The Role of Quantitative Analysis and Plant Bioassays for Investigations of Herbicide Contamination in Composts

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INTRODUCTION

A continuing discussion and evolution of viewpoint concerning presence and effects of herbicide residues in compost is evident, and dates from approximately summer of 2000, when events in Washington State first surfaced. In late 2002 and into 2003, newer and more specific sources of information became available. These include research results on threshold effects of clopyralid-containing compost (Brinton & Evans, 2002), pot-growth studies (Bary et al., 2002), field dissipation studies on turf-applied herbicide (Miltner at al, 2003) and a variety of informal scientific surveys of compost quality, including from California (CCQC, 2003), Washington (Nielson, 2003) and Oregon (2002, 2003). In addition, even more recent information is now available on half-lives of clopyralid in compost (Brinton et al., 2003; Blewett et al. 2003), and variations in compost quality that influence bioassays (Brinton & Evans, 2003). A continuing examination of more recent efforts and studies is essential to gaining a better understanding of the issue.

One recent study is of particular interest, as reported in January 2003 by Oregon Department of Environmental Quality (Roberts-Pillon, 2003). This was a survey of selected composts in the State and the researchers combined both plant bioassays with two types of quantitative lab work in one project, but conducted in two phases between September and December of 2002. During this same time, optimal bioassay methods were being discussed at the private lab level (Frank Shields, personal communication) and in at least two states, Maine and Washington (Hicks, 2002; WA-DOE, 2003). Staff of Woods End Lab were involved in several of these discussions, and were completing a variety of studies concurrently regarding bioassays and compost effects.

The Oregon study appears to be an excellent jumping off point to address a variety of scientific issues on the conduct of clopyralid investigations. In it, the authors grapple with a variety of factors or variables that are common to many similar investigations, and which may significantly influence the outcome of such studies.

In examining the data from the Oregon study, we make use of the on-line version of these reports, which contains appendices of supporting material. The published versions in *Biocycle* and *Composting Ne*ws were essentially extracts of the report (Rynk, 2003; McEnte, 2003). We have also examined Woods End Laboratory data, both published and internal, concerning expected dosage (ED) values for phytotoxicity symptomology for clover, peas and beans, the test plants employed in the OR-DEQ project. Similar plants are listed in the Draft WA-State protocol, to be released in fall of 2003 (Westcott, 2003). Other clopyralid bioassay methods we reviewed are summarized by the Integrated Waste Management Board (CIWMB, 2002) and we have also considered the uncertainty factors in plant toxicity assessment procedures (FIFRA, 2001).

ANALYTICAL METHODOLOGY FOR QUANTIFYING CLOPYRALID

There is common use of laboratory analysis for reporting presence of clopyralid. The Oregon report compared two types of analyses of clopyralid in compost, including the standard EPA 8151A method and a more recent method that the authors of the report call "GC/MS". All the lab work was subcontracted. The Oregon study is an important contribution because we know of no case outside our own and other private lab investigations where methods have been deliberately compared within the same project or for the same samples. Such comparisons necessarily require time and considerable expense. At the public level, there is both concern and confusion as to whether differing lab methods give comparable results, and if not, how this is to be interpreted (Public Testimony, CA-DPR, 2003). A study that compares methodology might shed light on interpretation differences. Since the inception of the issue in 2000, some labs have continued to evolve testing procedures, both by altering the extraction to take advantage of the chemistry of clopyralid, and also by modifying detection technology, such as changing from ECD to MS (*personal communication*, John Coddington, Anatek Labs).

Both laboratory methods compared in the OR-DEQ study are GC (gas chromatography) with a deliberate difference in the extraction method and a coincidental difference in the type of detector used. The terminology "GC/MS" as used in the OR-DEQ report is technically inaccurate and may be better termed a "Modified GC" method. EPA 8151A methods may also be of a GC/MS type, if a detector change is made, which is optional in some EPA methods. The report indicated that the particular modified GC method used was developed in collaboration with Dow AgroSciences. To provide appropriate credit, a residue method under development by Dow AgroSciences for the reregistration of clopyralid in Europe was provided to Morse Laboratories in Sacramento, which optimized the method for use on compost and other grass matrices, largely by altering the extraction procedure, not the detector.

