NEIC

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PESTICIDE PRODUCT LABORATORY PROCEDURES MANUAL

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National Enforcement Investigations Center, Denver

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF ENFORCEMENT

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This manual was prepared by Dean F. Hill of NEIC in cooperation with the Pesticides and Toxic Substances Enforcement Division, Office of Enforcement; and the Technical Services Division, Office of Toxic Substances.

NATIONAL ENFORCEMENT INVESTIGATIONS CENTER
Denver, Colorado

FOREWORD

This manual is the culmination of several years of work and many years of experience. The procedures set forth address those areas which play a large part in the overall quality assurance effort in the pesticide formulation enforcement laboratory. Safety and health will eventually be addressed also, although technically not directly related to quality assurance. Specific methodology is also not covered, primarily because of the other resources available and due to the wide range of chemicals and products involved. The NEIC Pesticide Formulation Methods Index should serve as an additional useful resource in the methods area.

The NEIC Pesticide Product Laboratory Procedures Manual is obviously incomplete, Chapters IX, XI, and XII not being fully ready yet. These sections and any revisions for other chapters will be automatically sent to all holders of this manual.

Any suggestions for changes, additions, or deletions are very welcome and should be forwarded to:

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CONTENTS

INTRODUCTION

I. INTRODUCTION

A State or EPA pesticide product laboratory is primarily an enforcement unit responsible for analyzing pesticide formulations (and related materials) under the authority of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), as amended, or a corresponding State law. State personnel also perform inspection and analytical services under delegated authority of FIFRA, as amended.

Pesticides are defined in FIFRA to include preparations intended for use as insecticides, fungicides, rodenticides, herbicides, nematocides, antimicrobial agents, amphibian/reptile/fish poisons and repellents, animal and invertebrate repellents, plant growth regulators, plant defoliants and plant dessicants. Pesticide formulations are derived from almost 1,000 different registered active ingredients in a wide variety of combinations. Pesticide formulations also exist in many application forms such as emulsifiable concentrates, wettable powders, dusts, pressurized containers, baits, encapsulations, suspensions, and ready-to-use impregnated plastics. In addition, there is now a trend towards sampling and analysis of use-dilution (or tank-mix) materials in connection with use investigations.

Potential violations deriving from laboratory analytical findings include deficiencies, over-formulations and cross-contaminations. Related physical measurements such as net contents, flammability and emulsifiability may also result in enforcement actions. A product that is chemically deficient will result usually in partial or total inefficacy. Also, a definite health hazard may also result from a chemical deficiency, as in the case of germicides, sanitizers and

disinfectants, particularly those used in hospitals and other public institutions. Over-formulations and cross-contaminations, in addition to contributing to an unnecessary environmental burden, may also: (1) create a potential applicator hazard by exceeding the label safety category, (2) give rise to illegal crop or other commodity residues, (3) give rise to adverse toxicity effects for formulations applied directly on animals and fowl, and (4) lead to phytotoxicity among desirable plant species. Undue flammability, particularly with respect to pressurized containers, can create an obvious safety hazard. Poor emulsification and other mixing problems, usually related to the inert ingredients in a pesticide formulation, can lead to efficacy and application problems.

Analytical testing is performed to determine whether an officially sampled pesticide formulation is correctly labeled, i.e., to determine if the actual contents are described by the active ingredient statement. Therefore, the label claims will be the basis for the laboratory to initiate analysis. The exception would be when suspected pesticides arrive at the laboratory with a missing, or obviously wrong, active ingredient statement. In such cases, the laboratory supervisor will have to determine the analytical level of effort based on the merits of the case.

Among the legal actions (state and federal) that can occur as a result of laboratory findings are: (1) stop-sale, (2) recall, (3) seizure, (4) civil action, (5) criminal action, and (6) cancellation and/or suspension or registration.

Thus it is imperative that all laboratory operating procedures and methodology be valid from both a scientific and legal point of view. All results and conclusions must not only be accurate, but defensible in a court of law. A single procedural or analytical

error uncovered during the development or conclusion of any enforcement action can severely erode the credibility of any laboratory. The entire laboratory staff must make every effort to adhere to established quality assurance criteria.

QUALITY ASSURANCE

II. QUALITY ASSURANCE

The primary purpose of quality assurance in any enforcement analytical program is to assure a maximum degree of accuracy and defensibility of data. This means not only providing certainty that analytical results are reliable, but also that criteria for chain of custody, record keeping, reporting and sampling are considered as integral parts of the overall quality assurance effort.

Quality assurance from a pesticide product analytical standpoint can best be maintained by adherence to certain procedural standards and participation in the analysis of inter-laboratory reference samples. Adherence to procedural standards is critical, particularly with respect to potential chemically violative products, because it is the analytical results for these samples that are likely to be contested. The guidelines and criteria as spelled out in this document, if followed rigorously in day-to-day practice, should establish a high degree of scientific and legal credibility for any laboratory.

Inherent in any meaningful quality assurance program is the continued need for upgrading of personnel, methods, instrumentation and even the quality assurance program itself. Methods development and collaboration should be continuously supported and encouraged. Methods are needed that are specific, efficient and broadly applicable to different formulation types and mixtures. Upgrading of methods to higher status for inclusion in published works such as: J.A.O.A.C or the EPA Manual of Analysis for Pesticides and Devices, and eventually, following collaborative testing, to the A.O.A.C. Methods of Analysis itself, is of utmost importance since the burden of proof can be substantially lessened. Attained experience and training are, of course, basic to any quality assurance effort.

Finally, the Quality Assurance Program itself must be flexible enough so that adjustments can be made for new needs and correction of shortcomings.

SAMPLE COLLECTION

III. SAMPLE COLLECTION

Pesticide product sample collection for EPA and State laboratories is normally not performed by analytical personnel; official samples are collected, documented, and transferred to the laboratory by trained inspectional staffs.

Most formulation samples will derive from Producer Establishment Inspections (PEI's) or the market place (wholesale or retail). However, samples can also be collected at ports-of-entry, points of application, and points of shipment.

Guidance for collection, documentation, and shipment for products collected under the authority of FIFRA, as amended, is presented in detail in Chapter 12 of the EPA Pesticides Inspection Manual. The single-most important piece of documentation for each sample is the Collection Report (CR), as it contains or references all the specific background information. The EPA CR's are numbered consecutively on a nationwide basis, thus precluding any chance of duplication.

Any observed sample problems relating to collection, such as container leakage, inadequate quantity, inadequate labeling, improper shipping, and so forth, should be brought to the attention of the laboratory supervisor who should notify the inspector or the supervisory inspector of the problem.

CHAIN OF CUSTODY

IV. CHAIN OF CUSTODY

INTRODUCTION

Any official pesticide product sample has the potential of being used as evidence in a legal proceeding. It is important that strict custody criteria be adopted and followed by all state and EPA laboratories acting in an enforcement capacity. This applies from the moment a sample is collected until its final disposition. Even products that appear to be "chemically satisfactory" may have labeling, efficacy or other deficiencies that could lead to enforcement actions.

This document will address Chain of Custody only from the time of receipt at the laboratory. If it appears to the laboratory staff that custody has been broken or improperly maintained during the inspectional phase, then such deficiencies should be brought to the attention of the laboratory supervisor, who will decide what action to take regarding analysis of the questionable sample(s). The supervisor should also notify the appropriate inspectional staff of the problem.

Chain-of-Custody procedures are necessary to ensure that the product collected has not been tampered with in the event of any subsequent legal action. Although actual tampering is very unlikely under most circumstances, it is very important, from a legal standpoint, to preclude any doubt whatsoever regarding sample integrity.

The legal requirement for Chain of Custody consists of two aspects: documentation and physical sample security. Criteria for these two elements are set forth in the following sections.

SAMPLE CUSTODIAN

If possible, one person (and an alternate) in the laboratory should be designated as Sample Custodian. This person may be a professional or non-professional depending on the the size and operations of each individual laboratory. This designated individual must be fully aware of the custody requirements and potential hazard of pesticide formulations. The Sample Custodian is responsible for officially receiving samples into the laboratory and for proper storage before and after analysis. The Sample Custodian may also perform other related duties such as sample delivery within the laboratory, preparation of documentation folders, maintenance of logbooks, disposal, and so forth.

RECEIPT OF SAMPLES AT THE LABORATORY

Upon receipt of officially collected pesticide samples, either from a freight agency, U.S. Mail, inspector or other source, the shipping or outer containers should be inspected as to their overall condition. Any leakage or other evidence of damage should be brought to the attention of the supervisor. The supervisor will report such conditions to the appropriate inspectional staff and make a decision regarding analysis or sample disposition based on the degree of damage and importance of the sample.

Any Freight Bills, Bills of Lading, or other documentation related to the incoming shipment should be initialed and dated by the Sample Custodian. Such shipping documentation may represent a large number of samples, thus a sheet of paper can be attached to the Freight Bill listing all sample numbers in that shipment. This information should then be retained in a secured laboratory file for future reference.

HISTORY OF OFFICIAL SAMPLE FORM

For those pesticide formulations that are collected under the authority of FIFRA, as amended, a single "History of Official Sample, EPA Form 3540-17(12-73)" should be initiated for each sample. For those samples collected under state authority, but for which legal action may eventually be taken by EPA, the state should employ the EPA form or a State counterpart conforming to EPA Regional requirements. This form documents the passage of the sample from its receipt into the laboratory until its final disposition. Whenever an official seal is broken, or the sample transferred between analysts or storage locations, it is mandatory that such action be documented on the History of Official Sample or its equivalent.

Items 1 to 14 (1st column) on the History of Official Sample are to be handwritten in neatly by the Sample Custodian upon receipt of an official sample into the laboratory (see Appendix A for examples). The specific items should be completed as follows:

Item 1. Official Sample Identification Number. Not a special laboratory identification number, which should be placed in the margin of the form, if necessary.

Item 2. Complete EPA Registration No., including state and distributor's designation, if applicable. Insert a dash if no EPA Registration Number. State Registration Numbers should be clearly identified as such on state custody forms.

Item 3. Name of Product. Include Company name if part of the title. If title is unreasonably long, write out the first five words followed by three dots. If several sizes of the same product have been collected as separate sample numbers, parenthesize the size after the title.

Item 4. Laboratory location or designation.

Item 5. Date received at the laboratory.

Item 6. Name of person receiving sample into the laboratory (Usually Sample Custodian or alternate).

Item 7. Name or title of person making delivery, e.g., "U.P.S. Delivery Person", "Mailperson", etc. If individual's name is known, particularly if local staff, then write in his or her name. Would be actual person making pick-up, if received directly from freight or Post Office.

Item 8. Name of commercial carrier, U.S. Postal Service or "by hand" if brought directly to the laboratory by inspector or other staff person.

Item 9. If sample condition appears to be satisfactory, that is, no apparent leaking, broken or disfigured containers, then write "OK." If sample exhibits leakage, damage, etc., then indicate as such and elaborate, if necessary, in Item 26, Remarks.

Item 10. The condition of the official seal should be noted. If filled out correctly and intact, write "OK." If the seal is broken, missing or incompletely filled out, indicate as such, and elaborate, if necessary in Item 26.

Any noteworthy observations regarding sample or seal condition should be brought to the attention of the supervisor who will determine the disposition of the sample.

Item 11. Person's name who sealed sample.

Item 12. Date sample was sealed. If other evidence indicates that this date is wrong, append the designation "(SIC)".

Item 13. Should reflect the number of individually sealed plastic outer bags and the number of subsamples within each bag, e.g. "1 x 2", "2 x 4" or "2 x 2 + 1 x 1".

Item 14. Should designate the shelf and/or cabinet where the sample is stored prior to analysis. If given directly to the analyst, write "Given to (name)."

The analyst who performs the initial analysis should fill out Items 15 to 19 upon receipt of the sample from storage or the Sample Custodian. Items 20 to 24 are completed after analysis has been finished.

Items 15 - 24 are completed as follows:

Item 15. Name of the person who assigns the sample to the analyst (usually the supervisor), senior chemist, or the analyst.

Item 16. Name of analyst.

Item 17. Person who delivered sample from storage.

Item 18. Date delivered to analyst.

Item 19. Total number of subsample containers received. Usually the same as the total in Item 13.

Item 20. Number of subsamples actually analyzed (including physical analysis).

Item 21. Date seal broken by analyst.

Item 22. Date resealed by analyst.

Item 23. Name of individual (usually the analyst) who reseals the sample.

Item 24. Storage shelf location.

Whenever the sample is reassigned to another analyst for check or additional analysis within the laboratory, then the second person will fill out Items 15 to 24 in the second column in similar fashion. Likewise, the third column or even additional sheets are used if necessary, to document every sample transfer within the laboratory. If more than one History of Official Sample is needed for an official sample, only the headings (Items 1 to 3) need to be completed with the word "(continued)" appended to the Sample Number.

Whenever a sample or a portion of it is transferred to another laboratory for chemical, physical or biological testing, then a note is made in Item 26. Remarks, under the Release Date regarding: (1) To whom the sample is being sent, (2) Why the sample is being sent, i.e. "Check Analysis", "Special Request", etc., (3) Date the sample is sent,

(4) Mode of transportation of sample, and (6) Initials of person who prepared the sample for shipment.

The original, or a photocopy of the History of Official Sample, should be forwarded to the receiving laboratory along with the remainder of the sample documentation and analytical results. The receiving laboratory will then fill out the second column, starting with Item 4, similar to the first column. This process continues for each laboratory that handles the sample.

Do not leave any spaces blank up to the point where the last Item is completed. Insert a dash or "NA" if necessary, to indicate that such a space was not overlooked.

STORAGE BEFORE ANALYSIS

Unless given directly to the analyst, official samples are stored in a locked sample cabinet or room, on the shelf noted in Item 14 of the History of Official Sample. Keys to the general sample storage area should be accountable and limited only to those persons directly concerned with handling the sample, i.e., the Sample Custodian, Laboratory Supervisor and certain analytical staff. If there are no unsealed samples in the storage area, it may be left unlocked for convenience purposes during working hours. If unsealed samples are being kept in the general storage area for any reason, then the cabinet or closet must be kept locked at all times except for adding or removing samples. If any keys are lost or unaccounted for, then the lock must be changed and new keys issued.

If it is anticipated that rodenticides or molluscide baits will be tested for efficacy upon completion of chemical analysis, then a separate locked storage cabinet must be provided. Separate storage for these materials will minimize the possibility of contamination by foreign chemical odors deriving from stored pesticides. Contamination of this nature

can affect the acceptability of the bait material by rodents or molluscs during subsequent efficacy testing.

HANDLING OF THE SAMPLE DURING ANALYSIS

When the analyst receives an official sample, Items 15 to 19 and 21 on the History of Official Sample are completed as described previously, and any internal laboratory logbooks or records are completed as necessary. The inspector's seal is broken by tearing, and then initialed and dated (see Appendix B). If possible, the plastic bag enclosing the sample should be opened in such a way as to preserve reuse of the bag after analysis is completed. If it is necessary to cut or tear the bag open, then it should be retained and stored with the remainder of the sample. Any observed discrepancies regarding the seal, sample identification or sample condition, not previously noted by the Sample Custodian, should be described, initialed and dated in the Remarks Section of the History of Official Sample and brought to the attention of the supervisor.

The broken and initialed seal should be removed, if possible, from the plastic bag and taped to the sample container, avoiding the concealment of any label wording. If the broken seal cannot be conveniently removed without tearing to pieces, the broken seal can be left attached to the neck of the plastic bag. In any event, all broken seals are to be retained with the original sample.

During the time that the sample is unsealed and undergoing analysis, it is very important that the sample be kept in a locked cabinet or room for which only a restricted number of personnel have access. When unsealed, the sample should be kept locked up at all times, except when actually being handled.

When the original analysis is complete, the analyst officially re-seals the samples in an inverted* polyethylene bag using a new seal. The EPA seal should be completed as shown in Appendix B. Any State seal should be completed according to local custom, but should contain at a minimum the analyst's name, title and date.

The use of polyethylene bags is preferable to direct sealing of bottles, jars and other containers. Their use should also be encouraged by inspectional staff. A sample enclosed in an officially sealed inverted polyethylene bag presents a defensible item of evidence; the sample cannot be reached without breaking either the seal or the bag. With the bag inverted, the heat seal normally at the bottom of the bag, will be enclosed as an additional measure of security. The use of clear plastic bags assures that the label and container markings can be observed without breaking open the bag each time.

Another advantage of polyethylene bags is that if a bottle or jar is leaking, or is accidentally broken, the bag will usually contain the bulk of pesticide until it can be safely disposed of. Undesirable odors often associated with pesticide samples are also minimized. Clear polyethylene bags are available in a wide variety of widths, lengths and thicknesses, but 6", 9" and 12" x 36" have been found to be the most useful. A 4 mil thickness is adequate to provide protection without being difficult to knot. Large clear trash bags are also desirable to have on hand if retail-size fertilizer/pesticide combinations are expected to be received for analysis.

The top of the bag can be knotted, folded and/or taped in a wide variety of ways, the critical factor being that the sample should not be attainable except by tearing or cutting the seal or bag. If it is necessary to use several bags for multiple samples, then the seals should be identical for each. Large fertilizer bags may be officially sealed, if

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* Turned inside-out.

large clean plastic bags are not available, by placing four identical seals over each of the four corners of the bag so as to cover the ends of all draw strings.

