TOWN OF SALINA LANDFILL

REMEDIAL INVESTIGATION/FEASIBILITY STUDY

SITE I.D. NO. 734036

TECHNICAL WORK PLAN
FOR
PHASE II INVESTIGATION

(VOLUME I)

PREPARED FOR:

TOWN OF SALINA
201 School Road
Liverpool, New York 13088

CHA Project No. 6967

JULY 14, 1999

PREPARED BY:

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1.0 INTRODUCTION

In September 1998, Clough, Harbour & Associates LLP (CHA) and Lawler, Matusky, and Skelly Engineers LLP (LMS) completed a first phase field investigation at the Town of Salina Landfill. Since that time, the data generated from the first investigation have been summarized and presented in a Data Summary Report, two Ecological Risk Assessment Memoranda and a Human Health Risk Assessment Memorandum. The general objectives of the first investigation were to characterize the nature and extent of contamination and to gather data to evaluate the potential risk to human health and the environment. More specifically, our objectives were to:

- determine the limit of waste;
- determine if an old sewer line crossing the site acted as a preferred contaminant pathway;
- determine if the landfill was still producing methane and whether the methane was migrating off-site;
- determine the presence/absence and thickness of soil cover over the waste;
- screen the surface soil cover for contamination on a limited basis, presuming the landfill will be capped in the future;
- determine if native soils beneath the waste acted as a source of residual contamination to groundwater;
- determine the nature and extent of contamination in shallow groundwater;
- determine if a deeper aquifer existed beneath the site and whether it had been impacted by the site;
- determine the hydraulic properties of the aquifer(s);
- determine the nature and extent of surface water and sediment contamination in Ley Creek;
- determine the character of leachate entering Ley Creek;
- perform a wildlife survey and evaluate the potential risk to the environment;

At the completion of the first phase investigation, the majority of these objectives were fulfilled. However, there are several questions that still remain. Specifically, the questions that remain include the nature and the limit of waste and the severity of groundwater contamination in one of the

downgradient monitoring wells. Additionally, based on discussions with the NYSDEC, the Town of Salina has been advised that the presumptive remedy for landfills, namely the placement of a cap constructed in accordance with existing Part 360 regulations, might be avoided if it could be shown that the soil cap placed on the landfill in the early 1980s was sufficient to provide protection to human health and the environment.

Accordingly, there are three primary tasks that have been identified for further investigation. They are:

- an expanded waste area investigation
- an expanded surface soil investigation
- an expanded groundwater investigation

There are also two secondary tasks that are necessary to complete the investigation. They are:

- site survey
- data validation

Our proposed plan to address each of these tasks is presented below.

2.0 PHASE II INVESTIGATION TASKS

For the purposes of project budgeting and accounting, Task 4 of the Remedial Investigation was initially set aside for the Phase II investigation, if a second phase was necessary. Accordingly, the five tasks identified above have been designated as subtasks to Task 4.

TASK 4.1 - EXPANDED WASTE AREA INVESTIGATION

Statement of Problem

The limit of waste for the landfill was characterized through excavation of more than 40 test pits. This work allowed for a fairly precise delineation of the limit of waste, with two exceptions. Test pits excavated on both banks of Ley Creek revealed that waste extended to the banks of the Creek. Review of historical aerial photography appears to indicate that prior to the early 1970s, waste was placed in the area where the Ley Creek channel is now located. Since a portion of the Ley Creek channel was moved in the early 1970s, it is possible that waste exists under the bed of Ley Creek. The bed of Ley Creek was not accessible during the first phase investigation.

Excavation of test pits in the northwest corner of the site shows that waste extends beyond the Town property boundary. However, the limit of waste was not completely characterized in this area because of access problems; a fence along a portion of the property line prevented further investigation during the first phase.

In addition to these two data gaps, it is important to note that CHA observed a black viscous liquid in a number of test pits. The results of a chemical analysis of this material collected from test pit TP-8 revealed high levels of contaminants, including PCBs. NYSDEC has requested that the Town collect additional information on this material.

Scope of Services

To determine if waste exists under Ley Creek, CHA proposes to drill two borings through the creek bed. After on-site consultation with our drilling subcontractor, American Auger and Ditching Co., we have determined that the best way to accomplish this task is to place a geoprobe rig on a barge within Ley Creek. The barge and geoprobe rig will be lowered into the creek using an excavator. The barge will have an opening in the center that will allow for the downhole penetration of the drilling tools. The drilling contractor will drive a 4 in-steel casing into the bed of the creek. This is necessary to keep surface water from migrating down into the borehole. Drilling will proceed through the casing to a target depth of 30 feet, or until a dense glacial till is encountered. (Drilling to this depth will not only confirm the presence or absence of garbage, but will add to our knowledge of the subsurface stratigraphy in the area). Soil samples will be collected continuously throughout the borehole. Samples will be logged by CHA personnel. The proposed boring locations are depicted in Figure 3. We anticipate this work will take approximately 3 days to complete. The majority of the time will be associated with setting up the drilling rig and moving to each location.

It should be noted that CHA has contacted several longtime employees of the Onondaga County Department of Drainage and Sanitation regarding the rechannelization of Ley Creek in the early 1970s. None of the individuals contacted could provide any details of the work or any recollections of whether the new channel was excavated through waste.

Our previous investigation of the limit of waste in the northwest corner of the site indicated that waste apparently exists beyond the town property. The adjacent property is owned by Onondaga County Resource Recovery Agency (OCRRA). Additionally, a fence in this area prohibited further exploration during Phase I. CHA will use American Auger and Ditching Company, under our existing agreement, to excavate a series of test pits in this area (see Figure 3). We anticipate that this work will take a little more than one day to complete. CHA will ensure that the Town Attorney secures access to the OCRRA property before the work starts and will reinstall the fence at the completion of work. CHA will use the same procedures previously described in the Field Sampling Plan to perform this work.

To further investigate the nature of the black viscous liquid identified in test pit TP-8, CHA will spend up to one additional day excavating test pits in the vicinity of test pit TP-8 (see Figure 3). If this material is encountered CHA will collect up to two additional samples of this material. Similarly, if this material is encountered in test pits excavated in the northwest corner of the site, on OCRRA's property, CHA will also collect up to two samples of the material from this area of the site.

The samples of the black viscous material will be submitted to the laboratory for analysis of full Target Compound List parameters including volatile organic compounds (VOCs), semi-volatile organic compounds (SVOCs), pesticides, PCBs, metals and cyanide. For quality control purposes, CHA will also collect and analyze one blind duplicate and one matrix spike/matrix spike duplicate pair.

The borings will be designated B-21 and B-22 and the test pits will be designated as before beginning with TP-42 and continuing numerically upward.

TASK 4.2 – EXPANDED SURFACE SOIL INVESTIGATION

Statement of Problem

CHA conducted a limited surface soil investigation on the landfill during the first phase investigation. The investigation was limited in extent with the understanding that the landfill would have a multi-layer cap constructed over it in the future (the presumptive remedy for landfills). The limited sampling of the surface soils revealed that the soil contains some contaminants that may pose a risk to the environment. However, based on informal discussions held with the NYSDEC, it was suggested that we should consider "no action" or limited action alternatives specifically associated with a landfill cap, depending upon the extent of the contamination.

Scope of Services

To evaluate a "no action" or limited action alternatives, specifically as they relate to risk assessment, CHA proposes to collect and analyze an additional 22 surface soil samples. This total would include 20 samples collected from the landfill and two samples collected off site. Combined with the existing data, this would bring the total number of samples collected on the landfill to 27 (approximately 1 sample for every 2 acres) and 3 background samples (to adequately characterize background). The proposed sample locations are depicted in Figure 4. The samples are to be collected in the same manner as those previously collected. The samples will be designated SS-20 through SS-41, continuing from the previous numbering system.

The additional background samples are proposed to be collected on the OCRRA property northwest of the site, beyond the area where waste exists. We have also proposed to collect two samples in wetland areas along the western and northern portions of the site, along with one sample from an area on top of the landfill that may be a seasonal wetland.

The soil samples will be submitted to the laboratory for analysis of Target Compound List SVOCs, PCBs, metals and cyanide. The samples will not be analyzed for VOCs because no VOCs were identified in surface soils in the first round of sampling.

For quality assurance/quality control (QA/QC) samples, we will collect and analyze one blind field duplicate and a matrix spike/matrix spike duplicate (MS/MSD) pair.

Our existing ecological risk assessment indicates that the Hazard Quotient for certain contaminants of concern (COCs) does not greatly exceed 1. To adjust bioaccumulation factors and to refine Hazard Quotient values, CHA proposes to conduct a biota analysis. The bioaccumulation of surface soil contaminants into the terrestrial food chain would be assessed by the collection and analysis of earthworms.

Earthworms were found on the Town of Salina Landfill during the site survey previously conducted. They live in the soil and filter nutrients from organic soil particles. Earthworms were selected for analysis because they are a lower level of the terrestrial food web and are commonly fed upon by shrews, robins and other terrestrial species.

Earthworms will be collected at six locations on the landfill by turning soil and searching for worms. The six locations will consist of two highly contaminated areas, two moderately contaminated areas, and two areas of lesser contamination. Proposed locations are identified on Figure 4. A minimum of ten (again weight/size dependent) specimens will be collected at six locations for the preparation of one composite earthworm sample at each location. Three replicates will be collected at each location totaling 18 composite samples. If the landfill soil is dry at the time of the sampling, water may be used to soak the soil and draw worms closer to the surface for capture. Each lot of 10 earthworms will be bagged, assigned an inventory number and transferred to the analytical laboratory under appropriate chain-of-custody protocols. Each lot of ten earthworms will be weighed at the time of collection to be sure they exceed the minimum weight requirement by 20%. Samples will be iced and shipped to the analytical laboratory where they will be logged in, processed, and analyzed. The 18 composite earthworm samples will be analyzed for SVOCs, pesticides/PCBs, metals and cyanide. Again, VOCs will not be analyzed since there were none found in surface soils.

The soil cap placed on the landfill in the early 1980s was reportedly constructed to comply with the regulations governing landfill closure in effect at the time. One reason to install a landfill cap is to limit infiltration of precipitation through the waste mass. To help evaluate the effectiveness of the existing soil cap in this regard, it would be helpful to know the permeability of the soil cap. To estimate the permeability (or hydraulic conductivity) of the existing cap material, CHA will use the following procedure in each of four (4) quadrants of the landfill:

- Attempt to push a Shelby tube into the cap material to obtain an undisturbed sample of the cap soil. If this is successful, a laboratory triaxial permeability test or falling head test will be performed on the undisturbed sample to determine its hydraulic gradient. Triaxial testing will be used if the cap soil is primarily fine grained and is judged to have a hydraulic gradient less than about 1×10^{-5} cm/s. For coarser grained, more permeable soil, a falling head test will be performed.

- If the cap soil contains gravel, roots, or other debris, it may not be possible to obtain undisturbed Shelby tube samples. For these conditions, CHA will measure the in-place density of the cap material using a nuclear density device and will recompact a grab sample in the laboratory to approximately the same density. This recompacted sample will then be tested in the laboratory using a triaxial permeability test or falling head test.

- Grain size analyses will be performed for each permeability test sample. This index data can be used as a baseline for comparison to other cap samples.

- Six (6) additional gradation tests will be performed via method ASTM D422 on samples taken from other random locations to establish cap consistency across the site.

TASK 4.3 – EXPANDED GROUNDWATER INVESTIGATION

Statement of Problem

The previous sampling results from the groundwater monitoring wells revealed that MW-10, a downgradient well installed adjacent to Ley Creek revealed high levels of various contaminants. The concentrations of contaminants measured in this well were significantly elevated compared to other on site monitoring wells. To confirm that the results from the previous sampling event are representative, NYSDEC has recommended that this well be resampled.

Assuming that the degree of groundwater contamination in the vicinity of MW-10 is confirmed, additional efforts in a limited area may be required to remediate the groundwater. One potential alternative that CHA will evaluate during the Feasibility Study is *in-situ* bioremediation. To evaluate this alternative, additional information will need to be collected.

Scope of Services

Well MW-10 will be sampled following the same procedures as were used previously. The well will be sampled for full Target Compound List Parameters including VOCs, SVOCs, pesticides, PCBs, metals, and cyanide. In addition, NYSDEC has requested that the sample from this well be analyzed for dioxin isomers.

The reason that dioxin isomers have been added to the list of analytical parameters is that dioxin isomers are often found associated with PCBs. However, dioxin isomers may also be created as combustion products and can be distributed throughout the environment by atmospheric deposition. To evaluate whether the presence of dioxin isomers, if any, in MW-10 is related to site contamination or to other off-site sources, CHA will also resample well MW-0. This well has been previously designated as a background well for the site. To provide a basis of comparison to previous sampling events, CHA will collect and submit a sample from well MW-0 for both total metals and for dioxin isomers.

To evaluate the potential for some type of *in-situ* bioremediation to be applied to a limited area in the vicinity of MW-10, CHA proposes to use American Auger & Ditching Company to advance two additional borings near MW-10 using a geoprobe rig. The borings will be advanced to a maximum of 30 feet below grade. CHA will collect three samples from each boring; one from the vadose zone, one near the water-table surface, and one in the saturated zone. These samples will be submitted to the laboratory for analysis of total metals, nutrient levels, and microbial activity. The analysis of the metals will provide information as to whether there are toxic levels of heavy metals in the soil. The analysis of nutrient levels and microbial activity will indicate if microbes are present in the soil naturally.

After review of the existing slug test data, CHA suspects that some of the permeability values may be biased high. This may be due to the influence of the filter pack in the monitoring wells. To perform an independent check on the estimates of permeability, CHA will also submit one soil sample from each of the two borings to a qualified laboratory for a grain-size analysis via

method ASTM D422. The samples will be obtained from the same interval that well MW-10 is screened in: an interval of fine to medium sand with coarse gravel. CHA will then use the grain-size distribution to estimate permeability based on available empirical methods.

TASK 4.4 – SITE SURVEY

This is a secondary task needed to track and identify new sampling locations added in the second phase field investigation. We anticipate that a two-person survey crew will take 1 day to survey the locations of the new sampling stations. The information will be added to CHA's base map for the site.

TASK 4.5 – DATA VALIDATION (DATA USABILITY SUMMARY REPORT)

Based on initial discussions with the NYSDEC, it was agreed that if a second round of data were collected during the RI, the data would not require full validation. Rather, the data would be reviewed in a less rigorous manner.

LMS will review each analytical data package for completeness (i.e., have all the analyses requested been performed?) and general protocol compliance, such as holding times, detection limits, spike recoveries, and surrogate recoveries. The results of this review will be summarized and included in the final report. If information is found to be missing from the data package the analytical laboratory will be contacted and requested to submit any omissions.

Upon completion of the data review, LMS will perform a data usability analysis on all analytical laboratory data. Using information on sampling protocols, transport, analysis, reduction, and reporting, and previous experience, LMS will whether the results of each analysis can be used for the purpose intended. It will be determined whether the final results can be used as reported, qualified to indicate limitations, or rejected outright. The results of this review will be summarized in a Data Usability Summary Report (DUSR), prepared according to NYSDEC DER DUSR Guidelines, rev. 9/97, and will be included in the final report.

It should be noted that the analyses of pesticides and PCBs associated with animal tissue will not be performed strictly following ASP methods. ULI will use extraction methods described in the Official Methods of Analysis (AOAC, 14th Edition, Chapter 29). The analyses will follow SW-846 methods. The extraction methods are included in Appendix A. The laboratory will produce a data package that contains all of the information necessary to validate the data. Additionally, it should be noted that the fat in animal tissue may cause problems with recovery of laboratory spikes, particularly with the acid-extractable semi-volatile organic compounds.

3.0 PROJECT STAFFING

CHA will continue to be responsible for overall project technical direction and administration. Staff from LMS, working under CHA, will be responsible for completing the ecological and human health exposure assessments. CHA will use the following subcontractors for the Phase II investigation:

SUBCONTRACTORS

TYPE OF SERVICE	NAME OF FIRM	MBE/WBE	CONTRACT VALUE
Drilling/Test Pits	American Auger	WBE	$9,700
Analytical Testing	Upstate Laboratories, Inc.		$21,641
Soil Testing	CME Associates		$1,800

The work performed by American Auger will be performed under the terms of our existing contract.

CHA is proposing to use Upstate Laboratories, Inc. (ULI), under a new subcontract agreement, for the second round of laboratory analyses. CHA has recommended to the Town of Salina that RECRA Environmental (RECRA) not perform the second round of laboratory analyses. RECRA performed the first round of analyses, but submitted all results late. Additionally, RECRA did not communicate well with CHA with regard to these schedule delays. ULI submitted the second lowest proposal for laboratory analyses during our initial request for proposals. ULI is an ASP-certified laboratory and is local to the area. ULI has agreed to match the prices offered by RECRA for the upcoming round of sampling for Target Compound List Parameters.

Note that ULI has supplemented their price quote, at our request, to include a fee of $35/sample associated with preparation of animal tissue samples for analyses. This bid item was not included in our original request for proposals, but is considered reasonable relative to the work required. It is also important to note that there are a limited number of laboratories that are qualified to perform dioxin analyses. Based upon verbal approval from NYSDEC, ULI is in the process of obtaining three quotations to subcontract the dioxin analyses to one of the qualified laboratories. At the present time, CHA is using a budget figure of $800 per sample until all

quotations are received. It is important to note that due to the volume of work being conducted nationwide and the limited number of qualified laboratories available to perform dioxin analyses, CHA has already been given an indication that we may not receive the laboratory data within 30 days of sampling.

CHA has also obtained three verbal quotations for soil testing. The lowest quotation was received from CME Associates, Inc. based on the work expected to be performed.

4.0 PROJECT DELIVERABLES

There are no specific deliverables specifically associated with the Phase II investigation. Data collected from the second phase investigation will be included in the preparation of a draft RI report. The draft RI report will include the ecological risk assessment.

The site- specific data obtained from the second phase RI sampling will be used to modify and expand the ecological risk assessment memorandum already submitted. A literature search will be conducted using the Internet and LMS' reference library for information on NOAEL values, LOAEL values, LC_{50} values, and other parameters. As appropriate, articles will be ordered or downloaded from the Internet and reviewed.

The Phase II new site data on soil, water and specimen contamination will be reviewed and revisions will be made to site contaminant summary tables, and text and tables discussing site distribution and concentrations. Revised Hazard Quotients will be calculated based on the NOAEL, LOAEL and LC values found in the literature review. New assessment endpoints will be determined based on revised Hazard Quotient values for earthworms. This will be done in the text as well as in tabular form.

A comparison of the calculated risks from this Phase II sampling effort with Phase I calculations will be provided to compare the accuracy of the original calculations, which were based strictly on literature values, and to extrapolate this comparison to the estimate of risk for other species.

Note that the dioxin data will be incorporated into the results presented within the RI Report. However, the data will not be incorporated or used in the ecological or human health risk assessments due to the limited amount of data to be collected.

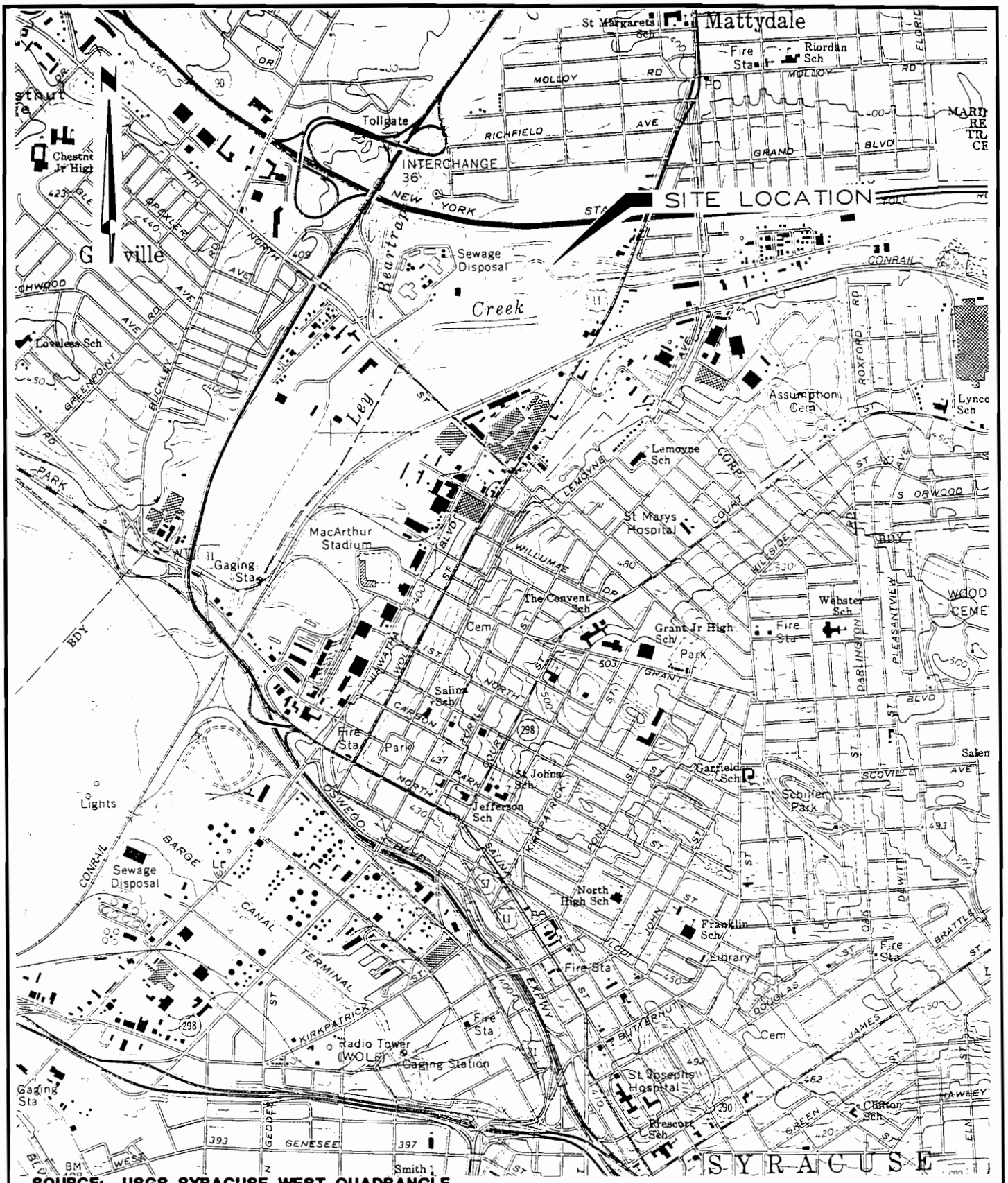
5.0 PROJECT SCHEDULE

A revised project schedule is summarized below.

TASK DESCRIPTION	START DATE	END DATE
WORK PLAN APPROVAL	July 30, 1999	
SITE CHARACTERIZATION		
Field Investigation (Round 2)	August 13, 1999	August 24, 1999
Laboratory Analysis (Round 1)	August 24, 1999	September 24, 1999
REMEDIAL INVESTIGATION RPT		
Data Usability Summary Report	September 24, 1999	October 24, 1999
Prepare Draft RI Report	October 24, 1999	December 3, 1999
DEC Review of Draft RI Report	December 6, 1999	December 31, 1999
Prepare Final RI Report	January 1, 2000	January 28, 2000
RISK ASSESSMENT		
Prepare Draft Risk Assessment Rpt.	October 24, 1999	December 3, 1999
DEC Review of Draft Report	December 6, 1999	December 31, 1999
Prepare Final Risk Assessment Rpt.	January 1, 2000	January 28, 2000
FEASIBILITY STUDY		
Prepare Screening Memo	February 1, 2000	February 29, 2000
DEC Review of Memo	March 1, 2000	March 31, 2000
Draft FS Report	April 1, 2000	May 31, 2000
DEC Review of Draft FS	June 1, 2000	July 31, 2000
Prepare Final FS Report	August 1, 2000	August 31, 2000
SUPPORT ACTIVITIES		
Citizen Participation	see CP Plan	
PRP Search	July 1, 1998	July 1, 1999

FIGURES

FIGURES



SOURCE: USGS SYRACUSE WEST QUADRANGLE

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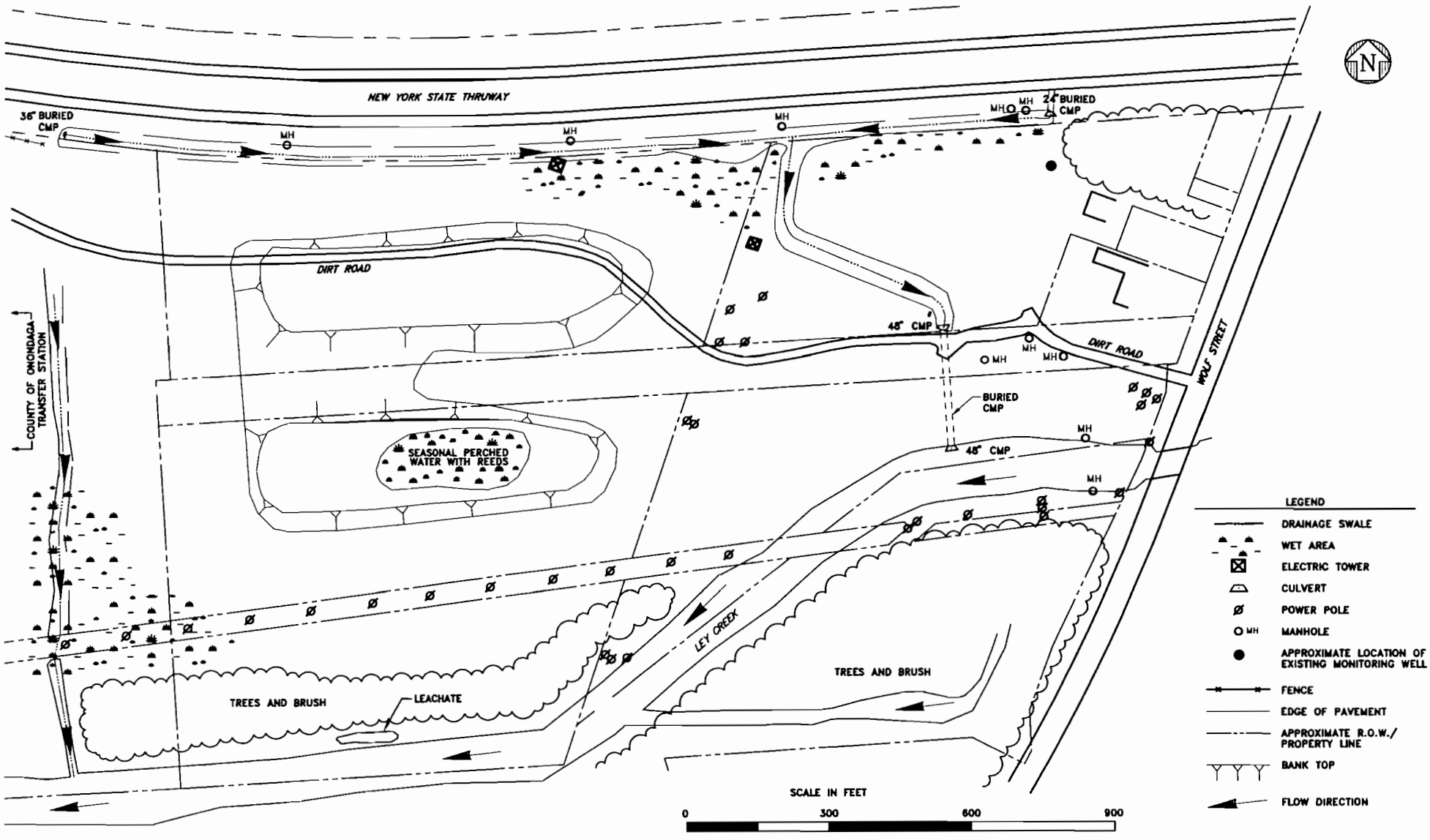
315-471-3920

CHA FILE NO. 6967 | SCALE: 1"=2000'

FIGURE 1
SITE LOCATION MAP
TOWN OF SALINA LANDFILL
SYRACUSE, NEW YORK

I:\6967\DRAWINGS\FIG1.DWG



SITE PLAN

FIGURE NO. 2	PROJECT NO. 6967	CLOUGH, HARBOUR & ASSOCIATES ENGINEERS, SURVEYORS, PLANNERS & LANDSCAPE ARCHITECTS 109 SOUTH WARREN STREET SYRACUSE, NEW YORK, 13202
SCALE: 1"=300'±	DATE: JUNE 1999	TOWN OF SALINA LANDFILL REMEDIAL INVESTIGATION/FEASIBILITY STUDY SALINA, NEW YORK