Oregon indicated that it had problems with the 8151A method. It was felt that it had limitations in detecting clopyralid in the ppb range. Therefore, they selected a second method and laboratory for Phase ll. In reading the report, one must grasp that more than one variable exists between the two phases of the study. The limits of detection were listed only for the Modified GC method. It is uncertain whether the same laboratory that ran the Modified GC method in Phase ll also ran the 8151A method during that phase. As a result, it is unclear whether the methods were being compared or different laboratories were being contrasted.

According to the Oregon study, the Modified GC method was more reliable because it could "detect clopyralid at ppb levels". This statement can be misleading. As we have pointed out, the extraction procedure was essentially altered. Given an adequate clean-up step, which is an internal procedure a lab uses to remove potential interference from the extract, either method should be sufficient if operating above the respective method's limit of detection. The use of a mass-spectrometer detector ("MS") is a preferred approach, of course, since this particular device is more selective to the analyte being detected. Yet, these changes alone may not make a method superior.

To demonstrate the problems that arise in comparing methods, we analyzed the data from the Oregon report and plotted it in a simple X-Y scheme (see Figure 1). From this, it may be concluded that the two respective methods agree quite well with each other. The comparison of the two methods shows a surprisingly good correlation of 0.888 (p=0.000). This means that one set of data statistically predicts the other to a high degree of confidence. There is also a good range of values from low to high clopyralid giving a satisfactory spread on the graph to result in a comfortable interpretation of the reliability as indicated in the r^2 value (0.79), which is an index of closeness of fit.

The question if there is actually superiority to the Modified MS method cannot be answered with the data presented. Furthermore, the Oregon report points out an additional factor by indicating that the greater quantities recovered in Phase II may actually result from more grass clippings in the compost samples, while remarking that the information about composition was not collected. This honest fact seriously dilutes the significance of the comparison. In Table 1 we show the range, mean and standard deviation for clopyralid residues reported in both phases of the study, where the same method is used, and cannot discern an appreciable difference in the values.

Table 1. Comparison of clopyralid levels in compost as found in Oregon Phase I vs. Phase II (EPA Method 8151A)

	Phase I	Phase II
	ppb dry weight	
Range	7.6 – 38	8.3 – 49
Average	20.2	23.3
Std. Dev.	13.5	14.2

The report continues by remarking “results of Phase II should not be used to conclude that clopyralid detection in Oregon is higher than in other states”. However, there is no way that the data presented in the study could ever support such a finding.

Scientifically, many confounding factors have appeared at once in this portion of the Oregon study. The careful reader has no way to interpret the findings in any direction save that revealed from our Figure 1, which tells us the two laboratory methods gave comparable results.

RELIABILITY OF BIOASSAYS

It has become customary to use plants that are sensitive to clopyralid to assess potential effects from compost containing it. A primary reason aside from cost is that analytical GC procedures do not directly translate into plant effects. Recognizing this, and particularly in view of the points raised above, it is prudent for studies to compare the relationship of bioassays to actual quantitative data. The Oregon project appears to be aware of this. Underlying this, a comparison of this nature becomes the means for environmental scientists to construct calibration assays. This is where a known amount of compound is dosed to a media and then plant trials are conducted. A second necessary level of calibration is simultaneously required here, and that is to determine the actual test amount dosed by reference to a specific analytical method. In all subsequent uses of the test, both these calibration factors are routinely noted. Only after

successful completion of all these steps, can a quantitative and accurate model be constructed relating test levels of a potential toxicant to a probable biological effect. To our knowledge, aside from the effort underway at Woods End Laboratory, no workers who have reported bioassay values for clopyralid have ever made reference to this protocol.

These steps satisfy, however, the calibration side of the scheme as far as a specific analytical test and specific plant procedure are concerned. A further level of calibration of bioassays to "real-world" conditions may also be required, if ultimately the question is asked how to interpret the data for composters and growers. Furthermore, if the only way or the preferred means to elicit a clear effect in a bioassay is by concentrating the media, for example with high percentages of compost containing an alleged contaminant, then normally the technician must note this. The burden is on them to show the calibration reference. This is common in ecological toxicity test protocol. Woods End made a step in this direction by reporting field calibration studies in the previous year (Brinton & Evans 2003). To our knowledge, outside of the work by Bary, et al., very little if any substantive effort elsewhere has been conducted to prepare similar calibrations. It should be noted that absence of such development would tend to prolong or exacerbate the debate and disagreement among workers as to interpretation of a potential environmental problem.

