parative blanks are 0.05 as against 0.13 p.p.m. and 0.08 as against about 8.00 p.p.m.

The recoveries from all the crops are satisfactory for the routine determination of residue hazards and replicates fall within ±5% of the mean, where the added concentration of schradan approaches toxicological significance. It would be satisfactory in such routine work to multiply observed results by a factor of 1.5, to get a measure of the schradan residue present in the crop.

The rather low recoveries obtained with lemons are probably explained by the lemon oil's acting as a relatively involatile solvent for schradan, thus preventing the efficient volatilization of the schradan onto the cold finger. Thus, if this method is to be completely satisfactory, plant-derived solvents of schradan must be removed before the distillation stage. This might be achieved by a preliminary extraction of the clarified aqueous macerate with petroleum ether or benzene, in which schradan is not very soluble, and from which the small proportion extracted can easily be removed by a back-wash with water.

The technique is obviously applicable to a number of other compounds of limited volatility, though compounds of much lower volatility than schradan are not likely to distill rapidly enough. It has been used successfully for the determination of mipafox (diisopropyl phosphorodiamidic fluoride) residues the heating bath temperature being 70° C. Compounds much more volatile are not easily separated by this technique, as re-evaporation from the condensing surface will lead to losses. This could be prevented by the use of a cooling system which keeps the surface at a lower temperature—solid carbon dioxide–acetone, for instance—or separation may be performed with a carrier (*8*).


### Summary

Both maceration of schradan-treated crops with water, followed by chloroform extraction of the clarified aqueous macerate, and reflux extraction with boiling chloroform are satisfactory methods of recovering the insecticide for residue assay. In a method for the determination of residues in a number of crops, the insecticide is separated from natural products by microdistillation. Recoveries and blanks are satisfactory.



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### Literature Cited


(1) Bennett, S. H., Thomas, W. D. E., 1st Radioisotope Technique Conference, vol. **1**, p. 439, H. M. Stationery Office, London, 1953; and private communication.

(2) Berenblum, J., Chain, E., *Biochem J.* **32,** 295 (1938).

(3) David, A., Hartley, G. S., Heath, D. F., Pound, D. W., *J. Sci. Food Agr.* **1,** 310 (1951).

(4) David, W. A. L., *Ann. Appl. Biol.* **38,** 508 (1951).

(5) *Ibid.*, **39,** 203 (1952).

(6) Dubois, K. P., Coon, J. M., *Arch. Ind. Hyg. Toxicol.* **6,** 9 (1952).

(7) Dubois, K. P., Doull, J., Coon, J. M., *J. Pharmacol. and Exptl. Therap.* **99,** 376 (1950).

(8) Dupée, L. F., Heath, D. F., Otter, I. K. H., J. Ag. Food Chem. **4,** 233 (1956).

(9) Hall, S. A., Stohlmann, J. W., III, Schechter, M. S., *Anal. Chem.* **23,** 1866 (1951).

(10) Heath, D. F., Casapieri, P., *Trans. Faraday Soc.* **47,** 1093 (1951).

(11) Heath, D. F., Lane, D. W. J., Llewellyn, Margaret, *J. Sci. Food Agr.* **3,** 60 (1952).

(12) Heath, D. F., Lane, D. W. J., Park, P. O., *Phil. Trans.* (**B**) **239,** 191 (1955).

(13) Klein, G., ed., "Handbuch der Pflanzenanalyse," vol. **I,** p. 317, J. Springer, Vienna, 1931.

(14) Otter, I. K. H., *Mikrochim. Acta*, in press.