Whenever a check or additional analysis is completed, or the plastic bag has been opened for any reason, the sample is then rebagged, resealed, stored and recorded just as performed for the original analysis.

STORAGE AFTER ANALYSIS

The sealed samples, after completion of analysis, are placed in a secured, well-ventilated sample storage area as designated in Item 24 on the History of Official Sample. All bags, container parts, sprayers, trays, caps or other remains of the original sample are to be retained and sealed. The general storage location for completed samples may be the same as that for incoming samples, as long as the two areas are segregated. Bait samples should be stored separately from other pesticides if efficacy testing is anticipated.

All official samples should be retained until notified by the appropriate legal or inspectional personnel that the case is no longer active, i.e., placed in Permanent Abeyance (PA'd).

SAMPLE SOLUTIONS AND STANDARDS

For the same reason that unsealed samples must be maintained under strictest security while in the analyst's custody, prepared sample and associated standard solutions should also be secured when not in actual use. From a legal point of view, these solutions are just as susceptible to tampering as the sample itself. This requirement is critical during the analysis of potentially violative formulations, as the laboratory

will likely be legally accountable for the analyses of these samples. Any solutions prepared for future analysis must be stored in the analyst's locked cabinet or other secure area. Such containers should be clearly identified as to contents and date prepared.

If it is necessary to leave a digestion or other mixture in a hood or unlocked refrigerator overnight, a completed official seal should be placed across the closed hood sash or door, then broken, initialed and dated upon reopening. This seal should be identified as "laboratory use" in the margin and retained with the other sample seals. The information regarding the sample and standard solution integrity should be recorded on the worksheet.

Analytical and technical pesticide standards, both bulk and working solutions should be kept secured along with any primary reagent standards. Reagents, solvents, reagent solutions, titrant, etc. may be kept in general storage in the laboratory (assuming adequate safety precautions are taken) as long as appropriate reagent blanks and titrant restandardizations are performed during the confirmation of any potentially violative sample results.

SAMPLE RECORDS

All records relating to any active official pesticide sample are to be handled essentially as confidential information: before, during and following analysis. Sample documentation and active laboratory records should be secured in a locked desk drawer or file cabinet when not in use. Sample records, including worksheets, graphs, notebooks, chromatograms, notes and other material should also be locked up when not in use.

It is up to laboratory custom as to the extent of record copies that need to be retained for any active official sample after the

analytical report is finished. It is critical, however, that all original laboratory data including worksheets, notes, chromatograms, graphs, and the related material be retained, along with the handwritten master copy of the Report of Analysis (or the State equivalent). It has been found useful to also keep a copy of the typed Report of Analysis, History of Official Sample and Collection Report for all samples, and in addition, a copy of the label and pertinent supplementary documentation for potentially violative samples.

When notified of final disposition, or a Permanent Abeyance notice is received by the laboratory, the physical sample may be disposed of, and the records placed in a general file for future technical reference.

SAMPLE ANALYSIS

V. SAMPLE ANALYSIS

SCOPE OF ANALYSIS

The basic task of the laboratory is to determine whether the label claim (or Registration disclosure) for any submitted formulation accurately describes the material actually in the container.

It is not necessary, and may even be impossible with current methodology, to analyze for all active ingredients in every pesticide formulation. Active ingredients that appear to be present more for their solvent or diluent properties than for their pesticidal activity normally need not be determined, e.g., petroleum distillates, alcohols, mineral oil, silica gel, and diatomaceous earth. Soaps, detergents, essential oils, creosote, complexing agents and polybutanes are examples of other active ingredients not ordinarily analyzed.

A qualitative test for the active ingredient(s) may be all that can be accomplished for some products such as low-percentage active ingredient bait materials and complex disinfectants. With such formulations, it is often difficult to substantiate beyond a reasonable doubt any deficiency or overformulation because of the methodology limitations. An efficacy test is usually more meaningful for these types of formulations.

Certain types of pesticide products are more effectively analyzed for their inert ingredients, e.g., pine oil-soap mixtures, creosotes and coal tar derivatives. Water and alcohol are the usual inert ingredients in such products, and can be determined easily by distillation.

Adherents and asphalt sprays are examples of products for which no chemical analyses are performed.

STATUS OF CHEMICAL METHODS

Although there is no official ranking of methods in EPA for the analysis of pesticide products, the current edition (and supplements) of the Official Methods of the Association of Official Analytical Chemists (AOAC) is generally accepted as containing the most valid methods from a scientific and enforcement viewpoint. The procedures have been tested collaboratively between laboratories, and their statistical reliability has been confirmed. These methods are termed "Official" and should be employed, whenever possible, when confirming potentially violative samples. It should also be kept in mind, however, that a particular AOAC method may not be applicable to all types and combinations of formulations for a particular pesticide, and that complete extraction should also be verified, if necessary.

The next most authoritative source of pesticide formulation methods are those in the EPA "Chemist's Manual", i.e., the Manual of Chemical Methods for Pesticides and Devices. The methods given in this manual and not listed as "Tentative" may be considered "Standard." Standard methods have proven reliable through wide use by regulatory analysts over the years, but have not necessarily been subjected to interlaboratory collaborative testing. Other methods that may also be considered as Standard for pesticide formulation analysis are those adopted by other authoritative testing groups such as ASTM, AWPA, CIPAC (except joint AOAC - CIPAC methods) and NBS. Applicable methods given in Scott's, Rosin, U.S. Pharmacopeia, National Formulary and recognized scientific journals (such as J.A.O.A.C.), may also be considered as Standard.

All other available methods should be considered Tentative. These include experimental, industrial and hoc methods, Tentative methods in the EPA Chemist's Manual, and methods in Zweig.

ASSAY OF SAMPLES

Initial Analysis of Routine Samples

Samples are selected by the supervisor or analyst with regard to expediting special or priority cases, maximizing efficiency and minimizing the average turn-around-time. The average process time for routine samples should be less than 30 days, whereas priority samples should be analyzed as soon as possible. Several laboratory practices which support these requirements are: (1) scheduling the analyst to begin samples requiring long digestions and extractions and during these operations completing samples which require close attention, (2) assigning to one analyst all samples containing the same ingredient, and (3) screening as many samples as possible for cross-contamination at one time.

The initial determination of an ingredient or combination of ingredients for a routine sample should be made by the most expeditious specific method available that can be expected to yield a reliable result. Thus, generally a non-AOAC procedure will be undertaken for most organic pesticides, i.e., gas or liquid chromatography using normal laboratory columns and conditions. Chromatography is rapid and specific, but there are few collaborated methods employing these techniques.

If more than one batch or code number is indicated as being represented for a single official sample, either from the container or the Collection Report, or there are other apparent label or physical differences between containers, then representative portions from each should be analyzed.

When the sample results for the initial analysis are satisfactory with respect to the Laboratory Verification Guidelines (see Appendix C) the final report is written up according to established procedures. It is a good practice, however, to verify with a duplicate analysis, results that are borderline, i.e., fall within 20% of the percentage limits of the Laboratory Verification Guidelines.

DEFECTIVE SAMPLES

Initial Results

If the results obtained upon first analysis indicate a potentially violative sample according to the Laboratory Verification Guidelines criteria, confirmatory procedures must be initiated. Each of the additional analyses performed by the original analyst to verify his or her original result (which indicated a defective sample) are termed "confirmatory analyses," in order to differentiate such work from "check analyses," which represents work accomplished by a second analyst.

If the initial method employed by an analyst for a defective sample is not Official or Standard, the analyst should confirm the original result by reanalyzing according to a more established method, if available. This analysis should be performed in true duplicate. A true duplicate analysis represents two separate determinations by exactly the same method, but utilizing two separate sample charges and standard solutions (if applicable). In addition, if more than one unit is available for the sample, then at least one other container (preferably from a different case) should be analyzed. Separate batch or lot numbers should be handled as completely independent samples.

When a titration is performed as the confirmatory or check analysis of a potentially violative sample, a restandardization of the titrant should also be performed in conjunction with the analysis. If a digestion, precipitation or other chemical reaction is carried out as part of a wet chemical technique, then a reagent blank must be performed and recorded.

If an AOAC or Standard Method is used initially for the assay of a defective sample, and there were no indications of interference or other analytical problems, then the initial result should be confirmed by repeating the analysis using a new sample charge and, if applicable, a new standard solution. The considerations previously mentioned regarding batch codes, multiple units, restandardization and reagent blanks still apply. When incomplete extraction is suspected to be a cause of low results for a sample using an Official or Standard procedure, the results should be verified by either lengthening the extraction time, significantly modifying the sample/solvent ratio or employing successive extractions. Soxhlet extraction or use of more polar solvents may also be used for verification of complete extraction, but extreme care must be taken to avoid thermal decomposition or co-extraction of normally insoluble inerts.

Overformulations using any technique, particularly chromatographic or spectrophotometric methods, should always be verified for absence of potential interferences by use of alternate methodology, or at a minimum using two significantly different GLC columns or HPLC conditions. Apparent overformulated quarternary ammonium chlorides, as analyzed by the AOAC chloride titration should also be confirmed using the Epton, ferricyanide or other specific method, due to the possible presence of free chloride.

Results for potentially violative samples using a Standard method (no applicable AOAC procedure) should be verified by an alternate

Standard method, if available and applicable. The alternate method should be distinctly different from the first, such as IR and GLC, UV and gravimetric, or HPLC and GLC, and not simply a variation in detector, column or solvent.

If only Tentative (including in-house or ad-hoc) methodology is available for a particular formulation, at least two, or if possible, three entirely different procedures are necessary for confirmation. Consideration must also be given to extraction, calibration, specificity and spiked sample recovery for all such cases, in addition to the need for duplicate analysis by at least one of the methods. Also at least two units should be analyzed, if available. It may be necessary to employ an elemental analysis to confirm violative samples in such cases, although normally such methods are discouraged due to lack of specificity. Supervisory discretion should be utilized when determining whether to declare a sample as volative using a single non-Official or non-Standard method.

Supervisory discretion and scientific judgement will also have to be exercised when results do not agree between units or when results between different methods fail to agree.

For all suspect violative samples where multiple active ingredients are present, and the methodology employed is not specifically applicable to such mixtures, then potential interferences should be positively ruled out or shown to have a negligible effect on results. This can be done by means of spiked samples, independent blanks using the co-ingredient(s) or by reference to previously developed information.

In any case where a single result appears to be anomalous, and probably due to some laboratory error, then the result should not be reported. However, a note should be added to the worksheet data stating "Not Reported," along with a brief reason if one is known or suspected.

The analytical report is completed and submitted to the supervisor when all analyses (including screening for cross-contaminants) are completed for a potentially violative sample, and the results are considered to be in good agreement. If the laboratory is using the EPA Report of Analysis form, the upper part of Item 11, Results of Analysis, should be left blank to be completed by the supervisor after the check analysis is performed.

The supervisor should review the initial results and Chain of Custody to this point and assign the sample to a second chemist for check analysis. If the laboratory has only one chemist experienced in pesticide product analysis, it may be necessary to have the check analysis performed in another laboratory. In any event, whether the sample is assigned to a second chemist within or outside the laboratory, strict custody is to be maintained.

CHECK ANALYSIS

All potentially violative samples, as determined by the original analyst's results and the Laboratory Verification Guidelines, are to be further verified by a "check analysis" performed by a second experienced chemist. The check analysis precludes any possible error or personal bias in laboratory procedures, methods, observations, or calculations.

Prior to starting actual analysis, the check analyst should check all the calculations of the original analyst and make a note of this by writing "checked" along with initials and date by each result on the worksheet. Discovered errors should be corrected by the original analyst along with initials and date.

The check analysis is usually just a single result obtained on a single container of the suspect product. The method employed may or

may not be the same as that used by the original analyst; however, if an Official or Standard method is available, but not employed by the first analyst for some reason, then it definitely should be used, preferably in true duplicate. Titrants, standard solutions and other prepared reagents used by the initial analyst are not to be used by the check analyst. Independent restandardizations and reagent blanks are also performed, if applicable.

In all cases where different containers yield substantially different (but verifiable) results, a check analysis should be performed on each.

In the event of a discrepancy between the second analyst and original analyst, the check analyst should first repeat his or her work to rule out possible laboratory error. If at this point the discrepancy still stands, the supervisor will have to use his or her discretion to resolve the difference.

FINAL REPORT

When the check analysis is completed and the results are in agreement with those of the original analyst, the sample is resealed and stored. The supervisor will then verify the check analyst's calculations, review the work in general to make sure the methodology was appropriate, and summarize the findings. On EPA Reports of Analysis, Items (top portion) 12 and 11 are completed as described in Section VII. The report is then ready for typing.

ANALYSIS BY TECHNICIANS

Initial results developed by non-professionals are defensible when: (1) the individual has had at least 1 year's experience in the

particular type of analysis being considered, (2) the individual works under the technical supervision of a chemist, and (3) all of the Chain of Custody and confirmation criteria as previously spelled out have been met.

If these conditions are not met, the technician, upon discovery of a potentially violative sample, should officially turn the sample over to a chemist or more experienced technician for completing the initial analysis.

RECORD KEEPING

VI. RECORD KEEPING

INTRODUCTION

It is imperative that detailed and specific notes be made regarding all sample analyses, manipulations and observations. This requirement is significant because many litigated cases do not reach the hearing or trial stage until long after the analysis has been performed, in some cases up to several years. Sufficient detail should be provided to enable not only oneself, but others to reconstruct any analysis step-by-step. Detailed notes not only serve litigation purposes, but will also assist in resolving disputed analyses, and provide specific methodology for future similar types of samples.

It is recommended that all analytical work, graphs, charts, notes, etc., be retained in a general laboratory locked file cabinet by sample number. This is not to say that each analyst should not keep a notebook or logbook, but the file system will assure that all primary information regarding a particular sample is in one location, i.e., it is not spread through a single analyst's notebook or through several analysts' notebooks. There is also less chance of loss.

If EPA Report of Analysis, 3540-5 (Rev. 5-76) is used, detailed information concerning analyses and observations should be kept on the back of the Lab Work Copy. As many sheets as necessary can be used as long as they are identified by sample number and analyst. The face needs to be completed for only one sheet, however. Different sheets should follow in chronological order and be sequentially numbered.

If laboratory notebooks are used, they should be of the "two-page" carbon or pressure-sensitive paper type. The originals are then removed from the notebook for retention with the laboratory records.

ANALYTICAL NOTES AND OBSERVATIONS

Careful notes should be made of the physical state, color, and other pertinent observations regarding the sample, particularly if there is phase separation, emulsification, non-uniformity, or other irregularities.

The preparation of the product for analysis should be accurately documented as to how and when (date) the material was mixed, subsampled, and treated. If the sample was ground, sieved, homogenized, filtered, or otherwise manipulated, give an accurate description of the amount of subsample, the technique used, and the duration of the activity. Also note any related observations, such as color, odor, or temperature change. All net contents measurements should be recorded in detail. If it is necessary to determine non-volatiles for a pressurized container, note the temperature and time of heating or standing, and whether any spraying or spillage of non-propellants occurred.

Each method of analysis used should be referenced, and specific notes made of any variations. Note should be made of all manipulations, reagents, and observations when the method used is not referenced. Each laboratory operation should be accurately documented as to date performed, particularly when an analysis or several analyses of a sample extend beyond one day. Time of starting and stopping should be recorded for all operations where duration is a factor, such as extractions, separations, centrifugations, color formations, and derivations.

Photographs should be taken, if possible, of any obvious physical abnormality, such as poor applicator or container design, extreme non-uniformity, layering and so forth, particularly if the condition could result in adverse safety, health, or environmental effects. Photographs or good photo copies should also be made of developed thin-layer chromatographic plates in cases of cross-contamination. All photocopies should be mounted on heavy paper and identified as to sample number, date, analyst, and subject matter.

Custody information and storage location should be documented if sample and/or standard solutions are stored overnight.

Reference standard information, including source, purity, and age should be recorded along with appropriate weighing and dilution data. If a reference standard is used that was prepared at an earlier date, then the original weighing and dilution data should be referenced.

All instrumental conditions should be recorded either on the worksheet or on an appropriate chart, graph or printout. All graphs, charts, and printouts should be identified by sample number, date, analyst and determination number.

Gas chromatography data should be recorded for each analysis at least to the following extent:

1. Gas Chromatograph	- Make, model and detector. Include designation if more than one of same model is available
2. Column	- Source and/or date prepared - Length, I.D., O.D. and composition - Packing (%, type and source)
3. Conditions	- Temperature of oven, injection port, detector, transfer lines, etc.

- Flow rates, composition and purity of carrier, detector and purge gases
- Electrometer conditions such as range, attenuation, voltage, amperage, etc.

4. Injection - Amount injected and size of syringe

5. Response - Digital integration (incl. make, model, slope sensitivity and other pertinent parameters) planimeter, peak height, cut and weigh, etc.

6. Internal Standard (if used) - Identification, source and concentration

7. Any conditioning or calibration

8. Recorder - Make, model, range and speed

HPLC data to be retained for each analysis should include at least the following:

1. Liquid chromatograph - Make, model, type and lab designation

2. Detector - Make, model, type and wavelength

3. Column
- Source and/or date prepared
- Length, I.D., O.D. and composition
- Packing (type, source and particle size)
- Pre-column, if applicable

4. Mobile phase
- Isocratic or gradient?
- Name and % of each solvent
- Degassed? Filtered?

5. Injector - Type, make and model
Amount injected

6. Temperature - Type of control and temperature

7. Sample handling - Filtration? Pore-size of filter

8. Response measurement	- Digital integration (incl. make, model and settings) planimeter, peak height, etc.
9. Recorder	- Make, model, range and speed
10. Internal standard	- Identification, source and concentration

Spectrophotometric data should be retained to the extent called for on the specific charts, along with any additional information as may be relevant to the measurement.