As a side note, Woods End has developed a bioassay procedure that has been recognized by the Maine Pesticide Control Agency. In it, technicians are required to use a compost + peat mixture adjusted to standard conductivity, using the official procedure that is a pre-requisite for plant toxicity and weed bioassays (USCC, 2002). This is a very basic precaution that the test results bear some resemblance to real-world application rates. Peas and clover are used as test plants, with a control that is insensitive to clopyralid (cress) and positive control compost containing a known amount of clopyralid. Most bioassay procedures include at least peas, use no negative control (or positive control) and do not make an adjustment of media concentration, but rather use a preset dilution, such as 50/50. A committee in Washington State will be proposing a standardized bioassay practice for gardeners and researchers (Wescott, 2003). This will require a fixed ratio of compost:peat of 3:1, which is a 66% compost rate, a very high concentration.

The OR DEQ study reported two bioassay groups: peas, clover, and beans for Phase I and peas and beans only in the second phase. There are two apparent sets of factors that emerge from this study and need to be separately discussed. On one level, the results reveal a significant difference in the plant damage responses for the two species when going from Phase I to Phase II. This suggests immediately an issue with either or both sensitivity and procedure, and possibly also an issue with time (this could implicate season, which is always a potential factor in greenhouse studies). This is not at all uncommon, but must be controlled. Should there be inter-variety differences in plants in a bioassay, researchers must identify it, since it would severely restrict universality of interpretation. Secondly, if differing researchers are ranking the plant damage, but using differing scales, this too could explain the shift and must be identified. Some plants are more difficult than others to rank.

As evidence of this large shift in bioassay rankings, we plotted the Oregon plant damage scores for both phases (Figure 2). In Phase I, a bean with a ranking of "moderate" or "2" on the scale,

corresponded to a "slight" or "1" on the pea scale. This indicates beans to be much more sensitive to clopyralid than peas. However, when the researchers continue the study in Phase II, a pea ranking of "Moderate=2" compares to a bean ranking of "Slight=1", indicating peas are now more sensitive than beans. This is a rather implausible shift. Previously, we had been led to believe that Phase II generally gave more satisfactory results on the analytical side, but from the plant data, new factors or variables are now evident. We do not know which of the two noted variables above explains the large change, and from the data, we are unable to decide which if any of the test plants is acceptable for continued use.

Another source of variation in the bioassays is identified in the calibration approach as noted previously. By plotting plant response on a numerical scale as given by the researchers, against tested clopyralid concentration in the media, we are able to surmise both how sensitive and accurately a plant may indicate the contaminant in question. Figure 3 plots the results of the pea response in Phase II, compared to *both* analytical methods used.

The calibration plot for 8151A suggests that at 50 ppb of clopyralid in the compost, peas are in the moderate to severe bracket (2-3). However, using the Modified GC method the calibration plot is approximately half the slope of the previous. What this means is that the interpretation resulting from the calibration plot must be adjusted by a factor of approximately 2. In other words, using the Modified GC method, plants now are half as sensitive to clopyralid, requiring 100ppb to elicit the same level of damage as the 8151A indicated at 50. This becomes enormously significant as we will show when extrapolating to field effects.

Figure 4 represent the same plot, now conducted for beans. A similar difference in calibration lines or slopes of the curves are seen. The Modified GC test indicates approximately one-half the calibration index as does the 8151A. In both cases, with peas and bean, the report neglects to note this large difference. If we keep in mind the previously identified variable of reversed sensitivity of beans and peas for Phase I and Phase II, then it is apparent that the calibration scales could be shifted by a second factor of two in either direction. This confirms enormous variability and unreliability in these particular bioassays.

The range of variables and factors seen in the Oregon study, which are not unusual, are extremely significant in understanding how and to what extent a reliable interpretation may be applied. Technically, the range of the variability implied in the calibration assays alone goes from about 25 to 100 ppb for a "slight" in the test plants. Secondly, if we note the high concentrations of compost that have been used for the bioassay, rates that are uncommon or non-typical of compost recommendations in the field, then this variability translated to the field scale appears to cover all possible or anticipated uses as falling within a margin of error. This would at least lead to a conclusion that extreme caution is required to interpret the data.