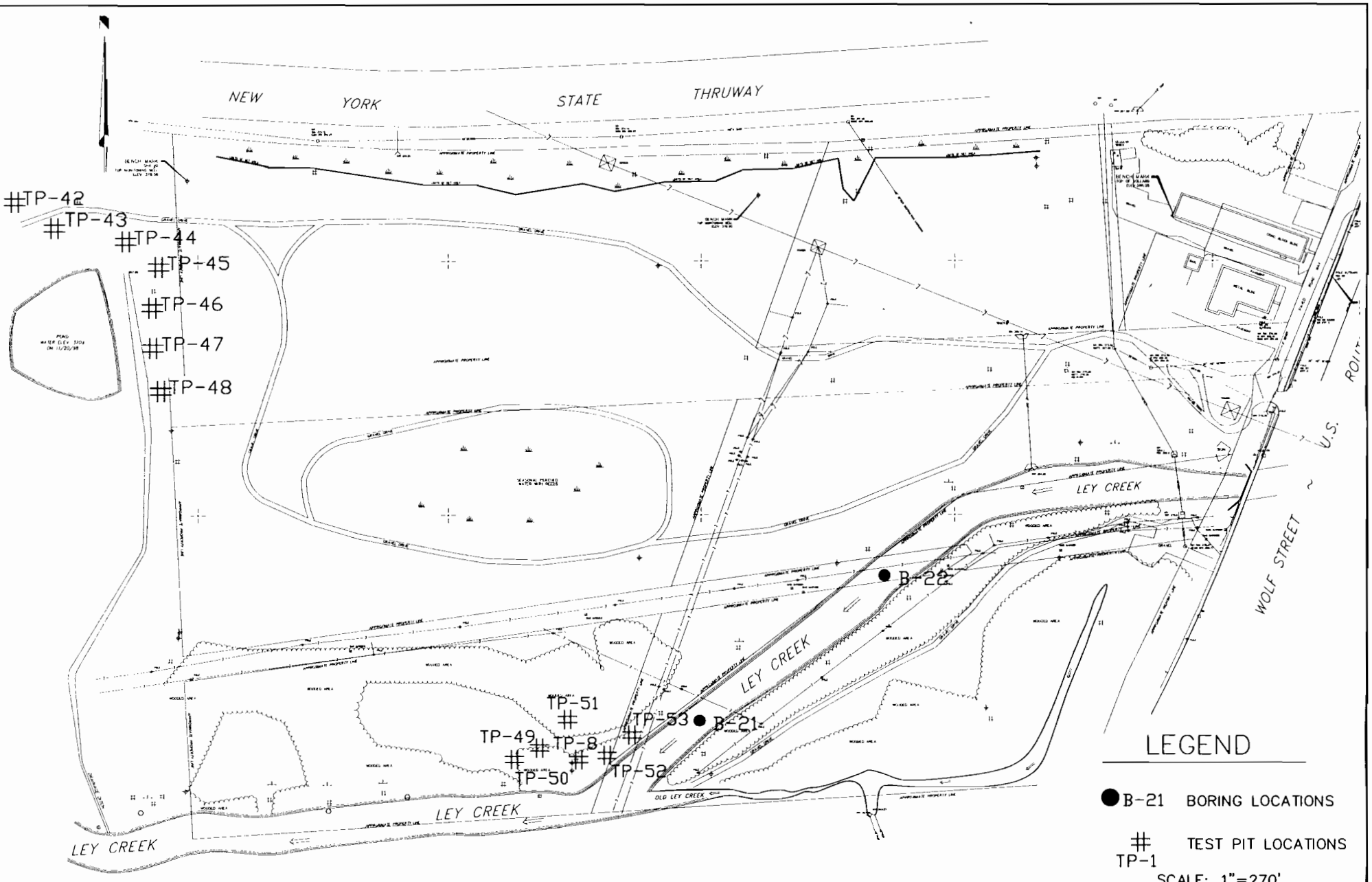


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FIGURE 3

TEST BORING MAP/TEST PIT LOCATION MAP

TOWN OF SALINA LANDFILL
SALINA, NEW YORK ONONDAGA COUNTY

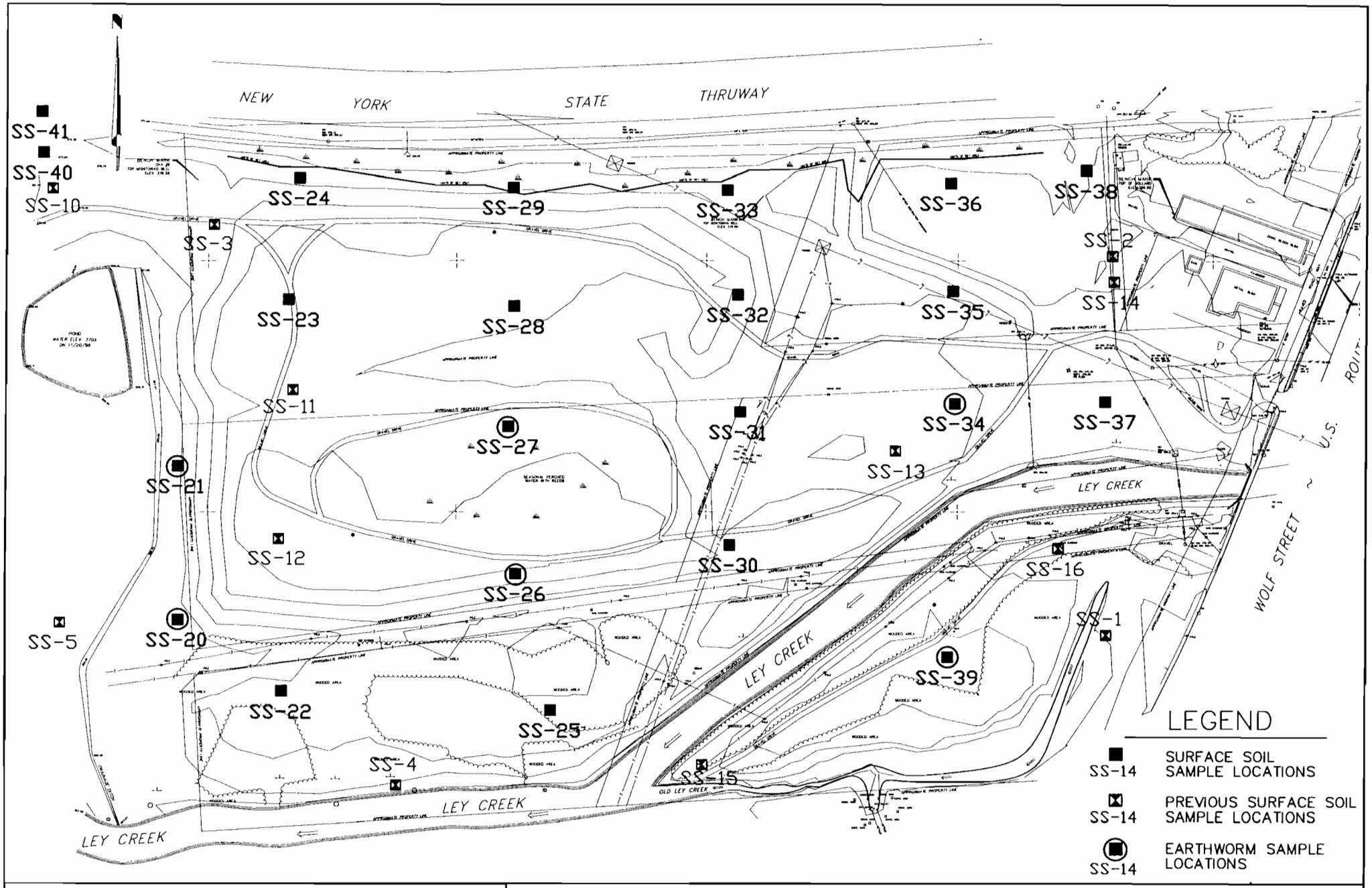

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FIGURE 4

SURFACE SOIL SAMPLE LOCATIONS

TOWN OF SALINA LANDFILL
SALINA, NEW YORK ONONDAGA COUNTY

APPENDIX A

29. Pesticide and Industrial Chemical Residues

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ORGANOCHLORINE AND ORGANOPHOSPHORUS PESTICIDE RESIDUES

Organochlorine and Organophosphorus Pesticide Residues

General Multiresidue Methods

29.001 ***Principle***

Thoroly mixed sample is extd with CH_3CN (high-H_2O foods) or aq. CH_3CN (low-H_2O or high sugar foods). Fat is extd from fatty foods and partitioned between pet ether and CH_3CN. Aliquot (nonfatty samples) or entire soln (fatty samples) of CH_3CN is dild with H_2O and residues are extd into pet ether. Residues are purified by chromatgy on Florisil column, eluting with mixt. of pet and Et ethers. Residues in concd eluates are measured by GC and identified by combinations of gas, thin layer, or paper chromatgy.

Analyst competence in applying method for trace residues should be assured before analysis. Recoveries of added compds thru method should be ≥80%.

Absence of interferences arising from laboratory and reagent contamination should also be assured by regular performance of reagent blanks. Solvs in particular, because of their concn during methods, can contribute significant interference if not sufficiently purified. Solvs of adequate purity are com. available from several manufacturers, but each batch must be tested under conditions of method in which it will be used.

Other reagents and app. (rubber, plastics, glass wool, etc.) are also potential source of interferences. *See* references for recoveries obtained during collaborative and validation studies and Table **29:01** for commodities approved. *See* **29.008**, introductory par., and **29.008**(c) for GC performance requirements: sensitivity, sepn capability, and linearity. Behavior of >200 pesticides and industrial chems in method is given in JAOAC **61**, 640(1978).

29.002 ***General Reagents***

Solvs must be purified and final distn conducted in all-glass app. (*Caution: See* **51.011**, **51.039**, **51.040**, **51.043**, **51.054**, **51.061**, and **51.073**.) *See* **29.001**.

Solvent purity test.—Electron capture GC requires absence of substances causing detector response as indicated by following test: Place 300 mL solv. in Kuderna-Danish concentrator fitted with 3-ball Snyder column and calibrated collection vessel, and evap. to 5 mL. Inject 5 μL conc. from 10 μL syringe into gas chromatograph, using conditions described in **29.008** (c). Conc. must not cause recorder deflection >1 mm from baseline for 2–60 min after injection.

(**a**) *Acetonitrile.—See solv. purity test.* Purify tech. CH_3CN as follows: To 4 L CH_3CN add 1 mL H_3PO_4, 30 g P_2O_5, and boiling chips, and distil in all-glass app. at 81–82°. Do not exceed 82°.

Some lots of reagent grade CH_3CN are impure and require distn. Generally vapors from such lots will turn moistened red litmus paper blue when held over mouth of storage container. Pronounced amine odor is detectable.

(**b**) *Acetonitrile saturated with petroleum ether.*— Sat. CH_3CN, (**a**), with redistd pet ether, (**m**).

(**c**) *Alcohol.*—USP, reagent grade, or MeOH, ACS.

(**d**) *Alcoholic alkali soln.*—2%. Dissolve 2 g KOH in alcohol, and dil. to 100 mL.

(**e**) *Eluting solvent, 6%.*—Dil. 60 mL Et ether, (h), to 1 L with redistd pet ether, (**m**).

(**f**) *Eluting solvent, 15%.*—Prep. as in (e), using 150 mL Et ether.

(**g**) *Eluting solvent, 50%.*—Prep. as in (e), using 500 mL Et ether.

(**h**) *Ethyl ether.—See solv. purity test.* Redistd at 34–35°, and stored under N. Add 2% alcohol. Must be peroxide-free by test in *Definitions of Terms and Explanatory Notes*, item (3).

(**i**) *Florisil.*—60/100 PR grade, activated at 675°C (1250°F), available from Floridin Co. When 675°C activated Florisil is obtained in bulk, transfer immediately after opening to ca 500 mL (1 pt) glass jars, or bottles, with g-s or foil-lined, screw-top lids, and store in dark. Heat ≥5 hr at 130° before use. Store at 130° in g-s bottles or in air-tight desiccator at room temp. and reheat at 130° after 2 days.

Prep. mixed pesticide std soln in hexane contg 1, 4, 1, 2, 1, 2, and 4 μg/mL, resp., of ronnel, ethion, heptachlor epoxide, parathion, dieldrin, endrin, and malathion.

Test each batch of activated Florisil by placing 1 mL mixed pesticide std on prepd column and eluting as in *Cleanup*, **29.015**. Conc. eluates from Florisil column to 10 mL. Inject aliquot (*see* **29.008**) of each eluate into gas chromatograph and det. quant. recovery of each compd as in **29.018**. Florisil that quant. elutes heptachlor epoxide, ronnel, and ethion in 6% eluate; dieldrin, endrin, and parathion in 15% eluate; and malathion in 50% eluate, is satisfactory.

Adsorptivity of lots of Florisil may be tested with lauric acid and size of column adjusted to compensate for variation in adsorptivity (JAOAC **51**, 29(1968)). Test adjusted column before use by performing elution test above.

(**j**) *Hexane.—See solv. purity test.* Reagent grade, redistd in all-glass app.

(**k**) *Magnesium oxide.*—Adsorptive magnesia (Fisher Scientific Co. No. S-120). Treat as follows: Slurry ca 500 g with H_2O, heat on steam bath ca 30 min, and filter with suction. Dry overnight at 105–130° and pulverize to pass No. 60 sieve. Store in closed jar.

(**l**) *Magnesia-Celite mixture.*—Mix treated MgO, (**k**), with Celite 545, 1 + 1 by wt. Pet ether ext of Celite should be free of electron-capturing substances.

(**m**) *Petroleum ether.—See solv. purity test.* Reagent grade, redistd in all-glass app. at 30–60°.

(**n**) *Sodium sulfate.*—Anhyd., granular.

Table 29:01 Compounds and Commodities to Which General Method Applies

Compound	Official Final Action
Dieldrin (CAS-60-57-1) Heptachlor epoxide (CAS-1024-57-3)	Group I nonfatty foods, dairy products, fish, vegetable oils, whole eggs
BHC (CAS-608-73-1) DDE (CAS-72-55-9) DDT (CAS-8017-34-3) TDE (CAS-72-54-8)	Group I nonfatty foods, dairy products, fish, whole eggs
Lindane (CAS-58-89-9) Methoxychlor (CAS-72-43-5) Ethylan (CAS-72-56-0)	Group I nonfatty foods, dairy products, whole eggs
Aldrin (CAS-309-00-2) Endrin (CAS-72-20-8) Heptachlor (CAS-76-44-8) Mirex (CAS-2385-85-5)	Group I nonfatty foods, whole eggs
o,p'-DDT (CAS-8017-34-3)	Group I nonfatty foods, dairy products
Diazinon (CAS-333-41-5) Ethion (CAS-563-12-2) Malathion (CAS-121-75-5) Me parathion (CAS-298-00-0) Parathion (CAS-56-38-2) Ronnel (CAS-299-84-2)	Group II nonfatty foods
PCBs (CAS-12767-79-2)	Poultry fat, fish, dairy products

Group I nonfatty foods: apples*, apricots, barley*, beets, bell peppers, broccoli*, cabbage*, cantaloupes, cauliflower*, celery, collard greens, corn meal and silage, cucumbers*, eggplant, endive, grapes*, green beans, hay, kale*, mustard greens*, oats*, peaches, pears, peas, plums, popcorn, potatoes*, radishes, radish tops, spinach, squash*, strawberries, sugar beets, sweet potatoes, tomatoes*, turnips*, turnip greens*, wheat*

Group II nonfatty foods: Group I nonfatty foods marked with asterisk (*) plus carrots, green peppers, and lettuce

29.003 *Reagents for Thin Layer Chromatography*

(a) *Aluminum oxide.*—Neutral Al_2O_3 G (Type E, MC/B Manufacturing Chemists, Inc., No. 1090), or equiv., for TLC.

(b) *Developing solvents for organochlorine pesticides.*—(*1*) *n*-Heptane, com. grade. (*2*) *n*-Heptane contg 2% reagent grade acetone.

(c) *Chromogenic agent for organochlorine pesticides.*— Dissolve 0.100 g $AgNO_3$ in 1 mL H_2O, add 20 mL 2-phenoxyethanol (Practical, Eastman Kodak Co.), dil. to 200 mL with acetone, add very small drop 30% H_2O_2, and mix. Store in dark overnight and decant into spray bottle. Discard after 4 days.

(d) *Developing solvents for organophosphorus pesticides.*—(*1*) *Immobile.*—15 or 20% *N,N*-dimethylformamide (DMF) in ether. Dil. 75 or 100 mL DMF to 500 mL with ether and mix. (*2*) *Mobile.*—Methylcyclohexane.

(e) *Chromogenic agents for organophosphorus pesticides.*—(*1*) *Stock dye soln.*—Dissolve 1 g tetrabromophenolphthalein Et ester (Eastman No. 6810) in 100 mL acetone. (*2*) *Dye soln.*—Dil. 10 mL stock dye soln (*1*) to 50 mL with acetone. (*3*) *Silver nitrate soln.*—Dissolve 0.5 g $AgNO_3$ in 25 mL H_2O and dil. to 100 mL with acetone. (*4*) *Citric acid soln.*—Dissolve 5 g granular citric acid in 50 mL H_2O and dil. to 100 mL with acetone.

29.004 ★ *Reagents for Paper Chromatography* ★

See **29.004**, 13th ed.

29.005 *General Apparatus*

(a) *High-speed blender.*—Waring Blendor, or equiv.

(b) *Chromatographic tubes.*—With Teflon stopcocks and coarse fritted plate or glass wool plug; 22 mm id × 300 mm.

(c) *Chromatographic tubes without stopcocks.*—22 mm id × 300 or 400 mm.

(d) *Filter tubes.*—Approx. 22 mm id × 200 mm with short delivery tube and coarse fritted plate or glass wool plug.

(e) *Kuderna-Danish concentrators.*—500 and 1000 mL with Snyder distilling column and 5 or 10 mL plain, vol., and graduated receiving flasks (Kontes Glass Co. No. K-570000, K-621400, and K-570050, or equiv.).

(f) *Separators.*—1000 and 125 mL with Teflon stopcocks.

(g) *Micro-Snyder column.*—2-ball (Kontes Glass Co. No. K-569001, or equiv.).

(h) *Micro-Vigreaux column.*—Kontes Glass Co. No. K-569251, or equiv.

29.006 *Apparatus for Thin Layer Chromatography*

(a) *Desaga/Brinkmann standard model applicator, or equiv.*

(b) *Desaga/Brinkmann standard mounting board, or equiv.*

(c) *Desaga/Brinkmann drying rack, or equiv.*—Accommodates ten 8 × 8″ plates.

(d) *Desaga/Brinkmann model 51 stainless steel desiccating cabinet, or equiv.*

(e) *Window glass.*—8 × 8″, double strength window glass plates of uniform width and thickness; smooth off corners and edges with file or other tool.

(f) *Chromatographic tank and accessories.*—Available from Arthur H. Thomas Co. No. 2749-F05, or equiv., with metal instead of glass troughs.

(g) *Dipping tank and accessories.*—Stainless steel, 8½ × 8½ × ¼–3/16″ inside width with metal supports and close-fitting U-shaped cover ca 9 × ½″. Capacity ca 300 mL (Arthur H. Thomas Co. No. 2749–H50, or equiv.).

(h) *Spotting pipets.*—1 μL (Kontes Glass Co. No. K-422520).

(i) *Spray bottle.*—8 oz (Arthur H. Thomas Co. No. 2753–J10 or SGA Scientific, Inc., No. JC 2850, 250 mL).

(j) *Chromatography spray flask.*—250 mL (Microchemical Specialties Co., 1825 Eastshore Hwy, Berkeley, CA 94710, No. S-4530-D).

(k) *Tank liner.*—Cut 2 pieces, 12¼ × 8¾″, from desk blotter, white or colored, and bend into L-shape to fit tank.

(l) *Strong ultraviolet light source.*—Such as germicidal lamps (General Electric Co., Nela Park, Cleveland, OH 44112), either (*1*) two 30 watt, 36″ tubes, No. G30T8, mounted in std 30 watt reflector fixture ca 20 cm above papers; or (*2*) two 15 watt, 18″ tubes, No. G15T8, mounted in std 15 watt desk lamp fixture placed ca 10 cm above papers. Shield to protect eyes and skin at all times.

29.007 ★ *Apparatus for Paper Chromatography* ★

See **29.007**, 13th ed.

29.008 *Apparatus for Gas Chromatography*

(*See also* JAOAC **47**, 326–342(1964); **49**, 8–21(1966).)

Gas chromatgc system when operated with column, (**b**), and approx. conditions described in *Gas Chromatography*, **29.018**, should be capable of producing ca ½ scale deflection for 1 ng heptachlor epoxide by electron capture detection and for 2 ng parathion by KCl-thermionic detection, and should resolve mixt. of heptachlor, aldrin, heptachlor epoxide, ethion, and carbophenothion into sep. peaks. Retention time for aldrin should be ca 4.5 min. Compds of interest must not be degraded by any part of GC system.

(a) *Gas chromatograph.*—Instrument consisting of on-column injection system, all-glass column in oven controlled to ±0.1°, electron capture and thermionic detectors, each with independent power supply, electrometer, and appropriate mv recorder.

(b) *Column.*—Glass, 1.85 m (6′) × 4 mm id packed with 10% DC-200 (w/w) on solid support: (*1*) 80–100 mesh Chromosorb W HP (Johns-Manville Products Corp., manufacturer, but available thru

★ Surplus method—*see* inside front cover.

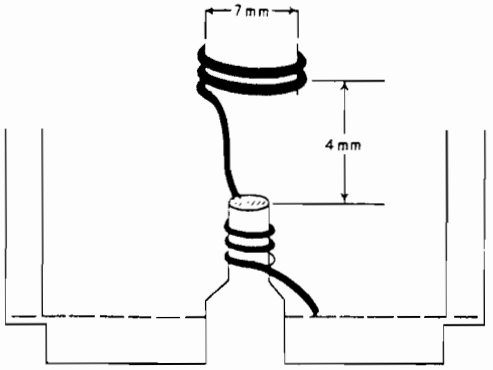

FIG. 29:01—KCl thermionic detector coil for in-series dual detection system

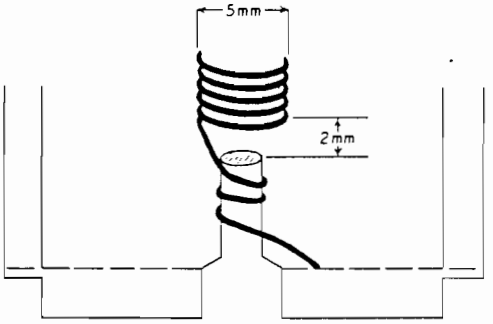

FIG. 29:02—KCl thermionic detector coil for parallel and in-series split dual detection systems

many GC distributors); (*2*) 80–100 mesh Gas-Chrom Q (Applied Science Laboratories, Inc.); (*3*) 80–90 mesh Anakrom ABS (Analabs, Inc.). DC-200 may be replaced by OV-101 (available from many GC distributors).

Weigh 2 g Dow Corning 200 silicone fluid (12,500 centistokes) or OV-101 into beaker. Dissolve in $CHCl_3$ and transfer to 300 mL Morton-type flask, using total of ca 100 mL $CHCl_3$. Add 18 g solid support, (*1*), (*2*) or (*3*), to flask. Swirl, and let stand ca 10 min. Place flask on rotary evaporator and remove solv. slowly with intermittent rotation, using 50° H_2O bath and slight vac. (Foaming may occur initially.) When solids appear damp, increase vac. Remove last traces of $CHCl_3$ without rotation or by air drying. Use only free-flowing material to fill column. Use care at all stages of column prepn to prevent fracturing solid support. Condition column at 250–260° with N flow of ca 100 mL/min ≥48 hr or until endrin exhibits single peak.

(**c**) *Electron capture detector (ECD).*—Concentric design, for use with dc voltage supply and ^3H source (ca 150 mCi ^3H, U.S. Nuclear Regulatory Commission license is required.)

Det. detector operating characteristics as follows: Apply dc voltage to detector. After system becomes stable (overnight), det. current-voltage relationship at various voltages between 200 and 0 v. (Current measurements at voltages of 200, 150, 100, 75, 50, 40, 30, 25, 20, 15, 10, 8, 6, 4, 2, and 1 provide points for smooth curve.) Slightly lower, stable, standing current may be obtained after detector has been at operating temp. several hr. This is probably due to loss of some easily removed radioactive material. Det. and plot response-voltage relationship at 1×10^{-9} amp full scale sensitivity for 1 ng injections of heptachlor epoxide at same voltages used in obtaining current-voltage curve. Select as operating voltage that voltage at which heptachlor epoxide causes ca 40–50% full scale recorder deflection. Check linearity of system from 0.2 to 2.0 ng heptachlor epoxide.

Other electron capture detectors may be substituted for dc voltage concentric design ^3H detector, which is no longer marketed. Const current, variable frequency ^{63}Ni electron capture detectors are acceptable substitutes when operated at conditions to produce stable, reproducible, linear responses. Optimum conditions may produce more sensitive response than from ^3H detector. To maintain same method limit of quantitation of ^3H detector, inject proportionately smaller equiv. sample wt into ^{63}Ni detector system. The ^{63}Ni electron capture detector may provide different relative responses for pesticides than those obtained with ^3H electron capture detector. Use of Ar-CH_4 carrier gas, as recommended for most ^{63}Ni detectors, precludes use of KClTD dual detection system, (**d**), (**h**)–(**j**).

(**d**) *Potassium chloride thermionic detector (KClTD).*—Flame ionization detector modified to incorporate coil with KCl coating prepd as in (*1*) or (*2*). Detector voltage is 300 v dc. Use in dual arrangement with electron capture detector.

All dual detector systems described are capable of comparable performance. In-series, (**h**), arrangement is preferred because of simplicity and ease of operation.

(*1*) *Coil with potassium chloride for in-series dual detector.*—*See* Fig. **29:01** (may be used with all detector arrangements). Wind Pt-Ir wire (B&S gage 26) on 7 mm diam. rod into 2 turn helix so that turns are touching. Approx. 5 mm below helix, continue to wind wire on 3 mm rod, or rod with same outside diam. as flame jet, making 3-turn spiral. Cut wire so that 7 mm helix is supported 4 mm above flame jet when 3 mm spiral is slipped over jet. Fill 30 mL tall-form Pt crucible ca ¼ full with KCl (ACS). Heat with Meker burner until all salt melts. Continue heating until bottom of crucible glows red, imparting pink glow to melt. Remove heat and begin dipping the 2-turn helix of coil into melt at 5 sec intervals as melt cools. (Make sure only 2-turn helix touches melt and do not raise coil above top of crucible.) When melt is at proper temp., salt clings to coil. Remove coil from melt. Place probe in center of coil while salt is molten. This causes crystn around probe tip. Remove center of coil. Remove any rough edges on coil coating by holding coil in burner flame 1 sec; id of properly coated coil is 5 mm. Position coil over flame jet.

(*2*) *Coil with potassium chloride for parallel and in-series split dual detectors.*—*See* Fig. **29:02**. Wind Pt-Ir wire (B&S gage 26) on 5 mm diam. rod into 5-turn helix so that turns are close together or touching. Continue to wind wire on 3 mm rod, or rod having same outside diam. as flame jet, making 3-turn spiral. Cut wire so that 5 mm helix is supported 2 mm above flame jet when 3 mm spiral is slipped over jet. Grasp formed wire by end opposite 5 mm helix with forceps. Dip 5 mm helix into satd KCl (recrystd twice from H_2O) soln, or apply KCl soln with dropper. Fuse in flame. (*Caution:* Use safety glasses; spattering occurs.) Repeat application of KCl soln 3–4 times until helix is coated with fused KCl. Coating should appear almost crystal clear. Position coil over flame jet.

(**e**) *Hydrogen.*—From generator or cylinder of compressed H gas (cylinder preferred). Equip cylinder with pressure drop of stainless steel capillary tubing (0.020″ id) to restrict H flow to ca 30 mL/min at 20 lb delivery pressure. Place H source close to detector and use gas lines with min. dead vol. to reduce outgassing time in lines. (For fine precise control of H flow, insert Nupro Very Fine Metering Valve, "S" series (Nupro Co., 4800 E 345th Street, Willoughby, OH 44094; Part Number B-1S) between exit end of capillary tubing pressure drop and inlet of detector H line. *Caution:* Do not use Nupro valve as shut-off valve. Repeated tightening damages needle.) Use Swagelok fittings for all connections.

(**f**) *Air.*—Min. air requirement for thermionic detector is 300 mL/min. Cylinder of compressed air or aquarium air pump is recommended.

(**g**) *Capillary T-tube.*—(*See* Figs. **29:03** and **29:04**.) Prep. 1:1 stream splitter (*B*) for parallel and in-series split dual detection systems. Fit two 4.5 cm lengths of stainless steel capillary tubing,

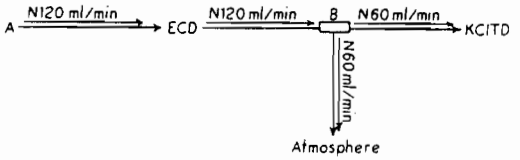

FIG. 29:03—In-series split dual detection system

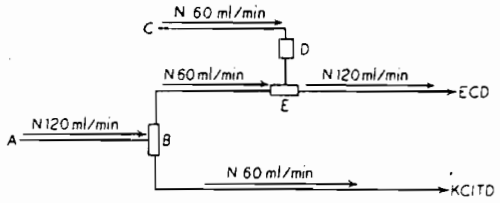

FIG. 29:04—Parallel dual detection system

0.010" id, 1/16" od, into 1 cm length of std wall, 1/8" stainless steel tubing. Fit 1" length of No. 16 hypodermic tubing at right angles in hole drilled into the piece of 1/8" tubing. Silver braze all connections. Prep. capillary T-tube (*E*) for introducing purge gas to parallel system. Fit two 2.5 cm lengths of No. 16 hypodermic tubing into 1 cm length of std wall, 1/8" stainless steel tubing. Fit 1 cm length of No. 16 hypodermic tubing at right angles in hole drilled into piece of 1/8" tubing. Silver braze as above.

(h) *Assembly of in-series dual detection system.*— Assemble as in Fig. **29:05**. Introduce column effluent (*A*) of 120 mL/min directly to ECD inlet. Connect ECD outlet directly to KCITD inlet, using No. 16 std wall Teflon tubing.

Note: For in-series, (h), and in-series split, (i), operation, thoroly check ECD for gas leaks, particularly at Teflon insulator.

(i) *Assembly of in-series split dual detection system.*—Assemble as in Fig. **29:03**. Introduce column effluent (*A*) of 120 mL/min directly to ECD inlet. Connect 1:1 stream splitter (*B*) between ECD outlet and KCITD inlet so that only 60 mL N/min enters KCITD and remaining 60 mL N/min exits to atm. Use No. 16 std wall Teflon tubing for all connections. *See Note* in (h).

(j) *Assembly of parallel dual detection system.*— Assemble as in Fig. **29:04**. Split column effluent (*A*) of 120 mL/min by passing thru 1:1 stream splitter (*B*) so that each detector receives 60 mL effluent/min. Increase flow to ECD by introducing 60 mL N/min from second N source (*C*) thru capillary T-tube (*E*). Preheat N from *C* by passing thru stainless steel capillary tube (*D*) (0.040" id) which extends 120 cm into column bath and returns to detector bath where addnl 35 cm of tubing is coiled into small helix. Connect capillary tubes and splitters to detectors with No. 16 std wall Teflon tubing. Measure flow at each end of splitter (*B*) to ensure exact 1:1 split.

(k) *Potassium chloride thermionic detector operation.*—Zero recorder with zero control before detector flame is ignited (no signal). Turn on H (ca 30 mL/min) and ignite flame. Adjust H with flame burning to give baseline current (BLC) of 0.2–0.8 $\times$ 10^{-8} amp. (Sensitivity to P compds is directly related to KCl temp., which depends on H concn in flame.) Select operational electrometer setting and adjust H concn to obtain 40–50% full scale recorder deflection for 2 ng parathion entering detector. When baseline has stabilized, measure BLC precisely, at electrometer setting of 1 $\times$ 10^{-8} amp full scale. Return to operational electrometer setting and zero recorder pen, using current balance control to "buck out" current generated by detector. Check linearity of system from 0.4 to 4.0 ng parathion. Monitor BLC frequently during operation. If drift occurs, readjust H concn to maintain same BLC. *For accurate quantitation, BLC must be identical during chromatgy of sample and std.*

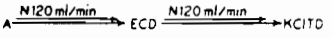

FIG. 29:05—In-series dual detection system

Concentration Technics

29.009 ***Purified Extracts***

(Never evap. purified exts to dryness.)

(a) *To approximately 5 mL or more.*—Evap. on steam bath in Kuderna-Danish concentrator fitted with 3-ball Snyder column and vol. flask or graduated collection tube; 20-mesh boiling chip is necessary.

(b) *To less than 5 mL.*—Evap. to ca 5 mL as in (a). Remove collection tube from concentrator and fit tube with 2-ball micro-Snyder or micro-Vigreux column. Evap. to slightly less than desired vol., permit condensate to drain into tube, and remove column. Min. attainable vol. is 0.2–0.4 mL.

29.010 ***Extracts Containing Fats, Oils, or Plant Extractives***

(a) *Kuderna-Danish concentrator.*—Fitted with 3-ball Snyder column and vol. flask or graduated collection tube. Use on steam bath.

(b) *Flash evaporator.*—Keep flask in H_2O bath at room temp.

(c) *Beaker.*—Evap. in beaker in H_2O bath at 35–40° under stream of clean, dry air. Remove from heat and air stream as soon as last of solv. evaps. Let residual H_2O evap. spontaneously. Solvs may be evapd from fats on steam bath for short periods.

Preparation of Sample and Extraction

29.011 ***Nonfatty Foods***

(*Caution: See* **51.004**, **51.011**, **51.039**, **51.040**, **51.043**, and **51.073**.)