REPORTING RESULTS

VII. REPORTING RESULTS

INTRODUCTION

Analytical results generated under the authority of FIFRA, as amended, should be reported using EPA Form 3540-5 (Rev. 5-76) or State equivalent as agreed to under the grant requirements.

The EPA Report of Analysis consists of a six page manifold, broken down as follows:

1. Sample Record Copy
2. Establishment Copy
3. Regional Office Copy
4. Laboratory Copy
5. Biological Lab Copy
6. Lab Work Copy

LAB WORK COPY

The Lab Work Copy is composed of heavy card stock and should be removed from the manifold prior to analysis. A group of cards can be removed and given to each analyst and the cardless manifolds forwarded to the typist.

The reverse side of the Lab Work Copy can be used for recording of all laboratory data as spelled out in Section VI. Upon completion of analysis, the front side of the Lab Work Copy should be filled out

by the analyst for use as the typist's master. When the typing is normally performed outside of the laboratory, a photocopy should be submitted for typing to minimize the chance of losing the original.

TYPING OF THE MANIFOLD

Typing should be performed on those manifolds from which the Lab Work Copy has been removed. The Establishment Copy is folded in half by inserting the bottom half under the top half so that only about 1 3/4" is exposed under Item 11. This will allow for further elaboration of results and comments on the in-house copies without carrying over this information, either in print or impression, to the Establishment Copy. Another alternative is for the typist to tear out the Establishment Copy after completion of the first part of Item 11.

Thus Item 11 of the Report of Analysis is subdivided into two sections, the bottom half of which is meant for in-house (State or EPA) use.

If it is necessary to prepare typewritten drafts, then blank manifolds may be torn apart and the pages used individually, except for the Establishment Copy.

COMPLETION OF REPORT OF ANALYSIS FORM

The analyst should complete the front of the Lab Work Copy except for Items 12, 13, 14 and 15 (see examples in Appendix D). The Supervisor's Summary in Item 11 for those samples that are potentially violative or otherwise noteworthy should also be left blank. Any items that are in doubt or unknown should be left blank and brought to the attention of the supervisor.

As much information as possible should be obtained from the actual product label rather than relying on information in the Collection Report, since the latter may contain typographical errors.

1. Sample No. - The official sample number.

2. Date Collected - Date on official seal.

3. Region - EPA Region in which the sample was collected.

4. EPA Reg. No. - The EPA Registration Number on the label itself, and should include the entire number including state designation (usually several letters), if any, and distributor designation. For example, the number could be 356-7," "356-7-AA" or "356-7-AA-67981." If no EPA Reg. No. insert a dash.

5. Establishment No. - The producing plant Establishment Number given on the label or container. This designation is a number followed by the state initials, followed by another number, e.g. 356-CA-1. The Establishment No. may not be directly discernable, i.e. it may be stamped on the bottom of the container or otherwise given as a perforated (or hand-marked) code. If there is no EPA Est. No. on the label or elsewhere on the container, insert a dash.

6. Description of Sample - Consists of two parts separated by a semicolon or slash mark. The first part should be a physical description of the exterior sample and the second should describe the physical appearance of the actual pesticide material. Examples are given as follows:

   2 x 1 lb. cardboard canisters/tan dust
   4 x 12 fl. oz. pressurized containers/yellow liquid
   1 x 1 gal. plastic jug/blue liquid
   2 x 1 pt. subsamples in glass bottles/amber liquid
   1 x 4 lb. cardboard carton/brown pellets

7. Name and Address - Name, title and address given on the Receipt for Sample, if available. If the information is not clearly identifiable from the Receipt for Sample, Collection Report or other documentation, it should be left blank to be completed by the inspectional office.

8. Product Name - Should be relatively short, yet yield enough information to clearly identify the product. The Company name should be included if it is an obvious part of the title. If more than one report is being prepared to reflect different size containers of the same product, the respective sizes should be appended to the product name in parentheses.

9. Lot or Code Number(s) - As given on the actual product, if present, otherwise as noted on the Collection Report for codes from shipping containers. Insert a dash if no code or batch numbers.

10. Name and Address of Producer - Actual producer or formulator of the product if not the same firm given for Item 7. This will require completion for non-PEI samples, such as those deriving from market surveillance, use/misuse, import, experimental-use and accident investigations. This information should include firm name, city, state and zip code if possible. If this item is the same as Item 7, then insert a dash or the words "Same as above."

11. Results of Analysis

    a. Upper Portion

        (1) For Chemically Satisfactory Samples

            Within the upper 1 3/4" of this Item, the statement "This sample has been analyzed and been found to be chemically satisfactory" can be made (see Appendix D). For those samples which have been analyzed and been found not to be particularly satisfactory, but for which no action should be taken due to sampling difficulties, poor methodology, borderline results or for some other reason, simply designate as "Passed."

            As an alternative, give a short summary of the results found as described below for chemically unsatisfactory samples.

        (2) For Chemically Unsatisfactory Samples

            Provide a brief summary of results, including method, ingredient and amount found. Quote the method reference only if AOAC, EPA or other recognized method source. In recording the amount found for deficiencies give only the highest average for all the work performed. If two or more methods were used and the results were comparable, then the highest result (or highest average)

should be reported for each method. For overformulations and cross-contaminations, report the lowest result or average for each method.

b. Lower Portion

(1) General

On the lower portion of Section II, the laboratory work is summarized in more detail, whether the sample is analytically satisfactory or not. Information is included concerning net contents, screening and any other pertinent information along with a more detailed summary of the analytical results than that given in the upper portion (see examples in Appendix D).

The lower section should be headed by the analyst's name and the date reported.

(2) Chemical Names

The chemical names employed in the report should be as listed on the label unless: (1) there is an obvious misspelling, (2) an ingredient is discovered that is not listed, or (3) the label makes no claim as to active ingredient(s). In all of the aforementioned cases, quote the prime referenced name listed in Acceptable Common Names and Chemical Names for the Ingredient Statement on Pesticide Labels, 3rd Edition (EPA-540/4-75-011, Dec. 1975). The common or official name of any pesticide is preferable if listed on the label.

(3) Methods Reporting

When reporting analytical methods, give a brief descriptive title of the technique and a reference source or number, e.g.:

Malathion (Colorimetric; AOAC 6.336)
Total phosphorus (Gravimetric; EPA-1)
Chlorpyrifos [GLC; J.A.O.A.C. 56, 1094 (1973)
Pyrolan (GLC; Zweig VI, p. 471)
Sodium Hydroxide (Titration; Rosin-5, p. 404)

If modifications were made to the referenced methods, append the abbreviation "mod" after the reference, and describe the modification as a brief note only for those samples that are potentially violative. Always fully describe any method modifications in the laboratory notes.

When a method used has no immediate reference, or has been adapted for a particular sample, describe the method in brief, but sufficient, detail so that another chemist reviewing the report can understand the essential steps of the method.

For GLC analysis, describe the column, oven temperature and internal standard (if used). Any derivitization should also be noted. Examples are given as follows:

Malathion (GLC; 3% OV-1 @ 180°)
p-Dichlorobenzene (GLC; 3% XE-60 @ 100°, I.S.-DDVP)
2,4-Dichlorophenoxyacetic acid (GLC; 3% OV-17 @ 170° as methyl ester)

HPLC analyses, not referenced elsewhere, should be described by the column, eluant and detector wave-length, e.g.:

o-Benzyl-p-chlorophenol (HPLC; μ Bondapak C_{18} CH_3OH/ 0.0025M H_3PO_4- 65/35 @ 238nm)
Thiram (HPLC; μ Bondapak C_{18}, CH_3CN/H_2O-55/45 @ 280 nm)

For spectrophotometric methods not given elsewhere, the wavelength and solvent should be reported, e.g.:

Phenothiazine (IR; 1300 cm^{-1} in CS_2)
2-(Naphthyloxy)acetic acid (UV; 272 nm in H_2O)
Dimethoate (NIR; 2930 nm in $CHCl_3$)

Derived methodology for potentially violative samples, should be further described in a note, giving such information as extraction conditions, clean-up, cell thickness, type of detector etc.

(4) Analytical Data

When a quantitative analysis is performed, the actual percentage of ingredient found should be reported. If more than one analysis by a single method on a single container is performed, and the results are comparable, the average should be reported in addition to the individual results. Results by different methods or on different containers should not be averaged.

When different containers are analyzed the results should be identified as sub 1, sub 2, etc. If the inspector has not marked the submitted samples as such, the analyst should mark each sub sample appropriately along with his initials and date.

When a semi-quantitative analysis is performed (e.g. TLC) give the interpreted result preceded by the designation "Est". TLC results are considered to normally reflect a precision of ±20%.

For qualitative tests, simply specify "Present" or "None Detected" as appropriate. When no ingredient is detected, the detection limit should be determined and given in a note.

Net contents, if estimated, should be reported as "satisfactory" or reported as given on the label. If a deficiency in net contents is detected, then the average of all containers should be reported under the "Found" column, and the individual weights listed below. The tare weight(s) should also be listed and identified.

Cross-contamination screening results for which no contaminants are discovered can be reported as:

"Screening (TLC; CL & P): Satisfactory"

If contaminants are detected and confirmed, write "(name of contaminant) detected" along with the other qualitative and quantitative results as described earlier. In such cases write "No claim" under the "Claim" column.

(5) Notes

Any notes regarding the description of the sample, analysis, label, etc. made by the analyst should be made in Item 11, if possible. Such notes should be followed by the analyst's initials to indicate they are his or her remarks, and should be hand initialed on the final typed copies. The analyst's notes should represent observations only, and not conclusions.

c. Reporting of Check Analysis

If a check analysis or any other additional work is performed on a sample by a second analyst, the results should be headed by the designation "Check Analysis" or "Additional Analysis," followed by the analyst's name and date reported. The results should be reported according to the previously described format, except that the "Claim" can be omitted if stated previously in the report (see example in Appendix D).

The check or additional analysis can be reported on the same page as the original analysis if there is enough room without crowding. For most samples, however, a second manifold is used, in which case only Item 1 need be filled out, followed by the word "(continued)." Items 2 through 10 can be left blank and the results reported in Item 11. The Establishment Copy should be discarded before typing, as it is not necessary if all the Establishment Copy information is on the first manifold.

d. Supervisor's Comments and Summary

Usually Item 12 is reserved for remarks or a summary made by the laboratory supervisor. Statements made by the supervisor should reflect or be based on observations and data of the analyst.

The supervisor should provide a brief statement summarizing the laboratory results for every potentially violative sample. The relative percentage deficiency or overformulation is stated so as to minimize the degree of variation from the label claims, i.e. to give the producer the benefit of the doubt. Cross-contamination should be summarized by quoting the lowest result found along with the identity and means of confirmation.

Typical summarizing statements are as follows:

1. Product is 11% deficient in malathion content.

2. Product is 30% overformulated with respect to thiram content.

3. Product contains 0.2% parathion not declared on the label. Confirmation by GLC (two different columns) and TLC (two different eluting solvents).

4. Product is 11% deficient in net contents for two containers.

5. Sub 1 is 15% deficient in toxaphene, sub 2 is 25% deficient. No apparent difference in lot numbers or physical appearance.

6. Product is at least 16% deficient in metaldehyde. Some variation in results due to non-uniformity of sample.

Any noteworthy conclusions based on the physical observations (whether sample is chemically violative or not) should also be stated, e.g.:

1. Strong odor indicates decomposition has occurred.

2. Product emulsifies poorly which could affect efficiency.

3. Non-homogeneity of sample may result in poor application.

4. Improper design of applicator may lead to undue human exposure.

Likewise, any conclusions based on the chemical results should also be summarized, e.g.:

1. Product appears to be 9% deficient in malathion content, but cannot confirm due to interferences.

2. Although 7 to 11% deficient in 2,4-dichlorophenoxy acetic acid, product is considered passed due to non-uniformity of sample.

3. Product appears to be 35% overformulated, however, considered passed since interferences cannot be definitely ruled out.

4. Product consists of two phases, the top phase being 10% deficient in malathion, the bottom phase 14% high in malathion. Malathion content of well mixed sample (no emulsification noted) yields a chemically satisfactory result for the sample as whole. No directions for shaking or mixing noted on the label.

The supervisor's name should be typed in Item 13 and the name of the laboratory in Item 14.

e. Typing the Analytical Report

After the supervisor has added his or her comments and reviewed the analyst's portion of the report, it should be typed.

When the typewritten manifold is received from the typist, it is proofread through Item 11 by the analyst, then initialed and dated, if correct, after his or her name. Any notes or comments should also be initialed by the analyst. The check analyst, if applicable, should check his or her typed portion and initial accordingly. Minor typographical errors in the bottom half of the report for chemically non-violative samples can be neatly erased or corrected by hand. Any errors in the top half of the report of any sample (i.e. that part that is sent to the establishment) or any errors at all in reports for potentially violative samples should be carefully corrected on the typewriter or the report should be retyped. The entire report for potentially violative samples should be very neat, showing no erasures, smudges or obvious corrections.

The supervisor then proofreads his or her remarks and scans the entire report for overall neatness and correctness. The supervisor should then sign in Item 13 and date stamp all copies. The jacket cover and History of Official Sample can also be dated at this time.

f. Final Disposition of Report

The manifold is then torn apart and the copies distributed as follows:

1. The Sample Record Copy, Regional Office Copy and Biological Lab Copy should be punched to fit the sample jacket and inserted on top just above the History of Official Sample.

2. The Establishment Copy should be paper-clipped inside the left-front cover of the jacket.

3. The Laboratory Copy should be retained in the laboratory and stapled together with the Lab Work Copy, a copy of the History of Official Sample, a copy of the Collecton Report and any other notes, charts, graphs, etc. related to the sample. Copies of any correspondence related to the laboratory handling of the sample should also be retained.

The sample jacket should then be returned to the designated Inspectional or Compliance Office, or if arrangements have been previously made, to some other Regional or Headquarters unit.

g. Labeling

A label review must be performed as part of the investigation for every sample collected under the authority of FIFRA, as amended. Thus the laboratory may have occasion to prepare review copies of the product label for inclusion on the jacket. The following is provided as guidance for proper label submission.

If the inspector submits a label obtained from a label bin at a producer establishment, and the label matches exactly that on the product (except for code numbers or net contents), then this label will be adequate for submission with the jacket. When the label differs, or the inspector's identification is incomplete or lacking, another copy of original label should be prepared from the container(s) and properly identified. Code number and net contents differences should be noted (with initials and date) on the margins of the photocopy or photograph, or in a note attached to the mounting paper.

Identification consists of sample number, date, and initials of the preparer somewhere on the label or copy surface so as not to cover up any wording.

Labeling may be submitted either as an original, as photographs, as a photocopy, as a typed copy, or a hand-written copy.

(1) If an original label from a container is submitted, a copy should be made to replace the original on the container. If metal cans have to be cut up for submission due to poor printing contrast, the corners should be rounded and the edges covered with heavy tape. If there is an odor associated with the label that can't be removed, the label(s) should be inserted in a transparent polyethylene bag and sealed with tape. All parts and panels should have their location on the original container indicated, such as "top," "right side," "front," etc.

(2) Photocopies and photographs may be submitted as long as all portions of the label are legible. Small or difficult to reproduce areas may be typed out separately or filled in by hand and initialed. The "Danger" warnings, skull and crossbones and background are to be identified as to their color for all Class "B" poisons.

It may be necessary to prepare a good photocopy by cutting up the legible portions of several photocopies and taping them together.

All photographs and photocopies should be mounted with tape or staples to a sheet of heavy construction paper.

Each photograph or photocopy is to be identified by ID number, date and analyst's initials.

a. Suitable photographs may be obtained with a Polaroid or other equivalent instant camera using black and white or color film. A close-up lens can be used for smaller print, whereas a portrait lens can be used for larger print.

b. In order to photocopy round containers a photocopier with a movable table and fixed light source is necessary. Apeco, Saxon and Savin each manufacture models with this feature. Round container copies can usually be suitably prepared by hand rolling or with the aid of a ruler.

(3) If it is impossible to submit an original, because of very poor printing contrast, (e.g., silk-screened glass jug), a typed or handwritten copy may be submitted as long as it is properly identified.

Originals or copies of all inserts, advertising literature and other related material asssociated with the product should be identified and included as part of the labeling.

When the labels are different for different size containers (except for the size designation) or different subsamples, all of the different labels or copies should be submitted.

The nine basic components which should be present on every product label are: (1) brand name, (2) active ingredient statement, (3) precautionary statement, (4) manufacturer's or distributor's name and address, (5) directions for use, (6) net contents, (7) assigned EPA Registration Number, (8) Establishment Registration Number and (9) use classification.

SCREENING

VIII. SCREENING

INTRODUCTION

EPA Headquarters guidance with respect to screening for cross-contamination is reproduced in Appendix E and should be accepted as the overall criteria by which to develop and maintain a screening program. The following information is provided as supplementary material to assist in the accomplishment of the screening function.