We have not addressed the matter of the use of red clover as a bioassay plant. In the first Oregon phase, five out of twelve samples showed beans and/or peas had more severe symptoms than red clover and in Phase II red clover suffered a crop loss. In Woods End work, red clover is consistently the most sensitive species to clopyralid, by almost an order of magnitude over peas followed by beans. This is a very surprising difference. Following a recent meeting with WSU

researchers, it was agreed to attempt to grow each lab's respective cultivars in an attempt to identify these and other differences in reporting results. Until this is resolved, additional effort is necessary to define reliable bioassay regimes and additionally to understand the correlation of these to analytical data. We have not drawn attention to the difficulty of correlating visual symptomology to what are termed economic field effects. To our knowledge, little work has been done in this area specific to clopyralid. Bary has reported tomato yields continuing to increase with increased symptomology of clopyralid (Bary et al., 2002). In another study, growers were invited to rank greenhouse plants affected by clopyralid. They distinguished economic effects at levels significantly higher than those researchers could distinguish in symptomology (Brinton & Evans, 2002; Brinton, 2003).

CONCLUSION

Test results reported by various groups, and a study performed by Oregon DEQ draw attention to classical analytical and interpretive issues for environmental investigations. A principal dilemma concerns a change from the traditional EPA 8151A to a modified GC procedure for detection of the compound in question. The Oregon DEQ study which compared both methods suggests that the modified GC method is an improved approach. However, due to several overlapping variables that the report notes, such as content of grass in the waste, and differing labs, it becomes impossible to reach a plausible, conclusive finding.

Another important feature identified in the Oregon study concerns calibration issues. By modifying a lab method and recovering more compound, the apparent sensitivity as related to bioassays with plants has been changed, and in this particular study, cut approximately in half. Applied to the field scale, such a change in calibration would mean that the concentration of clopyralid to reach a threshold effect must be predicted at twice the level previously thought.

Bioassay methods being used by a variety of researchers may have neither the consistency nor reliability that some believe they do, as clearly indicated in the Oregon DEQ study. There is a significant possibility of confusing artifacts, results due to the particular structure of an experimental study. Not all the uncertainty factors that are normally addressed in eco-toxicity protocols have been identified. Some of the researchers involved in that study have subsequently agreed to evaluate variability within plant varieties and between differing greenhouse conditions. The range in variation in the bioassays reported is large enough that when projected to a field scale, it may possibly eliminate any truly useful applications of the test. This, combined with the unresolved concerns about analytical methods, suggests that considerably more effort may be required to reach agreement on methodology and interpretation. ◘

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Figure 1. Comparison of GC/MS and 8151A Results