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**PESTICIDE RESIDUES**

# Determining Traces of Tetramethylphosphorodiamidic Fluoride (Dimefox) in Crops


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**A satisfactory method for the determination of dimefox residues in food crops must achieve a sensitivity of 0.1 p.p.m. or less. The most general method consists of macerating a 50-gram sample of the crop with water, filtering, extracting with chloroform, evaporating the chloroform to a low bulk, transferring to a microdistillation apparatus, and distilling in the presence of a few drops of glycerol-glycol mixture. The dimefox in the distillate is estimated as phosphate by the method of Berenblum and Chain. An abbreviated version can sometimes be used. The second method, used for oily crops, consists of distilling a macerate in oil and separating the dimefox from interfering compounds in the oily distillate. Satisfactorily low blanks were obtained on 15 crops, and satisfactory recoveries on 10 crops further investigated.**


Dimefox, $(Me_2N)_2POF$, commercially available under the trade name Hanane, is a systemic insecticide (*2, 4, 7, 10*) highly toxic to mammals (*3, 5*).

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Work which shows that dimefox is decomposed in plants to harmless products is being prepared for publication. The hazard in consuming treated crops therefore comes from their content of undecomposed dimefox. As tolerable residues are still subject to controversy, a target sensitivity of 0.1 p.p.m. has been adopted

No highly specific group reactions have yet been discovered for any organophosphorus systemic insecticide. Dimefox must therefore be estimated by separating it from natural interfering substances, decomposing it, and de-

termining a component atom or group. Estimation as phosphate, by the method of Berenblum and Chain (*1*), has been chosen. The sensitivity of this method is 0.02 p.p.m. of dimefox on a 50-gram sample, which is about ten times more sensitive than the alternative estimation as dimethylamine which has been used by Hall, Stohlmann, and Schechter (*6*) for schradan. An additional reason for estimating as phosphate is that most systemics contain phosphorus, but few contain dimethylamine.

For a given crop the variation in the blank derived from an untreated control sample is about the same as the average blank, so that blanks must be reduced below 0.1 p.p.m. if the target sensitivity is to be reached. The general method described in this paper for separating dimefox from natural products is by distillation in a carrier solvent under reduced pressure; dimefox is apparently stable in the presence of plant materials at temperatures below about 180° C.

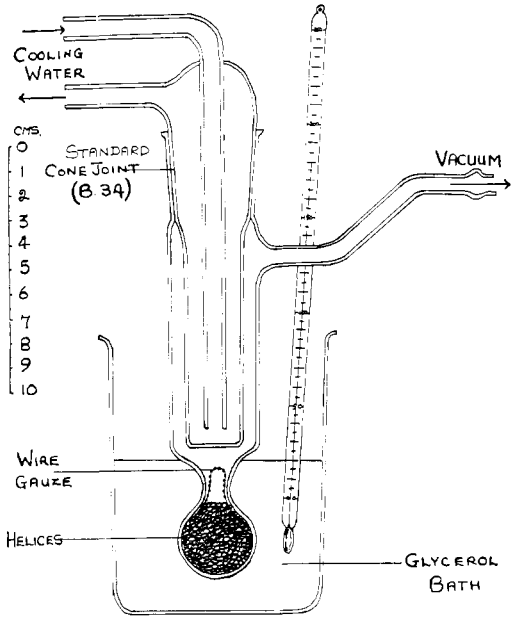


**Figure 1. Microdistillation apparatus for dimefox**

By choosing a carrier solvent with a somewhat higher boiling point than dimefox, such as glycerol or Shell Risella oil 17, it is possible to hold back natural phosphorus compounds, while providing a diluent for the dimefox; this prevents its re-evaporation from the condensing surface. It is not, however, usually adequate just to macerate the plant sample with the carrier and distill, as under this treatment many plant materials decompose to give colored distillates, the distillate frequently froths uncontrollable, and the blanks are sometimes not low enough. These difficulties can be overcome by first extracting the dimefox from a filtered aqueous macerate of the plant by chloroform. By methods described (*9*) it was shown that macerating the crop in water leads to almost complete extraction of dimefox into the aqueous solution. The insecticide is then partitioned into chloroform (the partition coefficient for chloroform-water is 17 to 1), and the chloroform extract is concentrated. Glycerol-glycol as carrier is added to the residue, and the mixture is distilled. In certain instances, the preliminary separation can be omitted.