CHLORINATED HYDROCARBON SCREENING

Although AOAC 6.026 should be used to verify the presence of any suspect chlorinated hydrocarbon contamination, it is preferable to use commercially prepared TLC plates for day-to-day routine screening. Aluminum oxide sheets (0.2 mm) of thin aluminum are available from several suppliers (EM, Brinkman or Eastman). The fluorescent indicator normally present does not affect the chromatography or detection and occasionally contributes to sample information. These sheets can be easily cut into the desired size with shears or a paper cutter to minimize waste.

Commercial plates, after elution, are sprayed with a fresh 0.2% silver nitrate solution in methanol, allowed to dry 5 to 10 minutes and then exposed for at least 1 hour under intense short-wave UV. Observation should be made every 15 minutes.

Once identity of a cross-contaminant has been established, a semi-quantitative estimate can be obtained from the spot intensity by applying enough different standards amounts to bracket the sample concentration.

Among the chlorinated hydrocarbons that can easily be detected and identified by TLC are: aldrin, dieldrin, heptachlor, chlordane, (tech. and AG), toxaphene, DDT, TDE, Perthane, BHC, gamma BHC, endrin, Kelthane, Dacthal, hexachlorobenzene, PCNB, chlorthalonil, erbon and p-dichlorobenzene. Spots at or near the origin should be followed up for suspect phenoxy herbicides.

Gas chromotography should be used, if possible, for both qualitative and quantitative verification. If specific AOAC or standard methods are applicable, they should be used, i.e., when the cross-contaminant is present in significant quantity or is the only ingredient present. Since most of the technical chlorinated hydrocarbons consist of several isomers or related compounds, identification can usually be definitely established by means of comparison of retention values on at least two substantially different TLC solvents systems plus one GLC column, or one TLC system and two different GLC columns. Several alternate TLC solvent systems are given in AOAC 6.027(b).

A gas chromatograph equipped with a Coulson or Hall electrolytic conductivity conductor and a venting valve will produce a specific response to chlorinated hydrocarbons in the presence of other non-chlorinated pesticides. Solvent and major ingredient peaks should be vented, if possible, to minimize contamination of the cell water. A 3% OV-1 column operated at 180° to 210° will be adequate for most situations. 3% XE-60 (OV-225) and 3% Carbowax 20M are also recommended for confirmation purposes. A flame-ionization detector will generally be adequate for analysis of contaminants present at greater than 0.1%. An electron-capture (EC) detector can also be used for chlorinated hydrocarbon contaminant analysis. However, one must be very careful in the interpretation of results, as the EC detector is not as specific and is more sensitive than the electrolytic conductivity detectors.

For products formulated with technical chlordane or toxaphene, TLC screening will normally only serve to confirm the identity of the active ingredient, rather than provide any useful information regarding contamination. However, the screening is still considered meaningful since non-specific total chlorine assays are often employed for these products.

Additional TLC solvent systems and relative retention data for most chlorinated hydrocarbons can be found in the EPA Manual of Chemical Methods for Pesticides and Devices and Volume VII of Analytical Methods for Pesticides and Plant Growth Regulators by Sherma and Zweig. Likewise, additional GLC data relative to contaminant identification and verification can be found in Volume VI of Analytical Methods for Pesticides and Plant Growth Regulators.

ORGANOPHOSPHATE SCREENING

Screening for organophosphates can be accomplished by several techniques. In addition to the methods outlined in AOAC 29.022 (for residues) and in the EPA Chemist's Manual, one may also employ the method recommended by McDaniel (NEIC-TLC-1) and adapted from J.O.A.C. 49 1171 (1966). Toluene should be substituted for benzene to minimize the health hazard.

Pre-coated silica-gel plates with fluorescent indicator (EM Silica Gel 60, F-254, 0.2 mm or equivalent) on aluminum or plastic sheets are preferable to laboratory prepared plates. They are more uniform and can be cut to different sizes as needed.

Parathion, methyl parathion, disulfoton, malathion, diazinon, phorate, ethion, EPN, fenthion, coumophos and trithion all can be detected with the referenced TLC systems. A suspected spot noted at the origin, when using benzene or toluene as eluting solvent, should

be further investigated due to the possible presence of TEPP, dibrom, dimethoate or mevinphos. Ethyl acetate is considered a good secondary solvent for eluting organophosphate spots off of the origin. Elemental sulfur will also show up as a spot in most of the described systems at R_f = 0.8 or greater.

Gas chromatography using a thermionic or flame-photometric (FPD) detector can be used as an organophosphate screening procedure of individual samples in addition to providing quantitation and qualitative confirmation. Screening by GLC is not advisable for products formulated with organophosphates since the detector could become overloaded and contaminated.

A 3% OV-1 column operated at 180° is recommended for screening, using a 1-3 micro-liter injections. The sample is prepared by extracting 0.5 grams of sample with acetone. The range and attenuation are adjusted to give 50% full scale deflection upon the injection of 1 nanogram of a typical organophosphate such as parathion. Any sample peaks not present in the technical ingredient standard(s) giving at least 50% deflection indicate possible contamination. This response will represent a contamination level of about 0.01% for most organophosphates of concern.

For qualitative confirmation, the parameters given above will normally be adequate for most organophosphates. However, for quantitative purposes, the sample and standard concentrations must be adjusted to within 5% of each other to minimize non-linearity errors.

Positive confirmation for organophosphates is generally more difficult than with chlorinated hydrocarbons, since most technical materials consist of a single major compound. Thus, two distinct TLC systems and two GLC columns should yield matched retention values to

assure identification. If identification is in any way in doubt, further confirmation should be obtained by other means such as IR or mass spectroscopy.

If the contaminant is present at levels greater than 0.1%, analysis can usually be accomplished by FID.

The EPA Chemist's Manual and Volumes VI and VII of Analytical Methods for Pesticides and Plant Growth Regulators by Zweig and Sherma list retention data for many TLC and GLC systems.

SCREENING FOR OTHER CLASSES OF PESTICIDES

At present there are no routine methods currently in use for screening of other classes of pesticides. If such is desired, different TLC systems are described by class in Volume VII of the previously mentioned reference for: carbamates, triazine herbicides, nitrophenols, substituted ureas and uracils, dithiocarbamates and phenoxyherbicides.

INSTRUMENTATION

IX. LABORATORY INSTRUMENTATION

INTRODUCTION

The pesticide product laboratory should maintain, at a minimum, the following equipment in order to adequately respond to the expected sample variety and to fully take advantage of contemporary technical innovations. Quality assurance considerations dictate that each laboratory be able to apply state-of-the-art methodology--which in today's terms means full capability for gas chromatographic and liquid chromatographic analysis. The quantities of each item and different combinations available for balances, spectrophotometers and chromatographs will be contingent on the number of analysts, space allocation, fund availability, and overall sample composition.

ANALYTICAL BALANCE

1. Specifications - Single pan (mechanical or digital)
   Precision: ± 0.05 mg
   Capacity: 160 to 200 grams

2. Maintenance and Calibration

   The balance should be located as level as possible in a draft-free area on a heavy shockproof, braced table or bench top to minimize external interferences. Spills should be cleaned up immediately, and the balance and surrounding area cleaned up in general after each use. Weights should not be left on the knife edge when the balance is not in use, nor should the balance be set in

the "read" position before having located the final "rough adjust" position. Weight adjusting knobs should be turned slowly. The balance should be cleaned thoroughly and calibrated at least annually (or as needed) by a factory representative or authorized agent. The accuracy should also be checked every three months, or more often, if necessary, by laboratory staff using NBS Class S calibrated weights. A log book should be retained for recording of all maintenance and calibrations.

3. Use

The analytical balance is used for all sample and standard weighings up to 10 grams. It will also be employed for all other preparations where accuracy is necessary, such as primary standard and titrating solutions.

TOP-LOADING BALANCE I

1. Specifications - Mechanical or digital
   Precision: ± 0.005 gr.
   Capacity: 1200 gr. (Tare optional)

2. Maintenance and Calibration

Generally, the same as given above for the analytical balance.

3. Use

The top-loading balance should be used for analytical weighings over 10 grams and for net contents determinations within its capacity. This balance can also be used for the preparation of most laboratory reagent solutions.

TOP-LOADING BALANCE II (Optional)

1. Specifications - Mechanical or digital, readable to 1 gram.

   Precision: ± 0.5 gram

   Capacity: 3 kg (tare optional)

2. Maintenance and Calibration

   Same as given previously for the analytical balance. IOLM standard weights are satisfactory for calibration above 200 grams.

3. Use

   Used for determination of net contents when gross weight is over 1 kg.

   Note: Satisfactory digital top-loading balances are also available that combine features of the high and low loading balances described above.

pH/MILLIVOLT METER

1. Specifications Range: 0-14 pH units or ± 1500 mv, accuracy: ± 0.01 pH unit or ± 1 mv, (± 2000 mv range and ± 0.1 mv accuracy, if specific ion analysis is to be performed). The meter should accomodate common electrode jacks and have temperature compensation capability.

   Electrodes necessary for analysis of pesticide formulation include the following: (1) standard calomel fiber junction with saturated potassium chloride electrolyte, (2) glass electrode (Fisher 13-639-3 or equivalent),

(3) silver billet (Corning 476065 or equivalent), (4) combination pH/reference electrode, (5) combination redox (platinum, Orion 96-78 or equivalent).

Automatic titrating units are available that are also satisfactory for routine laboratory use. These instruments (Fisher, Mettler, and Brinkmann, and others) are available with different degrees of automation from end-point detection to automatic sample switching. The most desirable feature of automatic titrators is the synchronous recorder option, which will yield a permanent record of the titration curve.

The acquisition of the automated titrator unit should be considered by those laboratories performing a high volume of titration samples; i.e., at least 50/month. The titrator unit will also serve as a routine pH meter, if necessary.

2. Maintenance and Calibration

The meter and electrodes should be maintained according to the manufacturer's recommendations.

Normally, the glass electrode and silver billet will be left attached to the instrument as the chloride determination will be the most prevalent use. These two electrodes should be left standing in clean distilled water. Periodically buff the silver billet tip with steel wool to enhance response.

Titration curves should actually be plotted for suspected violative samples and during reagent standardizations to verify end-point consistency. In most routine cases,

however, the determination can be performed by titrating in progressively smaller millivolt increments to a predetermined end-point value.

Buffers of pH = 3, 7, 10 should also be maintained for purposes of accuracy in pH measurements, particularly for certain colorimetric determinations, extraction steps and HPLC mobile phase adjustment. Exact calibration is not as critical for pH or mv titrations, since usually detection of the pH change at the end-point is all that is important.

Combination pH and redox electrodes are more convenient to use, and are just as accurate as dual electrode systems, when these type of measurements and/or titrations need to be performed.

3. Use

As mentioned above, the pH/millivolt meter will most often be used for the potentiometric determination of chloride or bromide.

The halide anion may derive directly, as with the determination of quaternary ammonium compounds, or indirectly after reduction of an organohalide with sodium, sodium biphenyl, Parr bomb or lime fusion. The chief interference will be sulfur or sulfide, which must be oxidized to sulfate with hydrogen peroxide before halide titration can be accomplished. In most cases, chloride and bromide can be determined individually in the presence of each other by carefully plotting mv vs. volume. However, neither ion should be present in great excess over

the other, or only total halide can be determined. The resulting curve should be similiar to the illustrated in Figure IX-1.

The pH meter will also be used for acid-base and redox titrations. Although chromatic chemical indicators are specified in many written methods and will serve for most general acid-base titrations, a potentiometric plot should be accomplished whenever the solution being titrated is suspected of being buffered, or thought to contain different species than claimed. A potentiometric titration will be particularly useful in titrating two or more species in a mixture of differing pk_a or pk_b values; i.e., phosphoric acid in the presence of a mineral acid such as HCl [see Figure IX-2].

Specific ion electrodes have yet to find extensive use in pesticide formulation analysis, however, this should not preclude their use on an experimental basis.

INFRARED SPECTROPHOTOMETER

1. Specifications

A research spectrophotometer is not necessary for routine pesticide formulation analysis. However, the instrument should be of double-beam/optical null design with grating optics and wavelength range of 4000 to 400 cm^{-1}. Ordinate repeatability should be at least 1% of full scale with an accuracy of ± 1% full scale. The chart paper grid width (ordinate) should be at least 15 cm wide to ensure accurate absorbance readings to three significant figures. Ordinate and abscissa expansion are desirable, but not necessary options.

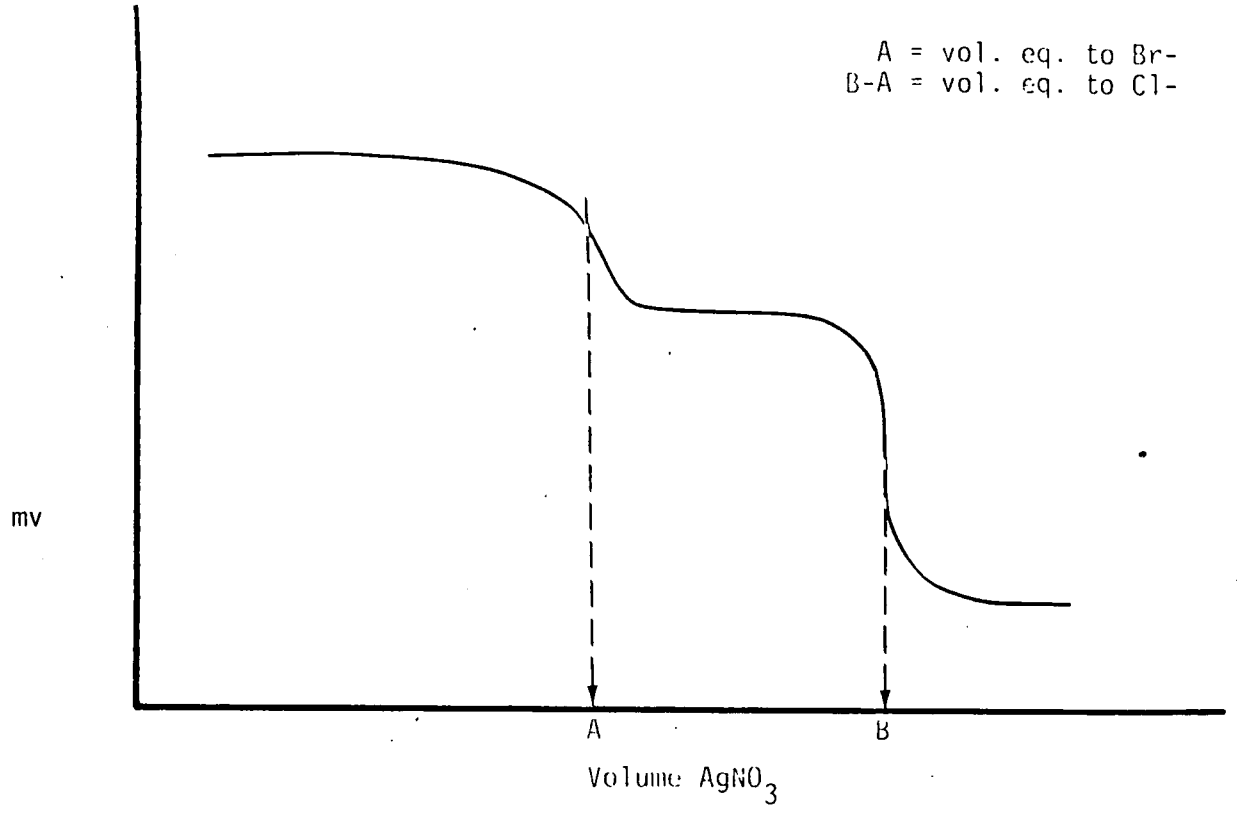


Figure IX-1. Titration of Br- and Cl- with $AgNO_3$

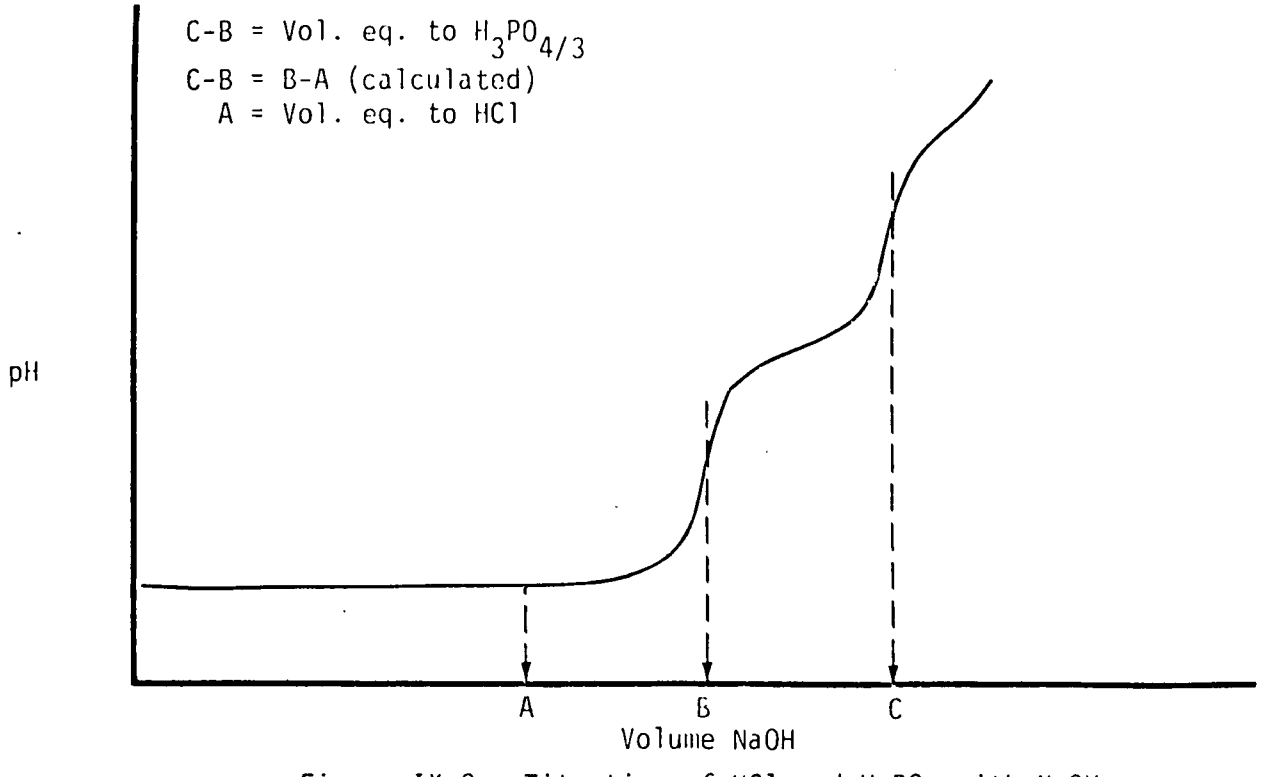


Figure IX-2. Titration of HCl and H_3PO_4 with NaOH

Matched potassium bromide cells should be available in 0.1, 0.2, 0.5, and 1.0 mm thicknesses. Potassium bromide pellet apparatus (press, die, holder, etc.) may prove useful on occasion for qualitative confirmation purposes.