Pit *soft fruits*, if necessary. Chop or blend representative sample of *leafy or cole-type vegetables, pitted soft fruits, firm fruits, and roots*. Mix thoroly to obtain homogeneous sample before taking portions for analysis. Grind *dry or low moisture products*, e.g., hays, to pass No. 20 sieve and mix thoroly. Proceed as in (a), (b), (c), or (d).

(a) *High moisture (more than 75% H_2O) products containing less than 5% sugar.*—*(1) Products other than eggs.*—Weigh 100 g chopped or blended sample into high-speed blender jar, add 200 mL CH_3CN and ca 10 g Celite, and blend 2 min at high speed. Filter with suction thru 12 cm buchner fitted with sharkskin paper into 500 mL suction flask. Transfer filtrate to 250 mL graduate and record vol. (*F*). Transfer measured filtrate to 1 L separator, and proceed as in (e). *(2) Whole eggs.*—Discard shells and blend combined yolks and whites at *low* speed ≥5 min or until sample is homogeneous. Low-speed blending will minimize foaming or "whipping" of sample. Weigh ≤25 g thoroly mixed yolks and whites into high-speed blender jar, and proceed with addn of CH_3CN as in *(1)*.

(b) *High moisture (more than 75% H_2O) products containing 5–15% sugar.*—Add 200 mL CH_3CN and 50 mL H_2O to 100 g sample in blender and proceed as in (a). Transfer ≤250 mL filtered ext (record vol. (*F*)) to 1 L separator, and proceed as in (e).

(c) *High moisture (more than 75% H_2O) products containing 15–30% sugar, e.g., grapes.*—Heat mixt. of 200 mL CH_3CN and 50 mL H_2O to 75°, add to 100 g sample in blender, and immediately proceed as in (a). Before filtered ext cools, transfer ≤250 mL (record vol. (*F*)) to 1 L separator. Let cool to room temp. and proceed as in (e).

(d) *Dry or low-moisture products, e.g., hays.*—Add 350 mL 35% H_2O-CH_3CN (350 mL H_2O dild to 1 L with CH_3CN) to 20–50 g ground sample in blender (if larger sample is required, add enough addnl extn mixt. to wet sample and permit thoro blending). Blend 5 min at high speed, and proceed as in (a), beginning "Filter with

suction . . ." Transfer ≤250 mL filtered ext (record vol. (*F*)) to 1 L separator, and proceed as in (e).

(e) *Transfer of residues to petroleum ether.*—Carefully measure 100 mL pet ether and pour into separator contg filtrate. Shake vigorously 1–2 min and add 10 mL satd NaCl soln and 600 mL H_2O. Hold separator in horizontal position and mix vigorously 30–45 sec. Let sep., discard aq. layer, and gently wash solv. layer with two 100 mL portions H_2O. Discard washings, transfer solv. layer to 100 mL g-s cylinder, and record vol. (*P*). Add ca 15 g anhyd. Na_2SO_4 and shake vigorously. Do not let ext remain with Na_2SO_4 >1 hr or losses of organochlorine pesticides by adsorption may result. Transfer soln directly to Florisil column, **29.015**, or conc. to 5–10 mL in Kuderna-Danish concentrator for transfer.

(f) *Calculation for fruits and vegetables.*—Calc. g sample as $S \times (F/T) \times (P/100)$; where S = g sample taken; F = vol. filtrate; T = total vol. (mL H_2O in sample + mL CH_3CN added − correction in mL for vol. contraction); P = mL pet ether ext; and 100 = mL pet ether into which residues were partitioned. When 50 mL H_2O is added to CH_3CN for extn of high sugar products, total vol., T, is increased by 45, *i.e.*, T = 325 instead of 280 for samples contg 85% H_2O.

Example: 100 g sample contains 85 g H_2O; 200 mL CH_3CN is added; vol. contraction is 5 mL. Total vol., T, is 280 mL. If vol. filtrate is 235 mL, vol. pet ether ext is 85 mL, and residue is transferred to 100 mL pet ether, then 100 × (235/280) × (85/100) = 71 g sample.

Consult refs on food composition for av. H_2O content. Water content of most fresh fruits and vegetables may be assumed to be 85%.

For 25 g whole eggs and 200 mL CH_3CN, use 215 as T.

(g) *Calculation for dry or low moisture products, e.g., hays.*—Calc. g sample as in fruits and vegetables, (f), except T = total vol. (mL H_2O in sample + mL 35% H_2O-CH_3CN added − correction in mL for vol. contraction). If H_2O content of sample is ≤10%, disregard and use vol. of extg mixt. as T.

29.012 *Fat-Containing Foods*

(After isolation of fat, proceed with CH_3CN partitioning, **29.014**.)

(a) *Animal and vegetable fats and oils.*—If solid, warm until liq. and filter thru dry filter.

(b) *Butter.*—Warm at ca 50° until fat seps and decant fat thru dry filter.

(c) *Milk.*—(*Caution: See* **51.011**, **51.039**, **51.054**, and **51.073**.) To 100 mL fluid milk (dil. evapd milk 1 + 1 with H_2O) in 500 mL centrf. bottle, add 100 mL alcohol or MeOH and ca 1 g Na or K oxalate, and mix. Add 50 mL ether and shake vigorously 1 min; then add 50 mL pet ether and shake vigorously 1 min. Centrf. ca 5 min at ca 1500 rpm. Blow off solv. layer with wash bottle device, **16.246**, *Notes*, into 1 L separator contg 500– 600 mL H_2O and 30 mL satd NaCl soln. Re-ext aq. residue twice, shaking vigorously with 50 mL portions ether-pet ether (1 + 1); centrf. and blow off solv. layer into separator after each extn. Mix combined exts and H_2O cautiously. Drain and discard H_2O. Rewash solv. layer twice with 100 mL portions H_2O, discarding H_2O each time. (If emulsions form, add ca 5 mL satd NaCl soln to solv. layer or include with H_2O wash.) Pass ether soln thru column of anhyd. Na_2SO_4, 50 × 25 mm od, and collect eluate in 400 mL beaker. Wash column with small portions pet ether and evap. solv. from combined exts at steam bath temp. under air current to obtain fat.

(d) *Cheese.*—Place 25–100 g (to provide 3 g fat) diced sample, ca 2 g Na or K oxalate, and 100 mL alcohol or MeOH in high-speed blender and blend 2–3 min. (If experience with product indicates emulsions will not be broken by centrfg, add 1 mL H_2O/2 g sample before blending.) Pour into 500 mL centrf. bottle, add 50 mL ether, and shake vigorously 1 min; then add 50 mL pet ether and shake vigorously 1 min (or divide between two 250 mL bottles and ext each by shaking vigorously 1 min with 25 mL each ether). Proceed as in (c), beginning "Centrf. ca 5 min at ca 1500 rpm."

(e) *Fish.*—(*Caution: See* **51.004**, **51.011**, **51.039**, and **51.073**.)

Weigh 25–50 g thoroly ground and mixed sample into high-speed blender. (If fat content is known or can be estd, adjust sample size so that max. of ca 3 g fat will be extd.) Add 100 g anhyd. Na_2SO_4 to combine with H_2O present and disintegrate sample. Alternately blend and mix with spatula until sample and Na_2SO_4 are well mixed. Scrape down sides of blender jar and break up caked material with spatula. Add 150 mL pet ether and blend at high speed 2 min. Decant supernate pet ether thru 12 cm buchner, fitted with 2 sharkskin papers, into 500 mL suction flask. Scrape down sides of blender jar and break up caked material with spatula. Re-ext residue in blender jar with two 100 mL portions pet ether and blend 2 min each time. (After blending 1 min, stop blender, scrape down sides of blender jar, and break up caked material with spatula; continue blending 1 min.) Scrape down sides of blender jar and break up caked material between extns. Decant supernate pet ether from repeat blendings thru buchner and combine with first ext. After last blending, transfer residue from blender jar to buchner, and rinse blender jar and material in buchner with three 25–50 mL portions pet ether. Immediately after last rinse, press residue in buchner with bottom of beaker to force out remaining pet ether. Pour combined exts thru 40 × 25 mm od column of anhyd. Na_2SO_4 and collect eluate in 500 or 1000 mL Kuderna-Danish concentrator with plain tube. Wash flask and column with small portions pet ether and evap. most of pet ether from combined exts and rinses in Kuderna-Danish concentrator. Transfer fat soln to tared beaker, using small amts pet ether. Evap. pet ether at steam bath temp. under current of dry air to obtain fat. When pet ether is completely removed, weigh and record wt of fat extd.

Record wt of fat taken for cleanup. ((Wt fat for cleanup/wt fat extd) × wt original sample = wt sample analyzed.) If it is known that ≤3 g fat will be extd from particular sample, do not isolate and weigh fat before CH_3CN partitioning. Detn is then on basis of wt of original sample.

29.013 *Soil (2)*

(Official final action for aldrin, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, dieldrin, endrin, heptachlor, heptachlor epoxide, lindane, and *p,p'*-TDE (DDD))

Weigh 10.0 g undried soil, sieved thru 2 mm sieve and mixed thoroly, into 250 mL erlenmeyer. Add 7 mL *0.2M NH_4Cl soln* (10.7 g/L) and let stand 15 min. Add 100 mL hexane-acetone (1 + 1), stopper tightly, and shake overnight (≥12 hr) on reciprocal or wrist-action shaker at 180 rpm.

Carefully pour supernate, avoiding aq.-clay phase, thru 2–3 cm column (22 mm id) of Florisil, **29.002**(i), and collect eluate in 1 L separator. Rinse flask and soil with two 25 mL portions hexane-acetone and decant thru column. Rinse column with 10 mL hexane-acetone.

Add 200 mL H_2O to separator and shake gently ca 30 sec. Drain aq. phase into second separator and ext with 50 mL hexane. Combine hexane layers in first separator and wash with 100 mL H_2O. Drain and discard H_2O. Pour hexane thru 2 cm column (22 mm id) Na_2SO_4, conc. to 100 mL, and make preliminary injection of 5–10 μL into gas chromatograph. If peaks are present at retention times of DDE or dieldrin, conc. to 10 mL in Kuderna-Danish concentrator, **29.010**(a), and sep. DDE or dieldrin as in **29.015**. (This cleanup may also be necessary with exts from high org. matter soils.) Proceed as in **29.018**, using ECD, (b). To calc. to dry basis, dry sep. sample of 10 g ca 16 hr at 105° to obtain % solids.

Refs.: JAOAC **56**, 728(1973); **57**, 604(1974).

Cleanup Technics

29.014 *Acetonitrile Partitioning*

(*Caution: See* **51.011**, **51.039**, and **51.073**. Different fats and oils may show varying tendencies to emulsion formation.)

Weigh ≤3 g fat into 125 mL separator, and add pet ether so that total vol. of fat and pet ether is 15 mL. Add 30 mL CH_3CN satd with pet ether, **29.002(b)**, shake vigorously 1 min, let layers sep., and drain CH_3CN into 1 L separator contg 650 mL H_2O, 40 mL satd NaCl soln, and 100 mL pet ether. Ext pet ether soln in 125 mL separator with 3 addnl 30 mL portions CH_3CN satd with pet ether, shaking vigorously 1 min each time. Combine all exts in the 1 L separator.

(If experience with particular sample (e.g., fish) indicates that cleanup may not be sufficient, perform partitioning as follows: Drain CH_3CN phase from first partitioning into second 125 mL separator contg 15 mL pet ether, shake vigorously 1 min, let layers sep., and drain CH_3CN into 1 L separator contg 650 mL H_2O, 40 mL satd NaCl soln, and 100 mL pet ether. Pass CH_3CN phase from each of 3 addnl partitionings thru same 15 mL pet ether in 125 mL separator. Shake vigorously each time and combine CH_3CN exts in the 1 L separator.)

Hold separator in horizontal position and mix thoroly 30–45 sec. Let layers sep. and drain aq. layer into second 1 L separator. Add 100 mL pet ether to second separator, shake vigorously 15 sec, and let layers sep. Discard aq. layer, combine pet ether with that in original separator, and wash with two 100 mL portions H_2O. Discard washings and draw off pet ether layer thru 50 × 25 mm od column of anhyd. Na_2SO_4 into 500 mL Kuderna-Danish concentrator. Rinse separator and then column with three ca 10 mL portions pet ether. Evap. combined ext and rinses to ca 10 mL in Kuderna-Danish concentrator for transfer to Florisil column.

29.015 *Florisil Cleanup*

(*Caution: See* **51.011**, **51.039**, **51.040**, **51.054**, and **51.073**.)

Prep. 22 mm id Florisil column, **29.005(b)**, contg 10 cm, after settling (or amt detd by lauric acid test, **29.002(i)**), of activated Florisil topped with ca 1 cm anhyd. Na_2SO_4. Prewet column with 40–50 mL pet ether. Place Kuderna-Danish concentrator with vol. flask or graduated collection flask under column to receive eluate. Transfer pet ether ext or conc. to column, letting it pass thru at ≤5 mL/min. Rinse containers and Na_2SO_4, if present, with two ca 5 mL portions pet ether, pour rinsings onto column, rinse walls of tube with addnl small portions pet ether, and elute at ca 5 mL/min with 200 mL 6% eluting solv., **29.002(e)**. Change receivers and elute with 200 mL 15% eluting solv., **29.002(f)**, at ca 5 mL/min. Change receivers and elute with 200 mL 50% eluting solv., **29.002(g)**, at ca 5 mL/min.

Conc. each eluate to suitable definite vol. in Kuderna-Danish concentrator. When vol. <5 mL is needed, use 2-ball micro-Snyder or micro-Vigreux column.

First eluate (6%) contains *organochlorine pesticides* (aldrin, BHC, DDE, DDD (TDE), *o,p'*- and *p,p'*-DDT, heptachlor, heptachlor epoxide, lindane, methoxychlor, mirex, and ethylan), *industrial chems* (polychlorinated biphenyls (PCB)), and *organophosphorus pesticides* (ethion and ronnel) and is usually suitable for GC directly. If further cleanup is necessary, repeat Florisil cleanup, using new column. Second eluate (15%) contains *organochlorine pesticides* (dieldrin and endrin) and *organophosphorus pesticides* (diazinon, Me parathion, and parathion). If further cleanup is necessary, det. organophosphorus pesticides by GC and TLC; then proceed with *Magnesia Cleanup*, **29.016**, and/or *Saponification*, **29.017**, which are applicable only to organochlorine pesticides in 15% eluate (organophosphorus pesticides are degraded). Third eluate (50%) contains *organophosphorus pesticide* malathion.

29.016 *Magnesia Cleanup*

(Applicable only to organochlorine pesticides in 15% eluate when addnl cleanup is necessary)

Transfer ca 10 g MgO-Celite mixt., **29.002(l)**, to chromatgc tube without stopcock, **29.005(c)**, using vac. to pack. Prewash with ca 40 mL pet ether, discard prewash, and place Kuderna-Danish concentrator under column. Transfer 15% Florisil eluate, concd to ca 5 mL, to column, rinsing with small portions pet ether. Force pet ether into column with slight vac. or pressure. Then elute with 100 mL pet ether. Conc. eluate to suitable vol. Proceed with detn. or saponification, if required.

29.017 *Saponification–First Action*

(Applicable only to those chems stable to hot alkali treatment. Use as supplemental cleanup if 15% eluate or MgO–Celite eluate is not substantially free from oily materials.)

Conc. pet. ether–ether (85 + 15) fraction under current of air to 2 mL, add 1 mL 2% alc. KOH, attach micro-Snyder column, and carefully reduce to ≤1 mL on steam bath. Reflux sample 15 min, remove, and cool. Add 2 mL alcohol–H_2O (1 + 1) and 5 mL hexane, and shake 1 min. Centrf. to sep. layers. Transfer as much hexane layer as possible to second tube, using disposable Pasteur pipet, and repeat extn with 5 mL hexane. Conc. combined hexane to appropriate vol. for GC analysis.

Detection Methods

29.018 *Gas Chromatography—Tentative Identification and Quantitative Measurement*

(Applicable to organochlorine pesticides, organophosphorus pesticides, and polychlorinated biphenyls (PCB). Method is applicable to PCB residues when present alone in sample. If pesticidal or other compds are detected in chromatogram of the PCB residue, other chemical or physical operations must be applied to eliminate or minimize their interference before PCB quantitation.)

Inject suitable aliquot (3–8 μL) of concd eluate from Florisil or MgO-Celite column contg amt of compd within linear range into gas chromatograph, **29.008**, using 10 μL syringe. Tentatively identify residue peaks on basis of retention times. Measure area or ht of residue peak(s) and det. residue amt by comparison to peak area or ht obtained from known amt of appropriate ref. material(s). To ensure valid measurement of residue amt, size of peaks from residue and ref. std should be within ±25%. Chromatograph ref. material(s) immediately after sample.

Measure PCB residues by comparing total area or ht of residue peaks to total area or ht of peaks from appropriate Aroclor(s) (Analabs, Inc.) ref. materials. Measure total area or ht response from common baseline under all peaks. Use only those peaks from sample that can be attributed to chlorobiphenyls. These peaks must also be present in chromatogram of ref. material. Mixt. of Aroclors may be required to provide best match of GC patterns of sample and ref.

Alternatively, det. PCB residues by individual peak area comparisons using Aroclor ref. material wt factors in Table **29:02**. Calc. each PCB peak against appropriate individual ref. peak with exactly same absolute retention. Sum individual peak values to obtain total ppm PCB. (This method is recommended for PCB residues with chromatgc patterns which are altered extensively from that of any Aroclor ref.)

(a) *Recommended operating conditions for 10% DC-200 or OV-101 column.*—Glass column, 1.8 m (6') × 4 mm id. Temps (°): injector, 225; column 200; ^3H electron capture detector, 210 max.; carrier gas flow, 120 mL N/min.

(b) *Electron capture detection (ECD).*—(Use for detn of organochlorine pesticides in fruits, vegetables, and food contg fats and for detn of PCB in foods and paperboard.) Select for ^3H electron

Table 29:02 Weight % Factors for Individual Gas Chromatographic Peaks in Aroclor Reference Materials (Peaks are identified by their retention time relative to *p,p'*-DDE = 100 at conditions consistent with 29.018(a) and (b))

	AROCLOR				
$R_{DDE}(100\times)$	1016 (77-029)[a]	1242 (71-696)[a]	1248 (71-697)[a]	1254 (71-698)[a]	1260 (71-699)[a]
11	0.2				
16	3.8	3.4	0.3		
21	8.1	10.3	1.1		
24	1.2	1.1	0.2		
28	16.8	15.8	6.0		
32	7.6	7.3	2.6		
37	18.5	17.0	8.7		
40	14.6	13.0	7.4		
47	11.6	9.9	15.7	7.1	
54	7.7	7.1	9.3	2.7	
58	6.4	4.4	8.3	1.2	
70	3.4	8.7	18.2	14.7	2.4
78		1.9	6.4		
84			4.6	18.6	3.6
98			3.4	8.3	2.8
104			3.3	14.1	
112			1.0		
117					4.4
125			2.3	15.6	11.0
146			1.2	9.0	13.3
160					5.5
174				7.4	10.0
203				1.3	10.9
232-244					11.2
280					12.5
332					4.2
360-372					5.4
448					0.8
528					2.0

[a] Food and Drug Administration Lot Nos. (Wt factors are valid only for these FDA Lot Nos.) Aroclor ref. materials are available from Food and Drug Administration, Division of Chemical Technology, HFF-420, 200 C St SW, Washington, DC 20204.

capture detector operating voltage that voltage (ca 50 v dc) at which 1 ng heptachlor epoxide produces 40–50% full scale recorder deflection at 1 or 3 × 10^{-9} amp full scale sensitivity.

Operate ^{63}Ni electron capture detector to produce stable, reproducible, linear response, and adjust amt of injected sample to accommodate differences in instrument sensitivity.

(c) *Potassium chloride thermionic and electron capture dual detection.*—(Use one of the 3 dual detection systems specified in **29.008(h)**, (i), (j), for detn of organophosphorus and organochlorine pesticides and PCB. In-series system, (h), is preferred because of simplicity and ease of operation.) *(1) In-series dual detection.*—Operate ECD as in (b). For KCITD, adjust H flow producing 0.2–0.8 × 10^{-8} amp baseline current and select electrometer setting at which 2 ng parathion produces 40–50% full scale recorder deflection. *(2) In-series split dual detection.*—Same as *(3), Parallel*, except ECD receives entire injection and KCITD receives ½ amt injected into column. *(3) Parallel dual detection.*— Same as *(1), In-series dual*, except column effluent is split; therefore, inject twice as much sample to obtain desired limit of quantitation.

Thin Layer Chromatography—Confirmation of Identity

Method I

(Applicable to organochlorine and organophosphorus pesticides except where indicated)

29.019 *Preparation of Adsorbent Layer*

Before coating, wash plates in hot soapy water and thoroly rinse with distilled H_2O. Press plates snugly into position on mounting board that has retaining ledge on one side and one end. Plastic board is mounted so that long side with raised ledge faces operator while short side with ledge is to right of operator. Before coating, wipe plates with few mL alcohol. Position applicator, trough open, with left edge 6 mm in from edge of first plate to be coated.

To coat 5 plates, weigh 30 g Al_2O_3 G, **29.003(a)**, into 250 mL ℨ erlenmeyer. Add 50 mL H_2O, stopper, and shake moderately 45 sec. Violent shaking produces bubbles, resulting in "pock-marked" layer.

Suspensions that contain adsorbents with binders set rapidly, and entire operation from prepn of slurry to final coating must be completed within 2 min.

After shaking, immediately pour slurry into applicator chamber. Rotate chamber by turning large lever handle thru 180°. After few sec, slurry begins to flow out of exit slit. Grasp applicator with both hands and pull it manually with steady motion across series of plates. Approx. 5 sec is required for actual coating operation. Immediately after application, tap edge of mounting board or shake entire board gently to smooth out slight ripples or imperfections in wet coating.

Let coated plates dry in position on mounting board 15 min. Then dry plates in forced-draft oven 30 min at 80°. Remove plates and cool.

Examine plates carefully in transmitted and reflected light for imperfections or irregularities in coating. Discard any plates showing extensive rippling or mottling of layer.

Prep. 5 more plates while first set is drying. Be sure applicator is thoroly cleaned and dried before reusing. The 10 coated and dried plates may be prewashed immediately.

29.020 *Prewashing of Adsorbent Layer*

Scrape 1 cm of adsorbent off edge of plate with razor blade. Pour 15 mL 50% aq. acetone into metal trough inside chromatgc tank. Cut out 2 × 20 cm strip of Whatman No. 1 filter paper, wet with solv., and place over scraped off portion with 6 mm overlapping adsorbent layer. Place plate in chromatgc tank, seal tank with masking tape, and develop with 50% aq. acetone to within 4 cm from top of plate (75–90 min). Remove plate from tank, remove filter paper wick, invert plate, and dry in hood 5 min. Dry plate 45 min at 80°. Remove plate from oven, cool, and store in desiccator. Use prepd plates within 1 week after prepn.

29.021 *Sample Spotting*

Make pencil mark 4 cm from bottom of plate at both sides. Imaginary line between the two points indicates sample spotting or origin "line." Draw line (which removes coating) completely across plate 14 cm from bottom edge; this line represents solv. front after development. On lower edge of adsorbent starting 2 cm in from left edge of plate, make 18 marks with pencil at 1 cm intervals. (Fewer marks with longer intervals may be used, if desired. Marks serve as horizontal guides to sample application. Identity of samples and stds may be etched directly into adsorbent layer above these marks above solv. front line.)

Imaginary spotting "line" is actually shadow line cast by strong light source from wooden ruler supported 2 cm above plate. Align ruler shadow on the two 4 cm marks on either edge of plate. Shadow line and 18 marks, resp., serve as vertical and horizontal guides for sample application.

For optimum semiquant. detn, spot aliquot of sample as follows:

(a) *Organochlorine pesticides.*—Adjust aliquot to give residue spot within range 0.005–0.1 μg. Spot stds and std mixts at 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, and 0.2 μg. Sample spots >0.2 μg are difficult to det. quant. and <0.005 μg may be difficult to distinguish. Spot all 6% Florisil eluates on one plate and 15% Florisil eluates on another plate.

(b) *Organophosphorus pesticides.*—Adjust aliquots of sample and

stds to give spot within range 0.1–0.5 μg. Spot 6, 15, and 50% Florisil eluates on same plate. Ronnel and ethion are not resolved; spot each std sep. Spot diazinon, Me parathion, and malathion sep. or as mixt.

Vol. of sample ext spotted should be ≤10 μL, if possible, and spotting should be done repeatedly with 1, 2, or 3 μL Kontes spotting pipet. Spot std and sample solns with same pipet. For best results, keep size of spotted samples as small as possible.

29.022 ***Development***

(a) *Organochlorine pesticides.*—Place liners and metal trough in tank, **29.006(f)**. Presat. liner by pouring 75 mL developing solv., **29.003(b)**, into bottom of tank ≥30 min before developing plate. Presatn decreases development time and improves uniformity of R_f values.

For plates spotted with 6% Florisil eluates, pour 50 mL *n*-heptane into trough. Place lower edge of plate in metal trough with top of plate leaning against side of tank. Place glass cover plate on tank and seal with masking tape.

For plates spotted with 15% Florisil eluates, use acetone-*n*-heptane (2+98) as developing solv.

(b) *Organophosphorus pesticides.*—Prep. chromatgc tank, **29.006(f)**, after samples and stds have been spotted on plate. Place liners and metal trough in tank. Pour 50 mL methylcyclohexane, **29.003(d)(2)**, into trough, and 75 mL into bottom of tank. Quickly fill dipping tank, **29.006(g)**, to within 4–5 cm from top with immobile solv., **29.003(d)(1)**. Invert plate and dip with uncoated side touching back wall of tank to prevent front wall from scraping the adsorbent layer during dipping operation. Dip plate *just* to spotting line, remove, and immediately place in metal trough, with top portion of plate leaning against side of tank. Place glass cover plate on tank and seal with masking tape.

When solv. front in (a) or (b) *just* reaches pencil line 10 cm above spotting "line," remove plate and dry in hood 5 min.

29.023 ***Spraying***

(*Caution: See* **51.017**.)

(a) *Organochlorine pesticides.*—Support plate on one side and spray fairly heavily with chromogenic agent, **29.003(c)**, using lateral motions of spray bottle perpendicular to direction of solv. flow. Spray until plate appears translucent or soaked with reagent. Underspraying will result in poor sensitivity. After spraying, dry plate in hood 15 min; then immediately place under UV light source and proceed as in **29.024**.

(b) *Organophosphorus pesticides.*—Immediately spray plate moderately heavily and uniformly with dye soln, **29.003(e)(2)**, using lateral motions of spray flask, **29.006(j)**, perpendicular to direction of solv. flow. Plate should be vivid blue after spraying. Using spray bottle, **29.006(i)**, overspray plate lightly and uniformly with $AgNO_3$ soln, **29.003(e)(3)** (at this point plate should be bluish purple and spots should be discernible).

After 2 min, overspray plate moderately and uniformly with citric acid soln, **29.003(e)(4)**, using spray bottle, **29.006(i)**. After spraying, thiophosphate pesticides should immediately appear as vivid blue or purple spots against yellow background. Color of spots reaches max. intensity ca 5–10 min after citric acid spraying. After ca 10 min, background begins to change from yellow to greenish blue, masking spots. At this point, respraying plate with citric acid soln changes background back to yellow and makes spots stand out as well as or better than originally. Evaluate chromatogram ≤10 min after respraying. Blue spots fade completely and irreversibly after 30–40 min from time of original citric acid spraying.

29.024 ***Exposure***

(*Caution: See* **51.016**.)

Expose plate to UV light until spot for std of lowest concn appears; 5 ng of most organochlorine pesticides should be visible after 15–20 min exposure with equipment described under **29.006(l)**. Exposure times >30 min will not harm plates. For best results, place plates 8 cm from bottom edge of lamps.

Method II

(Applicable only to organochlorine pesticides)

29.025 ***Preparation of Adsorbent Layer***

Weigh 40 g Al_2O_3 G, **29.003(a)**, into 500 mL centrf. bottle. Add 80 mL 0.2% HNO_3, shake well, and centrf. at ca 1200 rpm 1–2 min. Decant supernate into 100 mL graduate, and record vol. (35–40 mL should be recovered). Add 80 mL H_2O, breaking up material on bottom of centrf. bottle with glass rod, if necessary. Shake well and centrf. as before. Decant and record vol. supernate recovered (60–70 mL). Add 2 addnl 80 mL portions H_2O, shake well, centrf., and decant.

Weigh the Al_2O_3 and H_2O that has been retained. (Wt should be ca 100 g.) Add 10 mL 1% $AgNO_3$ soln and enough H_2O to make total wt 120–130 g. Shake well, place in applicator, and prep. plates as in **29.019**. Let plates air dry in position on mounting board 15 min. Place in metal drying rack, in vertical position, 30 min at 100°.

29.026 ***Sample Spotting***

Spot as in **29.021**. Draw line across plate 4 cm from top (which removes coating). Next, scrape 6 mm of coating from each side of plate. (Irregularities in thickness of coating on these outer edges cause uneven flow of mobile solv.) Make pencil mark at each side of layer 2.5 cm from bottom of plate; imaginary line between these 2 points indicates sample spotting line. Spot samples and stds at 1 cm intervals.

29.027 ***Development and Exposure of Plates***

(*Caution: See* **51.016**.)

Develop plates as in **29.022**, except use only 25–30 mL mobile solv. in trough, since spotting line has been lowered to 2.5 cm. Use *n*-heptane to develop 6% Florisil eluates, and acetone-*n*-heptane (2+98) for 15% Florisil eluates.

Plates may be exposed to UV light after short drying period (ca 5 min) after removal from tank. Spots of aldrin, DDE, and isomers of DDT will appear within 5–10 min after exposure; lindane, endrin, dieldrin, and all others will require more time. Plates may be exposed 1.5–2 hr without appreciable darkening of background.

29.028 ★ ***Paper Chromatography*** ★

See **29.028**, 13th ed.

Refs.: JAOAC **42**, 734(1959); **44**, 171(1961); **46**, 186(1963); **48**, 668(1965); **49**, 460, 463, 468(1966); **50**, 430, 623, 1205(1967); **51**, 311, 666, 892(1968); **52**, 1280(1969); **53**, 152, 355, 1300(1970); **54**, 325, 525(1971); **55**, 284(1972); **56**, 721, 1015(1973); **59**, 169(1976); **61**, 282(1978); **63**, 277(1980).

ORGANOCHLORINE RESIDUES

Organochlorine Pesticide and Polychlorinated Biphenyl Residues in Fish

Gas Chromatographic Method

First Action

29.029 ***Method Performance***

p,p'-DDE av. rec. at 0.5–2.5 ppm = 94% (S_r = 0.04–0.29, S_o = 0.03–0.16)

★ Surplus method—*see* inside front cover.

p,p'-DDD rec. at 1 ppm = 94% (S_x = 0.13, S_o = 0.11)

p,p'-DDT av. rec. at 0.9–2.4 ppm = 90% (S_x = 0.07–0.29, S_o = 0.07–0.26)

Dieldrin rec. at 0.2 ppm = 79% (S_x = 0.008, S_o = 0.008)

Heptachlor epoxide rec. at 0.20 ppm = 88% (S_x = 0.014, S_o = 0.014)

Aroclor av. rec. at 0.8–1.5 ppm = 96% (S_x = 0.08–0.16, S_o = 0.08–0.16)

29.030 ***Principle***

Chlorinated pesticides and polychlorinated biphenyls (PCBs) are extd from prepd fish sample with pet. ether, cleaned up on Florisil column, and detd by GC against ref. stds.