The aqueous macerates of some very oily crops, of which cocoa beans are an important example, are not easily extracted by chloroform, as the emulsions formed can be broken only with difficulty. The preliminary extraction has therefore to be omitted and the distillate from a macerate in Shell Risella 17 oil is treated to obtain low blanks. The dimefox is extracted from the oil by water, and the water is washed with petroleum ether, which extracts very little dimefox but most of the interfering compounds.

These two methods yield aqueous solutions from crops treated with pure dimefox in which dimefox is the only solute giving a positive reaction for phosphorus after hydrolysis. Commercial dimefox, however, contains a few per cent of nontoxic hexamethylphosphoramide, which is nearly as extractable as dimefox by chloroform (its chloroform-water partition coefficient is 6.7 to 1) and which boils at a temperature only about 40° C. higher. It is, however, much more stable to acid hydrolysis (*8*). Consequently it can be separated from dimefox by hydrolyzing the mixture with dilute perchloric acid under standard conditions and extracting the unhydrolyzed hexamethylphosphoramide with chloroform. The phosphorus of the dimefox remains in aqueous solution as phosphoric and fluorophosphoric acids.

Further acid hydrolysis converts any fluorophosphoric acid to phosphoric acid, which is then determined colorimetrically.

**Method 1** This is the general method suitable for all but a few very oily crops.

**Apparatus and Chemicals.** The microdistillation apparatus is shown in Figure 1. A macerator, centrifuge, condenser, water pump, glycerol bath, 4-mm. glass helices, and thermostat (25° ± 2° C.) are needed. Chemicals used include 1*N* sodium hydroxide, technical chloroform, glycerol of C.P. or equivalent grade, ethylene glycol of analytical reagent or equivalent grade, and 1*N* perchloric acid. [The apparatus used for estimation of schradan (*9*) is not satisfactory for dimefox.]

**Procedure.** Macerate a 50-gram sample with 100 ml. of water, filter, and wash the residue with water. Dilute the clear macerate to 150 ml. with water, add sufficient 1*N* sodium hydroxide to raise the pH to 8 to 10, and extract three times with 50 ml. of chloroform. Break any emulsions by centrifuging. Clarify by filtration and concentrate the bulked extracts to about 25 ml.

Transfer the chloroform concentrate to the outer container of the microdistillation apparatus, close the top with a stopper, and connect the side arm to a condenser. Distill off the chloroform, allowing some reflux, until the residue is nearly dry. With practice it is possible to arrange that all the residue is in the bulb of the container. Remove the stopper, add 0.25 ml. of glycerol, 2 drops of ethylene glycol, and sufficient helices to fill the lower bulb of the apparatus, and insert a metal clip in the neck. This acts as a splash head during subsequent operations. Remove the last traces of chloroform. Introduce the cooling finger, immerse the bulb of the apparatus in the glycerol bath, apply a vacuum of 15 mm. of mercury, and raise the temperature steadily to 180° C. in 10 minutes. Remove from the glycerol bath, release the vacuum, and allow to cool. Carefully remove the cold finger onto which the glycerol, ethylene glycol, and dimefox have been distilled. Wash the distillate from the finger into a beaker with a little distilled water, to obtain the dimefox in aqueous solution.

Add 4 ml. of 1*N* perchloric acid per 10 ml. of aqueous solution, shake, and keep at 25° ± 2° C. for 30 minutes. Extract three times with an equal volume of chloroform. Transfer the aqueous layer to a small Kjeldahl flask.

To the aqueous layer in the Kjeldahl flask add 4 ml. of 1*N* perchloric acid per 10 ml. of aqueous solution, and 10 drops of nitric acid. Evaporate to 1 to 2 ml., which serves to hydrolyze fluorophosphoric acid to phosphoric acid. Continue evaporation to the appearance of brown fumes, cool, add 5 ml. of water, and evaporate again. Cool, and continue with the development and photometric determination of the phosphomolybdate complex (*9*).

**Method 1, Abbreviated Version** This method is shorter than that given above. It is satisfactory for pineapples, but unsatisfactory for potatoes and cocoa beans.

**Apparatus and Chemicals.** Necessary apparatus includes a macerator, vacuum distillation apparatus with capillary leak, 500-ml. distilling flask, 25-cm. helices-filled fractionating column, and a short Liebig condenser. Glycerol, C.P. grade or equivalent, is used.