2. Maintenance and Calibration

Maintenance and routine operation should be performed according to the manufacturer's recommendations.

Wavelength calibration should be checked at least once every three months by means of a polystyrene film. Reproducibility should be verified, at least for all potentially violative samples analyzed by infrared, by repeating the specified wavelength scan in duplicate for both the sample and standard. The gain, slit program, sensitivity and scanning speed are to be adjusted according to the purpose of the particular scan; i.e., whether quantitative or qualitative.

The cells should be stored, when not in use, in a desiccator cabinet with indicating silica gel. Moisture contact with the cell surfaces is to be minimized with respect to handling, quality of solvents used and exposure of the cells to the atmosphere. After rinsing with solvent, the cells should be dried by use of drying tube and vacuum line. Care should be taken to prevent solvent from contacting the rubber gasket that binds the cell in the holder. The cells should be replaced when permanently fogged to the point resolution is intolerable or transmission for the blank cell(s) is less than 90%.

3. Use

Many Official, Standard and other methods are available utilizing IR techniques. For single component dusts, wettable powders and granules, IR is one of the most precise methods available from a quantitative standpoint. The relative precision for most tested IR methods is on the order of ± 1%.

Infrared spectroscopy can also be utilized to assay some emulsifiable concentrates, but its use is quite limited for the analysis of low percentage liquids and pesticide mixtures.

Generally, for powder and granules, a single shake-out for an hour followed by filtration or centrifugation will prepare the sample for analysis. For liquid formulations, an attempt is usually made to evaporate off as much solvent as possible by heating on a steam bath with a current of air prior to dilution; however, care must be taken to ensure that no active ingredient is lost through evaporation or decomposition. Chloroform and carbon disulfide have found the widest usage as IR solvents for quantitative purposes, although acetonitrile, carbon tetrachloride and acetone are also used for the extraction step and as the spectrophotometric solvent. Carbon disulfide, however, is a very poor extracting solvent, so that extraction usually must be carried out with acetone or a carbon disulfide-acetone (9:1 V/V) mixture. The extract is then evaporated prior to redilution with carbon disulfide for IR measurement.

It is desirable to match the absorbance of the sample and standard as closely as possible to minimize non-linearity effects, although most written methods have been verified for adherence to Beer's Law. One must be very cautious of deviations from linearity in regions of the spectra where hydrogen or other intermolecular bonding occurs, particularly in the near infrared.

ULTRA-VIOLET/VISIBLE SPECTROPHOTOMETER

1. Specifications

Double beam, single monochromator, ratio and energy recording.

Wavelength range: 190-3500 for UV/Vis./NIR
190-900 for UV/Vis.

Resolution: 0.2 mu at 220 nm

Photometric reproducibility: 0.5% Transmission

Wavelength accuracy: 0.4 nm, 1.5 nm, and 8 nm in the UV, visible and near infrared, respectively.

Variable scanning speeds: 1.8 to 1800 nm/min. depending on range

Oridinate and Abscissa expansion: optional

Source: H_2 discharge for measurements to 400 nm; Tungsten filament for 360 nm and above.

Detector: Photomultiplier for use to 700 nm
Lead sulfide cell for use above 360 nm

One cm matched infrared silica cells can be used for practically all routine determinations. Standard silica cells should be used when measuring absorption below 220 millimicrons. 0.1 cm silica cells may also be useful on occasion but are not necessary.

2. Maintenance and Calibration

Maintenance and calibration should be performed according to the Operator's Manual. The wavelength and response for a standard holmium oxide filter should be checked at least once every 3 months or whenever there is doubt regarding instrument performance. Annual cleaning and calibration by a factory representative is also recommended.

Reproducibility should be checked in duplicate for all potentially violative samples, and for all measurements taken in the near infrared. The hydrogen discharge lamp should not be left on any longer than necessary, as it has a limited life span and is expensive to replace.

Every precaution should be taken when cleaning and handling of the silica cells. Fingerprints, dust, deposits, and moisture on the cell windows are to be avoided. For cleaning purposes the cells should be rinsed with water and methanol and air-dried. Acetone should be avoided as any trace will strongly absorb in the UV Region. If enough sample or standard solution is available, the sample cell may be prepared for individual measurements during an analytical run by rinsing and discarding with solution to be measured three times prior to filling the cell for analysis. In such cases it is preferable to start with the weakest solution in a series and progress to the strongest.

3. Use

There are several pesticide formulation methods available utilizing the UV Region, fewer procedures that utilize the visible region, and very few utilizing the near infrared region. The accuracy and precision deteriorate as one

goes from UV to NIR, although the spectra become more specific. Many compounds (both pesticides and inerts) absorb in the UV region, thus any spectrophotometric results obtained should be carefully interpreted.

In the visible region, the results are generally more specific but care must be taken to match sample and standard in absorption intensity or prepare a full calibration curve; even though the concentration response curve may be linear, it does not always pass through the origin.

One must be extremely careful in making measurements in the near infrared; this is a relatively unstable spectrophotometric region from both an instrumental and molecular standpoint. If there is any doubt concerning the analytical results, another analysis should be performed using the infrared spectrophotometer or another method.

The response curve for most compounds in the near IR region is non-linear, or at best, linear over only a very limited concentration range. Thus either the standard and sample should be matched within 3% of each other, or a complete calibration curve should be prepared.

Also, since most of the measurements made in the NIR region result from the presence of an N-H moiety within the molecule, as is the case with carbamates, co-extratives will have an unpredictable effect on the band intensity due to hydrogen bonding and other intermolecular forces. If this situation appears to be the case, determinations should be made at two concentration levels and the results compared. If there are significant differences between the two results, an alternate procedure should be attempted.

For most spectrophotometric analyses, the following instrument parameters (or equivelent) will be optimum for quantitative determinations in the UV region:

Sensitivity:	10
Time constant:	0.2
Scanning speed:	36 nm/minute
Range:	0-1% T; 0-1A
Source:	Hydrogen lamp
Detector:	Photomultiplier 1x
Scale expansion:	1x

The instrument should be adjusted for 100% T at the wavelength of interest with reference solvent in both cells, and for 0% T with the sample shutter beam closed. The adjustments should be repeated if a different (more sensitive) range is going to be used. After the zero and 100% T have been adjusted in the transmittance mode, the unit may be switched to the absorbance mode. Usually, for UV determinations, the absorption is measured from 360 to 190 nm. This serves as an indication if any interferences are present and also allows determination of a good base point.

For use in the visible range the above settings should be retained, however, a tungsten source should be used instead of hydrogen lamp and a scanning speed of 360 nm/min employed. The peak of interest should be scanned for at least 100 mu on each side to verify that the measurement is being taken at the maximum absorption point and not on another peak shoulder. For use in the near infrared, the tungsten lamp and lead sulfide detector cell should be used.

GAS CHROMATOGRAPH

A gas chromatograph is an absolute necessity in the pesticide formulation analytical laboratory. Although there are many

limitations to the technique and a lack of official methods utilizing gas chromatography, there are too many occasions where it offers the only specific and/or rapid method available.

The basic requirements for the analysis of a pesticide by gas chromatography are for the compound of interest to be somewhat volatile and thermally stable.

1. Specifications

There are many commercial gas chromatograph models available offering a wide selection of detection systems, column configuration, programming capabilities, and data systems. The basic considerations when making a selection, however, are (1) adaptability to different detector systems, (2) overall system response and reproducibility, (3) simplicity of operation, (4) ease and availability of maintenance and service, and (5) adaption to data systems and auto samplers.

A research-oriented instrument is not required for most analytical needs, but at least one temperature programming unit should be available in the laboratory. The column oven should be stable to ± 0.2°C, and is a much more critical factor than the absolute temperature itself, although the latter figure should not vary by more than 5°C from its true value. Electrometer and recorder (1 mv) response should not vary by more than ± 0.5% each.

The following detectors are recommended for the pesticide formulation laboratory as part of the overall gas chromatographic capability:

(a) Flame Ionization Detector (FID)

The flame ionization detector is currently the most useful gas chromatographic detector to have available in the pesticide formulation laboratory for routine quantitative analysis. Its main features are: good sensitivity, non-selectivity (responds to all compounds except inorganic gases and water), and wide linearity range. The FID is relatively insensitive to minor thermal and flow fluctuations. The primary disadvantages are that it destroys the sample components during detection, and that it requires two additional gases in addition to carrier. Nitrogen or helium can be used as carrier.

A FID system should be maintained that is capable of being used as either a dual flame ionization unit or as two independent detectors. Dual flame operation will be rather rare in the pesticide formulation laboratory, thus two distinctly different types of columns can be maintained ready for use on each side of the FID. The dual-flame capability will only be required for high sensitivity or the temperature programming. If two columns are to be used simultaneously, however, two separate electrometers and recording channels will be necessary.

Although not specifically designed for this use, the flame photometric detector can also be used as a flame ionization detector. However, the flame will be a reducing flame rather than oxidizing, resulting in less overall sensitivity and reduced linear range. A solvent valve may be necessary for such usage, as any injections over 1 microliter may extinguish the flame.

Gases for FID operation are specified as follows:

Air -- Best quality medical breathing air or compressed air, both with filter-driers. Compressed air may prove to be unsatisfactory at very high sensitivities, however.

Hydrogren -- Prepurified with filter-drier.

Carrier -- 99.99% helium or pre-purified nitrogen, both with filter-driers.

(b) Thermal Conductivity Detector (TCD)

If a flame ionization detector is not available for quantitative analysis in the formulations laboratory, the second choice is a thermal conductivity (or hot wire) detector. An advantage of the TCD is that it is non-destructive; components may be collected from the exit port for further analysis. The TCD is also rather inexpensive and requires only carrier gas for operation. Unfortunately, the recommended carrier gas is helium, which is expensive and difficult to obtain in some locations. The disadvantages of the TCD are its lack of sensitivity and its relatively large response to minor temperature and flow variations.

The TCD should be protected with an over-heating or high-resistance switch to protect the detector from damage at high temperatures, particularly in the presence of oxygen or highly-oxygenated compounds. A TCD of low filament channel design is also preferable to minimize loss of resolution in the detector. This

will allow the use of 3.5 mm I.D. columns at relatively low flow rates.

Carrier gas should be 99.99% helium with a filter drier. Pre-purified hydrogen has also been used as carrier for the TCD, but is not recommended from a safety standpoint.

(c) Flame Photometric Detector (FPD)

The flame photometric detector (Melpar or Bendix) is also a useful addition to the gas chromatographic system in the pesticide product laboratory. The detector is specific for compounds containing either sulfur or phosphorus depending on the installed filter. Although not used routinely for quantitative purposes, the FPD is invaluable for cross contamination screening and for verifying the identity and concentration of cross-contaminants.

There is an occasional use for the FPD for routine sample assays when it is desirable to selectively detect an organophosphate or sulfur containing pesticide in the presence of oils or other interfering materials without resorting to clean-up procedures.

If possible, both phosphorus and sulfur filters should be mounted in separate photomultiplier tubes with dual electrometer and recorder channels. The two detector signals can then be monitored simultaneously.

Extreme caution should be taken if quantitative analysis is being performed in the sulfur mode. Sulfur response is logarithmic rather linear, so that it will

be necessary to prepare a calibration curve or to assure that sample and standard response are matched within 2% of each other.

The FPD may be equipped with a valve on the column exit to vent solvents and unwanted material.

The gases required for FPD operation are identical to those for FID, except that oxygen may be needed for fuel support. U.S.P. Oxygen with a filter drier performs satisfactorily. Newer models do not require oxygen.

(d) Electrolytic Conductivity Detector

The pesticide formulation analytical laboratory should also have available a gas chromatographic detector specific for halogen response. Of the available detectors that serve this purpose, microcoulometric, electron capture and electrolytic conductivity, the electrolytic conductivity detector (Hall) best fills the overall need.

Results from the electrolytic conductivity detector are relatively easy to interpret and use of the detector does not require an NRC license. The electrolytic conductivity detector will be primarily used for screening and verifying halogenated cross-contaminants. Although the electrolytic conductivity detector is not as sensitive as the electron-capture, this is not particularly a drawback in formulation analysis, as rarely is there any interest in components present at less than 50 ppm (0.005%).

The electrolytic detector can be modified to serve as a nitrogen specific detector by the insertion of an activated nickel wire and strontium hydroxide plug in the quartz tube. Newer versions are equipped with a nickel combustion tube.

The electrolytic conductivity detection system should be equipped with a valve so that solvents and other undesirable eluants can be vented to the atmosphere, thus minimizing contamination of the cell solution. On older model Coulson type detectors, by-pass carrier gas should be available directly to the valve and adjusted such that the flow rate through the cell is not disturbed significantly when in the vent mode, otherwise the siphon may be lost.

(e) Miscellaneous Detectors

Other detectors that may find occasional use in the pesticide analytical laboratory are the electron-capture, microcoulometric and thermionic (or alkali-bead) detectors.

The electron capture (either Tritium or Nickel-63) or microcoulmetric (Dohrmann) could, if necessary, substitute for the electrolytic conductivity detector, as all three are halogen specific, although electron-capture will also respond to many other atoms and groups. The alkali bead detector can substitute for either the flame-photometric (phosphorus mode) and/or the electrolytic conductivity (nitrogen mode).

Specific information on the use, maintenance and calibration of electron-capture and micro-coulometric detectors should be obtained from the manufacturer(s).

2. Maintenance and Calibration

Maintenance of the gas chromatograph is too lengthy and sophisticated to treat adequately here.

The Operator's Manual for most instruments treat preventive maintenance and trouble shooting in some detail. Other resources in this field include:

(a) EPA Manual of Analytical Quality Control for Pesticides in Human and Environmental Media; published by the Health Effects Research Laboratory, Research Triangle Park, NC, 27711, and

(b) Chromatographic Systems/Maintenance and Trouble Shooting, Walker, J. Q. et al, Academic Press, N.Y. 1972.

The principal criteria as to whether or not the gas chromatograph is operating satisfactorily are the peak shape and reproducibility of retention time and response. Excessive noise, poor response, and large solvent peaks are also causes for concern.

The gas chromatograph should be periodically checked against a mixture of known compounds using a standardized set of instrument parameters. A recommended audit procedure for the FID, FPD, NPD, and microconductimetric detectors is given in Appendix F.

3. Columns

The basic column inventory for pesticide formulation analysis should include the following:

(a) 3% OV-1 (or eq.) on Chrom WHP 80/100 (glass)
2 m x 3.5 mm

(b) 3% OV-210 (or eq). or Chrom WHP 80/100 (glass)
2 m x 3.5 mm

(c) 3% OV-225 (or eq.) on Chrom WHP 80/100 (glass)
2 m x 3.5 mm

(d) 3% Carbowax 20M (or eq.) on Chrom WHP 80/100 (glass)
2 m x 3.5 mm

The following columns will also find occasional use in the formulation laboratory.

(a) 3% OV-17 on Chrom WHP 80/100 (glass) 2 m x 3.5 mm
(b) Poropak Q. or Chromosorb 101 80/100 (glass) 2 m x 3.5 m

Although many other columns are listed in the pesticide formulation literature, usually one of the above columns will be equivalent. The OV and SP phases are quite superior, from a thermal stability and consistency standpoint, to the older phases listed in the literature.

It is recommended that the columns listed in "Report of the Committee on Gas Chromatography of Pesticide Formulations" [J.A.O.A.C. 50, 420, (1976)] be employed to the degree possible whenever developing new methodology.

Columns prepared for formulation use should never be employed for residue analysis due to possibility of contamination.

Stainless steel may be employed for the formulation analysis of many pesticides, but borosilicate glass is preferred, and is not that much more expensive for the advantages

gained. Glass is more inert, can easily be adapted for on-column injection, and is less likely to cause support fragmentation. It is also easier to observe voids and deterioration with glass columns. Bending of stainless steel columns after packing can crush support material and expose active sites which can lead to severe tailing problems even on new columns.