See **29.002, 29.005, 29.008–29.010** for general app., reagents, and technics.

29.031 ***Apparatus***

(a) *Gas chromatograph.*—With on-column injection system, 6 ft glass column (4 mm id), packed with 10% DC-200 on 80–100 mesh Chromosorb WHP, and electron capture detector. Other liq. phases such as 5% OV-101 on suitable supports may be substituted if known to give adequate resolution for compds present in samples.

Linearized ^{63}Ni detector capable of producing ½ scale deflection for 1 ng heptachlor epoxide is suggested; however, other equiv. electron capture detectors may be used. Operate GC in accordance with manuf. directions, adjusting to provide necessary response and resolution.

(b) *Chromatographic tube.*—10 mm id × 300 mm column with Teflon stopcock, coarse fritted disk, ȝ 24/40 top joint (Kontes Glass Co. K-420550, or equiv.).

(c) *Kuderna-Danish concentrators.*—Snyder distg column (Kontes K-503000-0121); 125 mL K-D flask (Kontes K-570001-9010) (special item) ȝ 19/22 lower joint; 10 mL concentrator tube (Kontes K-570050-1025).

(d) *Micro Snyder column.*—Kontes K-569251, ȝ 19/22.

29.032 ***Reagents***

(a) *Florisil.*—PR grade, 60–80 mesh (Floridin Co.). Must meet **29.002**(i) specifications.

(b) *Solvents.*—Pet. ether, ethyl ether, hexane, and alcohol, known to be suitable for pesticide residue detn.

(c) *Glass wool (Pyrex).*—Must be free of interference with electron capture detection.

(d) *Sodium sulfate.*—Anhyd., granular, reagent grade, free of interference with electron capture detection.

29.033 ***Extraction***

Weigh 20 g thoroly ground and mixed sample into metal blender cup. Moisten 40 g granular Na_2SO_4 with pet. ether and add to sample. Mix sample, using stirring rod, let stand 20 min, and mix again. Add 100 mL pet. ether to sample and blend 1–2 min. (Lourdes blender in series with rheostat set at 40–60%, or equiv., may be used.) Centrf. balanced sample cup 1–2 min at ca 2000 rpm to obtain clear pet. ether ext. Place glass wool plug in funnel, overlay with 20 g granular Na_2SO_4, and place funnel in 250 mL vol. flask. Decant pet. ether ext thru Na_2SO_4 into vol. flask. Mix sample again with stirring rod, add 100 mL pet. ether, and ext as before. Repeat using 70 mL pet. ether. Dil. to vol. with pet. ether.

Transfer 25 mL aliquot to tared 100 mL flat bottom extn flask. Place flask on steam bath to evap. solv., leave addnl 30 min on steam bath, remove, and cool. Weigh flask and det. % fat in fish.

For fish contg <10% fat, transfer 25 mL aliquot to 125 mL K-D concentrator. For fish contg >10% fat, take aliquot contg not >200 mg fat. Add several granules of 20–30 mesh carborundum and conc. to ca 3 mL on steam bath. Let cool and remove Snyder column. Rinse concentrator with two 1 mL portions of pet. ether and, using only current of air, conc. sample to 3 mL for transfer to Florisil column.

29.034 ***Florisil Cleanup***

Use 4 g Florisil adjusted for lauric acid value (JAOAC **51**, 29(1968)). Add Florisil to 300 × 10 mm id chromatgc tube and add Na_2SO_4 to ht 2 cm above Florisil. Completely open stopcock, tap tube to settle adsorbent, and mark tube 1 cm above Na_2SO_4 layer.

Add 20–25 mL pet. ether wash to Florisil column; as solv. level reaches mark, place 125 mL K-D flask under column. Using disposable Pasteur pipet, transfer 3 mL sample to column, and wash tube with 1 mL pet. ether and add wash to column. Solv. level must not go below mark. Temporarily close stopcock if necessary. Add 35 mL pet. ether–ether mixt. (94 + 6) and elute PCBs and DDT and its analogs. When solv. level reaches mark, change K-D flask, and add 35 mL pet. ether–ether (85 + 15) to elute compds such as dieldrin and endrin. Add several granules of carborundum to first concentrator, attach Snyder column, and carefully conc. on steam bath. Let concentrator cool, remove Snyder column and evap. solv. under air to appropriate vol. for GC detn. Fractions contg mixt. of PCBs and chlorinated compounds such as DDE may require addnl sepn technics.

29.035 ***Additional Cleanup***

Often addnl cleanup is required for second fraction (85 + 15) to prevent deterioration of GC column. Use **29.017**.

29.036 ***Gas Chromatography***

See **29.018**.

Ref.: JAOAC **66**, 969(1983).

Organochlorine Pesticide Residues in Poultry Fat

Gel Permeation Chromatographic Method

First Action

29.037 ***Method Performance***

p,p'-DDD av. rec. at 0.18–0.60 ppm = 96% (S_x = 0.016–0.063, S_o = 0.016–0.053)

p,p'-DDT av. rec. at 0.21–0.42 ppm = 97% (S_x = 0.026–0.045, S_o = 0.026–0.045)

Mirex av. rec. at 0.51–1.02 ppm = 90% (S_x = 0.038–0.144, S_o = 0.026–0.101)

HCB av. rec. at 0.19–0.39 ppm = 84% (S_x = 0.025–0.057, S_o = 0.024–0.44)

Methoxychlor av. rec. at 0.52–1.04 ppm = 85% (S_x = 0.066–0.114, S_o = 0.048–0.089)

Dieldrin av. rec. at 0.29–0.59 ppm = 98 % (S_x = 0.026–0.050, S_o = 0.026–0.029)

Oxychlordane av. rec. at 0.38 ppm = 85% (S_x = 0.055–0.060, S_o = 0.038–0.045)

p,p'-DDE av. rec. at 0.27–0.61 ppm = 89% (S_x = 0.027–0.050, S_o = 0.027)

o,p'-DDT av. rec. at 0.39–0.78 ppm = 95% (S_x = 0.041–0.135, S_o = 0.033–0.047)

α-BHC av. rec. at 0.31–0.50 ppm = 92% (S_x = 0.039–0.080, S_o = 0.029–0.030)

γ-BHC av. rec. at 0.21–0.42 ppm = 93% (S_x = 0.025–0.045, S_o = 0.013–0.034)

cis-Chlordane av. rec. at 0.28–0.56 ppm = 88% (S_x = 0.015–0.060, S_o = 0.009–0.060)

trans-Chlordane av. rec. at 0.18–0.54 ppm = 94% (S_x = 0.070–0.189, S_o = 0.030–0.189)

Endrin av. rec. at 0.15–0.42 ppm = 98% (S_r = 0.013–0.035, S_o = 0.013–0.035)

Heptachlor epoxide av. rec. at 0.42–1.04 ppm = 91% (S_r = 0.046–0.096, S_o = 0.046–0.067)

29.038 ***Principle***

Liq. poultry fat is dissolved in CH_2Cl_2-cyclohexane (1 + 1). Residues are sepd from lipid by gel permeation chromatgy (GPC), and identified and measured in concd eluates by GC-EC detection.

29.039 ***Reagents and Apparatus***

(a) *Solvents.*—CH_2Cl_2, cyclohexane (C_6H_{12}), isooctane. Must meet criteria in **29.002**.

(b) *Gel permeation chromatographic system (GPC).*—AutoPrep gel permeation chromatograph (Analytical Bio-Chemistry Laboratories, Inc., PO Box 1097, Columbia, MO 65205) or equiv. with 60 g BioBeads SX-3 resin, 200–400 mesh, in 60 × 2.5 cm id chromatgc tube, ca 48 cm bed length, elution solv. CH_2Cl_2-C_6H_{12} (1 + 1). Flow rate calibrated to 5.0 mL/min, operating pressure 7–10 psig.

(c) *Flash evaporator.*—Rotary evapn system (Calab, Emeryville, CA or equiv.) with 30° H_2O bath.

(d) *Gas chromatograph.*—EC detector (^{63}Ni) operated as in **29.008**. 1.85 m × 4 mm id column packed with 1.5% SP2250/1.95% S-2401 on 100–120 mesh Supelcoport (Supelco, Inc., Bellefonte, PA 16823). Operating conditions: injector 250°, column 200°, detector 350°, N flow 60–80 mL/min. Condition column 2 days at 250°.

29.040 ***GPC Calibration Procedure***

Chromatographic system will fractionate effluent from column into 23 equal fractions for elution calibration. (It is necessary to det. correct "dump" and "collect" times for desired residues, as function of pump flow rate.) Before fractionating, check flow rate with SX-3 gel column connected and adjust flow to 5.0 ± 0.2 mL/min (start pump ≥5 min before measuring flow to let flow equilibrate and improve accuracy). Fractionate vol. of eluant from 150 to 320 mL to ensure residue collection. Evap. fractions, resuspend in isooctane, and analyze to det. collection vol. for samples (fractionation procedure is described in instrument manual). Check calibration for quant. recovery with 2.0 g corn oil fortified with relevant compds.

29.041 ***Preparation of Sample***

Place ca 40 g representative poultry fat sample in glass funnel (8.0 cm) with glass wool plug. Place funnel in flint glass bottle or 250 mL beaker on hot plate at ≤110° until fat ceases to drip. Mix thoroly.

29.042 ***Cleanup***

Weigh 2.0 g liq. fat into 10 mL vol. flask. (Fortifications of corn oil to check recoveries may be made here with stds dild in CH_2Cl_2-C_6H_{12} (1 + 1).) Dil. to 10 mL with CH_2Cl_2-C_6H_{12} (1 + 1) and mix thoroly. Centrf. or filter if particulate matter is visible. Use ca 7 mL sample to load sample loops on precalibrated GPC (5 mL aliquot (1.0 g equiv. of sample) is accepted into sample loop). Process thru GPC system using dump/collect times from fractionation procedure and collect eluate in 250 mL boiling flask. Rotary-evap. to just dry at ≤30°. Transfer quant. with 10 mL isooctane or equiv. GC-EC compatible solvent into a precalibrated culture tube. Adjust vol. under gentle, dry N stream to 5.0 mL.

29.043 ***Gas Chromatography***

Inject 3–6 μL aliquots into a gas chromatograph operated as in **29.008** with ^{63}Ni ECD. Measure peaks (ht or area). If necessary, dil. sample to give residue concn approx. that of std soln. Inject aliquot of pesticide std soln (in same solv. as sample) and again measure peaks.

Each residue, ppm = concn std (μg/mL) × (peak size sample/peak size std) × (μL std/μL sample) × (diln vol/1.0 g sample).

(*Note:* Since only 5 mL of original 10 mL vol. contg 2.0 g fat is injected into GPC sample loop, only 1.0 g fat is analyzed.)

Ref.: JAOAC **67**, 284(1984).

Endosulfan, Endosulfan Sulfate, Tetradifon, and Tetrasul Pesticide Residues

Gas Chromatographic Method

Final Action

(Applicable to apples and cucumbers)

29.044 ***Principle***

Pesticides are extd with CH_3CN, partitioned with pet ether, eluted thru Florisil column with mixts of CH_2Cl_2, hexane, and CH_3CN, and detd by gas chromatgy. Method is variation of **29.001–29.018**, as it applies to nonfatty foods. Pesticides are eluted from Florisil column with different eluants to improve cleanup for these compds.

29.045 ***Apparatus***

See **29.005**(a)–(h) and **29.008**(a)–(c).

29.046 ***Reagents***

(a) *Florisil.*—*See* **29.002**(i).

(b) *Solvents.*—Hexane, CH_2Cl_2, and CH_3CN, all distd in glass and free from electron capturing substances (*see* **29.002**).

(c) *Eluant mixtures.*—(*1*) *Eluant I.*—20% CH_2Cl_2-hexane. Dil. 200 mL CH_2Cl_2 with hexane. Let mixt. reach room temp. and adjust vol. to 1 L with hexane. (*2*) *Eluant II.*—50% CH_2Cl_2-0.35% CH_3CN-49.65% hexane. Pipet 3.5 mL CH_3CN into 500 mL CH_2Cl_2, and dil. with hexane. Let mixt. reach room temp.; dil. to 1 L with hexane.

29.047 ***Preparation of Sample and Extraction***

See **29.011**(a), (b), (e)–(g).

29.048 ***Column Chromatography***

(*Caution: See* **51.011**, **51.043**, and **51.061**.)

Add wt activated Florisil detd from lauric acid absorption value, **29.002**(i), to 22 mm id chromatgc tube, **29.005**(b). Gently tap chromatgc column to settle Florisil. Top column with ca 12 mm anhyd. granular Na_2SO_4. Wet column with 40–50 mL hexane. Use Kuderna-Danish concentrator with volumetric or graduated tube to collect eluate. Transfer pet ether or hexane soln of sample ext to column, and let it elute at ca 5 mL/min. Rinse container (and Na_2SO_4, if present) with 2 ca 5 mL portions hexane, transfer rinsings to column, and rinse walls of chromatgc tube with addnl small portions of hexane. Elute tetrasul at ca 5 mL/min with 200 mL eluant I. Change receivers and elute endosulfan I and II, endosulfan sulfate, and tetradifon at ca 5 mL/min with 200 mL eluant II. Conc. each eluate to suitable definite vol. in Kuderna-Danish concentrator. For evapn <5 mL, use 2-ball micro Snyder or Vigreux column.

29.049 ***Determination***

See **29.008**(a)–(c).

Using the 10% DC-200 column, retention times relative to aldrin are ca 1.6 for endosulfan I, 2.2 for endosulfan II, 2.5 for tetrasul, 2.7 for endosulfan sulfate, and 5.4 for tetradifon.

Ref.: JAOAC **59**, 209(1976).

CAS-115-29-7 (endosulfan)
CAS-1031-07-8 (endosulfan sulfate)
CAS-116-29-0 (tetradifon)
CAS-2227-13-6 (tetrasul)

Polychlorinated Biphenyls in Paper and Paperboard

Gas Chromatographic Method

Final Action

29.050 ***Apparatus***

(a) *Gas chromatograph.*—Equipped with electron capture detector and 1.85 m (6′) × 4 mm id glass column contg either (*1*) 10% DC-200 or (*2*) 1 + 1 mixt. of 15% QF-1 + 10% DC-200 on 80–100 mesh Chromosorb W(HP). Operating conditions: temps (°)—column and detector 200, injector 225; flow rate, 120 mL N/min; concentric design electron capture detector operated at dc voltage to cause ½ full scale recorder deflection for 1 ng heptachlor epoxide when full scale deflection is 1×10^{-9} amp (*see* **29.008(c)**).

(b) *Chromatographic tubes.*—*See* **29.005(b)**.

(c) *Filter tube.*—*See* **29.005(d)**.

(d) *Kuderna-Danish concentrator.*—*See* **29.005(e)**, and (g).

(e) *West condenser.*—400 mm jacket length with ⅋ inner drip joint to fit 250 and 500 mL erlenmeyers.

29.051 ***Reagents***

(a) *Florisil.*—*See* **29.002(i)**.

(b) *Alcoholic potassium hydroxide soln.*—2% KOH in alcohol or redistd MeOH.

(c) *Petroleum ether.*—*See* **29.002(m)**.

(d) *Polychlorinated biphenyls.*—Com. mixts (Aroclors) for ref. in GC detn (Analabs, Inc.).

29.052 ***Extraction***

(*Caution: See* **51.039**, and **51.073**.)

Cut paper sample representative of lot into pieces ca 6 × 6 mm and mix thoroly.

Weigh 10 g sample into 250 mL erlenmeyer. Do not pack tightly. (*See* note below if vol. of 10 g sample is >50 mL.) Add 60 mL 2% alc. KOH, and fit flask with West condenser cooled with circulating cold tap H_2O. Reflux gently on steam bath 30 min. Rinse inside of condenser with small amt of alcohol. Transfer soln thru glass wool plug in small funnel, to 250 mL separator, avoiding transfer of any paper material. Rinse paper and flask with three 40 mL portions pet ether, combining rinses in separator. Add 60 mL H_2O to separator and shake vigorously 30 sec. Drain lower aq. layer into second 250 mL separator. Add 60 mL pet ether to second separator and shake vigorously 30 sec. Discard aq. layer and combine pet ether layers in first separator. Rinse second separator with several small portions pet ether, collecting rinses in first separator. Wash pet ether with three 40 mL portions H_2O, discarding each wash. Dry pet ether thru 50 mm column, (c), of anhyd. Na_2SO_4, collecting eluate in Kuderna-Danish concentrator. Rinse separator and then column with 3 ca 20 mL portions pet ether, collecting rinses. Conc. combined pet ether ext and rinses on steam bath to ca 5 mL. Ext is ready for cleanup on Florisil column, **29.053**. If experience with particular sample types indicates that Florisil column cleanup is not required, proceed to GC detn, **29.018**.

Note: Adequate extn of low density paper such as newspaper or tissue paper will require adjustment of either amt of sample to <10 g or vol. of reflux soln to >60 mL. Preferably, reduce sample to wt that is completely covered and wetted by 60 mL KOH soln. Increase in vol. of reflux soln >60 mL must be accompanied by proportional increases in vols of pet ether rinses of sample, H_2O diluent added to alc. reagent in separator, and size of erlenmeyers and separators.

Refs.: JAOAC **56**, 957(1973); **57**, 518(1974).

29.053 ***Florisil Cleanup***

Proceed as in **29.015**, pars 1 and 2, except prep. 10 g column, pre-wet column with 20 mL pet ether, and elute at ca 5 mL/min with 150 mL pet ether. Concd eluate is suitable for analysis by GC with electron capture detection, **29.018**.

Note: Waxes, if present in ext, can be removed before Florisil chromatgy by partitioning between pet ether and CH_3CN, **29.014**.

ORGANOPHOSPHORUS RESIDUES

Organophosphorus Pesticide Residues

Carbon Column Cleanup Method

Final Action

(CH_3CN extn and charcoal cleanup column using KCl thermionic or flame photometric detector for residues of parathion, paraoxon (diethyl *p*-nitrophenyl phosphate), carbophenothion and its O analog, and EPN (*O*-ethyl *O*-(*p*-nitrophenyl) phenylphosphonothioate) on apples and green beans)

29.054 ***Reagents***

(a) *Solvents.*—Redistd from glass (*see* **29.001**): EtOAc, CH_2Cl_2, benzene, hexane, CH_3CN, and isopropanol.

(b) *Acid-treated charcoal.*—Slurry 200 g Norit SG Extra (American Norit Co., Inc., 6301 Glidden Way, Jacksonville, FL 32208) or 100 g Nuchar C-190N (no longer marketed) with 500 mL HCl, cover with watch glass, and stir mag. while boiling 1 hr. Add 500 mL H_2O, stir, and boil addnl 30 min. Collect charcoal in buchner and wash with H_2O until washings are neut. to universal indicator paper. Dry at 130° in forced-draft oven.

(c) *Magnesium oxide.*—*See* **29.002(k)**.

(d) *Adsorbent mixture.*—Mix 1 part acid-treated charcoal, 2 parts hydrated MgO, and 4 parts Celite 545, acid washed. Keep sealed.

(e) *Pesticide std solns.*—Prep. solns contg 1 μg/mL EtOAc of each of following: parathion, paraoxon, carbophenothion, carbophenothion O analog, and EPN.

(f) *Eluting soln.*—CH_3CN-benzene (1 + 1).

Purity test.—Reagents must be free of substances causing KCl thermionic or flame photometric detector response, as indicated by following test: Carry reagents thru entire method, and inject 5 μL from final conc. into gas chromatograph, using conditions described in **29.064**. Conc. must not cause recorder deflection >1 mm from baseline for 2–60 min after injection.

29.055 ***Apparatus***

See also **29.005** and **29.060(a)**.

(a) *Vacuum adapter.*—Kontes Glass Co., No. K-954002, or equiv.

(b) *Gas chromatograph.*—With potassium chloride thermionic detector (see **29.060(i)** and **(k)**) or flame photometric detector (see **29.055(e)** and **(i)**).

(c) *Column.*—*See* **29.060(j)**.

(d) *Potassium chloride thermionic detector* (KCITD).—*See* **29.008(d)**(*1*) or (*2*), (e), (f), and (k). Also check linearity of GC system to paraoxon and carbophenothion O analog.

(e) *Flame photometric detector* (*FPD*).—With P selective optical filter for 526 nm wavelength (Tracor, Inc., 6500 Tracor Ln, Austin, TX 78721). Equiv. to KCITD for detn of organophosphorus pesticides in fruits and vegetables. (*Note:* Older commercial models of FPD may give rise to adsorption and/or degradation of O analogs of organophosphorus pesticides within detector's gas mixing chamber. Design changes of detectors manufactured after mid-1973 have generally corrected this problem. Flameout in FPD, on injection of sample, can be avoided by letting H enter detector (lower part) so that H and GC column effluent mix before burner area. Air-O enters detector thru upper part. This arrangement reverses that recommended by manufacturer. Specifications for physical modification of FPD to correct above problems are available from Division of Chemistry and Physics, Food and Drug Administration, Washington, DC 20204.) Use highly stabilized 0–750 v dc variable power supply capable of 10 ma output (Model 240 A; Keithley Instruments, Inc.,

28775 Aurora Rd, Cleveland, OH 44139, or equiv.), 6.3 v ac ignitor power supply, electrometer with bucking capability of 1×10^{-6} amp (Tracor, Inc., or equiv.), and variable transformer capable of delivering 150 watts to control temp. of flame housing. Strip chart recorder should be compatible with electrometer.

(f) *Hydrogen.*—From cylinder of compressed H gas. Equip cylinder with regulator, delivery line, and variable flow controller capable of 200 mL/min delivery. Metering shut-off valve is required sep. from controller.

(g) *Air.*—Cylinder of compressed air equipped as in (f) to deliver up to 100 mL/min. Sep. shut-off valve is not needed.

(h) *Oxygen.*—Cylinder of compressed O gas equipped as in (f) to deliver up to 50 mL/min. Combine with air using std Swagelok tee before detector inlet.

(i) *Flame photometric detector operation.*—Adjust temp. of burner housing to ca 170–180° before igniting flame. Temp. will rise 20–30° after ignition. Do not allow detector to exceed 220°. Adjust gas flows at controllers to ca 150–300 mL/min H, 50–100 mL/min air, and 5–20 mL/min O. Adjust column effluent flow, **29.018(a)**, to 120 mL N/min. Turn off H flow with metering shut-off valve (f). (*Caution:* Before attempting ignition, make certain H has been purged from detector with other gases. One min interval between ignition attempts is adequate.) Apply ca 750 v to photomultiplier tube from power supply. Zero recorder with electrometer set at appropriate sensitivity (ca 1×10^{-8} to 1×10^{-9} amp full scale). Push ignitor button and then slowly open H metering shut-off valve. Recorder pen will not return to zero baseline if flame ignites. If ignition is not effected, shut off H valve, increase O flow, and repeat ignition procedure. Establish proper baseline with buckout control after flame is lit. Operate at sensitivity that produces ½ full scale recorder deflection for 2 ng parathion. Reduce photomultiplier voltage to reduce sensitivity. Alternatively, use electrometer sensitivity and attenuator controls to achieve proper response. Check linearity of GC system to paraoxon and carbophenothion O analog.

29.056 *Preparation of Sample*

Blend and filter sample as in **29.011(a)**, or (b). Transfer aliquot of CH_3CN ext (30–35 mL) equiv. to ca 10 g sample from suction flask to 125 mL separator, add equal vol. CH_2Cl_2, shake vigorously 30 sec, and set aside 10–15 min to sep. Calc. g sample in aliquot as g sample × [mL aliquot/(mL H_2O in sample + mL extg solv. added − correction in mL for vol. contraction)].

29.057 *Charcoal Cleanup*

Fit 1-hole No. 5 rubber stopper onto tip of chromatgc tube, **29.005(b)**, add side-arm vac. adapter and ȝ 24/40 receiving flask, open stopcock, and connect app. to open vac. line. Place 1 g Celite 545 in tube, tamp, add 6 g adsorbent mixt., and tamp again. Add 2 cm glass wool plug on top of adsorbent. Prewash column with 100 mL eluting soln. Close stopcock when eluting soln is ca 2 cm above glass wool and maintain this head to ensure clean column. Disconnect vac., replace flask with 500 mL Kuderna-Danish flask equipped with 10 mL tube, **29.005(e)** (check calibration at 1 mL), and reconnect to open vac. line.

Drain lower CH_2Cl_2 layer in separator onto column, retaining H_2O layer (upper phase) in separator. Open column stopcock to vac. and adjust flow to ca 5 mL/min. Re-ext H_2O layer cautiously (do not shake vigorously) with two 10 mL portions CH_2Cl_2 and add exts to column. Discard H_2O phase. Elute column with 120 mL eluting soln (column may be taken to dryness). Disconnect app. and rinse column tip and vac. adapter with several mL EtOAc. Collect all rinses in same Kuderna-Danish concentrator with tube attached. Add 1 or 2 small boiling chips, attach Snyder column, and conc. cautiously over steam bath to ca 1 mL. (*Caution:* Begin heating very gently due to differences in densities and bps of individual solvs.) When cool, disconnect evaporative app. from Mills tube. Substitute column, **29.005(h)**, on Mills tube, add boiling chips again, and conc. solv. to <1 mL. While app. is still immersed in steam bath, add 3–4 mL isopropanol (to remove CH_3CN azeotropically) and distil under gentle reflux. Repeat isopropanol addn and conc. to ca 0.5 mL. Remove from heat, cool, remove column, and adjust vol. to 1.0 mL with EtOAc.

29.058 *Gas Chromatography*

Proceed as in **29.064**, using recommended operating conditions *1* specified for column, **29.060(j)**(*1*). (*See* **29.055(e)**–(i) if using flame photometric detector.

Refs.: JAOAC **54**, 513(1971); 57, 930(1974).

CAS-786-19-6 (carbophenothion)
CAS-2104-64-5 (EPN)
CAS-56-38-2 (parathion)
CAS-7173-84-4 (carbophenothion O analog)
CAS-311-45-5 (paraoxon)

Organophosphorus Pesticide Residues

Sweep Codistillation Method

Final Action

(Sweep codistillation cleanup for parent organophosphorus residues of carbophenothion, diazinon, ethion, malathion, Me parathion, and parathion in kale, endive, carrots, lettuce, apples, potatoes, and strawberries (fresh or non-sugared frozen); this cleanup is not adequate for electron capture gas chromatgc detector. Use only with KCl thermionic or flame photometric detector.)

29.059 *Reagents*

(a) *Ethyl acetate.*—Redistd from glass. Check suitability of reagent by concg 100 mL to 2 mL. Inject 5 μL into GC (KCl thermionic detector) with operating conditions specified in **29.060(i)**. Chromatogram should show no peaks to 20 min with chart speed of 1″/2 min.

(b) *Pesticide std soln.*—Prep. EtOAc soln contg 1 μg/mL of each of following: carbophenothion, diazinon, ethion, malathion, Me parathion, and parathion.

29.060 *Materials and Apparatus*

(a) *Glass wool.*—Silane-treated (available from Applied Science Laboratories, Inc.).

(b) *Anakrom ABS.*—80–90 mesh. Remove fines by stirring with EtOAc, decanting several times, and drying.

(c) *Teflon tubing.*—AWG No. 16, std, natural.

(d) *Disposable glass capillary pipets.*—145 mm long, 6 mm id, with capillary stem (Arthur H. Thomas Co. No. 7760-B10, or equiv.).

(e) *Syringes.*—1 mL Tuberculin Luer-Lok and 2 mL Luer-Lok with Luer-Lok 2″ No. 25G needles.

(f) *High-speed blender.*—400 mL capacity. Omnimixer (available from DuPont Instruments/Sorvall, Peck's Ln, Newtown, CT 06470), or equiv.

(g) *Sweep codistillation apparatus.*—*See* Fig. **29:06**. Following tubes are required: (*1*) *Storherr tube.* —24.5 cm long, 6 mm id (Kontes Glass Co., No. K-898600, or equiv.). (*2*) *Concentrating tube.*—10 mL calibrated to 0.5 mL. (*3*) *Adapter for extension of concentration tube.*—7 cm long, ȝ 19/22 (Kontes No. K-570100 (K-500750 part 355), or equiv.).

(h) *Kuderna-Danish concentrators.*—500 mL with Snyder distilling column and 5 mL vol. and 10 mL graduated (Mills tube) receiving flasks, ȝ 19/22 (Kontes Glass Co., No. K-570000, K-621400, and K-570050, or equiv.).

(i) *Gas chromatograph with potassium chloride thermionic detector.*—*See* **29.008(a)**. Only thermionic detector, **29.008(d)**, is required.

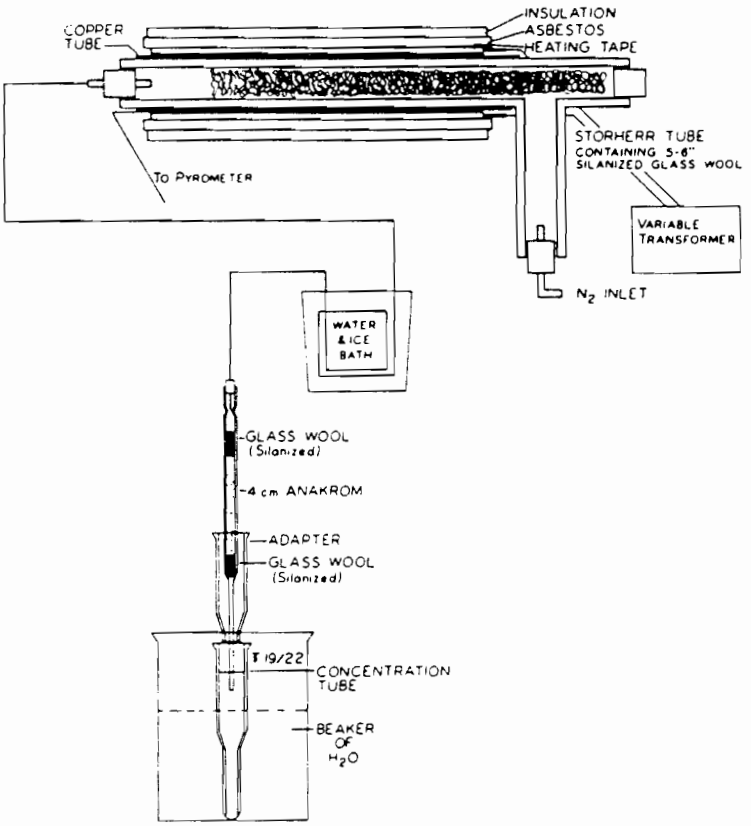

FIG. 29:06—Sweep codistillation apparatus

Following conditions are important in operation of GC and KCl thermionic detector:

(*1*) Every day before starting work change silane-treated glass wool plug insert in injection port of GC column. Remove and replace only that portion affected by syringe.

(*2*) Every week before starting work reheat KCl spiral over gas burner and reinsert into detector. Detector must then equilibrate ca 2 hr before use.

(j) *Column.*—10% DC 200 or OV-101 on 80–100 mesh Chromosorb W HP in glass column 1.85 m (6′) × 4 mm id; *see* **29.008**(b). Adjust column temp to give retention time for parathion of ca 5 min.