**Procedure.** Macerate a 50-gram sample with 150 ml. of glycerol. Transfer to the distilling flask and fractionate at 15 to 20 mm. of mercury pressure. Continue distillation until about 10 ml. of distillate have been collected. The hexamethylphosphoramide is separated

and dimefox estimated as phosphate in this distillate as in Method 1. The distillate consists mainly of water from the sample, and the glycerol does not interfere.

**Method 2** This method is used for oily crops, and especially for the determination of dimefox in cocoa beans.

**Apparatus and Chemicals.** The apparatus required includes a macerator, vacuum distillation apparatus with capillary leak, 3-liter long-necked distilling flask, efficient splash head, and Liebig condenser. Shell Risella oil 17, petroleum ether, boiling points 40° to 60° C., 5*N* sodium hydroxide, analytical grade chloroform, and 1*N* perchloric acid are used.

**Procedure.** Macerate a 50-gram sample in 150 to 200 ml. of Shell Risella oil 17. Transfer to the distilling flask, and distill under a pressure of 15 to 20 mm. of mercury with a Bunsen burner or 450-watt heating mantle, regulated so that the water distills over rapidly. Take special care during the expulsion of the last traces of water, as considerable frothing may occur. The risk of carry-over may be greatly reduced by heating the flask judiciously near the surface of the boiling liquid. When 30 to 40 ml. of oil have distilled over, release the vacuum and wash the condenser down with a little water, avoiding any sublimate in the upper part of the condenser.

Transfer the total distillate, oil and water, to a separating funnel, washing in with the least quantity of water. Shake vigorously, and allow to settle. Run off the aqueous layer, and wash the oil with a further 10 ml. of water. Wash the water layer and washings with an equal volume of petroleum ether. Wash the petroleum ether extract with 10 ml. of water. Combine the washings with the aqueous layer, add 1 ml. of 5*N* sodium hydroxide, and extract immediately with 20 ml. of chloroform and then twice with 10 ml. of chloroform. The first extraction must be carried out within 2 minutes of making the solution alkaline to avoid hydrolyzing significant quantities of dimefox. Combine the chloroform layers, add 10 ml. of water, and boil off the chloroform. Continue with separation from hexamethylphosphoramide and photometric estimation, as in Method 1.

### Blanks on Control Crops

The first criterion of a method is that the blanks from control crops be below 0.1 p.p.m. The blanks obtained experimentally are given in Table I.

The blanks are satisfactorily low, except for new potatoes. Method 2 gives markedly lower blanks on cocoa beans than Method 1, and is also faster. Although Method 2 was used for Brussels sprouts, results on schradan suggest that Method 1 would also work (*9*).

### Recovery Tests

Recovery tests as normally carried out consist of adding known quantities of compound to the crop at some early stage of the analysis (*9*). Thus, in Method 1, dimefox is added to the water used for maceration, and to the glycerol and oil, respectively, at the maceration stage in the abbreviated Method 1 and Method 2. This assumes that extraction from the crop into the liquid phase of the macerate is complete. This has been justified for Method 1, and is likely to be true for the abbreviated version of that method. The solubility of dimefox in the oil derived from cocoa beans is relatively low. Therefore the efficiency of the preliminary extraction must be proved for Method 2.

Unripe pods were left for 3 days with their stems in a dilute solution of radioactive dimefox, containing about 2% hexamethylphosphoramide impurity. The pods were then opened and the beans removed and divided into a number of similar samples with a riffle sampler. Four 50-gram samples and three 25-gram samples were obtained. The four 50-gram samples were analyzed by Method 2, except that no separation from hexamethylphosphoramide was performed, and the phosphorus was estimated by counting the phosphorus-32. One 25-gram sample was macerated with 400 ml. of 0.5*M* phosphate buffer, and refluxed for 1 hour. Two 25-gram samples were macerated with water. All three were filtered, and the radioactivity of the filtrates was determined. The refluxed sample contained radioactivity equivalent to 4.64 p.p.m. of dimefox in a 50-gram sample of beans; the macerated samples contained 4.01 and 4.03, respectively. All three were then extracted with chloroform, and the chloroform extracts were concentrated and counted.