All columns should be conditioned and operated according to standard gas chromatographic technique. One must be cautious when raising the column oven temperature so as not to exceed the temperature maximum of other columns in the oven. The outside of the column oven should be clearly marked as to what columns are on each injection port and the date they were installed.

It is recommended that each laboratory buy pre-coated column packings in a fairly large quantity (i.e., 50 grams) to save time and eliminate variability from column to column. Applied Science, Analabs, Supelco, and Altech all sell most standard packings that are needed for pesticide product work. Specialty columns and new packings can be prepared in the laboratory, however, using fluidizer, rotary evaporation, or other standard technique.

A column should be abandoned and repacked when evidence of deterioration appears such as non-reproducibility and severe tailing. Generally, silylating techniques have not improved chromatography in such cases so as to be worthwhile.

4. Typical Analysis

(a) External Standard Method - A typical routine external

standard quantitative determination by gas chromatography, utilizing the FID, may be accomplished as described below.

Approximately 25 mg of standard (ca. 1 drop for liquids) should be weighed out to the nearest tenth of a milligram in a 25 ml volumetric flask, dissolved in, and diluted to volume with acetone or other suitable solvent. This will yield a final standard concentration of ca. 1 mg/ml.

Enough sample (according to the label claim) should be weighed out so as to match the standard concentration as closely as possible for a 25 ml final volume. Liquids may be diluted directly in a volumetric flask. Solids should be shaken out for at least one hour on a reciprocating shaker or ultra-sonicated for 20 min. after addition of 25 ml of acetone by pipet. A 50 ml screw-top erlenmeyer flask with a Poly-seal cap is recommended. After shaking or ultra-sonicating, the extracted material should be filtered (avoiding evaporation) or centrifuged for several minutes to yield clear supernatant. One of the columns listed earlier should be selected for analysis, usually either the 3% OV-1 or 3% OV-225, according to the nature of the material being analyzed. The column oven temperature should be adjusted to give elution of the desired component in 4 to 5 minutes, if possible. Many pesticides elute satisfactorily in the temperature range 180 to 220°C. If a specific or appropriate method is not available, the relative retention times for many pesticides can be found in the following references:

(1) Analytical Methods for Pesticides and Plant Growth Regulations, Vol. VI Gas Chromatographic Analysis, Ed. by G. Zweig, Academic Press N.Y.

(2) EPA Manual of Quality Control for Pesticides in Human and Environmental Media, published by the Health Effects Laboratory Research Laboratory, Research Triangle Park, N.C. 27711.

(3) FDA Pesticide Analytical Manual, published by U.S. Dept. HEW, Food and Drug Administration, Appendix to Volumne I, Transmittal 79-1.

The injection port temperature is maintained at least 10°C, but not more than 25°C, above the column oven temperature. The FID temperature should be adjusted to 230 to 250°C. The carrier flow should be between 50 and 70 ml/min for ¼-in column, hydrogen at about 40 ml/min and air at 350 to 400 ml/min. Three microliters should be injected (by total injection technique) for both sample and standard. If the response of the sample is significantly different (>5%) from the standard, the sample (if apparently over-formulated) or standard (if apparently deficient) should be diluted to establish a more equivalent response, rather than the varying injection volume or attenuation. A calibration curve may also be established by injecting 3 µl of various concentrations, which should include at least one value on either side of the sample response.

The input/output (or range/attenuation) should be adjusted to give 50 to 70% full scale recorder response. Standard and sample should be injected alternately

until each pair reproduce to within ± 2% of each other. The output or attenuation should be used to adjust recorder response, if necessary, rather than the input or range adjustment.

The retention times of sample and standard should also be compared, and if there is any doubt as to their not being identical, a spiked sample should be injected. Two peaks or a visible shoulder will indicate that the compounds are different.

Peak areas or peak heights should be averaged for the sample and standard, and the percent active ingredient calculated as follows:

$$\frac{\text{Response}_{\text{sample}}}{\text{Response}_{\text{standard}}} \times \frac{\text{conc.}_{\text{standard}}}{\text{conc.}_{\text{sample}}} \times \%\ \text{purity of std.} = \%\ \text{active ingred.}$$

(b) Internal Standard Method

A typical internal standard analysis may be accomplished in a similar manner by adding an identical amount of a previously prepared internal standard solution to both sample and standard.

For liquids, 10 ml of internal standard solution is added to sample and standard previously weighed out in a 25 ml volumetric flask; the solutions are then diluted to the mark for GC analysis. The internal standard generally needs to be in a more concentrated form to account for this dilution.

For dusts, powders and granules, the internal standard solution is used as the extracting solvent. In this

case, the standard for the assayed ingredient should be diluted by pipet in a screw-top flask to minimize volumetric error that would occur by diluting "to volume" in a volumetric flask.

The internal standard response should be within ± 1% for sample and standard, or else the sample/standard should be reprepared and/or the sample should be checked for an internal standard co-eluter. In the latter case, a different internal standard should be selected or an external standard method employed.

It is recommended that a non-pesticide internal standard be selected when developing new methodology. This will minimize cross contaminant interference, minimize handling of concentrated toxic pesticides and save valuable standard material. Some typical retention times for suggested internal standards are given in Table IX-1.

The percent active ingredient for an internal standard method can be manually calculated as follows:

$$\text{Adjusted sample response} = \text{sample response} \times \frac{\text{internal std. response (standard)}}{\text{internal std. response (sample)}}$$

$$\frac{\text{Adjusted sample response}}{\text{Standard response}} \times \frac{\text{conc. std}}{\text{conc. sample}} \times \text{\% purity of std.} = \text{\% active ingred.}$$

HIGH-PERFORMANCE LIQUID CHROMATOGRAPH

Although high-performance liquid chromatography (HPLC) is a relatively new quantitative technique, many published methods for

Table IX-1
NON-PESTICIDE INTERNAL STANDARD RETENTION TIMES

Column: 3% OV-1 on Chrom WHP 80/100 6' x 4 mm ID (glass)

Carrier: N_2-60 ml/min.

	140°C		160°C		180°C		200°C	
	RT	RRT*	RT	RRT*	RT	RRT*	RT	RRT*
p-Bromophenol	1.2	0.08	-	-	-	-	-	-
Biphenyl	1.85	0.12	0.9	0.10	-	-	-	-
Dimethyl Phthalate	2.45	0.16	1.2	0.13	0.7	0.16	-	-
Diethyl Phthalate	4.7	0.30	2.0	0.22	1.05	0.24	-	-
Benzophenone	5.4	0.35	2.4	0.27	1.25	0.29	-	-
Dipropyl Phthalate	9.0	0.45	3.4	0.44	1.7	0.47	0.9	0.50
Benzyl Benzoate	9.9	0.63	4.0	0.44	2.0	0.47	1.1	0.51
Aldrin	15.65	1.00	9.0	1.00	4.3	1.00	2.15	1.00
Diisobutyl Phthalate	16.15	1.03	6.05	0.67	2.7	0.63	1.4	0.65
Dibutyl Phthalate	-	-	8.7	0.97	3.7	0.86	1.8	0.84
Triphenylmethane	-	-	9.8	1.09	4.35	1.01	2.2	1.02
Dipentyl Phthalate	-	-	18.7	2.08	7.3	1.70	3.3	1.53
Diphenyl Phthalate	-	-	-	-	21.6	6.00	8.4	4.67
Dioctyl Phthalate	-	-	-	-	-	-	11.9	5.53

Column: 3% OV-225 on Supelcoport 80/100 6' x 4 mm ID (glass)

	RT	RRT*	RT	RRT*	RT	RRT*	RT	RRT*
p-Bromophenol	6.2	0.31	2.6	0.33	1.3	0.34	0.7	0.39
Biphenyl	2.2	0.11	1.0	0.13	0.6	0.16	-	-
Dimethyl Phthalate	6.6	0.33	2.75	0.34	1.4	0.37	0.7	0.39
Diethyl Phthalate	10.8	0.54	4.3	0.54	2.0	0.53	1.0	0.56
Benzophenone	11.6	0.58	4.8	0.60	2.3	0.61	1.1	0.61
Benzyl Benzoate	19.3	0.97	7.4	0.93	3.3	0.87	1.6	0.89
Aldrin	20.0	1.00	8.0	1.00	3.8	1.00	1.8	1.00
Diisobutyl Phthalate	28.7	1.44	10.1	1.26	4.2	1.11	1.8	1.00
Dipropyl Phthalate	22.3	1.12	8.1	1.01	3.4	0.89	1.6	0.89
Dibutyl Phthalate	-	-	16.0	2.0	6.3	1.66	2.7	1.50
Triphenylmethane	-	-	14.8	1.85	6.1	1.61	2.7	1.50
Dipentyl Phthalate	-	-	32.4	4.05	11.85	3.12	4.75	2.64
Dioctyl Phthalate	-	-	-	-	36.4	9.58	12.8	7.11

formulation analysis are now available. HPLC appears to be the dominant trend in the field of formulation analysis and most new methodology utilizes the technique. HPLC has the advantage over GC in that non-volatile compounds lend themselves to direct analysis (without derivatization) and that thermally labile compounds can be easily chromatographed. The disadvantages are high cost, non-uniformity of column technology and large amounts of spent solvents to dispose of. HPLC still finds only limited use in the residue area due to detector limitations.

1. Specifications

A full purpose liquid chromatograph for the formulation laboratory should be equipped for dual solvent gradient operation. With the wide variation in columns available, particularly reverse phases, the gradient capability will help considerably in establishing the ideal mobile phase composition. Solvent composition will often need to be varied from that given in referenced methods, particularly when the column is not exactly the same as that specified. The gradient should be capable of reproducing retention times to within 1% for like injections so that analyses can be conducted directly in the gradient mode.

The liquid chromatograph should be equipped with either a fixed-loop injector or autosampler for maximum reproducibility of injection for formulation analysis. With formulation analyses, it is usually very easy to adjust sample and/or standard concentration to adapt to a 5 or 10 microliter loop. If both residue and formulation analyses are to be performed on the same instrument, it will probably be more practical to utilize a syringe or some other variable type injector.

The chromatograph should also be equipped with a variable wavelength UV detector. The variable wavelength option will allow for maximum sensitivity and for "tuning out" of potential interferences. A continuous (200 to 700 nm) wavelength detector is ideal, but the multiple wavelength type with 30 nm interval filters will also be satisfactory for most cases. The detector should produce minimal drift in the isocratic mode, and possess a 10^4 linear dynamic range with 1 or 10 mV recorder output. The refractive index detector has not found wide use in the pesticide formulation laboratory.

A column oven or water-jacket is recommended whenever there is appreciable variation in ambient temperature or there are instrument heating effects. Column temperature regulation is also necessary for working with ion-exchange and gel exclusion packings. The solvent-flow rate should be adjustable to at least 0.1 ml/min and be capable of producing at least 5 ml/min with analytical grade columns and 15 ml/min for preparative grade columns.

2. Maintenance and Calibration

Preventive maintenance trouble shooting should be performed according to the manufacturer's recommendations. The most important maintenance requirements are lubrication of mechanical parts and periodic replacement or cleaning of the in-line filters. The loop injector will also require periodical adjustment to prevent leakage due to wear.

Corrosive mobile phase such as acids and bases should not be allowed to stand on the instrument when not in use.

Acidic and basic mobile phases can hasten deterioration of analytical reverse phase columns and stainless steel lines if allowed to sit in contact for extended periods of time.

Care must also be taken when using reverse phase columns to avoid precipitating any of the sample ingredients, as compounds of interest may be lost or the column can become plugged. When changing from one solvent to another that is immiscible with the first, an intermediate solvent should first be flushed through the system to eliminate the last traces of the initial solvent. Acetone and p-dioxane are considered good solvents for this purpose.

A calibration mixture should be established for each column that can be analyzed periodically or whenever malfunction is suspected. A suggested phthalate mixture and HPLC conditions for a C_{18} Bondapak (Waters) are given in Appendix F, Table 1. Injection of such a calibration mixture will assure proper response and separation when actual samples are analyzed. Such calibration injections should be recorded in the instrument logbook.

3. Typical Analysis

In practice, a typical HPLC analysis will closely parallel a gas chromatographic analysis. There are two distinct differences, however, that must be kept in mind, (1) to improve GC separation, a change is generally made in the column and/or temperature, whereas in most cases during liquid chromatography a change will be made in the mobile phase. A column change is generally a last resort. (2) The UV detector response for HPLC will vary considerably from compound to compound, whereas, response with the GC/FID system, there is relatively little response change from compound to compound.

A good introduction to HPLC analysis of pesticides (both formulations and residues) can be found in:

> Analytical Methods for Pesticides and Plant Growth Regulators, Vol. VII, Ed. by G. Zweig and J. Sherma, Academic Press, N.Y.

With few exceptions, most pesticide formulation methods today are being carried out on reverse phase microparticulate columns. These columns offer good resolution for a wide range of polar and non-polar compounds. Acidic and basic pesticides can be analyzed in most cases directly by extraction and/or dilution using a paired ion in the mobile phase.

As in GC, the internal standard method should be utilized whenever applicable although with loop injectors, the need is minimized. Also it is desirable to use non-pesticide internal standards; however, such compounds with known retention values for HPLC are not as available as for GC. Thus, it may take considerable scouting to come up with a good non-pesticide internal standard for a lot of pesticides. Some suggested non-pesticide internal standards are given in Table IX-2.

Normally, the internal standard can be made up in concentrated form in either the particular mobile phase to be used or one of the make-up solvents. Identical amounts of internal standard can then be added to both standard sample extract (or dilution) as done for GLC. Extraction of active ingredients from solid matrices usually is effected by shake-out and/or ultrasonic treatment with one of the mobile phase solvents, or at a minimum a solvent that is miscible in the mobile phase and of low UV absorptivity at

Table IX-2

HPLC INTERNAL STANDARDS

RETENTION TIMES IN MINUTES

REVERSE PHASE MEOH/H_2O VARIAN 5000 ON MCH-10 @ 2ml/min 254 nm

Internal Std.	% Mobile Phase as MeOH/H_2O							
	100/0	85/15	75/25	65/35	60/40	50/50	40/60	25/75
Phenol	1.40	1.60	1.70		2.18	2.95	4.15	7.48
Dimethyl Phthalate	1.57	1.70	2.10	2.70	3.10	5.30	11.50	
Acetophenone	1.65	1.83	2.20	2.90	3.30	5.05	9.40	
p-Bromophenol	1.50	1.71	2.25	3.20	3.83	6.75	13.61	
Diethyl Phthalate	1.60	1.95	2.60	4.35	5.50	12.30		
Benzophenone	1.80	2.25	3.45	6.50	8.80	20.90		
Benzyl Benzoate	1.90	2.70	4.95	11.83	17.75			
Biphenyl	1.91	2.95	5.80	13.35	19.70			
Diisobutyl-phthalate	1.60	2.70	5.80	17.38	29.15			
Dibutyl Phthalate	1.81	2.94	6.30	19.45	33.5			
Triphenyl-methane	1.90	4.40	10.45	39.7				
Dipentyl Phthalate	1.95	3.85	11.95					
m-Diphenoxy-benzene	2.05	4.35	12.60					
Dioctyl Phthalate	2.33	12.6						

the analytical wavelength. If relatively strong acids or bases are necessary for extraction, then the filtered or centrifuged extract should be neutralized prior to injection.

For all extraction solutions, it is a good idea to filter the sample through at least a 0.2 μ filter prior to injection to minimize clogging of the column (and pre-column filter). Metricel (Gelman) filters (0.2 micron) or equivalent work very well with most organic solvents; they are available to fit a 13 mm Swinny adaptor for quick filtration with a syringe.

The working range for most pesticides is in the 0.5 to 2 mg/ml which will generally yield mid-range 0.5 to 1.0 attenuation (aufs) for a 10 μ injection. For baits, plant growth regulations and other low percentage formulations, it may be necessary to go to a lower concentration and/or increase sensitivity. The flow rate normally runs from 0.5 to 2.0 ml/min. The wavelength ideally should be adjusted to an absorption peak for maximum sensitivity, reproducibility and specificity. "End absorption", i.e., 220 nm or lower may be necessary for some poorly absorbing material.

Most contemporary HPLC instruments will allow acceptable quantitative analyses to be performed in the isocratic mode by mixing solvent <u>in situ</u>, rather than having to pre-mix solvents. Most instruments are also now capable of giving good reproducible results in the gradient mode, however, the analyst should be certain of obtaining at least as good precision in the gradient mode as expected isocratically.

Standard and sample should be injected alternately until duplicate injections reproduce to within ± 1% for loop

injectors or ± 2% for syringe injectors. Auto-injected samples and standards should reproduce to within ± 1%. Response values for each of the two valid sample and standard injections should be averaged and the percent active ingredient calculated as given for GC analysis.

CHROMATOGRAPHIC DATA HANDLING

Various techniques are available to measure peak area from the gas or liquid chromatograph. These techniques are summarized as follows:

1. Planimetry

The planimeter is a mechanical instrument used to measure the area of any irregular shape. A baseline is extrapolated under the peak and the pointer, which attached to a movable arm, is used to carefully trace the area of interest. As the pointer traverses the peak or peaks, a dial and vernier drum will rotate. The difference between the initial and final readings on the scales gives the area of the peak(s). The method is somewhat time-consuming and can yield as much as ± 5% error for small peaks. Reproducibility between analysts is poor. Precision can be improved by tracing several times and taking an average. This method is useful for measuring total response for compounds such as chlordane, and toxaphene, if an electronic integrator is not available.