(*1*) *Recommended operating conditions: I.*—Temps (°): injection 225, column 200, detector 210; N flow 120 mL/min. Split column effluent with 1:1 stream splitter so that only 60 mL N/min enters KCITD. (*2*) *Recommended operating conditions II.*—Temps (°): column 220, injector and detector 240; N flow 60 mL/min.

(k) *Potassium chloride thermionic detector (KCITD).*—*See* **29.008**(d)(*1*) or (*2*), (e), (f), and (k).

29.061 *Preparation of Apparatus*

App. is constructed in 3 parts: removable Storherr tube, permanent heating coil, and distillate collector (*see* Fig. **29:06**).

(a) *Preparation of removable Storherr tube.*—Pack Storherr tube with silane-treated glass wool. Use silane-treated glass wool as received. Do not pack glass wool too tightly; otherwise removal for cleaning is difficult. Only 13–15 cm portion from injection end requires packing. Insert injection septum and two 1-hole septums. Tube is now ready for use. Use clean tube for each sample. Clean tubes thoroly with soap and H_2O after use, rinse with acetone, and dry. Soak tubes difficult to clean in chromic acid cleaning soln before cleaning with soap and H_2O.

(b) *Preparation of permanent heating coil.*—Attach bimetallic wires of calibrated pyrometer directly to outside middle area of Cu tube (length 20 cm (8″) × 11 mm (7/16″) id). (Thermometer with stem covered with Al foil may also be used for temp. measurement.) Wrap heating tape (60 × 1.3 cm) uniformly around outside of Cu tubing and over bimetallic wires or thermometer, and secure ends. Cover heating tape with asbestos tape and secure with glass tape or glass thread. Cover asbestos with several layers of Al foil and secure with tape.

Place heating assembly on ring stand, using asbestos-covered 3-prong clamp. Orient and use heating coil in near horizontal position. Attach heating tape leads to variable transformer. Adjust transformer so pyrometer reads 180–185°. Use this setting or temp. for all crop cleanup.

Add N flow, 600 mL/min, to sidearm of Storherr tube. (For N pressure gage to give meaningful readings, add stainless steel capillary tube to reduce gas flow. Connect capillary tube directly to 1-hole septum in sidearm of tube with short length Teflon tubing.) Measure N flow with gas flow gage, and calibrate pressure regulator gage by this means.

(c) *Sample distillate collector.*—Construct in 3 parts: cooling coil, scrubber tube, and concn tube with extended adapter.

(*1*) *Cooling coil.*—Cut 120 cm length of Teflon tubing. Form this tubing into three 7 cm diam. loops having 2 arms of ca 20 cm lengths. Attach Teflon cooling coil and 1-hole septum directly to Storherr tube. Place coils in 250 mL beaker contg ice and H_2O. Place 250 mL beaker inside 400 mL beaker for insulation.

(*2*) *Scrubber tube.*—Insert silane-treated glass wool plug in constricted end of disposable pipet. On outside of pipet place marks 4 and 6 cm above top of glass wool plug. Add Anakrom ABS to 6 cm mark and pack Anakrom to 4 cm mark by compressing with 3 mm rod. Place silane-treated glass wool plug on top of packed Anakrom and 1-hole septum in pipet top. Connect exit arm of Teflon cooling coil directly into 1-hole septum in scrubber tube and extending ca

2 cm below septum. Secure scrubber tube on sep. ring stand with 3-prong clamp. Scrubber tube must be lower than cooling bath, especially in rinsing step.

(*3*) *Concentration tubes.*—Use 10 mL calibrated tubes, **29.060(h)**. Adapter, (**g**)(*3*), is needed for insertion into concn tube to prevent splash during sweep and rinsing steps. Place tip of scrubber thru adapter and into concn tube. If possible, place scrubber tip against wall of concn tube. Hold tube in place with clamp.

Adjust heat to 180–185° and N flow to 600 mL/min (measure before entering Storherr tube). Flush several 0.5 mL EtOAc injections thru entire system, using 2 mL syringe (used for all rinsings). Replace concn tube with clean tube and insert adapter; assembly is ready for use.

29.062 *Extraction*

Ext all crops with EtOAc in *exact* order as follows: To high-speed blender, add 25 g chopped sample, 125 mL EtOAc from pipet, and 25 g anhyd. granular Na_2SO_4. Blend 5 min at slow speed; then 5 min at high speed with mixer cup immersed in ice-H_2O bath. Decant liq. thru 2.5 cm silane-treated glass wool plug contained in short glass chromatgc tube. (Do not add solids to glass wool plug.) Collect EtOAc ext (ca 100 mL) in 125 mL flask or bottle. Remove 50 mL aliquot (equiv. to 10 g original sample) and place in Kuderna-Danish concentrator with Snyder column, calibrated Mills tube, or 5 mL vol. receiving flask, and conc. to ca 5 mL. Adjust vol. to exactly 5.0 mL, using air jet or adding EtOAc. Use 1 mL aliquots (2 g sample) for sweep codistn cleanup.

Store all stds and crop solns at ≤0° when not in use. Warm to room temp. ca 1 hr before use.

29.063 *Sweep CoDistillation Cleanup*

Assemble app. as in Fig. **29:06**, except position Storherr tube and heating unit so exit end of Storherr tube is ca 10° below horizontal to avoid backup of sample into N inlet arm. If sample backs up, *discard detn.* Check temp. (180–185°), N flow (600 mL/min), and receiver tube. Inject 1 mL (2 g) sample, using 1 mL Luer-Lok tuberculin syringe. Immediately follow sample with injection of 0.5 mL EtOAc sweeping solv. and repeat 0.5 mL EtOAc injection every 3 min for 21 min. After last injection wait 1 min until solv. has cleared cooling coil and scrubber tube; then disconnect cooling coil arm with septum from Storherr tube. Disconnect septum with attached cooling coil arm from scrubber tube and rinse 2 cm Teflon projection, collecting rinse in scrubber tube (still in position in concn tube). With septums in place on disconnected cooling coil arms, *reverse* coil arms and place that end formerly in Storherr tube into scrubber tube. Make certain that Teflon tubing in this arm extends 2 cm into scrubber tube below inserted septum (similar to position when cleaning up sample). Slowly inject 1 mL EtOAc rinse from 2 mL syringe directly into open end of cooling coil arm formerly in scrubber tube. Gently force rinse, using N flow from disconnected Storherr tube, thru cooling coil into scrubber tube and into concn tube. Repeat 1 mL EtOAc rinse 1–2 addnl times. Rinse scrubber tip end and inside of adapter, remove scrubber, disconnect adapter, and rinse ⅓ joint. Collect all rinses in concn tube. Rinse down sides of concn tube and conc. to 1 mL, using N or air jet. Prevent H_2O condensation inside tubes by placing tube in room temp. H_2O bath during this step. If cleaned up soln is too concd for GC detn (>2 μg/mL), dil. soln to 5 or 10 mL with EtOAc. If calibrations of Mills tube are incorrect (most usually are except for 1 mL mark), quant. transfer the concd soln to 5 or 10 mL vol. flask, using disposable pipet with attached rubber bulb. Rinse inside of tube with EtOAc and transfer rinse in same pipet. Repeat this rinse of tube and pipet several times; then rinse inside of pipet into flask, using EtOAc. Dil. to vol. Further diln with EtOAc or concn may be necessary to bring concn within measurement range.

Anakrom scrubber tube is used repeatedly without change. Final EtOAc rinses after each run keep it clean. However, if Anakrom becomes discolored, prep. new tube.

29.064 *Determination by Gas Chromatography*

Operate chromatograph under conditions specified for column, **29.060(j)**. Inject 3–8 μL aliquot concd, cleaned-up soln contg amt of pesticide within linear range of gas chromatgc system, (i), using 10 μL syringe. Tentatively identify residue peaks on basis of retention times. Det. amt of pesticide by comparing area under peak with that from known amt of appropriate std pesticide. For accurate detn, baseline current of sample and std must be identical during chromatgy.

Injections <3 μL are difficult to reproduce; injections >8 μL may cause flame blow-out. Sample wt is not critical—use injections equiv. to <1 mg or several hundred mg. Inject appropriate std immediately after every sample. Peak ht also may be used for detn, but only if ht of ref. std is ca same ht as sample unknown (width of base should then be same).

Ref.: JAOAC **51**, 662(1968).

CAS-786-19-6 (carbophenothion)
CAS-333-41-5 (diazinon)
CAS-563-12-2 (ethion)
CAS-121-75-5 (malathion)
CAS-298-00-0 (methyl parathion)
CAS-56-38-2 (parathion)

Organophosphorus Pesticide Residues

Single Sweep Oscillographic Polarographic Confirmatory Method

Final Action

(Applicable to diazinon, malathion, Me parathion, and parathion)

29.065 *Apparatus*

(Wash all glassware with hot HNO_3 (1 + 1) and rinse with H_2O.)

(**a**) *Polarograph.*—Any voltammetric or polarographic instrument capable of linear sweep voltammetry at 10 ng pesticide/mL cell soln (equiv. to 0.01 ppm based on 1 g sample in 1 mL cell soln).

(**b**) *Silver wire electrode.*—Deposit very thin coating of AgCl on No. 20 or 22 gage Ag wire as follows: Dip wire in 10% HNO_3, rinse in H_2O, and then let stand 10 min in 1*N* HCl.

29.066 *Reagents*

(*See* statement regarding solvs, **29.001**.)

(**a**) *Acetonitrile.*—Distd in glass at 82 ± 1°.

(**b**) *Acetone.*—Distil at 56.5° with 0.25 g $KMnO_4$/L. Distn must be performed as directed.

(**c**) *Ethyl acetate.*—Distd in glass at 77 ± 1°.

(**d**) *Petroleum ether.*—Distd in glass at 30–60°.

(**e**) *Nitrogen.*—Prepurified, H_2O-pumped.

(**f**) *Tetramethyl ammonium bromide.*—Eastman No. 670, or equiv.

(**g**) *Electrolyte solns.*—(*1*) *For diazinon.*—Dissolve 7.7 g Me_4NBr in 300 mL H_2O. Add 115 mL HOAc and dil. to 500 mL with H_2O. (*2*) *For malathion.*—Dissolve 15.4 g Me_4NBr in 300 mL H_2O. Add 0.2 g LiCl and 4.1 mL HCl, and dil. to 500 mL with H_2O. (*3*) *For methyl parathion and parathion.*—Dissolve 2.2 g NaOAc .$3H_2O$ and 1.17 g NaCl in 100 mL H_2O and adjust to pH 4.8 with HOAc, using pH meter.

(**h**) *Pesticide std solns.*—(*1*) *Stock solns.*—Prep. individual solns contg 1.00 mg pesticide/mL EtOAc. Store at 0°. (*2*) *Intermediate solns.*—0.2 mg/mL. Transfer 5 mL stock soln to 25 mL vol. flask and dil. to vol. with pet ether for diazinon, MeOH for malathion, and acetone for Me parathion and parathion.

29.067 ***Preparation of Standard Curves***

(a) *Diazinon.*—Transfer 0.0, 1.0, 2.0, 3.0, 4.0, and 5.0 mL intermediate std soln of diazinon to individual 100 mL vol. flasks and dil. to vol. with pet ether. Transfer 1.0 mL of each soln to sep. 50 mL erlenmeyers and evap. to incipient dryness under gentle jet of dry air. Evap. remaining solv. with warmth of hand. Dissolve residue in 5.0 mL electrolyte soln, (g)(*1*). Transfer soln to polarographic cell, adjust to 25 ± 1°, and bubble N thru soln 5 min. Polarograph between −0.70 and −1.2 v against either Hg pool or Ag wire ref. electrode.

Peak potential for diazinon at 25° is −0.90 ± 0.05 v against either electrode. Plot μg diazinon/mL cell soln against peak ht in units × instrument sensitivity setting.

(b) *Malathion.*—Transfer 0.0, 1.0, 2.0, 3.0, 4.0, and 5.0 mL intermediate std soln of malathion to individual 25 mL vol. flasks and dil. to vol. with MeOH. Transfer 2.0 mL of each soln to sep. 50 mL erlenmeyers and add 1.0 mL 0.1*N* KOH. After 3 min, add 2.0 mL electrolyte soln, (g)(*2*), mix well, and let stand 5 min. Transfer to polarographic cell, adjust to 25 ± 1°, and bubble N thru soln 5 min. Polarograph between −0.5 and −1.0 v against either Hg pool or Ag wire electrode.

Peak potential for malathion at 25° is −0.82 ± 0.05 v against Ag wire and −0.85 ± 0.05 v against Hg pool ref. electrodes. Plot μg malathion/mL cell soln against peak ht in units × instrument sensitivity setting. (*Note:* Compd actually polarographed is fumaric acid resulting from basic hydrolysis of malathion.)

(c) *Parathion and methyl parathion.*—Transfer 0.0, 1.0, 2.0, 3.0, 4.0, and 5.0 mL intermediate parathion (or Me parathion) std soln to individual 100 mL vol. flasks and dil. to vol. with acetone. Transfer 5.0 mL aliquots of each soln to sep. 50 mL erlenmeyers, add 5.0 mL electrolyte soln, (g)(*3*), mix well, and transfer ca 5 mL to polarographic cell. Adjust to 25 ± 1°, bubble N thru soln 5 min, and polarograph between −0.4 and −0.9 v against either Hg pool or Ag wire ref. electrode.

Peak potential for parathion and Me parathion at 25° is −0.68 ± 0.05 v against Hg pool and −0.70 ± 0.05 v against Ag wire ref. electrodes. Plot μg pesticide/mL cell soln (10 mL) against peak ht in units × instrument sensitivity setting. Cell soln vol. = 5 mL sample soln + 5 mL electrolyte soln.

29.068 ***Preparation of Sample Solution***

Prep., ext. and clean up samples as in **29.011–29.015**. Conc. 15% and 50% eluates from Florisil column to suitable definite vol. in Kuderna-Danish concentrator. All eluting solvs must be peroxide-free by test in *Definitions and Explanatory Terms*, item (3).

29.069 ***Determination***

(a) *Parathion and/or methyl parathion.*—Transfer aliquot of concd 15% Florisil eluate, equiv. to 5 g crop, to 50 mL erlenmeyer. Carefully evap. to dryness under gentle jet of air at room temp. Dissolve residue in 3.0 mL acetone. (*Note:* Since good polarograms can be obtained by using as little as 0.5 mL soln in cell, min. of 0.25 mL acetone can be used to dissolve residue.) Add 3.0 mL electrolyte soln, (g)(*3*), mix well, transfer to polarographic cell, and adjust to 25 ± 1°. Bubble N thru soln 5 min and polarograph as in **29.067**(c). Measure ht of wave whose peak potential corresponds to that of parathion, and det. concn from freshly prepd std curve or by comparing wave hts of sample soln with those of std soln polarographed immediately before or after sample. (Latter method is recommended for greater accuracy.)

Calc. μg/mL as follows:

$$C_{sample} = [(WH_{sample}) \times (IS_{sample}) \times (C_{std})]/[(WH_{std}) \times (IS_{std})]$$

where C = μg pesticide/mL cell soln; WH = wave ht; IS = instrument sensitivity setting.

$$ppm = (C_{sample} \times \text{mL sample soln})/\text{g sample}$$

Limit of quant. detn is 0.01 ppm based on 1 g crop in 1 mL cell soln.

Me parathion, parathion, and paraoxon polarograph at ca same peak potential. If any one of these pesticides is present as indicated by multiple residue methods, it should be polarographed against that std. If these pesticides are present together, use mixed std contg ratio of pesticides as estd from analysis by multiple residue method. (Paraoxon will not be recovered by cleanup specified.)

Other pesticides known to give polarographic peak potentials similar to parathion are pentachloronitrobenzene (PCNB), 1,2,4,5-tetrachloro-3-nitrobenzene (TCNB, tecnazene), and *O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothioate (EPN). PCNB and TCNB are recovered in 6% Florisil eluate and will not interfere. Verify presence or absence of EPN by GC or TLC.

(b) *Diazinon.*—Transfer aliquot of concd 15% Florisil eluate, equiv. to 5 g crop, to 50 mL erlenmeyer. Carefully evap. just to dryness, using gentle jet of dry air at room temp. Dissolve residue in 5.0 mL electrolyte soln, (g)(*1*). Transfer soln to polarographic cell and adjust to 25 ± 1°. Bubble N thru soln 5 min and polarograph as in **29.067**(a). Calc. amt of diazinon present as in (a).

Limit of quant. detn is 0.2 ppm based on 1 g crop sample in 1 mL cell soln.

(c) *Malathion.*—Transfer aliquot of concd eluate from 50% Florisil eluate, equiv. to 5 g crop, to 50 mL erlenmeyer. Carefully evap. just to dryness under gentle jet of dry air at room temp. Dissolve residue in 2.0 mL MeOH, add 1.0 mL 0.1*N* KOH, and let stand 3 min. Add 2.0 mL electrolyte soln, (g)(*2*), mix well, and let stand 5 min. Transfer to polarographic cell, adjust to 25 ± 1°, bubble N thru soln 5 min, and polarograph as in **29.067**(b). Calc. amt of malathion present as in (a).

Limit of quant. detn is 0.3 ppm based on 1 g crop in 1.0 mL cell soln.

Note 1: If polarogram cannot be obtained because of high residual currents, check concd eluate for peroxides. If peroxides are present, transfer 5 mL concd eluate to small separator contg 25 mL 3% $FeSO_4$ soln; shake well and discard aq. layer. Transfer 1.0 mL ether layer to 50 mL erlenmeyer and proceed as in (a), (b), or (c).

Note 2: All glassware used for polarographic detns should be thoroly washed with hot HNO_3 (1 + 1) and rinsed with distd H_2O.

Ref.: JAOAC **52**, 811(1969).

CAS-333-41-5 (diazinon)
CAS-121-75-5 (malathion)
CAS-298-00-0 (methyl parathion)
CAS-56-38-2 (parathion)

★ Organophosphorus Pesticide Residues ★

Cholinesterase Inhibition Method

First Action

29.070 *See* **29.049–29.055**, 12th ed.

FUMIGANT RESIDUES

Volatile Fumigants in Grain

Gas Chromatographic Method

Final Action

(Applicable to $CHCl_3$, CCl_4, Cl_2CCClH, and BrH_2CCH_2Br in wheat and corn grain)

29.071 ***Apparatus and Reagents***

(a) *Column.*—4 m × 2.2 (id) mm stainless steel packed with 15% polypropylene glycol (LB 550X, Ucon fluid) on 60–80 mesh Chromosorb W.

★ Surplus method—*see* inside front cover.

(b) *Gas chromatograph.*—Isothermal with source-heated electron capture detector and glass-lined heated injection block. (100–200 mCi ^3H with Ar as β-ionization detector is more useful for multiresidue detns than ^{63}Ni and N.) Use 1 mv recorder with max. response time of 1 sec and chart speed of 0.5 cm/min. Operate electron capture detector with N at 25 psi (173 kPa) at 95° with polypropylene glycol column for CCl_4 (retention time, ca 6 min). Use 120° for $CHCl_3$ (3 min), Cl_2CCClH (4 min), and BrH_2CCH_2Br (8 min).

(c) *Acetone.*—Check for interfering peaks by gas chromatgy before use.

29.072 ***Determination***

(*Caution: see* **51.046.**)

Store sample at ≤5°. Quickly weigh 50 g and immerse in 150 mL acetone-H_2O (5 + 1) in 250 mL g-s flask, and stopper. Let stand 48 hr in dark at 20–25°, swirling at 24 hr. Decant 10 mL supernate into 25 mL g-s graduate, add 2 g NaCl, stopper, and shake vigorously 2 min. Let stand until layers sep. Pour 5 mL clear upper layer into 10 mL g-s graduate, add 1 g anhyd. $CaCl_2$, stopper, and shake 2 min. Let stand 30 min with occasional shaking.

Withdraw 0.5 μL aliquots from upper layer into 1 μL syringe. Inject into gas chromatograph. Dil. 10× or 100× with dry acetone, if necessary to avoid overloading detector. Inject all solns in triplicate and average results.

Construct calibration curve daily of peak hts against ng fumigant/125 mL acetone for suitable range.

Ref.: Analyst **99**, 570(1974).

CAS-56-23-5 (carbon tetrachloride)
CAS-67-66-3 (chloroform)
CAS-106-93-4 (ethylene dibromide)
CAS-79-01-6 (trichloroethylene)

CARBAMATE RESIDUES

N-Methylcarbamate Insecticide Residues

Gas Chromatographic Method

Final Action

(Carbanolate, Carbaryl, Carbofuran, and Propoxur)

(Applicable to apples, cabbage, collards, corn kernels, green beans, kale, and turnip tops. Rinse all glassware with acetone and then distd H_2O before use.)

29.073 ***Principle***

Residue is extd from crop with CH_3CN, and ext is purified by partitioning with pet ether and coagulating with H_3PO_4-NH_4Cl soln. Phenolic impurities are largely eliminated by partitioning CH_2Cl_2 ext with KOH soln. Carbamate residues are treated with 1-fluoro-2,4-dinitrobenzene to form ether derivative. Residues may be detd at levels ≥0.05 ppm. Recoveries range from 90 to 110%.

29.074 ***Reagents***

(a) *Borax.*—5% aq. soln.

(b) *Diatomaceous earth.*—Wash thoroly with acetone and dry 2 hr at 110°.

(c) *Coagulating soln.*—(*1*) *Stock soln.*—Dissolve 20 g NH_4Cl and 40 mL H_3PO_4 in 360 mL H_2O. (*2*) *Working soln.*—Dil. 100 mL stock soln to 1 L for coagulation.

(d) *1-Fluoro-2,4-dinitrobenzene soln.*—(Eastman Kodak Co.) Redistil at 128° and 1 mm pressure. Dissolve 1.5 mL in 25 mL acetone.

(e) *Pesticides.*—Best quality obtainable from manufacturer; anal. grades when available.

(f) *Potassium hydroxide soln.*—0.5*N* aq. soln.

(g) *Sodium chloride soln.*—30% aq. soln.

(h) *Solvents.*—Acetone, CH_2Cl_2, isooctane, CH_3CN, and pet ether (distd in glass; *see* statement regarding solvs, **29.001**); acetophenone and MeOH (anal. grade).

29.075 ***Gas Chromatographic Apparatus***

Gas chromatograph equipped with ^3H electron capture detector and 46 × 0.64 (od) cm (18 × ¼") glass column contg 10% DC-200 (12,500 cst) on 60–70 mesh Anakrom ABS (Analabs, Inc.). Porous Teflon end plugs for ¼" od glass tubing (Chemical Research Services, Inc., 852 Westgate Dr, Addison, IL 60101) are preferable, but glass wool can be used at outlet and omitted at inlet if necessary. (Glass wool at inlet tends to adsorb derivatives gradually and to release them later, giving rise to "ghost images" of compds.)

Equilibrate column 2 days at 250° and 2 weeks at 212°. Operating conditions: temps (°)—column 212, detector 218, standby temps 190 and 200, resp.; N carrier gas 60 mL/min; sensitivity 1 × 10^{-9} amp full scale; and detector potential either 25 or 50 V, depending on response level needed (⅓ to ⅔ full scale peak ht with injections of 4 ng carbamate).

Alternatively, use instrument with ^{63}Ni detector and 1.8 m (6′) × 4 mm id glass column contg 10% DC-200 on 60–70 mesh Anakrom ABS. Do not use glass wool at beginning of column. Operating conditions: temps (°)—column 232, detector 250, N carrier gas 80 mL/min, sensitivity 1 × 10^{-9} amp full scale, and detector potential 50 or 75 V.

29.076 ***Extraction of Pesticides***

(*Caution: See* **51.004**, **51.043**, and **51.073**)

Place 100 g sample and 200 mL CH_3CN (add 50 mL H_2O with fruit or other samples contg 5–15% sugar) in sq screw-top jar, and macerate in blender operated 2 min at moderate speed. Filter with suction into 500 mL r-b flask thru rapid paper in 11 cm buchner. Transfer aliquot equiv. to 40 g crop (mL aliquot = (mL H_2O in sample + mL CH_3CN added + mL H_2O added − 5 mL vol. contraction) × 40/100) to 250 mL separator. Shake 10 sec with 25 mL NaCl soln. Drain and discard aq. phase. Repeat with fresh NaCl soln. Add 100 mL pet ether, and shake 30 sec. Drain CH_3CN into 1 L separator. Strip pet ether by shaking 20 sec with 50 and 10 mL portions CH_3CN, draining each into the 1 L separator. Add 300 mL H_2O, 25 mL NaCl soln, and 50 mL MeOH. Ext mixt. with 100 mL and two 25 mL portions CH_2Cl_2, shaking each 20 sec, and drain lower layer into 500 mL r-b flask. Add 2 drops acetophenone, and evap. in rotary evaporator connected to aspirator pump. During evapn, keep H_2O bath within 40–50° range and remove flask from H_2O bath when ext vol. has been reduced to few mL, so that final evapn to dryness takes place at low temp.

Add 5 mL acetone, and swirl flask to dissolve residue. Add 50 mL coagulating soln, swirl to mix, add 1–2 g diat. earth, and swirl again to mix. Pour soln into 150 mL suction filter of medium porosity packed with 6 mm (¼") diat. earth, and collect filtrate in 500 mL r-b flask. Break vac. immediately after liq. is drawn into diat. earth layer. Rinse sides of flask with 5 mL acetone, swirl, and repeat coagulation. Rinse flask with 20 mL coagulating soln, and add rinse to filter just after liq. of second coagulation is drawn into diat. earth layer. After filtration is complete (ca 5 min), transfer filtrate to 250 mL separator. Ext carbamates by shaking 20 sec with three 25 mL portions CH_2Cl_2, rinsing filter flask with each portion before adding to separator. Drain CH_2Cl_2 (lower) ext into another 250 mL separator. Soln may be held overnight at this point. Add 40 mL H_2O and 10 mL 0.5*N* KOH, mix briefly by gentle swirling, and shake 20 sec. Drain CH_2Cl_2 thru granular anhyd. Na_2SO_4 supported by glass wool in filter funnel, and collect filtrate in 250 mL erlenmeyer. Add 10 mL CH_2Cl_2 to separator, swirl gently, and drain org. phase. Repeat once. Rinse filter with two 10 mL portions CH_2Cl_2. Add 2 drops acetophenone, and evap. with same technic used in first evapn.

29.077 ***Determination***

Add 100 mL H_2O, 2 mL 0.5*N* KOH, and 1 mL 1-fluoro-2,4-dinitrobenzene soln. Stopper, and mix 20 min at high speed on mech. agitator. Add 10 mL 5% borax, swirl to mix, and heat on steam bath 20 min. Cool to room temp. by placing flasks in shallow H_2O bath 10 min. Add 5 mL isooctane, stopper, shake 3 min at high speed, and pour into 250 mL separator. Drain aq. phase, and rinse twice with H_2O. Drain isooctane soln thru funnel contg 6 mm glass wool plug into g-s test tube. Soln may be held overnight at this point. Inject 10 μL sample into gas chromatograph. If necessary to dil. sample, transfer 1 mL of isooctane ext to another test tube, dil. to exact vol. with isooctane, and shake to mix. Chromatograph std and sample solns at approx. same level of response.

Methylcarbamates, ppm

= concn std × (peak ht sample/peak ht std)
× (μL std/μL sample) × (diln vol./aliquot vol.) × 5/40

29.078 ***Preparation of Standard Curves***

Dissolve 50 mg each carbamate in 100 mL benzene and store in brown bottles. Dil. 5 mL aliquots from these solns to 50 mL with benzene, and store in brown bottles. Transfer 50 μL to 250 mL erlenmeyer, and derivatize as in **29.077**. After extn of derivatives, solns will contain equiv. of 0.5 ng each carbamate/μL. Chromatograph 4, 6, 8, and 10 μL and plot mm response against ng carbamate. If response is nonlinear, adjust GC parameters and/or prep. more dil. ref. soln, *e.g.*, equiv. of 0.25 μg carbamate/mL, and establish suitable linear working range.

Refs.: JAOAC **56**, 713(1973); **58**, 562(1975).

CAS-671-04-5 (carbanolate)
CAS-63-25-2 (carbaryl)
CAS-1563-66-2 (carbofuran)
CAS-114-26-1 (propoxur)

INDIVIDUAL RESIDUES

★ Benzene Hexachloride Pesticide Residues ★

Colorimetric Method

Final Action

29.079 *See* **24.101–24.105**, 10th ed. (*Caution: See* **51.011**, **51.039**, **51.049**, and **51.054**.)

★ Lindane and Technical BHC Pesticide Residues ★

Distinguishing Method

First Action

(*Caution: See* **51.004**, **51.011**, **51.016**, **51.030**, **51.031**, **51.039**, and **51.061**.)

29.080 *See* **24.107–24.110**, 10th ed.

Biphenyl Pesticide Residues in Citrus Fruits

Thin Layer Chromatographic–Spectrophotometric Method

Final Action

29.081 ***Principle***

Biphenyl is extd from blended peel or pulp by steam-liq.-liq. extn. Ext is subjected to TLC and biphenyl zone is completely scraped from developed plate. Biphenyl is eluted from adsorbent with alcohol for spectrophtric detn.

★Surplus method—*see* inside front cover.

29.082 ***Reagents***

(a) *Silica gel.*—GF-254 (Brinkmann Instruments, Inc. No. 7730).

(b) *Biphenyl std solns.*—(*1*) *Stock soln.*—Approx. 0.5 mg/mL. Dissolve ca 50 mg accurately weighed biphenyl in *n*-heptane and dil. to 100 mL with *n*-heptane. (*2*) *Limit soln.*—Approx. 0.01 mg/mL. Dil. 5 mL stock std to 250 mL with *n*-heptane.

Use stock std soln for spectrophtric quantitation after TLC step. Limit std soln aids in locating biphenyl zone and in estg small amts.

29.083 ***Apparatus***

(a) *Thin layer apparatus.*—See **19.041**; use 8 × 8" glass plates.

(b) *Spotting pipet.*—100 μL (Kontes Glass Co. No. K-763800, or equiv.).

(c) *Tank liner.*—Whatman 3MM paper cut to fit tank.

(d) *Moisture test apparatus.*—Similar to lighter-than-H_2O volatile oil trap, **30.020(a)**, Fig. **30:01**, with cold finger condenser (SGA Scientific, Inc. No. JM-8590, or equiv.).

29.084 ***Preparation of TLC Plates***

Mix 40 g silica gel with 80 mL H_2O, shaking vigorously few sec, and finally swirling ca 30 sec to eliminate air bubbles. Spread slurry 0.3 mm thick over 5 plates. Let plates air dry in place ca 1 hr. Put plates in drying rack and place in 100° oven 2 min. Remove plates and store in desiccator over silica gel or $CaCl_2$ until used. Plates may be stored up to 30 days.

29.085 ***Preparation of Sample***

Sort out and discard rotten units. Completely peel ≥6 whole fruits (include all white material under peel in peel portion). Weigh peelings and peeled fruit, and calc. wt ratio of peelings to peeled fruit.

(a) *Peel.*—Grind combined peel in food grinder. Blend 200 g ground peel with 400 g H_2O at high speed 5 min (or in five 1 min increments if blender becomes very warm), using high-speed blender. (Larger batches may be blended with large blender as long as peel-H_2O ratio is same.)