As the counts were low, because most of the radioactive compounds were not extractable by chloroform, the chloroform extracts were bulked, further concentrated, and counted. The dimefox and hexamethylphosphoramide found was 0.046 p.p.m. on the original sample, compared with 0.043, 0.043, 0.044, and 0.045 p.p.m. for the four samples treated by Method 2. The error on the counts was less than 0.002 p.p.m. in each determination. Repeat experiments using unripe beans containing more dimefox showed that Method 2 normally gave results about 5% lower than those obtained by refluxing.

As ripe beans do not take up dimefox through the cut stems of the pods, some were soaked in a 0.1% solution of radioactive dimefox, and dried. One 50-gram sample was extracted by refluxing an aqueous macerate, a second was analyzed by Method 2, and the third was analyzed by Method 2 after the beans had been moistened with water and left in a closed jar overnight. These methods gave 6.9, 4.2, and 6.1 p.p.m. of dimefox, respectively. Several recovery tests were also performed on ripe beans in which dimefox was added to the oil (see Table II). The recoveries were about 75%. It therefore appears that about 25% is decomposed during distillation. It is concluded that extracting beans by distillation of a sample left in contact with moisture overnight is as efficient as extracting by refluxing a macerate in water. Distilling beans without moistening may, however, lead to low recoveries.

The results of recovery tests performed are given in Table II. Recovery tests

**Table I. Blanks on Untreated Crops**

| Crop | Method[a] | Blanks Estimated as P.P.M. Dimefox |
|---|---|---|
| Potatoes (stored) | 1 | 0.01, 0.01 |
| Potatoes (new) | 1 | 0.10, 0.20 |
| Pineapples | 1 abbv. | 0.01, 0.04, 0.02 |
| Oranges (fruit) | 1 | 0.005, 0.015 (100-gram samples) |
| Sugar mangolds (roots) | 1 | 0.04, 0.03, 0.05, 0.02, 0.03, 0.03 |
| Sugar mangolds (foliage) | 1 | 0.02 |
| Mangolds (roots) | 1 | 0.06 or less (10 samples) |
| Mangolds (foliage) | 1 | 0.02, 0.05 |
| Cocoa beans | 1 | 0.09, 0.07, 0.08 |
| | 2 | <0.02 (10 samples) |
| Cocoa yams (tubers) | 2 | 0.09, 0.08 |
| Cocoa yams (leaves) | 2 | 0.07, 0.08, 0.10, 0.11 |
| Bush yams (tubers) | 2 | 0.04, 0.08, 0.07, 0.10 |
| Wheat | 2 | <0.02 (1 sample) |
| Brussels sprouts | 2 | <0.02 (1 sample) |
| Coffee beans | 2 | <0.02 (2 samples) |

[a] In practice some modifications were made to the preliminary extractions used in Method 1, in attempts to shorten centrifugation. Thus oranges were pulverized with Solid $CO_2$ and chloroform and the product was warmed to volatilize the carbon dioxide. Mangold and sugar mangold roots were cut up fine, and extracted with chloroform for 2 hours in a Soxhlet apparatus. The results (*9*) indicate that these methods are likely to be as effective for extracting dimefox as Method 1. Method 1 as given in the text is practicable for these crops, but may take a little longer.