2. Height x Width and Half-Height

Since normal peaks approximate a triangle, one can approximate the area by multiplying peak height by the width at

the half-height. The baseline needs to be extrapolated. This method is not very good for peaks that significantly tail, are poorly resolved, or are rider peaks. For good gaussian-shaped peaks, this method is fast and simple. If half-height widths are identical for sample and standard, their peak height alone can be used. Width measurement can be improved by increasing chart speed.

3. Triangulation

For gaussian-shaped peaks, the area of the triangle formed by the baseline and tangents at the inflection points is equal to height x 1/2 base width. This method is more time-consuming than height x width at half-height, but is as accurate if peak shape allows significant measurements.

4. Cut and Weigh

Peak areas are measured by cutting out the chromatographic peak and weighing on an analytical balance. An extrapolated baseline is required. Thickness and moisture content of paper must be constant. This method is time-consuming and destroys the chromatogram (unless photocopies are cut up), but is fairly precise.

5. Disc Integrator

This type of integrator operates from the output of the recorder-servo system, and is also coupled to the chart movement. The area of a given peak is proportional to the product of these two parameters and is obtained by counting the total number of oscillations, which are recorded directly on the chart paper. Extrapolated baselines must be corrected for by proportional readings on either side of

the peak. One can obtain high precision and accuracy for well-resolved peaks, but the technique is somewhat time-consuming. Good reproducibility can be obtained between analysts.

6. Electronic Integrator/Computer

The chromatographic signal is fed into a voltage to frequency converter (or an analog to digital converter) which generates an output pulse rate proportional to the peak area. This method is very precise, quick, and accurate. Generally no attenuation is needed, as "off-scale" peaks can be accurately measured. This technique also ties in effectively with data storage capability. The technique requires relatively expensive equipment and a high degree of operator skill. It is recommended that the integrator stop-and-start be reflected by markers on the chromatogram so as to be able to "see" what is being integrated. It is also important to know what kind of baseline the integrator is using for any given peak.

Integrators vary from very simple "add-on" modules that simply yield an area measurement to those that will calculate answers according to pre-programmed methods. Data systems are available today that will also store peak information for recalculation or other future manipulation(s). If BASIC capability is available, one can program standard deviation and/or deviation from label claim. Printer-plotters are also now available that combine recorder, integrator and programming modes all in one module.

Table IX-3 shows the precision expected out of the various integration techniques.

Table IX-3

PRECISION OF VARIOUS INTEGRATION TECHNIQUES

Type	Precision
Height times half-height	3%
Triangulation	4%
Cut and Weigh	2%
Planimeter	4%
DISC® Integrator	1%
Electronic Integrator	0.5%
Computer	0.5% and better
Peak Height	1-4%

REFERENCE STANDARDS

X. REFERENCE STANDARDS

INTRODUCTION

Analytical reference standards should be maintained at the laboratory for all normally encountered pesticides. It is also desirable, but not necessary, to maintain standards of as many inert ingredients, decomposition products and by-products as possible.

Both analytical and technical grades (if different) of pesticide standards should be retained. Analytical grade standards of known purity will be required for most assay procedures, whereas technical grade material will be necessary for cross-contamination screening purposes. The technical grade standard will usually allow identification of normal by-products so that they won't be mistaken for cross-contaminants.

SOURCES

Possible sources of pesticide standards and related materials include the following:

1. U.S. Environmental Protection Agency
   Office of Toxic Substances, OPP Benefits & Field Studies Division, CBIB Chemistry Laboratory, Bldg. 306, ARC-East Beltsville, MD 20705 (Primarily for formulation analysis)

2. Quality Assurance Section Analytical Chemistry Branch, ETD/HERL (MD-69) U.S. Environmental Protection Agency Research Triangle Park, NC 27711 (Primarily for residue analysis, catalog available)

3. Basic manufacturers.

4. Private commercial sources Chem Service, Applied Science, Poly Science, Nanogen and chemical reagent sources (Eastman, Baker, etc.)

The EPA Beltsville Chemistry Laboratory should be considered as the primary source for technical and analytical standards for pesticide formulation analysis. Many of their standards have been independently assayed, either by differential scanning calorimetry or by assay against other reliable primary standards. Quantities are usually adequate for formulation analysis, although some standards are always in short supply. There is no charge for their service.

The EPA Triangle Park (RTP) primarily provides pesticide and related metabolite standards for residue analysis. Quantities are very limited, but the standards are quite reliable. All standards are provided free of charge.

Standards from the basic manufacturer are usually reliable, however, quality assurance considerations dictate that their purity should be confirmed, whenever possible, by independent assay, normally against EPA Beltsville or RTP standards. Most companies provide standards of their materials free of charge.

Standards from commercial supply houses should only be obtained as a last resort, and used with a great deal of care. These standards should be independently assayed whenever possible. Shelf stocks of these materials should be replaced when other sources become available. Technical material is often sold as analytical grade. The cost of standards from these sources can be exorbitant. However, commercial sources may be the only recourse for some chemicals.

Storage

All technical and analytical standards should be retained under custody conditions as done for official samples, i.e. under lock and key with controlled access.

Standards should be inventoried by card file or log book showing compound, type, source, date of acquisition, purity, date of purity determination and any other significant information regarding the standard.

Organophosphates and other labile/volatile standards should be kept refrigerated (2-4°C). The refrigerator can be equipped with a hasp and padlock or special locking device. The refrigerator may also be placed in a controlled access storage area. Other pesticides may be kept in refrigerator, if space permits, or kept at ambient temperature. Freezing should be discouraged due to condensation and frost problems. Even upon refrigeration, however, standards should be allowed to equilibrate to ambient temperature before opening the container to minimize condensation.

Non-refrigerated standard storage areas should be directly vented. A storage cabinet with a duct to an external blower provides an adequately ventilated storage area. Most cabinets can also be easily locked for custody purposes.

Diluted analytical standards propared for quantitative analysis should be retained for no longer than 1 month. Qualitative technical standards prepared primarily for TLC cross-contamination screening (1 and 10 mg/µl in acetone) can be kept for up to one year unless decomposition is evident. Both quantitative and qualitative solutions can be stored at ambient temperature.

Any retained diluted standards should be labeled as to ingredient, solvent, concentration, and date of preparation. Fresh standard solutions are always to be prepared when verifying results on potentially violative samples.

REPLACEMENT

Organophosphates and other labile compounds should be replaced or reassayed every two years, unless prior decomposition is evident. Other pesticides can be kept for up to 4 years before replacement or reassay. If reassay indicates decompositon of more than 2% relative then the standard should be replaced.

Table X-1 contains a list of 50 common pesticides that are recommended for replacement every 2 or 4 years according to type. Any other routinely used pesticide standard should also be replaced or reassayed in this time period. Other less commonly encountered chemicals are to be replaced or reassayed only on verifying results for a defective samples.

Table X-1

Pesticide Reference Standards

1.	p-tert-Amylphenol	26.	Malathion
2.	Atrazine	27.	MCPA
3.	o-Benzyl-p-chlorophenol	28.	Mecroprop
4.	Bromacil	29.	Methyl Parathion
5.	Captan	30.	Mevinphos
6.	Carbaryl	31.	MGK-264
7.	Carbofuran	32.	Monuron
8.	Chlordane	33.	Naled
9.	Chlorpyrifos	34.	Paraquat
10.	Crotoxyphos	35.	Parathion
11.	2,4-D	36.	Pentachlorophenol
12.	2,4-D, butoxyethanol ester	37.	o-Phenylphenol
13.	Dacthal	38.	Piperonyl Butoxide
14.	Diazinon	39.	Propoxur
15.	Dicamba	40.	Pyrethrins
16.	Dichlorvos	41.	Resmethrin
17.	Dicofol	42.	Ronnel
18.	Dimethoate	43.	Rotenone
19.	Dinocap	44.	Silvex
20.	Diphacinone	45.	Strychnine
21.	Disulfoton	46.	2,4,5-T
22.	Endrin	47.	Toxaphene
23.	EPN	48.	Trichlorofon
24.	Heptachlor	49.	Trifluralin
25.	Lindane	50.	Warfarin

APPENDIX A

HISTORY OF OFFICIAL PRODUCT

HISTORY OF OFFICIAL SAMPLE		1. SAMPLE NUMBER: 100001	2. REGISTRATION NUMBER: 75620-333
		3. PRODUCT: Fly-Away	
4. LABORATORY	Denver		
5. DATE RECEIVED	6-6-66		
6. RECEIVED BY	Sal Hepatica		
7. RECEIVED FROM	Crown Freight Del. person		
8. SENT VIA	Crown Freight		
9. SAMPLE CONDITION	ok		
10. CONDITION OF SEALS	Intact		
11. SEALED BY	Porteous D. Frisbee		
12. DATE SEALED	5-27-66		
13. PIECES RECEIVED	1 x 1		
14. PLACE STORED	5-7		
15. ASSIGNED BY	Alexander Beedle		
16. ASSIGNED TO	Walter Scott		
17. DELIVERED BY	Walter Scott		
18. DATE DELIVERED	6-9-66		
19. NUMBER SUBS RECEIVED	1		
20. SUBS ANALYZED	1		
21. DATE SEAL BROKEN	6-10-66		
22. DATE RESEALED	6-13-66		
23. RESEALED BY	Walter Scott		
24. PLACE STORED	44		
25. DATE JACKET SENT OUT			

26. REMARKS

HISTORY OF OFFICIAL SAMPLE		1. SAMPLE NUMBER 164001	2. REGISTRATION NUMBER 32201-784
		3. PRODUCT Wipe Out Pestikill	
4. LABORATORY	San Francisco		Corvallis
5. DATE RECEIVED	7-7-77		7-22-77
6. RECEIVED BY	Clark Kent		Max Wax
7. RECEIVED FROM	Lloyd Boyd		U.P.S. Del. Person
8. SENT VIA	Hand		U.P.S.
9. SAMPLE CONDITION	Leaking *		OK
10. CONDITION OF SEALS	OK		OK
11. SEALED BY	Lloyd Boyd		Dennis Stennis
12. DATE SEALED	7-6-77		7-17-77
13. PIECES RECEIVED	2x2		1x4
14. PLACE STORED	S-3		Given to REP
15. ASSIGNED BY	Florence Lawrence	Florence Lawrence	Richard Pritchard
16. ASSIGNED TO	Neil Dieldrin	Dennis Stennis	" "
17. DELIVERED BY	Clark Kent	Clark Kent	" "
18. DATE DELIVERED	7-7-77	7-15-77	7-22-77
19. NUMBER SUBS RECEIVED	4	4	4
20. SUBS ANALYZED	2	1	2
21. DATE SEAL BROKEN	7-10-77	7-16-77	7-22-77
22. DATE RESEALED	7-14-77	7-17-77	8-29-77
23. RESEALED BY	Neil Dieldrin	Dennis Stennis	Richard Pritchard
24. PLACE STORED	S-3	S-3	35
25. DATE JACKET SENT OUT		7-17-77	9-4-77

26. REMARKS

Corvallis 7-20-77
via U.P.S. / 4 subs
Efficacy Request
ck.

* one sample leaking due to loose cap. ck.

APPENDIX B

OFFICIAL SEAL

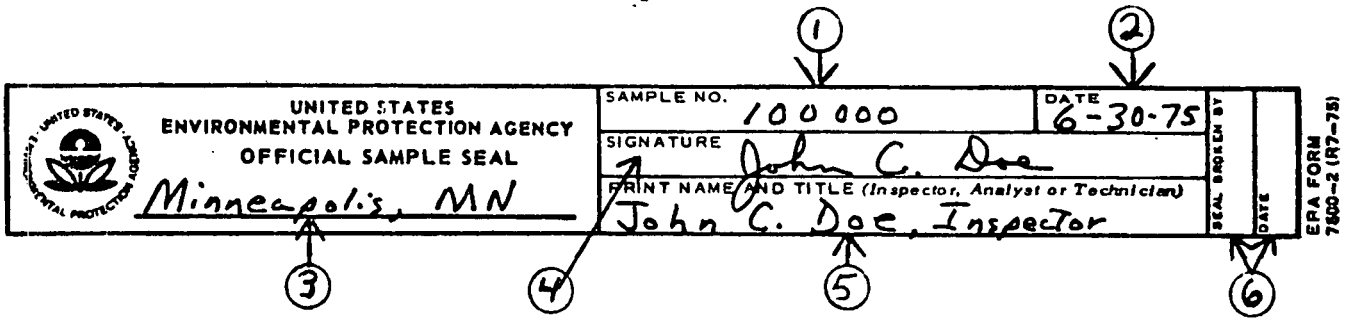
3. Sealing of Sample

a. Preparing Sample

All official samples shall be sealed with official EPA seals. Other samples should also be sealed when it is likely that they will be used directly as evidence. Samples may best be sealed by placing in an inverted plastic bag, tying a knot and turning the excess amount of bag back over the knot and taping the excess bag below the knot. Bulk samples should be placed in glass or metal containers before being sealed in plastic bags. The seal is then placed just below the knot in such a manner that it cannot be slipped over. It is important that the sample's label may be read without opening the sealed unit. If more than one glass unit is to be sealed in the same bag, it will be necessary to adequately wrap with packing material all but one unit in order to prevent breakage. If samples are wrapped before sealing in such a manner as to hide the label, it will be necessary to identify the samples as instructed in Section 12G1 for bulk samples.

b. Preparing Seal - EPA Form 7500-2

Pesticide samples will be sealed with EPA Form 7500-2. This seal will be completed as shown below:



(1) Insert sample number

(2) Insert date sealed. Use figures, month, day, year.

(3) Print location of collector's station.

(4) Signature of person sealing sample.

(5) Print name (same as signature) and title of sealer.

(6) When seal is broken for any purpose, initial here and enter the date broken. Submit broken seal with sample records.

APPENDIX C

LABORATORY VERIFICATION GUIDELINES

LABORATORY VERIFICATION GUIDELINES

CHEMICAL DEFICIENCY

The following criteria should be used by the initial analyst to determine whether to follow up with confirmation analysis after the first result is obtained, and to determine if a check analysis is required by a second analyst.

Active Ingredient Label Claim	Minimum Content
Less than 0.51%	80% of label claim
0.51 - 1.00%	85%
1.01 - 5.00%	90%
5.01 - 9.99%	92%
10.00 - 50.00%	94%
50.01 - 100%	96%

A sample result outside these guidelines is not decisive if it appears that there may have been uncertainties either in the sampling or analysis of any material. In such cases, the supervisor will decide whether or not a sample will pass. The allowable deviation below the label claim for several special cases is given below:

1. Fertilizer/pesticide mixtures, pressed blocks and non-uniform baits.

Active Ingredient Content Claimed	Minimum Content
Less than 1.26%	67% of label claim
1.26 - 5.00%	80%
over 5.00%	85%

2. Rotenone, pyrethrin and other natural product formulations.

Active Ingredient Content Claimed	Minimum Content
Less than 0.51%	70% of label claim
0.51 - 1.25%	80% of label claim
over 1.25%	85% of label claim

OVERFORMULATION

The overformulation limit depends on the original label claim, as follows:

Active Ingredient Content Claimed	Maximum Content
Less than 0.51%	150% of label claim
0.51 - 5.00%	140% of label claim
5.01 - 10.00%	130% of label claim
10.01 - 50.00%	125% of label claim
50.01 - 100%	115% of label claim

Any overformulation should be verified if any of the following situations are determined to apply:

a. An illegal residue would result if used according to directions.

b. An additional hazard to the applicator or user would result because of increased toxicity.

c. If the product contains label claims 40% sodium fluoride, 2% sodium arsenite or 1.5% arsenic trioxide, and is intended for household use, then the active ingredient content should not exceed the label claim by more than 10% relative.

d. Overformulation would result in damage to non-target organisms or the environment (for instance, lawn products overformulated with herbicides that may damage lawn grass).

(NOTE)

Some products are intentionally overformulated at the time of production because of restrictive shelf-life. This is considered to be a permissible manufacturing process within certain limits. Sodium hypochlorite solutions, DDVP sugarbaits and zinc phosphide preparations are some typical formulations of this type. If there is any doubt as to what action to take or not to take in such a situation, guidance should be obtained from the Region EPA Office or the National Enforcement Investigations Center Laboratory.

CROSS-CONTAMINATION

The following paragraph is extracted from Appendix F, Contaminant Screening Guidelines, prepared by PTSED.

> "The presence of any pesticide other than what is declared on the label and present in quantities equal to or greater than .05%; however, highly toxic material* (e.g. endrin or sulfotepp) if present in an amount equal to or greater than .01%, and undeclared herbicides which may cause plant damage at a level greater than .001% would be considered contaminants."

These guidelines should be followed in deciding whether or not to confirm and check any suspect contaminants.

All cross-contaminants that have been cancelled or suspended should be reported, such as DDT, aldin, dieldrin, heptachlor and chlordane, 2,4,5-T or silvex, if present at greater than 0.01%.

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* Highly toxic materials are those materials classified as Toxicity Category I by the proposed Section 3 guidelines.