(b) *Peeled fruit.*—Cut peeled fruit into small pieces and blend at high speed 5 min (or in five 1 min increments if blender becomes very warm).

29.086 ***Extraction***

Accurately weigh ca 300 g recently blended peel slurry or ca 100 g recently blended peeled fruit, and transfer to 1 L r-b ⅄ 29/42 flask with enough H_2O to yield total vol. of ca 500 mL; add few boiling chips (6 mesh granular SiC is convenient). Connect extn unit of moisture test app. to flask and fill side arm with H_2O to overflowing. Place ca 3 mL *n*-heptane on top of H_2O layer and insert cold finger cooled with very rapid flow of cold H_2O. Gradually heat flask with mantle (controlled by variable transformer) until even boiling is obtained, then intensely enough to maintain vigorous boiling. Continue extn 3 hr from time mixt. starts boiling. (Wrap exposed portion of flask and connector arm between flask and extn unit with Al foil.) Initial carry-over of froth does not interfere. After 3 hr, discontinue heat and drain entire contents of extractor into 125 mL separator. Discard lower layer and drain heptane ext thru 2.5 cm column of granular anhyd. Na_2SO_4 (8–10 mm id column) into 10 mL vol. flask. Rinse separator with 1 mL *n*-heptane and add rinse to column. Rinse cold finger and extn unit with five 2 mL portions alcohol, collecting successive rinses in separator. Add 5 mL *n*-heptane to separator and shake vigorously few sec; add 50–75 mL H_2O and shake moderately few sec. Let layers sep. (lower layer may remain slightly cloudy) and discard lower layer. Pass heptane layer thru same Na_2SO_4 column into vol. flask. Rinse separator and column with enough *n*-heptane to dil. to vol.

29.087 ***Thin Layer Chromatography***

Pre-sat. tank contg liner with *n*-heptane ≥1 hr before use. Establish imaginary spotting line 3 cm from bottom edge of plate. For each intended spot, use tip of 100 μL pipet to scratch mark in adsorbent layer just size of pipet tip. (Space spots evenly with max. of 7 spots including blank.) Spot 100 μL each stock and limit std solns on extreme spots (one on far right and one on far left of plate). Spot 100 μL *n*-heptane as blank and 100 μL sample between std spots. Use same pipet for all spots, rinsing thoroly with *n*-heptane between applications. Keep size of spots uniform at 1.5–2 cm diam. by using following technic: Fill 100 μL pipet past mark with soln to be spotted. Carefully drain excess into absorbent towel until soln is at exact vol. mark. Press pipet tip against exposed glass in center of spotting mark on plate (hold pipet in vertical position at all times). Regulate size of spot by holding finger over top of pipet and pressing tip tightly against plate. Blow across spot (orally) only when necessary to regulate size of spot and never lift pipet from place once spotting is begun.

Pour 10–15 mL *n*-heptane in tank trough, insert plate, and seal tank. Develop until solv. is within 2.5 cm from top of plate (ca 30 min). Remove plate, air dry few min, and view under UV light. Biphenyl appears as bright blue spot on yellow background.

If no biphenyl appears in sample, end analysis at this point. If biphenyl is found, remove spots from plate without delay. Score upper and lower extremes of biphenyl zone horizontally across plate. Score vertical lines in adsorbent between biphenyl spots to include approx. equal area in each rectangle, scribing same area for ref. spot. Use razor blade to scrape off, and discard all adsorbent below biphenyl zone and outside extreme vertical lines. Use absorbent tissue and alcohol to clean exposed glass thoroly. Carefully scrape adsorbent from one extreme rectangular zone onto glazed paper and transfer to funnel inserted in 10 mL vol. flask; do not use solv. to rinse paper. Rinse off razor blade into funnel with small portion of alcohol. Tip plate at angle to facilitate rinsing of scraped area into funnel and rinse with several small portions alcohol. Rinse funnel and finally dil. to vol. with alcohol. Shake mixt. vigorously and let stand 5 min, shaking occasionally. Remove each biphenyl spot same way, working inward from each side of plate and cleaning and drying each previously removed zone. Filter each mixt. thru Whatman No. 44 paper, or equiv., and store filtrate in stoppered vessel for spectrophtric detn.

29.088 ***Spectrophotometry***

Det. *A* of each soln at 248 and 300 nm in 1 cm cell with alcohol as ref.

ppm Biphenyl = $(\Delta A_{248}$ sample/ΔA_{248} std) × (μg std spotted/g sample spotted), where $\Delta A_{248} = A_{248} - [A_{300} \times (A_{248}$ blank/A_{300} blank)].

Ref.: JAOAC **50**, 934(1967).

CAS-92-52-4 (biphenyl)

⋆ 2-(*p-tert*-Butylphenoxy)-1-Methylethyl ⋆ 2-Chloroethyl Sulfite Pesticide Residues

Colorimetric Method

Final Action

(*Caution: See* **51.037**, **51.039**, **51.045**, and **51.069**.)

29.089 *See* **29.067–29.071**, 12th ed.

⋆ Surplus method—*see* inside front cover.

Captan Pesticide Residues

Spectrophotometric Method

Final Action

(Applicable to firm fruits such as apples, pears, peaches, and plums and to green vegetables)

29.090 ***Principle***

Captan is extd from crop with benzene; H_2O, color, and appreciable amts of waxes are removed, and red color is developed by fusion of captan with resorcinol at 135°; color changes to yellow on addn of HOAc.

29.091 ***Reagents***

(a) *Resorcinol.*—Must be free of discoloration and pass following tests: Fuse 0.5 g and dissolve in 25 mL HOAc. *A* at 425 nm is ≤0.015, against HOAc. 1.00 g should not lose >2 mg in 4 hr over H_2SO_4; if more is lost, dry over H_2SO_4 until test is satisfactory.

(b) *Cleanup mix.*—10 parts Nuchar, 5 parts Hyflo Super-Cel, and 5 parts anhyd. Na_2SO_4.

(c) *Captan std solns.*—(*1*) *Stock soln.*—3 mg/mL. Transfer 150 mg pure captan (available from Chevron Chemical Co., 940 Hensley St, Richmond, CA 94804) to 50 mL vol. flask and dil. to vol. with benzene. (*2*) *Intermediate soln.*—300 μg/mL. Pipet 10 mL stock soln into 100 mL vol. flask and dil. to vol. with benzene. (*3*) *Working soln.*—30 μg/mL. Pipet 10 mL intermediate std soln into 100 mL vol. flask and dil. to vol. with benzene.

29.092 ***Preparation of Sample***

(*Caution: See* **51.039**, **51.040**, and **51.045**.)

(a) *Fruits.*—Accurately weigh ca 500 g sample into clean, dry jar with screw cap faced with sheet cork gasket covered with wet filter paper, or other solv.-tight lid, and add 500 mL benzene. Multiples of sample-to-benzene ratio can be used. Agitate 15 min, drain benzene into container, and transfer to separator. (Transfer to separator may be omitted where there is no separable aq. layer.)

Transfer ca 100 mL sepd benzene layer to 250 mL g-s flask, and decolorize and dehydrate with 3–4 g cleanup mix, (b), by shaking vigorously ca 5 min. Filter thru folded paper, rejecting first 10–15 mL.

(b) *Green vegetables.*—Chop sample in food chopper such as Hobart Food Cutter, mix, and transfer 100 g to explosion-proof blender. Add 200 mL benzene and blend 2 min; add 20 g anhyd. Na_2SO_4 and blend 2 min more. Pour mixt. into 500 mL centrf. bottle, stopper with *cork*, and centrf. at ca 1400 rpm 5–10 min. Decant benzene layer into 250 mL g-s erlenmeyer, add ca 6 g cleanup mix, (b)/100 mL benzene, and shake vigorously ca 5 min. Filter thru folded paper, discarding first 10 mL. If water-white soln does not result, repeat cleanup treatment. Pipet 50 mL into 100 mL vol. flask and dil. to vol. with benzene.

29.093 ***Determination***

(*Caution: See* **51.018**, **51.040**, **51.041**, and **51.045**.)

Pipet 5 mL filtrate, **29.092**(a), or aliquot, (b), into 25 × 200 mm test tube and add 0.5 ± 0.1 g resorcinol. Heat 20 min in oil bath at 135 ± 5°, cautiously at first to evap. benzene; then immerse reaction tubes to depth of ca 5 cm but do not let them touch bottom of bath. Remove, and immediately add 10–15 mL HOAc, followed by rapid immersion in H_2O at room temp. Transfer quant. to 25 mL vol. flask, using HOAc, dil. to vol. with HOAc, and mix.

Det. *A* at 425 nm in 1 cm cell against HOAc within 1 hr. Calc. ppm from std curve.

29.094 ***Preparation of Standard Curve***

Prep. std curve simultaneously with samples. Pipet 0, 2, 4, and 5 mL aliquots of working std soln into 25 × 200 mm test tubes and add benzene to make total vol. of 5 mL in each tube. Add 0.5 ± 0.1 g resorcinol and continue as in detn, beginning "Heat 20 min in oil bath . . ."

Note: One drop H_2O in reaction tube will cause apparent loss of ca 20% captan. Do not leave benzene aliquots in unstoppered reaction tubes where condensation of moisture will take place.

Refs.: JAOAC **40**, 219(1957); **46**, 143, 241(1963).

CAS-133-06-2 (captan)

Carbaryl Pesticide Residues

Colorimetric Method

Final Action

29.095 ***Reagents***

(a) *Acetone.*—Redistd.

(b) *Coagulating soln.*—Dissolve 0.5 g NH_4Cl in 400 mL H_2O contg 1 mL H_3PO_4.

(c) *Color reagent.*—Dissolve 25 mg *p*-nitrobenzene-diazonium fluoborate in 5 mL MeOH and add 20 mL HOAc. Prep. just before use.

(d) *Methylene chloride.*—Redistd CH_2Cl_2.

(e) *Alcoholic potassium hydroxide soln.*—0.1*N* in MeOH.

(f) *Polyethylene glycol soln.*—Dil. 1 mL polyethylene glycol to 100 mL with CH_2Cl_2.

(g) *Carbaryl std solns.*—Ref. std material is available from Union Carbide Corp., PO Box 1906, Salinas, CA 93901. (*1*) *Stock soln.*—0.5 mg/mL. Place 50.0 mg in 100 mL vol. flask and dil. to vol. with CH_2Cl_2. (*2*) *Intermediate soln.*—50 μg/mL. Transfer 10 mL stock soln to 100 mL vol. flask and dil. to vol. with CH_2Cl_2. (*3*) *Working soln.*—5.0 μg/mL. Transfer 10 mL intermediate soln to 100 mL vol. flask and dil. to vol. with CH_2Cl_2.

29.096 ***Apparatus***

Evaporative concentrator.—*See* Fig. **29:07**. Vac. manifold connected thru stopcock to antisurge column, 250 × 19 mm od, contg glass marble, or Snyder column, attached to ⅀ 24/40 erlenmeyer. Use surgical tubing wherever contact with sample is likely.

29.097 ***Preparation of Sample Solution***

Transfer 50 g sample to high-speed blender and add 150 mL CH_2Cl_2 and 100 g powd anhyd. Na_2SO_4. Blend at high speed 2 min and let settle 1 min. Decant solv. into 9 cm buchner fitted with Whatman No. 1, or equiv., paper covered with thin coat of Hyflo Super-Cel, or equiv., filter aid. Cautiously apply vac. until all solv. has filtered. Repeat extn with two 100 mL portions CH_2Cl_2. Treat combined filtrates as in (a) or (b):

(a) Transfer combined filtrates to 500 mL ⅀ erlenmeyer and add 1 mL polyethylene glycol soln. Connect to evaporative concentrator, place flask in H_2O bath at 25–30°, and carefully reduce pressure to ca 20 mm (2.7 kPa). After solv. evaps, immediately disconnect antisurge column from manifold. Rinse down walls of column and flask with 5 mL acetone from pipet, swirl flask, and warm gently under hot H_2O tap 30 sec. Add 50 mL coagulating soln thru column, and swirl. Remove column, let mixt. stand 30 min, and filter with vac. thru 3 mm layer of Super-Cel in No. 1 buchner. Wash flask and pad with two 15 mL portions coagulating soln.

Transfer filtrate to 125 mL separator, add 25 mL CH_2Cl_2, shake well, and let sep. completely. Drain lower layer into ⅀ 250 mL erlenmeyer. Repeat extn of aq. layer with 25 mL CH_2Cl_2, adding

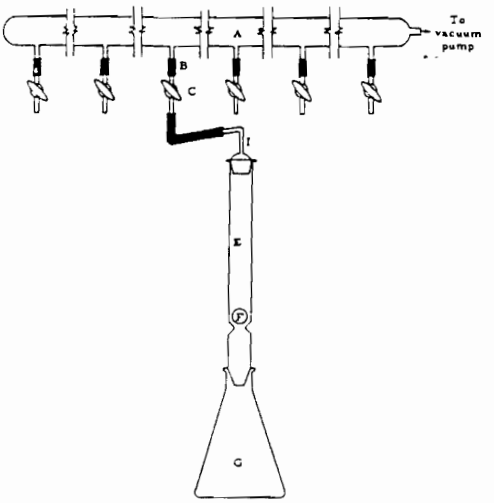

FIG. 29:07—Evaporative concentrator. A, glass manifold. B, pressure tubing. C, stopcock. D, adapter, ⅀ 24/40. E, antisurge column, 25 cm × 19 mm od. F, glass marble. G, erlenmeyer, ⅀ 24/40, 250 mL.

ext to same 250 mL erlenmeyer. If combined exts are cloudy, add 5–10 g granular anhyd. Na_2SO_4, and shake. Decant solv. into clean 250 mL ⅀ erlenmeyer, rinsing with small portion CH_2Cl_2. (If residue is expected to be >2 ppm, dil. exts to vol. in 100 mL vol. flask, and use appropriate aliquot.)

(b) Add 1 mL polyethylene glycol. Stopper, carefully reduce pressure to ca 150 mm, and warm on steam bath. When vol. is ca 5 mL, remove from steam bath and swirl until dry. Release vac., remove stopper, and let cool. Continue as in (a), beginning "Rinse down walls . . ." except column is not present.

29.098 ***Determination***

To soln in erlenmeyer add 1 mL polyethylene glycol soln and connect to column and evaporator. Evap. solv. as before, immediately disconnect, and remove column. Rinse down walls of flask with 2 mL 0.1*N* KOH in MeOH from pipet, rotating to ensure complete contact. Let stand 5 min, add exactly 17 mL HOAc, and with swirling add 1 mL color reagent. Let stand exactly 1 min and det. *A* in 1 cm cell at 475 nm against reagent blank processed along with sample as ref. Det. μg from std curve.

ppm Carbaryl = (μg/g sample) × (diln factor if aliquot was used)

29.099 ***Preparation of Standard Curve***

(*Caution: See* **51.018** and **51.041**.)

Pipet 0, 1, 3, 5, and 10 mL aliquots working std soln to 500 mL ⅀ erlenmeyers, add 300 mL CH_2Cl_2 to each, and proceed as in **29.097**, beginning "Treat combined filtrates as in (a) or (b):"

Plot *A* against μg carbaryl to obtain std curve.

Refs.: JAOAC **47**, 283(1964); **48**, 676(1965).

CAS-63-25-2 (carbaryl)

Carbaryl Pesticide Residues

Qualitative and Semiquantitative Method

Final Action

(Applicable to apples and spinach)

29.100 ***Reagents***

(a) *Adsorbent.*—Al_2O_3 G (contains 10% $CaSO_4$). *See* **29.003**(a).

(b) *Coagulating soln.*—*See* **29.095**(b).

(c) *Chromogenic spray soln.*—Sat. diethylene glycol-alcohol soln (1+9) with *p*-nitrobenzene-diazonium fluoborate (practical grade, ca 25 mg/100 mL) by stirring ca 2 min. Filter, keep cold during use, and store in refrigerator. Do not use after 3 days.

(d) *Diethylene glycol soln.*—Dil. 10 mL diethylene glycol to 100 mL with redistd CH_2Cl_2.

(e) *Carbaryl std.*—Mp 141–142°. *See* **29.095**(g). Recrystallize from alcohol and H_2O, if necessary.

29.101 ***Apparatus***

(a) *TLC apparatus.*—App. suitable for 8 × 8" plates. *See* **29.006**.

(b) *Evaporative concentrator.*—Two chamber, 24/25, micro-Snyder column (Kontes Glass Co. K-569001); with 10 mL Mills tube, graduated (Kontes K-570050).

29.102 ***Extraction and Cleanup of Sample***

Transfer 25 g sample to blender. Add 150 mL CH_2Cl_2 and 100 g powd (150 g granular) anhyd. Na_2SO_4. Blend 2 min at low speed and let settle. Attach 9 cm buchner contg rapid paper to 500 mL filter flask. Cover paper with thin coat of Hyflo Super-Cel prepd as slurry in CH_2Cl_2. Decant ext into buchner and cautiously apply vac. Rinse blender with 50 mL CH_2Cl_2 and filter. Return residue to blender. (Complete sepn of residue from Super-Cel is unimportant.) Add 150 mL CH_2Cl_2, re-ext, filter, and rinse again with 50 mL CH_2Cl_2. Add 1 mL diethylene glycol soln to filter flask. Place flask with buchner contg original filter pad attached on steam bath and apply vac. When vol. in flask is ca 5 mL, remove flask from steam bath and swirl until dry. Release vac., remove buchner, and let flask cool.

Rinse down side of flask with 3 mL acetone from pipet and swirl to dissolve residue. While gently swirling flask, add 15 mL coagulating soln and let stand >10 min with occasional swirling. Filter, using vac., thru small fritted glass funnel, medium porosity, contg ca 6 mm layer of Hyflo Super-Cel and receive filtrate in 30 mL test tube. Wash ppt with three 2 mL portions acetone-H_2O soln (1+9), letting each washing remain in contact with ppt ca 15 sec before applying vac. Transfer filtrate and washings to 25 mL vol. flask, dil. to vol. with acetone-H_2O soln (1+9), and mix.

29.103 ***Determination***

(*Caution: See* **51.017**.)

Transfer 10 mL sample soln to 125 mL separator. Ext soln with two 5 mL portions CH_2Cl_2, shaking 5–10 sec each time. Combine exts in Mills tube, add small SiC chip (<0.01 mL vol.), fit with micro-Snyder column, and evap. to 0.1 mL on steam bath. (*Caution:* Samples may be lost by vigorous ebullition.)

Prepare 8 × 8" TLC plates coated with 250 μm layer Al_2O_3 adsorbent. Dry plates in forced-draft oven 30 min at 80°. Store in desiccator cabinet. Using 1 μL pipet, spot aliquots equiv. to 2 g sample and carbaryl stds (in CH_2Cl_2) to cover expected range.

Place trough in chromatgc tank lined with blotting paper. Add ca 50 mL acetone-benzene soln (1+4) to bottom of tank to sat. atm., and then add 50 mL same soln to trough. Place plate in trough and seal tank with masking tape. Develop plate until solv. front just reaches line drawn 10 cm from origin. Dry plate ca 15 min in hood. Spray moderately with 1.0*N* alc. KOH soln. Then spray moist plate with chromogenic soln. Blue spot with R_f value same as std carbaryl spot indicates carbaryl (R_f range, 0.52–0.60). Compare size and intensity of sample and std spots for semiquant. estn of amt of pesticide. It is possible to distinguish, for example, between 0.2 and 0.4 μg, but not between 0.3 and 0.4 μg. Optimum range for quant. estn is ca 0.1–0.4 μg. For amts >0.4 μg, spot smaller aliquot of remaining 80 μL soln. Then spot same vol. of std soln for valid comparison.

Ref.: JAOAC **51**, 679(1968).

CAS-63-25-2 (carbaryl)

★ *p*-Chlorophenyl Phenyl Sulfone Pesticide Residues ★

Spectrophotometric Method

Final Action

(*Caution: See* **51.039** and **51.043**.)

29.104 *See* **29.075–29.081**, 11th ed.

Ethylan (Perthane) Pesticide Residues

Gas Chromatographic Method

Final Action

(For low levels (less than ca 50 ng/GC injection) and for confirmatory quantitation of higher levels of ethylan previously detd by **29.001–29.018**)

29.105 ***Principle***

Method is extension of general method for multiple residues, **29.001–29.018**. After electron capture GC detn of ethylan and other organochlorine and organophosphorus pesticides, ethylan in 6% mixed ether eluate, **29.015**, is dehydrochlorinated to its olefin. Ethylan olefin is extd from reaction mixt. into hexane and portion of hexane is injected into gas chromatograph with electron capture detector. Ethylan olefin produces 10-fold increase in electron capture detector response over that of parent compd.

29.106 ***Reagents***

(a) *Hexane.*—*See* **29.002**(j).

(b) *Carborundum chips.*—SiC, ca 20 mesh.

(c) *Alcoholic potassium hydroxide soln.*—Dissolve 2 g KOH in 100 mL alcohol.

(d) *Ethylan std soln.*—50 μg/mL hexane. Ethylan available from Rohm & Haas Co.

(e) *Ethylan olefin std soln.*—5 μg/mL hexane.

29.107 ***Apparatus***

(a) *Graduated centrifuge tube.*—With No. 13 glass stopper (Corning Glass Works, No. 8084, or equiv.).

(b) *Oil bath.*—100 mL beaker contg 80 mL paraffin oil. Heat on mag. stirrer hot plate calibrated to maintain oil temp. at 100 ± 5°.

29.108 ***Determination***

After electron capture GC of ethylan and other organochlorine and organophosphorus pesticides, **29.001–29.018**, pipet entire 6% eluate, **29.015**, or aliquot contg ≤30 μg ethylan into reaction tube. Carefully evap. to dryness under gentle air current. Add 2 mL alc. KOH soln and 2–5 SiC chips. Place reaction tube in 100° oil bath to depth of ca 1.0 mL graduation and let soln reflux 15 min. (Conduct reaction in hood. Air flow thru hood will cool upper part of tube, which serves as condenser. Hood also removes odors escaping from hot oil.) Remove tube from oil bath, cool to room temp., and add 3 mL H_2O. Pipet vol. hexane (but ≥1 mL) into tube to give concn ca 5 μg ethylan olefin/mL. Shake vigorously ca 30 sec and let layers sep. Det. ethylan olefin in hexane layer as in **29.018**.

$$\text{ppm Ethylan} = (R/R') \times (W'/W) \times (307.25/270.78)$$

where R and R' = responses to ethylan olefin in sample and std, resp.; W' = ng std injected; W = mg equiv. sample injected; and 307.25 and 270.78 = MW ethylan and ethylan olefin, resp.

Refs.: JAOAC **55**, 1042(1972); **56**, 721(1973).

CAS-72-56-0 (ethylan)

★ Surplus method—*see* inside front cover.

★ DDT Pesticide Residues ★

Colorimetric Method

Final Action

29.109 *See* **29.097–29.102**, 13th ed.

Dichlone Pesticide Residues

Spectrophotometric Method

Final Action

(Applicable to fresh fruits and vegetables)

29.110 ***Reagents***

(a) *Dichlone std soln.*—0.2 mg/mL. Dissolve and dil. 40 mg dichlone (Eastman Kodak Co. No. 3836, or equiv.) to 200 mL with benzene.

(b) *Dimethylamine.*—25% aq. soln (Eastman Kodak Co. P601 or equiv.).

(c) *Florisil.*—60/100 mesh, PR Grade, activated at 1250°F (Floridin Co.). Heat ≥4 hr at 130° and store in stoppered flasks in desiccator prior to use.

29.111 ***Preparation of Standard Curve***

Place 0, 1.00, 2.00, 3.00, 4.00, and 5.00 mL dichlone std soln in 25 mL g-s graduates and dil. each to 10 mL with benzene. To each graduate add isopropanol to 20 mL mark and mix. Add 1 mL 25% Me_2NH soln, dil. to 25 mL with isopropanol, and mix.

Read *A* of stds against blank in covered 1 cm cells at 495 nm, and plot *A* against mg dichlone (0–1.0 mg range). Color is stable >1 hr.

29.112 ***Preparation of Column***

Fill 15 × 300 mm chromatgc tube, fitted with fritted glass disk or glass wool plug, with Florisil to ca ⅓ its length. (No stopcock is required.) Prewet Florisil with 30 mL benzene.

29.113 ***Determination***

(*Caution: See* **51.011**, **51.039**, **51.040**, and **51.045**.)

Strip weighed sample (ca 1 kg) with 500 mL benzene by gently turning or tumbling 10 min in suitable container (ca 4 L; 1 gal.). (Avoid breaking plant tissue.) Drain benzene into 1 L flask thru folded paper (ca 32 cm) contg ca 50 g anhyd. Na_2SO_4.

Add 200 mL dried benzene strip soln to prepd chromatgc column. Discard benzene eluate. Elute dichlone from column with 100 mL acetone-benzene eluting mixt. (1 + 99). Collect eluate in beaker and evap. to ca 15 mL. (Do not let sample overheat or go to dryness.) Rinse sample into graduate and dil. to 20 mL with benzene. Develop color in 10 mL of this soln as in **29.111**.

mg Dichlone from std curve × 5 = ppm dichlone (for 1 kg sample)

If visible color is present in benzene eluate, simultaneously develop color in remaining 10 mL aliquot, omitting Me_2NH and adding 1 mL H_2O. Subtract this blank *A* from that of developed sample to correct for sample blank.

Ref.: JAOAC **48**, 759(1965).

CAS-117-80-6 (dichlone)

★ Azinphos Methyl Pesticide Residues ★

Spectrophotometric Method

First Action

(Applicable to cole-type crops and to apples, plums, peaches, grapes, apricots, and cherries.)

29.114 *See* **29.102–29.107**, 12th ed.

Dodine Pesticide Residues

Spectrophotometric Method

Final Action

(Applicable to apples, peaches, pears, pecans, and strawberries)

29.115 ***Reagents***

(a) *Bromocresol purple soln.*—Recrystallize indicator-grade bromocresol purple from boiling toluene (ca 2 g/100 mL). Dissolve 0.4 g recrystd material in 75 mL 0.01*N* NaOH; if necessary, add addnl 0.01*N* NaOH to bring pH to 6.0–6.1. Filter, if necessary, and dil. to 500 mL with CO_2-free H_2O. Store in brown bottle.

(b) *Buffer soln.*—pH 5.5. Dissolve 15.2 g $Na_2HPO_4.7H_2O$ and 74.0 g $NaH_2PO_4.H_2O$ in CO_2-free H_2O and dil. to 1 L.

(c) *Dodine (DDGA) std solns.*—(*1*) *Stock soln.*—130 μg/mL. Dissolve 32.5 mg Ref. Std (available from American Cyanamid Co.) in MeOH and dil. to 250 mL with MeOH. (*2*) *Working soln.*—13 μg/mL. Dil. 25 mL aliquot stock soln to 250 mL with MeOH.

29.116 ***Preparation of Sample***

Grind sample in high-speed blender with MeOH-$CHCl_3$ (2 + 1) in ratio of 400 mL solv./100 g sample. Filter with suction thru 2 Whatman No. 1, or equiv., papers in buchner, and wash pulp with MeOH-$CHCl_3$ (2 + 1), using 100 mL/100 g sample. Det. vol. of ext and transfer portion equiv. to 50 g sample to 400 mL beaker.

29.117 ***Determination***

Add several glass beads and 1 mL HCl to beaker, and evap. to 50 mL on steam bath. Add 30 mL 30% NaCl soln and 100 mL MeOH. Cool, transfer to 500 mL separator, and ext *gently* with 50 mL CCl_4 by inverting separator 6–8 times. Let phases sep. and discard CCl_4 layer. Repeat with 50 mL CCl_4, inverting separator ca twice as many times. Discard CCl_4; then ext with 50 mL CCl_4, shaking gently 30 sec. Finally, ext with 50 mL CCl_4, shaking vigorously 1 min, and again discard CCl_4.

Adjust pH of soln to ca 5.5 with 4*N* NaOH (pH meter), and add 20 mL pH 5.5 buffer and 20 mL bromocresol purple soln. Re-adjust pH to 5.5 and ext complex with two 50 mL portions $CHCl_3$, shaking 2 min each time. Shake combined ext 30 sec with 25 mL pH 5.5 buffer, and transfer $CHCl_3$ layer to another separator. Shake 1 min with 25 mL pH 5.5 buffer, let stand 10 min, and transfer $CHCl_3$ to another separator. Shake with 20 mL 0.05*N* NaOH to remove all combined indicator and any org. acids which may persist. Recomplex dodecylguanidine (in $CHCl_3$ as free base) by shaking 3 min with 5 mL bromocresol purple soln and 20 mL pH 5.5 buffer. Wash $CHCl_3$ with three 15 mL portions pH 5.5 buffer, shaking 1 min each time. Transfer $CHCl_3$ to dry 250 mL separator and shake 2 min with 20 mL 0.05*N* NaOH, measured by pipet. Read *A* of indicator in aq. soln at 590 nm, using Beckman spectrophtr, or equiv. Obtain μg DDGA from std curve.

29.118 ***Preparation of Standard Curve***

Add 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mL std soln to series of separators contg 100 mL MeOH, 50 mL H_2O, 30 mL 30% NaCl soln, 20 mL bromocresol purple soln, and 20 mL pH 5.5 buffer. Adjust pH of each soln to 5.5 and continue as in **29.117**, par. 2, beginning ". . . and ext complex with two 50 mL portions $CHCl_3$,

★ Surplus method—*see* inside front cover.

shaking 2 min each time." Read *A* of each aq. soln at 590 nm and plot against μg DDGA. No blank correction is necessary for stds.

ppm DDGA = μg DDGA in aliquot/g sample in aliquot

Ref.: JAOAC **47**, 300(1964).

CAS-2439-10-3 (dodine)

Ethylenethiourea Pesticide Residues

Gas Chromatographic Method

Final Action

(Applicable to potatoes, spinach, applesauce, and milk. *Caution: See* **51.056** and **51.066**.)

29.119 *Apparatus*

(a) *Chromatographic tube.*—Glass, 300 × 22 (id) mm, with coarse fritted disk and Teflon stopcock.

(b) *Filter paper.*—Sharkskin (Arthur H. Thomas Co., or equiv.).

(c) *Gas chromatograph.*—With flame photometric detector (Meloy Laboratories, Inc., 6715 Electronic Dr, Springfield VA 22151, or equiv.) contg S filter and 1.8 m × 4 (id) mm coiled glass column packed with 5% Carbowax 20M plus 2.5% KOH (prepd in MeOH) on 80–100 mesh Chromosorb W(HP). Condition new column 2 days at 210°. Typical operating conditions—temps (°): column 180, injection port 185, detector 185; flow rates (mL/min): N carrier gas 60, O 15, air 125, H 200; electrometer sensitivity 1 × 10^{-9} amp full scale deflection with 1 mv recorder. Approx. retention time of S-butylated ETU under these conditions is 4 min; 12 ng gives ca 50% full scale deflection. Change glass wool plug in injection port daily before use, and clean out inside of column at injection port weekly.

(d) *High-speed blender.*—Waring Blendor, or equiv. (*Caution: See* **51.004**.)

(e) *Pipets.*—Disposable glass capillary pipets, 145 × 6 (id) mm (Arthur H. Thomas Co., or equiv.).

(f) *Rotary evaporator.*—Use with 150 mm ⅀ 24/40 Vigreux column and place vac. release valve in line. (*Caution: See* **51.004**.)

