 times higher than normally used under field conditions. Some of the radioactivity associated with the roots is possibly due to the adsorption of the BDN to the root surface.

The highest levels of uptake and transport occurred in the hydroponic study. Like the preceding experiments, the roots had higher levels of radioactivity than the shoot tissues. The lack of soil-binding sites probably contributed to higher levels throughout the plant. It is still not clear if significant BDN breakdown occurs in solution (bottles wrapped in foil). Even under these hydroponic conditions and with 10,000 times the normal rate of application, the amount of radioactivity and calculated levels of BDN found in the shoots is only in the 2 to 3 ppm range.

Very little compound (>0.000002%) was translocated to the developing seed head of the wheat plant, when radioactive BDN was applied directly to the flag leaf (Table 4) to simulate the effect of pellet lodging during the broadcast application for rodent control. This application rate was even greater than that used for the soil treatments. As expected, most of the radioactivity remained at the application site. The levels found below this site may have occurred from surface movement down the ligule or leaf sheath (which extended down the stem about 7.6 to 12.7 cm) following application.

The higher levels of radioactivity in clover and wheat shoot tissue than apple tissue may be partially due to the greater translocation distance in the latter. It may reflect the woody nature of the apple roots.

In summary, it seems that BDN is not readily leached through the soil. Therefore, it would not be expected to contaminate water supplies and aquifers. Furthermore, the uptake and transport of this chemical by plants, even under the best of conditions, is expected to be very low at the recommended field-use concentrations. No attempt was made to determine the nature of radioactivity translocated in the plants or remaining in the soil after 16 days. Based on previous studies, however, it is anticipated that a large percentage of the BDN would have decomposed to several nontoxic products during this time (Askham et al. 1985) and that much of the radioactivity found in the roots and shoots is not BDN. The experiments reported here were to establish the risk of using BDN or its degraded products in commercial crop production under maximum transport conditions. It appears that there is a minimum probability of significant translocation and/or accumulation under these conditions.

One fact, however, remains. No one can foresee all the implications of this study. Additional tests still need to be completed on these materials as well as others before it is known what they do in the operational environments in which they are placed. It is critical that the effects of these chemicals upon the ecosystems in which they are placed are known as far as possible and within reasonable constraints before they are released for general use. Let is not be forgotten that the agricultural environment in which this material is placed, as well as others of similar constitution, is much different than that of the commensal environment for which they were developed.

ACKNOWLEDGMENTS

A special note of thanks is extended to the various people who helped with this project. In particular, the author would like to thank C & 0 Nurseries, Wenatchee, Washington, for providing the trees; Jim Brainard, who did all of the "grunt" work; Mike Godfrey, for the advice and review; Bob Wample and S. Gurusiddiah for technical support and coauthorship on some sections of this paper; and Chempar Products, a division of Lipha Chemicals, Inc., for their faith, patience, and financial support.

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