APPENDIX D

REPORT OF ANALYSIS

UNITED STATES
ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

REPORT OF ANALYSIS

1. SAMPLE NO.	2. DATE COLLECTED
152088	8-15-78
3. REGION	**4. EPA REG. NO.**
4	777-888
5. ESTABLISHMENT NO.	
-	

6. DESCRIPTION OF SAMPLE
1-1 Gallon metal can; colorless liquid

7. NAME AND ADDRESS OF ESTABLISHMENT WHERE SAMPLE WAS COLLECTED *(Include ZIP code)*

S. Smith, Manager
Magic Pesticides Inc.
88 Bugsa Way
St. Louis, MO 32210

8. PRODUCT NAME
Perf Turf

9. LOT OR CODE NUMBER(S)
B2435-9

10. NAME AND ADDRESS OF PRODUCER *(If different from 7 above)*
-

11. RESULTS OF ANALYSIS

Sample was analyzed and found to be chemically satisfactory.

Analyst: Delbert Portnoy DP 9-1-78

	Claim	Found
Cadmium (AA; EPA-1)	12.3%	12.6%
Screened (TLC; Cl & P): Satisfactory		

12. LABORATORY COMMENTS

No EPA Est. No. on label or elsewhere on container. SG SG

13. SIGNATURE OF LAB SUPERVISOR		14. LABORATORY	15. DATE
Sylvia Ginsburg	Sylvia Ginsburg	Bay St. Louis	9-3-78

UNITED STATES
ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

REPORT OF ANALYSIS

1. SAMPLE NO.	2. DATE COLLECTED
144999	10-10-77
3. REGION	**4. EPA REG. NO.**
5	13566-78-AA
5. ESTABLISHMENT NO.	
13566-OH-1	

6. DESCRIPTION OF SAMPLE
1-1 pint bottle of subsample; clear liquid

7. NAME AND ADDRESS OF ESTABLISHMENT WHERE SAMPLE WAS COLLECTED *(Include ZIP code)*

Bill Lipservice, Manager
Babe Ruthless & Co.
800 Seymore Street
Kalmazoo, MI 47880

8. PRODUCT NAME
Fungaway

9. LOT OR CODE NUMBER(S)
-

10. NAME AND ADDRESS OF PRODUCER *(If different from 7 above)*
Armstrong & Doolittle, Cleveland, OH

11. RESULTS OF ANALYSIS

Passed

Analyst: Gerald Booth GB 10-19-77

	Claim	Found
2,2-Methylenebis (4-chlorophenol) (IR; NEIC-563-1)	1.45%	1.2%

Screening (TLC; Cl & P): Satisfactory

12. LABORATORY COMMENTS

Considered chemically satisfactory for this type of sample. DFJ 10/20/77

13. SIGNATURE OF LAB SUPERVISOR		14. LABORATORY	15. DATE
Dennis F. Johnson	Dennis F. Johnson	New York	10-20-77

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UNITED STATES
ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

REPORT OF ANALYSIS

1. SAMPLE NO.	2. DATE COLLECTED
131311	10-27-76
3. REGION	**4. EPA REG. NO.**
8	7890-33-AA
5. ESTABLISHMENT NO.	
-	

6. DESCRIPTION OF SAMPLE
1 - 1 Gallon plastic jug; blue liquid

7. NAME AND ADDRESS OF ESTABLISHMENT WHERE SAMPLE WAS COLLECTED *(Include ZIP code)*

Archimedes O'Toole, Vice-President
Alchemist Corp. of America
222 Turngold Way
Boulder, CO 81334

8. PRODUCT NAME
Germigone

9. LOT OR CODE NUMBER(S)
L61067

10. NAME AND ADDRESS OF PRODUCER *(If different from 7 above)*
-

11. RESULTS OF ANALYSIS

Method	Ingredient	Found
Titration (AOAC 6.377)	n-Alkyl(50%C_{14}, 40%C_{12}, 10%C_{16}) dimethyl benzyl ammonium chloride	3.1%

Analyst: Edward R. Green ERG 11-5-76

	Claim	Found
Net Contents (Est.)	1 Gal.	1 Gal.
n-Alkyl(*) dimethyl benzyl ammonium chloride (Titr'n; AOAC 6.377)	7%	3.06% 3.08% 3.1% Avg.

* 50%C_{14}, 40%C_{12}, 10%C_{16}

12. LABORATORY COMMENTS

Product is 56% deficient in n-alkyl (50%C_{14}, 40%C_{12}, 10%C_{16}) dimethyl benzyl ammonium chloride content, based on total chloride. DFH DFH

13. SIGNATURE OF LAB SUPERVISOR		14. LABORATORY	15. DATE
Daniel F. Hall	Daniel F. Hall	Denver	11-17-76

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ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

REPORT OF ANALYSIS

1. SAMPLE NO.	2. DATE COLLECTED
131311 (Continued)	
3. REGION	4. EPA REG. NO.
5. ESTABLISHMENT NO.	

6. DESCRIPTION OF SAMPLE

7. NAME AND ADDRESS OF ESTABLISHMENT WHERE SAMPLE WAS COLLECTED *(Include ZIP code)*

8. PRODUCT NAME

9. LOT OR CODE NUMBER(S)

10. NAME AND ADDRESS OF PRODUCER *(If different from 7 above)*

11. RESULTS OF ANALYSIS

Check Analysis: Arthur Brown *ab* 11-16-76

n-Alkyl (50%C_{14}, 40%C_{12}, 10%C_{16}) dimethyl benzyl ammonium chloride (Titration; AOAC 6.377): 3.07%, 3.09%
Avg.: 3.1%

12. LABORATORY COMMENTS

13. SIGNATURE OF LAB SUPERVISOR		14. LABORATORY	15. DATE
Daniel F. Hall	Daniel F. Hall	Denver	11-17-76

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UNITED STATES
ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

REPORT OF ANALYSIS

1. SAMPLE NO.	2. DATE COLLECTED
113172	4-29-75
3. REGION	**4. EPA REG. NO.**
10	1683-111-AA
5. ESTABLISHMENT NO.	
1683-OR-2	

6. DESCRIPTION OF SAMPLE
1 - 1/2 Pint bottle of subsample; amber liquid

7. NAME AND ADDRESS OF ESTABLISHMENT WHERE SAMPLE WAS COLLECTED *(Include ZIP code)*

Idaho Tree Service
56 Ballantine Road
Boise, ID 88333

8. PRODUCT NAME
Power Lindane Spray

9. LOT OR CODE NUMBER(S)
-

10. NAME AND ADDRESS OF PRODUCER *(If different from 7 above)*
Power Chemical Co., Portland, OR

11. RESULTS OF ANALYSIS

Method of Analysis	Ingredient	Found
GLC	Lindane	42.1%
GLC	DDT	0.1%

Analyst: Hazel Smith HS 5-14-75

	Claim	Found
Lindane (GLC; 10% OV-1 @ 190°)	40%	42.1%
DDT (GLC; 10% OV-1 @ 220°)	No Claim	0.11%
		0.12%
		0.1% Avg.

Screening (TLC-Cl; AOAC 6.026): DDT detected - Ca. 0.1%
(TLC-P): No organophosphate contamination detected.

Note: Presence of DDT confirmed on additional GLC column (3% XE-60 @ 180°) and one additional TLC system ($CHCl_3$ on Al_2O_3) HS HS

12. LABORATORY COMMENTS

Product contains 0.1% DDT not declared on the label. Confirmation is by three GLC systems and two TLC systems. DFH DFH

13. SIGNATURE OF LAB SUPERVISOR		14. LABORATORY	15. DATE
Darlene F Horton	Darlene F. Horton	New York	5-20-75

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UNITED STATES
ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

REPORT OF ANALYSIS

1. SAMPLE NO.	2. DATE COLLECTED
113172 (Continued)	
3. REGION	4. EPA REG. NO.
5. ESTABLISHMENT NO.	

6. DESCRIPTION OF SAMPLE

7. NAME AND ADDRESS OF ESTABLISHMENT WHERE SAMPLE WAS COLLECTED *(Include ZIP code)*

8. PRODUCT NAME

9. LOT OR CODE NUMBER(S)

10. NAME AND ADDRESS OF PRODUCER *(If different from 7 above)*

11. RESULTS OF ANALYSIS

Check Analysis: Ned Ball NB 5-16-75

DDT (GLC; 2% DEGS @ 180°): 0.10%, 0.12% Avg.: 0.1%

12. LABORATORY COMMENTS

13. SIGNATURE OF LAB SUPERVISOR		14. LABORATORY	15. DATE
Darlene F. Horton	Darlene F. Horton	New York	5-20-75

EPA Form 3540-5 (Rev. 5-76) PREVIOUS EDITIONS ARE OBSOLETE. SAMPLE RECORD COPY

APPENDIX E

CONTAMINANT SCREENING GUIDELINES

CONTAMINANT SCREENING GUIDELINES

Prepared by:

Scientific Support Branch
Pesticides and Toxic Substances
Enforcement Division

February 1977

Contaminant Screening Guidelines

Purpose

Pursuant to Section 23(a) of the Federal Insecticide, Fungicide, and Rodenticide Act, as amended in 1972 and 1975, the Assistant Administrator for Enforcement gave notice, published in 41 FR 32778 (Thursday August 5, 1976), that the Environmental Protection Agency would be implementing a pesticides enforcement grant-in-aid program, for fiscal year 1977, to certain State lead agencies whose responsibility is to enforce State pesticide laws. Included in the grant contract is a clause which requires that State participants in the pesticides enforcement program screen pesticide formulations for contaminants. It is therefore the intent of this document:

1) To provide the participating States the necessary guidelines to screen for contaminants.

2) To provide guidelines which non-participating States may adopt in order to enhance their pesticides enforcement program.

Objectives

1) To ensure that contaminated products having the potential for hazardous contact with man, domestic animals, and the environment are routinely screened to help eliminate incidents which may jeopardize public safety;

2) To enhance the pesticides enforcement program by detecting contaminants that would otherwise go unnoticed;

3) To provide guidelines which are designed to alert responsible State lab supervisors to potential problems and to allow them to proceed on each contamination case based on their judgment as to residues, exposure and handling hazards.

Background

A basic concern of FIFRA, as amended, is to ensure that pesticides registered for use within the United States perform their functions without causing undue hazards to man and the environment. Numerous provisions within FIFRA and its regulations have been created to allow for this compatibility. One such provision is paragraph 162.10(g)(5) of FIFRA Section 3 Regulations which requires that pesticides contain as precise as possible those percentages of ingredients that are represented on the product label. If the product does not meet these specifications, it is said to be adulterated, and its resultant quality may be such as to render it ineffective, unsafe, and unacceptable.

EPA labs routinely screen certain pesticides for contamination in an effort to minimize any deleterious impact on man or the environment. One EPA lab has reported that 5% of all pesticide samples analyzed at any given time are contaminated. If these pesticides go unscreened, contaminants go undetected and the concomitant enforcement actions (i.e., Notice of Warning, Civil Complaint, Criminal Prosecution, Stop Sale, and/or Recall) are not taken. The dividend from screening for contaminants outweigh the minimal amounts of time, cost, and equipment expended and the results are vital to a good enforcement program.

Discussion and Definition of Contamination

For the purpose of these guidelines, contamination does not include impurities that arise from reactions that occur during the manufacture of a product. Sources of contamination in pesticides may originate from such activities as failure to clean equipment between production runs and the reuse of inadequately cleaned or uncleaned pesticide containers.

Based on practical reasons i.e., time, cost, and resources, the following definition has been used for establishing that point at which the analytical chemist should run further analysis to identify and quantify the contaminant:

> The presence of any pesticide other than what is declared on the label and present in quantities equal to or greater than .05%; however, highly toxic material* (e.g. endrin or sulfotepp) if present in an amount equal to or greater than .01%, and undeclared herbicides which may cause plant damage at a level greater than .001% would be considered contaminants.

Requirements

1) Products to screen.

   a. Those pesticides intended for use on agricultural commodities (before or after shipment to the public market place) which will be used for human or animal consumption.

   b. Those pesticides used for home gardening.

   c. Those pesticides used to control pests on pets.

* Highly toxic materials are those materials classified as Toxicity Category I by the proposed Section 3 guidelines.

d. Those pesticides used in or around the home. (Not to include disinfectants such as quaternary compounds, bleaches and swimming pool treatments).

e. Those pesticides used in processing plants and institutions.

f. Those pesticides used on humans.

2) Contaminant detection.

a. The presence of a contaminant should be determined based on the definitions given in these guidelines.

b. If the contaminant is significant, its identity and percentage should be determined based on standard analytical methods, i.e., gas chromatography, mass spectrometry, etc.

3) Thin Layer Chromatography (TLC) methods to screen for the presence of contaminants. (TLC will not detect all contaminants that may be present but is adequate for routine screening.)

a. TLC for organophosphorus pesticides - AOAC 29.022.

b. TLC for chlorinated hydrocarbon pesticides - AOAC 6.030.

c. TLC section within U.S. EPA Manual of Chemical Methods for Pesticides and Devices.

d. Other methods approved by EPA.

If the method of analysis used in determining the product formulation reveals contaminants (e.g. gas chromatography), then the analyst need not use any of the TLC methods.

4) Materials and Equipment to use in determining the presence of a contaminant.

a. Technical grade standards and <u>not</u> analytical grade standards need to be used for comparison purposes.

b. Pre-coated plates are generally available from most chemical supply houses and may be used instead of homemade plates.

c. For chlorinated pesticides: refer to AOAC-6.026-6.029.

d. For organophosphorus pesticides: refer to AOAC-6.026-6.29.

e. Refer to TLC section within U.S. EPA Manual of Chemical Methods for Pesticides and Devices.

5) Report of Analysis.

The results of the contaminant screening and the identity and percentage of each contaminant, if determined, should be reported on the standard EPA Sample Summary Analytical Report (EPA Form 8500-4).

Note: It is generally not necessary to screen for inorganic contaminants. However, if the analyst suspects inorganic contamination, then atomic absorption spectroscopy should be used to search for the most probable contaminants, i.e., arsenic, lead, and cadmium.

APPENDIX F

INSTRUMENT AUDITS

INSTRUMENT AUDITS

In order to ascertain the operational status of the gas chromatographic and liquid chromatographic systems (instruments, columns, and detectors) used for analytical quantitation, the following audits should be performed once/week or whenever instrument malfunction is suspected.

Suggested Gas and Liquid Chromatographic parameters are given in Tables 1 and 2. Specific parameters will have to be established according to available instrumentation.

Once equilibrated, 2 µl of the appropriate test standards (e.g. Hall Cell, FID, FPD-NPD) are injected. The components of the test standards are listed in Table 3.

All audit results should be recorded and filed in the instrument log book and compared to previous test standard injections. The retention times and sensitivities should be within 5% of the original recorded value. If the results fall below these limits, several more injections should be made by the analyst until the values fall within the limits. Quantitative analyses should not be performed on the instrument if it fails to meet the audit limits. If, after three injections, the values still do not fall within the specified limits, the analyst should ascertain the problem and/or consult with the supervisor prior to continuing on with analysis.

Table 1

GAS CHROMATOGRAPHIC PARAMETERS

Detector	Column	Col. No.	Col. Flow N_2	Col. Temp °C	Attn. Input	Attn. Recorder	Temp. Inj. °C	Temp. Detector	Flow Air	Flow H_2	Inj. Vol.	Comments
FID-1	3% Carbowax 20M	4	4x40	200°C	10	2^8	225	300	0.8	30	2 ul	
FID-2	3% OV-1	2	3x40	20°/min 150(2^a)→210(2^a)	10	2^8	225	300	0.8	30	2 ul	
FID-A	3% XE-60	8	20x69	30°/min 150(2^a)→200(4^a)	100	2^3	250	250	Pre-set		2 ul	
FID-B	3% OV-1	7	30x69	30°min 150(2^a)→210(2^a)	100	2^2	250	250	Pre-set		2 ul	Split 1:1
NDP	3% OV-1	7	15x69	190	10	2^7	250	250	Pre-set		2 ul	Split 1:1
FPD Ch.=Ph. Ch.=S.	3% OV-1	2	6x40	190	10^4	(1)256 (2) 64	225	200	125	70	2 ul	Bucking=6
Hall Cell	3% OV-1	1	5x40	200	10	2^8	225	860	-	50	2 ul	

FIDs 1 & 2 on MT-222
FIDs A & B on PE Sigma 1
a Hold Time (min.)

Table 2

LIQUID CHROMATOGRAPHIC PARAMETERS

HPLC Conditions

Column: μ Bondapak C_{18} (Waters)

Col. Temp. = 28°C

Flow: 2.0 ml/min

Reservoirs: A = H_2O B = CH_3CN

AUFS = 0.2 Attn. = 2

λ = 254 nm

Gradient Profile

Time	Code	Value
.0	Flow	2.0
.0	% B	60
2.5	% B	60
3.0	% B	100
7.0	% B	100

Table 3

TEST STANDARDS

	Component	Approx. Conc. (Accurately Weighed)
FID Test Std.	Dimethyl Phthalate	1 mg/ml
	Diethyl Phthalate	1 mg/ml
	Dibutyl Phthalate	1 mg/ml
Hall Cell Test Std.	Gamma-BHC	10 ug/ml
	Aldrin	10 ug/ml
FPD-NPD Test Std.	Diazinon	10 ug/ml
	Parathion	10 ug/ml
	Methyl Parathion	10 ug/ml
HPLC Test Std.	Dimethyl Phthalate	1 mg/ml
	Diethyl Phthalate	1 mg/ml
	Dibutyl Phthalate	1 mg/ml