(g) *Silanized glass wool.*—Applied Science Laboratories, Inc., or equiv.

29.120 *Reagents*

(a) *Aluminum oxide.*—Fisher No. A-540, or equiv., 80–200 mesh, for chromatgc adsorption. (Available from Fisher Scientific Co. as "Alumina, Adsorption, Fisher.") Use as received.

(b) *1-Bromobutane.*—Fisher Scientific Co., or equiv. Redistil between 101 and 101.5°.

(c) *Diatomaceous earth.*—Celite 545. Do not acid-wash.

(d) *Eluant.*—4% alcohol in $CHCl_3$. Dil. 40 mL alcohol to 1 L with $CHCl_3$, and mix well.

(e) *Solvents.*—$CHCl_3$, MeOH, and toluene, distd in glass (*see* statement regarding solvs, **29.001**).

(f) *Ethylenethiourea std solns.*—(*1*) *Stock soln.*—10 μg ETU/mL. Transfer 100 mg ETU ref. std (available from Pesticide Reference Standards Section, Registration Division, Environmental Protection Agency, 401 M St, SW, Washington, DC 20460) to 100 mL vol. flask, and dil. to vol. with H_2O. Pipet 1 mL this soln into another 100 mL vol. flask, and dil. to vol. with H_2O. Prep. fresh monthly. (*2*) *Working soln.*—2 μg ETU/mL. Pipet 10 mL stock soln into 50 mL vol. flask, and dil. to vol. with H_2O. Prep. fresh weekly.

29.121 *Extraction*

(Samples must be started and completed on same day.)

(a) *For crops, canned goods, and milk.*—Blend 100 g chopped crop (vegetables and fruits) or 100 g milk, 150 mL H_2O, 15 g NaCl, 10 g diat. earth, (c), and 200 mL MeOH in high-speed blender 2 min. Filter with vac. thru 1.3 cm bed of diat. earth spread dry and evenly on 9 cm double sharkskin filter paper in 91 mm (id) buchner. Transfer 87 mL (20 g) aliquot to previously weighed 500 mL ⅀ 24/40 r-b flask. Add 50–70 mL MeOH, insert Vigreux column into flask, and conc. on rotary vac. evaporator immersed in 60–65° H_2O bath. If substantial initial frothing occurs, add 4–5 drops octanol. If much frothing occurs during last stages of concn, add addnl 4–5 drops octanol, 25 mL alcohol, or both. Conc. to ca 10 g. Disconnect flask, weigh, and add enough H_2O to bring wt to 13 g. Proceed immediately as in **29.122**.

(b) *Optional extraction for crops.*—(Applicable when presence of parent ethylenebisdithiocarbamate (EBDC) fungicide is suspected in sample. Provides measure of potential ETU residues which may be converted from EBDCs in home cooking.) Place 100 g chopped crop, 150 mL H_2O, and 1 mL NH_4OH in 1 L beaker, and record total wt. Cover with large watch glass, place on 600–720 watt hot plate turned to high, and heat 15 min, reducing heat to low after initial boiling. Cool, remove watch glass, and reweigh. Add H_2O to beaker to restore to original wt. Transfer quant. to high-speed blender, using 200 mL MeOH. Add 10 g diat. earth, (c), and blend 20 sec. Proceed as in (a), beginning "Filter with vac. thru 1.3 cm bed . . .", except conc. sample to ca 8 g and add H_2O to bring wt to 10 g. Proceed immediately as in **29.122**.

(c) *Optional extraction for milk.*—(Applicable when presence of parent EBDC fungicide is suspected in sample.) Place 100 g milk, 25 mL H_2O, 5 g NaCl, and 1 mL NH_4OH in 1 L beaker, and record total wt. Proceed as in (b), beginning "Cover with large watch glass, . . ." except use 275 mL MeOH, stir thoroly 1 min, and, after filtering, transfer 80 mL (20 g) aliquot to r-b flask. Proceed immediately as in **29.122**.

29.122 *Cleanup*

Add 10 g Gas-Chrom S to sample ext. stopper, and shake vigorously until lump-free (30–60 sec), tapping on cork ring, if necessary. Add 50 mL eluant, (d), stopper, and shake 1–2 min. Pour mixt., including as much of Gas-Chrom S as possible, into chromatgc tube, **29.119**(a), contg 4–5 g Al_2O_3, (a), held in place with 1.3 cm glass wool plug. Rinse flask with 3 addnl 50 mL portions eluant, adding each rinse to tube. Collect eluate in 500 mL ⅀ 24/40 r-b flask contg 10 mL H_2O and 20 mL alcohol. Conc. eluate to ca 20 mL using rotary vac. evaporator with Vigreux column. Add 5 mL H_2O and 20 mL alcohol, and conc. to 10 mL to eliminate $CHCl_3$. Rinse column and flask with 5 mL H_2O followed by 20 mL alcohol. Proceed immediately as in **29.123**.

29.123 *Derivatization*

(a) *Sample.*—To sample flask add 1.5 g KOH, 2 mL 1-bromobutane, (b), and 5–6 boiling chips. Reflux on pre-heated hot plate 10 min, using cold H_2O condenser and clamp to support flask. Cool, and transfer quant. to 250 mL separator with 10 mL H_2O followed by 50–60 mL $CHCl_3$. Shake 1–2 min, and let layers sep. completely (ca 5 min). Collect lower $CHCl_3$ layer in clean 250 mL ⅀ 24/40 r-b flask. Add 2 drops HCl and evap. to near dryness on rotary vac. evaporator, using Vigreux column and 60–65° H_2O bath. Rinse neck of flask with 2–3 mL MeOH, and evap. again on rotary evaporator. Remove flask and evap. to dryness, using air jet for 2–3 min. *All MeOH must be removed by this final evapn or it will interfere with GC detn.* Remove flask from air jet, pipet in 1 mL toluene and add 1.0–1.5 mL 10% KOH, stopper, and shake 1–2 min. Remove 0.5–0.7 mL of toluene layer with disposable pipet, (e), without collecting any of lower aq. layer. Place toluene sample ext in clean, dry calibrated test tube, and record vol. At this point, 1 mL ext is equiv. to 20 g sample. However, if final ext is dild for GC, vol. recorded with respect to original 1 mL toluene must be used for sample wt. calcns:

Wt sample in final diln

= [(vol. obtained from 1 mL)/(1 mL toluene)] × 20 g

Do not conc. sample ext at this point due to volatility of butyl derivative of ETU.

(b) *Standard.*—Pipet 1 mL ETU working std soln into 500 mL ŝ 24/40 r-b flask. Add 15 mL H_2O, 20 mL alcohol, 1.5 g KOH, 2 mL 1-bromobutane, and 5-6 boiling chips, and proceed as in (a), beginning "Reflux on preheated hot plate . . ."

29.124 ***Determination***

Initially, inject 4–6 μL std ext, **29.123**(b), into gas chromatograph, and then inject 5 μL sample ext, **29.123**(a). Adjust injection vol. of sample ext until peak hts of std and sample are approx. equal, and continue with alternate injections of sample and std. (S detector is non-linear; therefore, do not prep. std curve.)

$$\text{ETU, ppm} = (PH/PH') \times (W'/W)$$

where PH = peak ht of sample, PH' = peak ht of std, W' = ng ETU in std aliquot, and W = mg sample represented by sample aliquot.

29.125 ***Regeneration of Gas-Chrom S***

Shake all used Gas-Chrom S from cleanup column, **29.122**, into large beaker or flask. Discard Al_2O_3 and glass wool plug. Wash thoroly with H_2O, and decant after each wash. Wash thoroly with MeOH, decant, and vac. dry in large buchner. Air dry in hood and transfer to 80° oven overnight. Gas-Chrom S may now be re-used.

Refs.: JAOAC **60**, 1105, 1111(1977).

CAS-96-45-7 (ethylenethiourea)

Glyodin Pesticide Residues

Spectrophotometric Method

Final Action

(Applicable to apples and pears. Not applicable to fruits with extensive softening or decomposition. All glassware must be free of soap or detergent.)

29.126 ***Reagents***

(a) *Bromophenol blue soln.*—Prep. just before use. Transfer 50 mg bromophenol blue powder into 500 mL vol. flask with small amt of H_2O. Add 2 mL HOAc and swirl until dye is completely dissolved. Dil. to vol. with H_2O, and mix.

(b) *Glyodin std solns.*—Prep. from 2-heptadecyl glyoxalidine, purified grade (available from ICN-K&K Laboratories). (*1*) *Stock soln.*—1 mg/mL. Dissolve 100.0 mg 2-heptadecyl glyoxalidine in $CHCl_3$ in 100 mL vol. flask, dil. to vol. with $CHCl_3$, and mix. (*2*) *Working soln.*—0.05 mg free base/mL. Transfer 5.0 mL stock soln to 100 mL vol. flask, dil. to vol. with $CHCl_3$, and mix.

29.127 ***Preparation of Standard Curve***

Add 0, 2, 4, 6, 8, and 10 mL working std soln to six 50 mL vol. flasks. Add exactly 1 mL HOAc to each flask and dil. to vol. with $CHCl_3$. Place 25 mL of each std, measured in graduate or fast-flow pipet, in 125 mL separator. Add 25 mL bromophenol blue soln, (a), from graduate or fast-flow pipet to each separator, and shake vigorously 1 min. Let sep. ≥20 min. Filter $CHCl_3$ layer thru pledget of glass wool in stem of separator into small g-s erlenmeyer. Det. *A* at 415 nm in spectrophtr, using 1 cm cells and 0 std as ref. Plot *A* against mg 2-heptadecyl glyoxalidine.

29.128 ***Preparation of Sample***

Fill tared wide-mouth gal. (3.8 L) glass jar with whole fruit so that little or no slack is present (to prevent battering of fruit). Weigh, and add 250 mL isopropanol. Screw cap on tightly with double thickness of cellophane placed over mouth of jar before cap is screwed on to help prevent leakage. Tumble or shake 10 min. Filter into 500 mL vol. flask thru small layer of glass wool in funnel. Drain off as much liq. as possible. Repeat stripping with second 250 mL portion of isopropanol, and filter into vol. flask. Wash glass wool and funnel with small portions of isopropanol and dil. to vol.

29.129 ***Determination***

Transfer 25 mL aliquot of strip soln to 50 mL beaker and evap. to dryness on steam bath under air jet. To residue add exactly 1 mL HOAc, allowing acid to drip slowly down sides of beaker so that all residue is wetted. Cover beaker with watch glass and heat gently on steam bath with swirling, until residue at bottom loosens and disintegrates. Thoroly rinse down sides with few mL $CHCl_3$ and transfer to 50 mL vol. flask. Rinse beaker with 4 addnl small portions $CHCl_3$, and transfer to vol. flask. Dil. to vol. with $CHCl_3$ (disregard turbidity and slight color in soln).

Transfer 25 mL $CHCl_3$ soln, measured in graduate or fast-flow pipet, to 125 mL separator. Proceed as in **29.127**, beginning, "Add 25 mL bromophenol blue soln, (a), . . ."

Perform detns along with prepn of std curve, using 0 std as ref. when detg sample *A*. Det. amt of 2-heptadecyl glyoxalidine in aliquot from std curve.

Glyodin (2-heptadecyl glyoxalidine acetate)
= 2-heptadecyl glyoxalidine × 1.195

Ref.: JAOAC **46**, 238(1963).

CAS-556-22-9 (glyodin)

Hexachlorobenzene and Mirex Pesticide Residues in Fatty Products

Gas Chromatographic Method

Final Action

29.130 ***Reagents***

(a) *Solvents.*—Hexane, CH_2Cl_2, CH_3CN, and pet ether. *See* **29.002**.

(b) *Florisil.*—60–100 mesh PR grade. (*1*) *Unactivated, for partition chromatography.*—Use as received from manufacturer. (*2*) *Activated.*—*See* **29.002**(i).

(c) *Eluant mixture.*—For Florisil column cleanup. Dil. 200 mL CH_2Cl_2 with hexane. Let reach room temp. and adjust to 1 L with hexane.

29.131 ***Apparatus***

(a) *Gas chromatograph.*—With electron capture detector. *See* **29.008**(a) and (c).

(b) *Column.*—1.85 m × 4 (id) mm glass column with 80–100 mesh Chromosorb W (HP) support, N flow 120 mL/min, and injection temp. 220°. For HCB analysis, use 15% OV-210 liq. phase; for Mirex, 3% OV-101. For HCB, use column temp. 180°, detector, 200°; for Mirex, use column temp. 210°, detector 220°. Operate ^3H electron capture detector at dc voltage which produces half scale deflection for 0.5 ng HCB or Mirex when electrometer sensitivity is 1×10^{-9} amp. Or, operate ^{63}Ni detector to produce stable, reproducible, linear response and adjust amt of injected sample, **29.018**, to accommodate difference in instrument sensitivity.

(c) *Chromatographic tubes.*—(*1*) *Plain.*—250 × 22 (id) mm. (*2*) *With stopcocks.*—*See* **29.005**(b).

(d) *Kuderna-Danish concentrators.*—*See* **29.005**(e).

(e) *Micro-Vigreux Column.*—*See* **29.005**(h). Use for concn to vols < 5 mL.

29.132 ***Extraction of Fat***

Ext ≥3 g fat as in **29.012**. For products reported on fat basis, use 3 g fat. For products reported on as-is basis, record wt, *W*, of fat

extd. Corresponding wt sample analyzed = (wt fat taken for cleanup/*W*) × wt original sample.

29.133 ***Cleanup***

(*Caution: See* **51.043** and **51.073.**)

Weigh 3 g fat into 250 mL beaker, add 20 g unactivated Florisil, and stir with spatula or glass rod until free-flowing powder is obtained. Place glass wool plug in bottom of plain chromatgc tube and add 3 g unactivated Florisil. Completely transfer fat-Florisil mixt. to tube. Settle column by repeatedly tapping tube. Place glass wool plug on top of Florisil. Place 1 L separator under column as receiver. Elute with 150 mL CH_3CN by gravity.

When elution is complete, add exactly 100 mL pet ether to separator, and shake vigorously 1–2 min. Add 10 mL satd NaCl soln and 500–600 mL H_2O, and shake vigorously 1 min. Let sep. and discard aq. (lower) layer. Wash pet ether with two 100 mL portions H_2O. Discard washings, transfer pet ether to 100 mL g-s graduate, and record vol., *P*. Calc.

Wt sample in eluate = (wt sample taken for cleanup × *P*/100

where 100 = mL pet ether added.

Complete cleanup on column of activated Florisil, **29.015**, using amt Florisil detd from lauric acid test, **29.002**(i). Sample ext must be dry and free from polar solvs when placed on column. Elute at ca 5 mL/min with 200 mL eluant mixt., (c), and conc. Det HCB and Mirex as in **29.018**, using column, **29.131**(**b**).

Refs.: JAOAC **58**, 557(1975); **60**, 229(1977).

CAS-118-74-1 (hexachlorobenzene)
CAS-2385-85-5 (mirex)

Hexachlorobenzene and Mirex Pesticide Residues in Adipose Tissue

Gas Chromatographic Method

Final Action

(*Caution: See* **51.037**, **51.041**, **51.061**, and **51.072.**)

29.134 ***Method Performance***

HCB av. rec. at 20–49 ppb = 89.9% (S_r = 3.7–8.7, S_o = 3.7–8.7)

HCB derivative av. rec. at 18–41 ppb = 78.5% (S_r = 4.4–10.2, S_o = 4.0–8.1)

Mirex av. rec. at 160–490 ppb = 90.5% (S_r = 19–102, S_o = 18–62)

29.135 ***Reagents***

(a) *Hexane, 2-propanol.*—See solv. purity test, **29.002**.

(b) *Sodium sulfate.*—Anhyd. granular, Soxhlet-extd with hexane.

(c) *Sodium sulfate.*—2% aq. soln.

(d) *Florisil column.*—Packed with 100 mm Florisil and 12 mm Na_2SO_4 on top. Hold in 130 ± 2° oven ≥16 hr prior to use. (Remove stopcocks before placing columns in oven.) Prewash with 50 mL hexane immediately before use. Predetermine HCB and mirex elution on each lot of Florisil.

(e) *"Keeper" soln.*—1% paraffin oil in hexane.

(f) *Pyridine.*—Burdick and Jackson, or equiv., suitable for liq. and gas chromatgy.

(g) *Potassium hydroxide soln.*—10% KOH in 2-propanol.

29.136 ***Apparatus***

(a) *Gas chromatograph.*—With ^3H or ^{63}Ni electron capture detector and 1.8 m × 4 mm id borosilicate glass columns packed with 1.5% OV-17/1.95% OV-210 or 5% OV-210 on 80–100 mesh Gas-Chrom Q. *Typical operating conditions.*—Carrier gas flow rate 60 mL/min, column temp. 200° (OV-17/OV-210); 180° (5% OV-210).

(b) *Glassware.*—300 × 25 mm od Chromoflex column for Florisil (Kontes, K-420530), Kuderna-Danish (K-D) concentrator assembly (K-570000) fitted with 25 mL graduated tube (K-570050, size 2525, 19/22, 25 mL), modified micro Snyder column (K-569250), disposable pipets.

29.137 ***Determination***

Accurately weigh ca 0.5 g rendered or extd fat in tube. Dissolve fat in ca 0.5 mL hexane and transfer quant. to Florisil column prewashed with hexane. Rinse tube with two 0.5 mL portions hexane. Let column elute until solv. level is just at top of Na_2SO_4. Rinse column sides with 2–3 mL hexane. Elute with 200 mL hexane at 5 mL/min.

Collect eluate in K-D flask equipped with 25 mL concentrator tube. Evap. on steam bath to ≥10 mL. Use warm (50°) H_2O bath and gentle N stream for further vol. reduction.

Inject 3–8 μL stds and samples into OV-17/OV-210 column. Alternatively, OV-210 column at 180° may be used for mirex quantitation. Adjust sample vol. as required to produce major peak responses. Peak hts of stds and samples should not vary >25%. Do not quantitate <20% full scale deflection. Amts injected must fall within linear range of detector. Work at same attenuation.

29.138 ***Confirmation of HCB***

Prep. sample for derivatization by evapg hexane ext to 0.1–0.2 mL with warm H_2O bath and gentle N stream. Derivatize ≥3 stds along with samples. (HCB stds must bracket HCB concn in samples as detd by initial GC analysis. Response of HCB stds must be linear.) Add 2–3 drops 1% paraffin oil in hexane to stds as "keeper" before evapg to 0.1–0.2 mL.

Add 0.5 mL 10% KOH in 2-propanol and 0.2 mL pyridine to each sample and std. Gently tap tube to mix. Place modified micro Snyder columns on concentrator tubes and place tubes in boiling H_2O bath 45 min. Remove tubes and cool under cold tap H_2O. Add ca 10 mL 2% Na_2SO_4 soln to each tube and exactly 2 mL hexane. Mix vigorously 1 min. Let solv. phases sep. completely. Inject 3–8 μL hexane phase into gas chromatograph for quantitation. Adjust hexane vol. as required. Calc. concn HCB. Mirex will not be recovered thru derivative procedure.

Refs.: JAOAC **63**, 1128(1980); **64**, 531(1981).

CAS-118-74-1 (hexachlorobenzene)
CAS-2385-85-5 (mirex)

★ Malathion Pesticide Residues ★

Colorimetric Method

First Action

29.139 *See* **29.116–29.120**, 12th ed.

Maleic Hydrazide Pesticide Residues

Spectrophotometric Method

Final Action

(Applicable to whole, dehydrated mashed, and frozen french fried potatoes, and potato chips; whole cranberries, onions, and peaches; and tobacco dust)

29.140 ***Principle***

Sample is boiled in alk. soln to drive off volatile basic interferences. Distn with Zn with N sweep expels hydrazine liberated from maleic hydrazide. Hydrazine is reacted in acid soln with *p*-dimethylaminobenzaldehyde to form yellow compd.

★ Surplus method—*see* inside front cover.

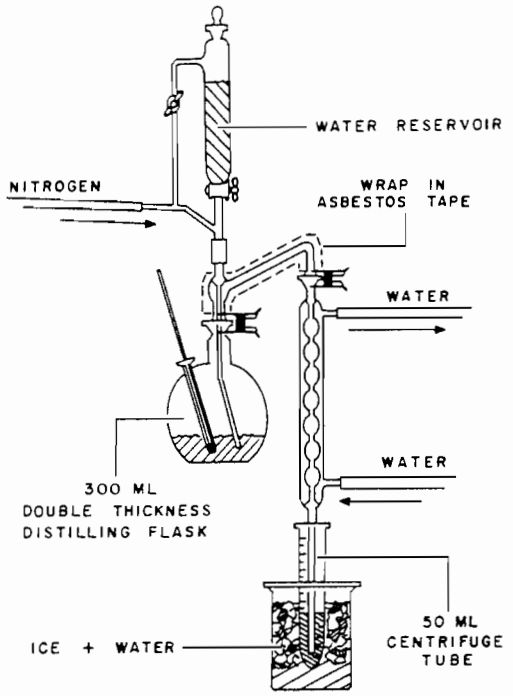

FIG. 29:08—Distillation apparatus for maleic hydrazide determination

29.141 ***Apparatus***

(a) *Distillation apparatus.*—*See* Fig. **29:08**. Flask is 300 mL capacity, flat-bottom, double thickness, with thermometer well. Thermometer is 90–220° (Tinius Olsen No. 718636 "Yellow Bak," or equiv. in temp. range and length; available on special order from Accuracy Scientific Instrument Co., 335 E Chew Ave, Philadelphia, PA 19120). Use 5" wire gauze with 4" diam. asbestos center. Centrf. tube receiver (50 mL) is graduated in 1 mL divisions.

(b) *Spectrophotometer.*—Beckman Model DU, or equiv.

29.142 ***Reagents***

(a) *p-Dimethylaminobenzaldehyde soln.*—Dissolve 2 g in 100 mL 1*N* H_2SO_4. Soln is stable.

(b) *Zn granules.*—"10 mesh."

(c) *Maleic hydrazide std soln.*—10 μg/mL. Dissolve 0.0100 g maleic hydrazide in 100 mL 0.1*N* NaOH and dil. to 1 L. Soln is stable.

29.143 ***Preparation of Sample***

Grind sample to soup-like consistency in high-speed blender, adding measured wt of H_2O if necessary. Prepd samples may be frozen for storage.

29.144 ***Determination***

Transfer wt ground sample specified in Table **29:03** to 300 mL distn flask. Dry socket neck joint, and add 50 g NaOH pellets, antifoam agent indicated in Table **29:03**, and 40 mL H_2O. Add 1 mL high bp oil to thermometer well and insert thermometer. Heat flask on high-temp. hot plate and swirl ca every 20 sec until pellets dissolve and gentle boiling begins. When temp. reads 160°, (180° with cranberries), remove flask and let cool 5 min. Wipe socket joint clean and dry; add 0.5 g *ferrous chloride* and 5 mL (equiv. to ca 5 g) Zn.

Quickly grease socket joint with light film of high-vac. silicone grease and attach flask to app. (Fig. **29:08**). Center flask firmly on asbestos pad. Place 4 mL *p*-dimethylaminobenzaldehyde soln in 50 mL centrf. tube (ice-cooled) and immerse condenser tip. Adjust N flow (*dry N*) to 3 bubbles/sec in receiver. With rapid flow of condenser H_2O, heat flask with Bunsen burner, centering tip of outer cone of flame on asbestos pad. When boiling begins, adjust distance of burner so foaming contents fill ca ⅔ of flask. Distil until temp. reads 173°, *slowly* add H_2O from reservoir until temp. drops to 168°, turn off H_2O, and distil to 173°. Continue H_2O addn and distn at these temps until receiver contains ca 40 mL. Remove receiver. (If during distn receiver soln becomes turbid or ppt appears, add 2 drops H_2SO_4 and shake.)

Record vol. of distillate and det. *A* at 430, 460, and 490 nm, using 1 cm cells and 4 mL *p*-dimethylaminobenzaldehyde soln dild to 40 mL as ref.

After distn, remove hot distg flask from app. with heat-resistant gloves, remove thermometer, and seal well with small cork. Rinse N-H_2O inlet tube free of caustic with HCl from plastic squeeze bottle followed by H_2O. Then (with gloves and safety glasses) pour molten contents of distg flask into Fe can in sink to trap Zn granules. Rinse flask 3 times with H_2O and 2 times with HCl to remove encrusted caustic and Zn granules. Fill flask with HCl (1+9) to remain until next use. Rinse 3 times with H_2O before reuse. (Careful removal of *all* Zn granules with HCl is essential because residual Zn would cause premature destruction of maleic hydrazide in precook of next sample. Because of corrosion by the caustic soln, flasks may last for only ca 30 detns.)

Table 29:03 Sample Weights of Commodities and Use of Antifoam Agents

Commodity	Wt Sample, g	Antifoam Agent
Cranberries (whole)	2.0	1 g paraffin wax + 1 mL Antifoam A
French fries (frozen)	1.0	none
Mashed potatoes (dehydrated)	1.5	1 g paraffin wax + 1 mL Antifoam A
Onions (whole)	4.2	1 mL Antifoam A
Peaches (whole)	5.0	1 g paraffin wax + 1 mL Antifoam A
Potatoes (whole)	2.5	0.5 mL vegetable oil
Potato chips	1.1	none
Tobacco (dust)	1.0	1 g paraffin wax + 1 mL Antifoam A

29.145 ***Preparation of Standard Curve***

To clean 300 mL distn flasks, add 50 g NaOH pellets, 40 mL H_2O, and std soln equiv. to 0, 5, 10, 20, 30, 50, 100, 150, and 200 μg maleic hydrazide. Precook, distil, and measure *A* as in **29.144**. Det. ΔA for each std as follows:

$$\Delta A = [A_{460} - ((A_{430} + A_{490})/2)] \times \text{mL color soln}/40$$

Plot ΔA of each std against μg maleic hydrazide to obtain std curve. If desired, derive simple factor from curve slope, *K*, converting ΔA to μg maleic hydrazide; thus, μg maleic hydrazide = $\Delta A \times K$.

29.146 ***Calculations***

Multiply ΔA of sample by *K* to derive μg maleic hydrazide; ppm = μg/sample wt (g).

Refs.: JAOAC **46**, 261(1963); **48**, 744(1965); **49**, 87(1966); **64**, 394(1981).

CAS-6915-15-7 (maleic hydrazide)

★ Methoxychlor Pesticide Residues ★

Colorimetric Method

Final Action

29.147 *See* **24.149–24.152**, 10th ed. (*Caution: See* **51.011**, **51.039**, and **51.073**.)

★ Surplus method—*see* inside front cover.

Monofluoroacetic Acid Pesticide Residues

Qualitative Test

Final Action
(Sodium salt, "1080")

(Monochloroacetic acid also responds to this test. Confirm presence of org. F by **29.150–29.156**.)

29.148 ***Reagents***

(a) *Decolorizing carbon.*—*See* **16.027(b)**.

(b) *Thiosalicylic acid soln.*—Dissolve 300 mg thiosalicylic acid (Eastman P2805 is suitable) in mixt. of 2 mL 1*N* NaOH and 18 mL H_2O.

(c) *Potassium ferricyanide soln.*—Dissolve 1 g $K_3Fe(CN)_6$ in H_2O and dil. to 50 mL with H_2O.

29.149 ***Test***

Prep. sample and ext as in **29.153–29.154**. If convenient, ext large enough sample to obtain 2–10 mg 1080. With very low levels of 1080, *e.g.*, 1–5 ppm, ext large enough sample to obtain ≥0.5 mg 1080.

Sep. ether ext from any aq. sludge which may have been carried over in extn, add ca 5 g anhyd. Na_2SO_4 and 0.5 g decolorizing C/100 mL ether, and shake vigorously. Let stand ca 15 min at room temp. with occasional shaking, and decant thru fluted paper into separator. Add ca 25 mL H_2O and enough NaOH soln (ca 1*N*) to make aq. layer alk. after vigorous shaking (outside test paper). Drain aq. layer into 125 mL erlenmeyer and aerate to remove dissolved ether. Using pH test paper and ca 1*N* solns of H_2SO_4 and NaOH, adjust to pH 4–6. Add 0.5 g C and place on steam bath for 15 min.

Cool under tap and filter thru fluted paper into ca 25 × 150 mm test tube. Add 1 mL thiosalicylic acid soln and 2 drops NaOH (1 + 1), and mix. Conc. soln to small vol. by placing on steam bath under gentle air current. Completely dry residue in oven at 130° or, if time is not factor, in 100° oven. (When convenient, overnight drying is satisfactory, with or without prior concn of soln.)

Dissolve *thoroly* dry residue in 2–3 mL H_2O, add 1 mL $K_3Fe(CN)_6$ soln, and mix. Red ppt, which forms at once when ≥1 mg 1080 is present, or upon standing when only fraction of mg is present, is pos. test for 1080.

Employ chromatgc instead of C purification in following cases:

(*1*) With pineapple juice when <2 mg 1080 can be extd.

(*2*) With grape juice even when ≥2 mg of 1080 can be conveniently extd.

(*3*) With any food or material when 1080 is strongly suspected and neg. test is obtained using C purification technic.

For chromatgc purification, follow **29.155** for sepg 1080 from other acids. Discard forerun, which may contain HOAc and other extraneous materials. Collect fraction large enough to contain all the 1080 as detd by preliminary detn. Ext fluoroacetic acid from eluate with 25 mL H_2O and enough alkali to cause aq. layer to retain alky after vigorous shaking (outside test paper). Drain org. layer and discard. Drain aq. layer into 125 mL erlenmeyer and aerate to remove $CHCl_3$. Pour soln into test tube and continue as above, beginning "Add 1 mL thiosalicylic acid soln . . ."

Refs.: JAOAC **32**, 788(1949); **33**, 608(1950); **34**, 827(1951); **37**, 581(1954).

CAS-144-49-0 (monofluroacetic acid)

Monofluoroacetic Acid Pesticide Residues

Quantitative Method

Final Action
(Sodium salt, "1080")

29.150 ***Principle***

After suitable sample prepn, acid is extd with ether and sepd from inorg. fluorides (partially ether-sol.) by partition chromatgy on silicic acid, using 0.5*N* H_2SO_4 as immobile solv. and $CHCl_3$ contg 10% *tert*-amyl alcohol or *n*-BuOH as mobile solv. Monofluoroacetic acid in eluate is converted to its Na salt, and quantity is detd by micro F detn, **25.081(a)**, **25.082**, and **25.083**.

29.151 ***Apparatus***

(a) *Chromatographic tubes.*—18 mm od × 250 mm long, prepd from Pyrex tubing.

(b) *Pressure source.*—Compressed air or cylinder of N or CO_2, and means of keeping pressure const. such as Hg column or diaphragm-type pressure regulator.

(c) *Mixer.*—High-speed blender.

29.152 ***Reagents***

(a) *Silicic acid.*—Mallinckrodt analytical reagent grade pptd powder, or equiv.

(b) *Mobile solvent.*—Add 100 mL *tert*-amyl alcohol or *n*-BuOH to 900 mL $CHCl_3$, and mix.

(c) *Phosphotungstic acid soln.*—Dissolve 20 g in H_2O and dil. to 100 mL.

29.153 ***Preparation of Sample***

This will vary with type of material. Dissolve sugars in H_2O, acidify with H_2SO_4, and ext directly. Following methods for different type materials will be suggestive. Simple H_2O wash may be adequate to prove contamination of certain foods.