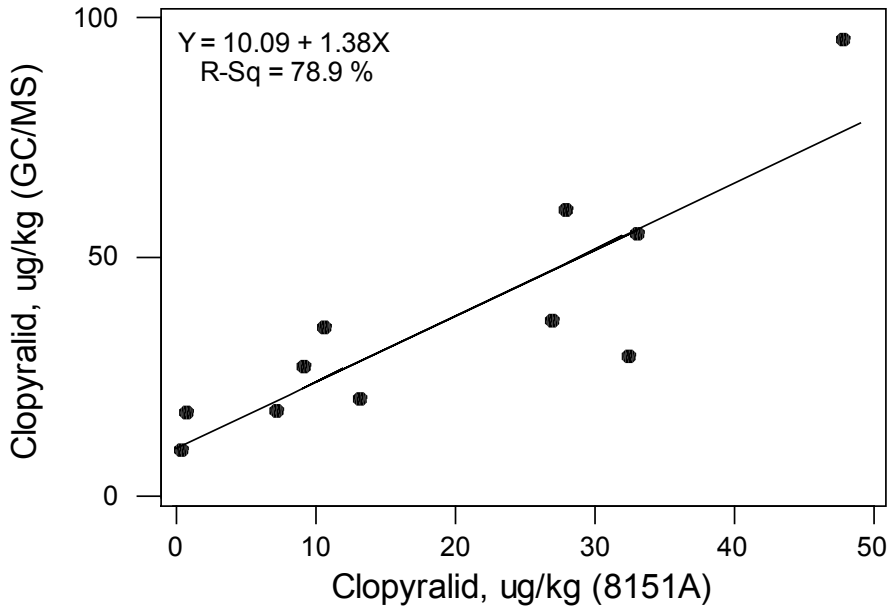

Figure 2. Comparison of Bioassay Ratings from Phase I and Phase II

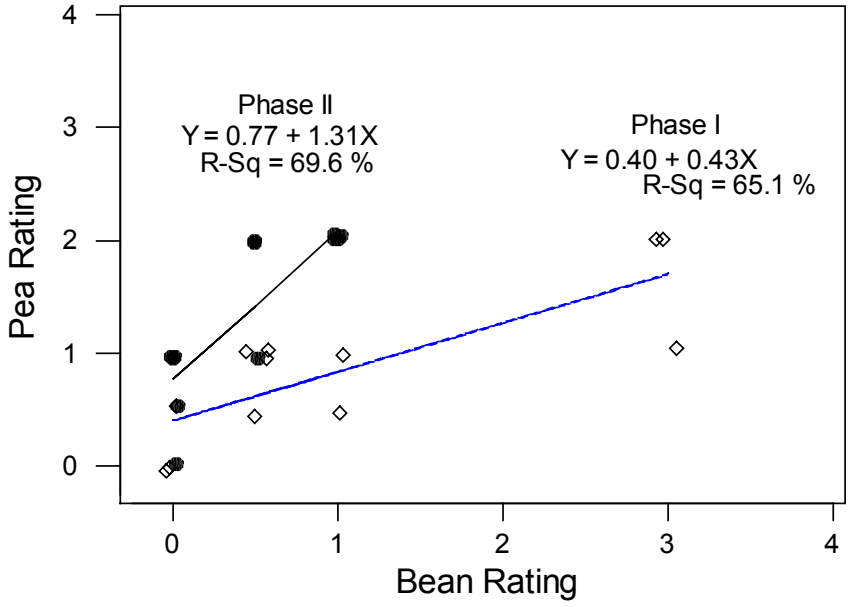

Figure 3. Comparison of Pea Bioassay Rating and Analytical Method (8151A or GC/MS)

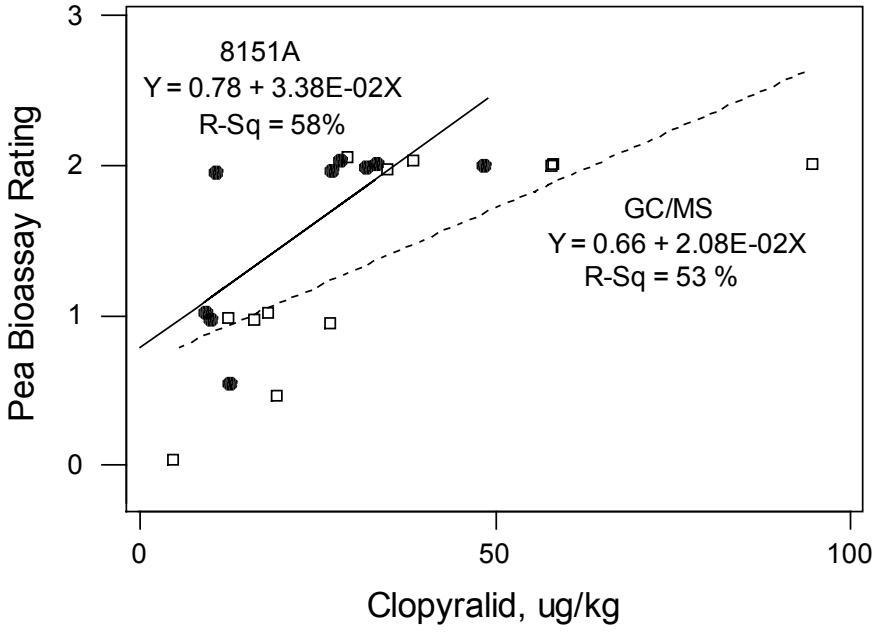

Figure 4. Comparison of Bean Bioassay Rating and Analytical Method (8151A or GC/MS)