(a) *Sugar.*—Dissolve 100 g sample in enough H_2O to give ca 350 mL.

(b) *Flour.*—Place 100 g sample in mixer, add 400 mL H_2O and 5 g *pancreatin*, and comminute ca 2 min. Adjust to pH 7–8, using *satd* $Na_2PO_4.12H_2O$ *soln* and suitable indicator paper. Transfer comminuted material to tared 1 L erlenmeyer, washing mixer with three 25 mL portions H_2O. Incubate mixt. ≥3 hr at 35–40°. Add 5 mL H_2SO_4 (1 + 1) and swirl. Add 20 mL phosphotungstic acid soln and swirl again. Dil. to 750 g with H_2O, stopper, and shake vigorously ca 2 min. Filter thru fluted paper or with suction thru buchner (16 cm size is convenient). Or, more quickly, centrf. and decant supernate. Use ≥375 g aliquot of filtrate. (Since sp gr of filtrate is very close to 1, measuring out aliquot in graduate is satisfactory.)

(c) *Wheat.*—Finely grind sample in suitable mill, such as Wiley mill. Proceed as in (**b**).

(d) *Corn meal.*—Proceed as in (**b**), except omit pancreatic digestion.

(e) *Corn.*—Grind sample and proceed as in (**d**).

(f) *Peanuts.*—Grind sample finely (like peanut butter) and proceed as in (**d**), except use 100 mL phosphotungstic acid soln. If necessary, refilter thru folded paper to remove oil.

(g) *Cheese.*—Proceed as in (**d**), except use 40 mL phosphotungstic acid soln.

(h) *Other foods such as chili peppers, cacao beans, etc.*—Treat in manner similar to one of preceding foods.

(i) *Biological tissue.*—If material is tough or fibrous, grind it twice thru food chopper. (Soft tissues, *e.g.*, brain and liver, need not be ground.) Place 100 g ground tissue in 800 mL beaker, add ca 300 mL H_2O, cover with watch glass, and boil gently ca 30 min. Transfer material to mixer, rinsing beaker with two 25 mL portions H_2O, and comminute thoroly (ca 2 min). Transfer comminuted material to tared 1 L erlenmeyer, rinsing mixer with two 25 mL portions H_2O. Add 5 mL H_2SO_4 (1 + 1) and mix. Add enough phosphotungstic acid soln (50–75 mL) to ppt all proteins, then H_2O to make 600 g. Shake vigorously ca 2 min, and filter thru fluted paper or with suction thru buchner. If material does not filter rapidly, return mixt. to flask, add ca 10 mL addnl phosphotungstic acid soln, shake vigorously, and refilter.

Alternative method.—Place 100 g ground tissue in mixer, add 300 mL H_2O and 15 g pancreatin, and comminute thoroly (ca 2 min).

Adjust to ca pH 8 with *satd $Na_3PO_4.12H_2O$ soln*, using suitable indicator paper. Transfer comminuted material from mixer to tared erlenmeyer, washing mixer with two 25 mL portions H_2O and incubate ca 3 hr at 35–40°. Ppt proteins and make to wt as directed previously.

29.154 ***Extraction***

Transfer soln (of sugar) or wt-aliquot of protein-free filtrate (of protein-contg materials) to 200 mL continuous extractor, Fig. **16:02**. (Tube is 115–120 cm long and 33–34 mm od, side arm, attached ca 63 cm from bottom, is 15–16 mm od; and inner tube is 12–13 mm od flared at top to ca 25 mm diam.; 1.5 L extractors of this type have been used successfully. Extra coarse fritted filter tip on bottom end of inner tube aids in getting smaller droplets of extg solv.) For each 50 g soln, add 1 mL H_2SO_4 (1+1). Ext with ether until all fluoroacetic acid has been extd (detd by preliminary experiment; usually 3–4 hr with 400 mL extractor). Transfer ether ext to separator of appropriate size.

To extn flask add ca 20 mL H_2O, 2 drops phthln, and enough 1.0*N* NaOH from buret to give strong alk. color of indicator after swirling. Pour rinse soln into separator and add addnl alkali until alk. color of indicator persists in aq. phase after vigorous shaking. Record vol. alkali required. Drain aq. layer into 100 mL beaker and wash ether with two 10 mL portions H_2O, rinsing extn flask each time with the H_2O before pouring it into separator. Add washings to beaker. Carefully adjust alky of ext just to alk. color of phthln with 0.1*N* H_2SO_4 and NaOH solns. Evap. neutzd ext to dryness on steam bath (current of air hastens evapn). If during evapn alk. color of indicator should disappear, add just enough 0.1*N* NaOH to give alk. color again. Do not continue heating after residue is apparently dry. Slightly moist residue is permissible.

29.155 ***Chromatography***

To 5 g silicic acid, (a), in mortar, add max. amt of 0.5*N* H_2SO_4 that it will hold without becoming sticky (50–80% of its wt). Mix well with pestle; then add ca 35 mL of the mobile solv. and work up into smooth slurry. (If SiO_2 agglomerates in solv., too much H_2SO_4 was used.) Place small cotton plug in bottom of chromatgc tube and pour in slurry, tilting tube slightly to avoid air bubbles. Let silicic acid pack down under 2–10 lb (14–69 kPa) pressure applied thru gas pressure regulator. When excess solv. has drained thru (column firm and viscous enough to resist pouring when tipped), column is ready for use. In prepg column take care to avoid cracking or drying out of the gel caused by leaving pressure on after column packs down and all solv. sinks into gel.

To dry or slightly moist residue in 100 mL beaker add enough H_2SO_4 (1+1) (ca 18*N*), usually 0.5–1.0 mL, to give excess of ca 0.25 mL over vol. necessary to convert all salts to free acid, as calcd from amt of 1*N* NaOH required to neutze acid extd by the ether. Wet salts *thoroly* with the acid, using small, narrow blade spatula (steel or Monel metal) to loosen salts from glass, and using flat-end glass rod to break up solid particles and mix resulting slurry. Add 5–10 g anhyd. granular Na_2SO_4 to take up excess liq. Stir well with tamping rod, breaking up any lumps. Add 10 mL mobile solv., (b), stir thoroly, and decant solv. carefully onto column.

Catch eluate in graduate. Apply pressure until all solv. sinks into gel; then release pressure. Add 5 mL mobile solv. to beaker and again stir thoroly. Carefully decant solv. onto column and, with aid of narrow-blade spatula, transfer bulk of material in beaker, mostly Na_2SO_4, to column. Renew pressure. When solv. passes ca halfway thru Na_2SO_4, release pressure. Rinse out beaker with addnl 5 mL solv. and transfer to column. After this washing sinks ca halfway into Na_2SO_4, fill tube with mobile solv. and complete collection, under pressure, of enough eluate to obtain all monofluoroacetic acid, as detd by test run on silicic acid used (ca 50 mL). Collect dropwise; 3–4 mL/min is convenient rate.

Transfer eluate to 125 mL separator; add ca 20 mL H_2O and enough 1.0*N* NaOH to give alk. color of phthln (phthln is present in eluate and no further addn is required) in aq. phase, after vigorous shaking. Drain aq. layer into 125 mL erlenmeyer and return solv. layer to separator. Wash solv. with two 10 mL portions H_2O and add washings to erlenmeyer. Aerate soln with current of air to remove traces of $CHCl_3$. (If excess $CHCl_3$ is not removed, excessive Cl may complicate F distn in next step.)

29.156 ***Determination***

Transfer aq. ext to Pt dish with little H_2O and mix with ca 20 mL lime suspension, **25.080(a)**, evap. to *dryness*, and ash 15–20 min at 600°. (Little C in ash will not interfere in detn.) Proceed as in **25.081(a)**, beginning "When clean ash is obtained, . . ." and **25.082–25.083** (100 mL Nessler tubes are preferable). Convert F results to fluoroacetic acid (×4.11) or to Na monofluoroacetate (1080) (×5.26) as desired, and correct for aliquot taken, if any, in extn. Ignore vol. occupied by insol. solids.

Refs.: JAOAC **32**, 788(1949); **33**, 608(1950); **34**, 827(1951); **37**, **581**(1954).

CAS-144-49-0 (monofluoroacetic acid)

Naphthyleneacetic Acid Pesticide Residues

Spectrophotometric Method

Final Action

(Applicable to apples and potatoes)

29.157 ***Apparatus***

(a) *Spectrophotometer.*—Cary 15 (current Model Cary 219, Varian Instruments), or equiv., with 5 cm cells.

(b) *Chromatographic tube.*—Glass, 22 mm id × 200 mm.

(c) *Food chopper.*—Hobart No. 84141 (Hobart Manufacturing Co., 711 Pennsylvania Ave, Troy, OH 45373), or equiv.

(d) *Blender cups.*—Stainless steel, 1 L capacity, with air-tight screw cover (Scientific Products, Inc. No. S8390) for high-speed blender.

29.158 ***Reagents***

(a) *Sodium phosphate soln.*—0.5*M*. 134 g $Na_2HPO_4.7H_2O$ or 70.5 g anhyd. salt/L.

(b) *Permanganate soln.*—0.02*M*. 31.6 g $KMnO_4$/L.

(c) *Florisil.*—60–100 mesh PR grade activated at 675° (1250°F) (Floridin Co.); use as received.

(d) *Naphthyleneacetic acid (NAA) soln.*—0.1 mg α-NAA/mL $CHCl_3$.

29.159 ***Extraction***

Chop sample in food chopper and transfer 200 g to blender cup. Add 20 mL 1*N* H_2SO_4 and 400 mL $CHCl_3$, screw top on blender, and blend 2 min at low speed. Pour mixt. into 500 mL centrf. bottle and centrf. 10 min at 1600 rpm. Take 200 mL aliquot from $CHCl_3$ layer.

29.160 ***Cleanup***

(a) *Apples.*—Place glass wool plug into chromatgc tube, add 10 cm Florisil, and top Florisil with glass wool plug. Transfer 200 mL $CHCl_3$ ext to column with min. amt $CHCl_3$. Rinse inside of tube twice with ca 5 mL $CHCl_3$. Elute column, in order, with 100 mL portions of CH_3CN, ether, NH_3-satd $CHCl_3$, and $CHCl_3$ and discard eluates. Using 500 mL separator as receiver, elute NAA with 100 mL 1% HOAc in $CHCl_3$ followed by 100 mL $CHCl_3$. Discard column, add 50 mL 1*N* H_2SO_4 to separator, and shake vigorously. Transfer

$CHCl_3$ layer to 250 mL separator contg 50 mL H_2O and shake vigorously. Transfer $CHCl_3$ layer to 250 mL separator contg exactly 50 mL 0.5*M* Na_2HPO_4, shake vigorously, and discard $CHCl_3$ layer.

(b) *Potatoes.*—Proceed as in (a). Add 2 mL 85% H_3PO_4 and 2 mL 0.02*M* $KMnO_4$ to separator contg Na_2HPO_4 phase, mix, and let stand exactly 5 min. Ext NAA with two 25 mL portions $CHCl_3$, transfer $CHCl_3$ exts to 125 mL separator contg exactly 50 mL 0.5*M* Na_2HPO_4, shake vigorously, and discard $CHCl_3$ layer.

29.161 ***Determination***

(a) *Apples.*—Transfer 1 mL NAA std soln to 125 mL separator, add exactly 50 mL 0.5*M* Na_2HPO_4 and 50 mL $CHCl_3$, and shake vigorously. Let layers sep. and discard $CHCl_3$ layer. Obtain UV spectra (230–330 nm) of cleaned up apple ext and NAA std ext, using 5 cm cells, against 0.5*M* Na_2HPO_4. Use peak at 283 nm to compare apple ext and NAA std ext, correcting for baseline *A*, and calc. ppm NAA present.

(b) *Potatoes.*—Transfer 1 mL NAA std soln to 125 mL separator, add 50 mL 0.5*M* Na_2HPO_4 and 50 mL $CHCl_3$, and shake vigorously. Let layers sep. and discard $CHCl_3$ layer. Add 2 mL 85% H_3PO_4 and 2 mL 0.02*M* $KMnO_4$ to separator, mix, and let stand exactly 5 min. Ext NAA with two 25 mL portions $CHCl_3$, transfer $CHCl_3$ exts to 125 mL separator contg exactly 50 mL 0.5*M* Na_2HPO_4, shake vigorously, let layers sep., and discard $CHCl_3$ layer. Obtain UV spectrum and calc. ppm NAA as in (a). (If there is excessive interference in sample spectra, repeat 5 min oxidn for both sample and std, beginning with "Add 2 mL 85% H_3PO_4 . . .")

Ref.: JAOAC **53**, 149(1970).

CAS-86-87-3 (naphthaleneacetic acid)

Nicotine Residues

Spectrophotometric Method

Final Action

(Applicable to apples, cabbage, and spinach)

29.162 ***Reagents***

(a) *Dilute hydrochloric acid.*—Approx. 0.05*N*. Dil. 4.1 mL HCl to 1 L.

(b) *Nicotine std solns.*—(*1*) *Stock soln.*—1 mg/mL. Dil. 100 mg nicotine (Eastman Kodak Co. No. 1242, or equiv.) to 100 mL in vol. flask with ca 0.05*N* HCl. (*Caution:* nicotine is very toxic.) (*2*) *Working soln.*—0.01 mg/mL. Pipet 1 mL stock soln into 100 mL vol. flask and dil. to vol. with ca 0.05*N* HCl.

(c) *Stripping soln.*—Dil. 20 mL NH_4OH to 2 L in vol. flask. Prep. at time of use.

Leafy Crops

29.163 ***Preparation of Sample***

(*Caution: See* **51.040**, **51.045**, and **51.056**.)

Weigh 500 g chopped sample (spinach, cabbage) into clean, dry jar (3–5 gal.; 11–20 L). Add 800 mL benzene, 200 mL $CHCl_3$, and 10 mL NH_4OH. Close, tumble or roll ca 10 min, and drain soln as completely as possible into 1 L beaker. Filter thru folded 38.5 cm paper into flask and proceed immediately with detn.

29.164 ***Determination***

Place 400 mL filtered soln in 500 mL separator. Add 25 mL ca 0.05*N* HCl and 2 mL HCl, and shake vigorously. Let phases sep. (ca 5 min) and drain lower layer into 250 mL separator. Swirl large separator, let stand ca 2 min, and drain any addnl ext into 250 mL separator. Repeat several times. Then ext soln with 25, 25, 15, and 10 mL portions ca 0.05*N* HCl, repeating swirling as above. Drain all acid exts into 250 mL separator. Make exts just alk. to litmus with 10% NaOH soln. Ext with two 50 mL and four 25 mL portions $CHCl_3$, combining exts in 250 mL separator.

Add 2 mL HCl to exts and make sure soln is acid to litmus. Ext with 25, 25, 20, 10, and 5 mL portions ca 0.05*N* HCl, combining all exts in short-stem 125 mL separator. Wash exts with 15 mL pet ether. Drain aq. layer into second 125 mL separator and wash pet ether with 5 mL ca 0.05*N* HCl, adding wash to combined acid soln. Ext soln with another 15 mL pet ether, drain aq. layer into 100 mL vol. flask, and wash pet ether with 5 mL ca 0.05*N* HCl. Drain acid into vol. flask and dil. to vol. with ca 0.05*N* HCl. Mix, pour portion into 50 mL beaker, and let stand 10–15 min. Det *A* at 236, 259, and 282 nm with ca 0.05*N* HCl as ref. Confirm presence of nicotine by reading at 2 nm intervals and plot absorption curve, or use recording spectrophtr. Det. *A* of std nicotine soln against ca 0.05*N* HCl as ref.

Waxy Crops

29.165 ***Preparation of Sample***

Weigh 2–2.5 kg apples into clean, dry, jar (3–5 gal.; 11–20 L). Add 1 L stripping soln, tumble or roll ca 10 min, and drain carefully into 1 L beaker. Filter thru folded 38.5 cm paper into flask and proceed immediately with detn.

29.166 ***Determination***

Place 400 mL filtered soln in 500 mL separator. Add 50 mL $CHCl_3$, invert separator back and forth gently ca 2 min, and let phases sep. Drain clear portion of ext into 250 mL separator. (With fruits, emulsions may form which are very hard to break. Break emulsions by drawing $CHCl_3$ layer into *dry* 125 mL separator and shaking vigorously. Separator must be dry.) Let phases sep. and drain clear portion into the 250 mL separator. Add 35 mL $CHCl_3$ to the 125 mL separator, shake gently, and drain into the 500 mL separator. Ext as above and combine clear ext in the 250 mL separator. Ext with 35, 35, and 10 mL $CHCl_3$, combining exts in the 250 mL separator. Add ≥1 mL HCl to exts until definitely acid to litmus. Then ext with three 15 mL portions ca 0.05*N* HCl, combining acid exts in a 125 mL separator. Wash the 250 mL separator with 10 mL ca 0.05*N* HCl after each extn and add to 125 mL separator used to break emulsions. Shake, but do not attempt to break any emulsions in this separator. Combine all acid exts in 125 mL separator and shake with 15 mL pet ether. Let stand ca 5 min and drain aq. layer into another 125 mL separator. Wash pet ether with 5 mL ca 0.05*N* HCl (do not shake vigorously) and add washings to separator. Repeat washing with 15 mL pet ether and drain aq. ext into 100 mL vol. flask. Wash pet ether as before, add washings to flask, and let stand 10–15 min. Dil. to vol. with ca 0.05*N* HCl and det. *A* at 236, 259, and 282 nm, against ca 0.05*N* HCl as ref. Confirm presence of nicotine as in **29.164**.

Take *A* of std soln as:

$$A_{std} = A'_{259} - 0.5(A'_{236} + A'_{282})$$

and *A* of sample soln as:

$$A_{samp.} = A_{259} - 0.5(A_{236} + A_{282})$$

Then:

$$\text{mg Nicotine} = (A_{samp.}/A_{std}) \times 2.5$$

Ref.: JAOAC **47**, 303(1964).

CAS-54-11-5 (nicotine)

★ Parathion Pesticide Residues ★

Colorimetric Method

Final Action

29.167 *See* **29.139–29.144**, 11th ed.

★ Surplus method—*see* inside front cover.

Piperonyl Butoxide Residues

Spectrophotometric Method

Final Action

(Applicable to Alaska peas, barley, hulled rice, oats, pinto beans, and wheat)

29.168 ***Principle***

Strong H_2SO_4 liberates HCHO, which is detd colorimetrically with chromotropic acid.

29.169 ***Reagents***

(a) *Chromotropic acid reagent.*—Dissolve 100 mg Na 1,8-dihydroxynaphthalene-3,6-disulfonate/mL of H_2O, filter, and keep in dark. Prep. daily. (1 mL required for each detn.)

(b) *Dilute sulfuric acid.*—Carefully mix 5 vols H_2SO_4 with 3 vols H_2O. Cool to room temp. and store in tight g-s container.

(c) *Methanolic potassium hydroxide.*—Dissolve 1.4 g KOH in 5 mL H_2O and add 95 mL MeOH (HCHO-free).

(d) *Methanol.*—If necessary, purify as follows: Reflux 1 L MeOH 1 hr with ca 10 g powd Al and ca 10 g NaOH and distil ca 800–900 mL.

(e) *Hexane.*—Redistd.

(f) *Chloroform.*—Reagent or redistd (for wheat extn).

(g) *Piperonyl butoxide std solns.*—*(1) Stock soln.*—1 mg/mL. Dissolve 0.1000 g in 100 mL benzene. *(2) Intermediate soln.*—100 μg/mL. Dil. 10 mL stock soln to 100 mL with benzene. *(3) Working soln.*—20 μg/mL. Dil. 20 mL intermediate soln to 100 mL with benzene.

29.170 ***Preparation of Standard Curve***

Add 0, 20, 40, 60, 80, and 100 μg piperonyl butoxide, resp., to each of 6 g-s test tubes (15 × 150 mm) (25–50 mL g-s centrf. tubes are also satisfactory) and evap. on steam bath with small air jet. Evap. last 1–2 mL benzene without heat.

Into each tube pipet both 1 mL chromotropic acid reagent and 5 mL dil. H_2SO_4, (b). Stopper loosely and place tubes in beaker of boiling H_2O 45 min, remove, and cool in beaker of cold H_2O. When cool, pipet 5 mL H_2O into each test tube, mix well, and read *A* in spectrophtr at 575 nm against reagent blank prepd similarly. Plot μg piperonyl butoxide against *A*.

29.171 ***Determination***

Ext 25 g sample with $CHCl_3$ in soxhlet app. or shake larger samples with suitable amts of $CHCl_3$ in centrf. bottle, centrf. and decant solv. Repeat extn once or twice according to sample size. Measure total vol. of exts. With current of air, evap. 25 mL (or suitable size aliquot) ext in small beaker just to dryness. Add 5 mL methanolic KOH. Warm gently just enough to melt wax (do not boil). Let stand 30 min, swirling vigorously at ca 10 min intervals. Transfer to small separator, rinse beaker with two 5 mL portions H_2O, and add to separator. Add 15 mL hexane to separator, shake vigorously 1 min, and let sep. Drain aq. layer and discard. Quant. transfer hexane layer to g-s test tube or centrf. tube and evap. to dryness with air jet. Small amt of heat may be used, but evap. last 1–2 mL with air alone. (Warmth of hand at this point is enough.)

Into dried residue pipet both 1 mL chromotropic acid reagent and 5 mL of the dil. H_2SO_4. Swirl vigorously to ensure that reagent contacts all of sample and place test tube in boiling H_2O bath. Stopper tube, lightly at first and then tighten. After 45 min in H_2O bath, remove, and cool to room temp. in beaker of cold H_2O. Pipet in 5 mL H_2O, mix well, and measure *A* in spectrophtr at 575 nm against reagent blank prepd similarly. From std curve, calc. piperonyl butoxide in aliquot.

Refs.: JAOAC **43,** 707(1960); **46,** 244(1963).

CAS-51-03-6 (piperonyl butoxide)

Table 29:04 Common Names and Chemical Names of Pesticides in Chapter 29

Common Name	Chemical Name
Aldrin	1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-*exo*-1,4-*endo*-5,8-dimethanonaphthalene, not less than 95%
Amitrole	3-Amino-1,2,4-triazole
Aroclor	Polychlorinated biphenyl compound
Azinphos-methyl	*O,O*-Dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl]phosphorodithioate
Benfluralin	*N*-Butyl-*N*-ethyl-*a,a,a*-trifluoro-2,6-dinitro-*p*-toluidine
BHC	1,2,3,4,5,6-Hexachlorocyclohexane (mixture of isomers)
Captan	*cis*-*N*-[(Trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide
Carbofuran	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate
Carbanolate	6-Chloro-3,4-xylyl-methylcarbamate
Carbaryl	1-Naphthyl *N*-methylcarbamate
Carbophenothion	*S*-[(*p*-Chlorophenylthio)methyl] *O,O*-diethyl phosphorodithioate
Chloramben	3-Amino-2,5-dichlorobenzoic acid
Chlordane	1,2,4,5,6,7,8,8-Octachlor-2,3,3a,4,7,7a-hexahydro-4,7-methanoindane
DDE	Dichlorodiphenyldichloroethylene
Diazinon	*O-O*-Diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothiote
Dichlone	2,3-Dichloro-1,4-naphthoquinone
Dieldrin	3,4,5,6,9,9-Hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphth(2,3-b)oxirene
Dodine	*n*-Dodecylguanidine acetate
Endosulfan	6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide
Endosulfan sulfate	1,4,5,6,7,7-Hexachloro-5-norbornene-2,3-dimethanol cyclic sulfate
Endrin	Hexachloroepoxyoctahydro-endo, endo-dimethanonaphthalene
EPN	*O*-Ethyl *O*-(4-nitrophenyl) phenylphosphonothioate
Ethion	*O,O,O,O*-Tetraethyl *S,S*-methylene bisphosphorodithioate
Ethylan	1,1-Dichloro-2,2-bis(4-ethylphenyl) ethane
Glyodin	2-Heptadecyl-2-imidazoline acetate
HCB	Hexachlorobenzene
Heptachlor	1,4,5,6,7,8,8-Heptachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanoindene
Heptachlor epoxide	Oxidation product of heptachlor
Lindane	Gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane (BHC)
Malathion	*O-O*-Dimethyl *S*-(1,2-dicarbethoxyethyl) phosphorodithioate
Maleic hydrazide	6-Hydroxy-3(2*H*)pyridazinone
Methoxychlor	2,2-Bis(*p*-methoxyphenyl)-1,1,1-trichloroethane
Methyl parathion	*O-O*-Dimethyl *O*-*p*-nitrophenyl phosphorothioate
Mirex	Dodecachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta(cd)pentalene
NAA	Naphthalene acetic acid
Nicotine	3-(1-Methyl-2-pyrrolidyl) pyridine
Oxychlor epoxide (also oxychlordane)	1-*exo*,2-*endo*-4,5,6,7,8,8-Octachloro-2,3-*exo*-epoxy-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene
Paraoxon	*O-O*-Diethyl *O*-*p*-nitrophenyl phosphate
Parathion (same as ethyl parathion)	*O,O*-Diethyl *O*-*p*-nitrophenyl phosphorothioate
PCBs	Some mixture of chlorinated biphenyl compounds having various percentages of chlorine
Piperonyl butoxide	α-[2-(2-Butoxyethoxy)ethoxy]-4,5-methylenedioxy-2-propyltoluene
Propoxur	2-(1-Methylethoxy) phenol methylcarbamate
Ronnel	*O,O*-Dimethyl *O*-(2,4,5-trichlorophenyl)phosphorothioate
TDE	1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethane
Tetrasul	4-Chlorophenyl 2,4,5-trichlorophenyl sulfide
Thiram	Bis(dimethylthiocarbamoyl) disulfide

Thiram Pesticide Residues

Spectrophotometric Method

Final Action

(Applicable to corn, apples, tomatoes, strawberries, celery, and similar fruits and vegetables)

29.172 ***Principle***

Thiram is extd from sample with $CHCl_3$. Treatment with solid CuI results in formation of brown, $CHCl_3$-sol. Cu dimethyldithiocarbamate, and its *A* is measured at 440 nm. Other commonly used pesticides do not interfere, with exception of metal dithiocarbamates sol. in $CHCl_3$, such as ferbam or ziram. Moderate amts of color, waxes, and other extd plant matter do not interfere.

29.173 ***Reagents***

(a) *Chloroform.*—Either reagent or tech. grade may be used.

(b) *Thiram std solns.*—*(1) Stock soln.*—500 μg/mL. Dissolve 50.0 mg thiram (available from E. I. du Pont de Nemours & Co., Biochemicals Dept., 1007 Market St, Wilmington, DE 19898) in $CHCl_3$ and dil. to 100 mL with $CHCl_3$. *(2) Working soln.*—25 μg/mL. Dil. 5 mL stock soln to 100 mL with $CHCl_3$.

(c) *Cuprous iodide.*—If not available, prep. as follows: To soln of 10 g $CuSO_4.5H_2O$ in ca 100 mL H_2O, slowly add excess of KI soln. Remove liberated I_2 by adding $Na_2S_2O_3$ soln in slight excess. Filter, and wash ppt thoroly with H_2O and with alcohol. Dry at room temp. and crush to fine powder.

(d) *Attapulgus clay.*—Available from MC/B Manufacturing Chemists, No. AX1799.

29.174 ***Apparatus***

(a) *Spectrophotometer.*—Suitable for measuring *A* in UV and at 440 nm.

(b) *Glassware.*—Avoid contamination by rinsing with $CHCl_3$ and drying before use. Rinse app. that may have contained CuI from previous detns with dil. acid, H_2O, alcohol, and $CHCl_3$.

29.175 ***Preparation of Standard Curve***

(To minimize errors due to evapn of solv., keep flasks closed as much as possible, and cover funnels with watch glasses during filtrations.)

Using buret, add 2.0, 5.0, 10.0, and 15.0 mL working std soln to 25 mL vol. flasks. Dil. to vol. with $CHCl_3$, and mix. Solns contain 2, 5, 10, and 15 μg thiram/mL, resp.

Transfer ca 10 mL portions of std solns to 125 mL g-s erlenmeyers, add 10 mg CuI to each, stopper, and let stand 1 hr with occasional mixing. Filter, using 9 cm quant. paper, and read *A* at 440 nm against $CHCl_3$ as ref. Plot *A* against thiram concn in μg/mL.

29.176 ***Isolation***

(Avoid contact of solv. with rubber.)

(a) *Corn.*—Ext 200 g by shaking with 100 mL $CHCl_3$ 5 min in 500 mL g-s erlenmeyer. Decant ext thru small funnel (to retain corn kernels) into flask.

(b) *Apples, pears, and similar firm fruits.*—Weigh 2–3 kg into clean, dry jar (ca 3 gal.; 11 L). Add 500 mL $CHCl_3$ and stopper with tight-fitting cork, wooden bung, or plastic screw cap faced with gasket of sheet cork or other suitable solv.-resisting material. Ext 5 min by tumbling or other agitation. Decant ext into flask.

(c) *Tomatoes, berries, and similar soft fruits and vegetables.*—Weigh 1–3 kg into suitable container. Add 500 mL $CHCl_3$ and stopper with solv.-resisting closure. Ext 5 min by gentle shaking and decant into g-s erlenmeyer thru loose plug of glass wool.

(d) *Celery.*—Cut 2–3 kg into 3–8 cm pieces. Mix thoroly and ext 500 g sample with 500 mL $CHCl_3$ as above.

Add anhyd. Na_2SO_4, ca 5 g/100 mL, to decanted ext. Stopper flask, shake 5 min, and filter thru folded Whatman No. 12, or equiv., paper.

29.177 ***Determination***

(Thiram in $CHCl_3$ soln, particularly in presence of plant extractives, may decompose. Make detns as soon as possible.)

Transfer ca 10 mL filtered ext to g-s erlenmeyer and develop color as in **29.175**, beginning ". . . add 10 mg CuI . . ." As ref., use another portion of filtered ext., untreated with CuI. From std curve, obtain thiram concn in μg/mL. If developed color is too intense, dil. with $CHCl_3$, making similar diln of ref. ext. and multiply thiram value found by appropriate diln factor.

ppm Thiram = (μg thiram/mL) × mL $CHCl_3$ used for extn/g sample.

29.178 ***Qualitative Test***

Adjust concn of ext, **29.176**, if necessary, to 10–15 μg thiram/mL by evapn on steam bath or by diln with $CHCl_3$. Add 0.25–1.0 g Attapulgus clay, depending on color of ext, to 50 mL of adjusted ext in beaker. Mix well and filter thru Whatman No. 12 folded paper, or equiv. Transfer 25 mL filtrate to g-s erlenmeyer, add 0.2 mL ca 0.1*N* $AgNO_3$ to ppt thiram and other $CHCl_3$-sol. dithiocarbamates, stopper, and shake vigorously 30 sec. Add ca 1 g anhyd. Na_2SO_4 and shake 30 sec. Let settle, decant carefully into 1 cm quartz cell, and *use as ref. soln*, adjusting to 0 *A* at 350 nm. Det. UV absorption curve over range 250–350 nm on clarified and filtered ext untreated with $AgNO_3$. Thiram gives curve with plateau at 270–283 nm, dropping sharply after peaking at ca 283. Ferbam and ziram give characteristic curves distinguishable from thiram.

Refs.: JAOAC **42**, 545(1959); **45**, 410(1962).

CAS-137-26-8 (thiram)

SPECIAL REFERENCES

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