**Table II. Recovery of Dimefox from Crops**

| Crop | Method | Dimefox, P.P.M. Added | Dimefox, P.P.M. Recovered | % Recovery |
|---|---|---|---|---|
| Potatoes (stored) | 1 | 0.75 | 0.57 | 76 |
| Potatoes (new) | 1 | 1.41 | 1.03 | 72 |
| Pineapples | 1 abbv. | 1.50 | 1.38 | 93 |
| Oranges (fruit) | 1 | 0.30 | 0.21, 0.22, 0.23 | 73 |
| Sugar mangolds (roots) | 1 | 0.95 | 0.92, 0.89 | 85 |
| | | 0.39 | 0.36, 0.37 | 94 |
| Mangolds (roots) | 1 | 0.93 | 0.69 | 80 |
| | | 0.67 | 0.59, 0.62 | 90 |
| | | 0.37 | 0.33, 0.35 | 92 |
| Cocoa beans | 2 | 200 | 144.0, 150.0, 150.0, 136.0 | 73 |
| | | 1.40 | 1.06, 1.06, 1.00 | 74 |
| | | 1.00 | 0.81, 0.76, 0.82, 0.68 | 77 |
| | | 0.20 | 0.20 | 100 |
| Coffee beans | 2 | 0.25 | 0.17 | 68 |
| | | 0.10 | 0.10 | 100 |
| | | 0.05 | 0.04 | 80 |
| Brussels sprouts | 2 | 1.00 | 0.73, 0.70 | 71 |

The accuracy of the final determination of phosphate limits the accuracy of any method to ±0.02 p.p.m. In only one instance are replicates not within ±5% of the mean.

have not been performed on all crops for which blanks were obtained.

Recoveries from different crops are probably significantly different. To obtain the accuracy required for research purposes, it would be necessary to find the percentage recovery more accurately than has been attempted here. It appears from the good reproducibility that this should not prove difficult. The authors have, however, used these methods only to assess the hazards to consumers of the crops. For this, high accuracy is not required, but it is essential that the results quoted should not be low. Therefore a recovery of 67% can be assumed, and the results multiplied by 1.5 will give an estimate of the dimefox. This is justified by the results in the table; no result is significantly below 70% and only one is significantly above 90%.

The methods described give satisfactory and reproducible recoveries and satisfactorily low blanks. The method which starts with macerating the sample in oil or glycerol does not, however, seem to be generally applicable, several crops giving high blanks if they are treated in this way. Distillation from oil is the least satisfactory in practice, because it is difficult to stop carry-over from the distilling flask to the condenser, and frothing is always a serious problem. This method may eventually be superseded entirely by methods using other solvents, but it is at present the best method for oily crops.


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### Literature Cited


(1) Berenblum, J., Chain, E., *Biochem. J.* **32,** 295 (1938).
(2) Bond, J. A. B., *Bull. Entomol. Research* **44,** 97 (1953).
(3) British Intelligence Objectives Sub-Committee, D.S.I.R. London, Final Rept. **1095,** 48 (1948).
(4) David, W. A. L., *Ann. Appl. Biol.* **39,** 203 (1952).
(5) Dubois, D., Coon, J. M., *Arch. Ind. Hyg. Toxicol.* **6,** 1 (July 1952).
(6) Hall, S. A., Stohlmann, J. W., III, Schechter, M. S., *Anal. Chem.* **23,** 1866 (1952).
(7) Hanna, A. D., Heatherington, W., Judenko, E., *Nature (London)* **169,** 334 (1952).
(8) Heath, D. F., Casapieri, P., *Trans. Faraday Soc.* **47,** 1093 (1951).
(9) Heath, D. F., Cleugh, Joan, Otter, I. K. H., Park, P. O., J. AGR. FOOD CHEM. **4,** 230 (1956).
(10) West, J., Report Cocoa Conf., p. 86, Cocoa, Chocolate, and Confectionary Alliance, Ltd., London, 1951.



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**PESTICIDE RESIDUES**

# Residual Properties of the Systemic Insecticide *O,O*-Dimethyl 1-Carbomethoxy-1-propen-2-yl Phosphate


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SUBSTITUTED-VINYL PHOSPHATES have been of considerable current interest to both entomologists and chemists because of their high insecticidal activity (*2, 5–7, 10, 11, 14, 20, 25–27, 32, 33, 36–38*). Their adaptability as insecticides may be somewhat limited by a mammalian toxicity in the range of parathion (*22–24, 27, 32*), whereas other organophosphate insecticides of lower mammalian toxicity have been recently introduced (*12, 13*). However, their very high insecticidal toxicity and the almost unique systemic properties of certain of these compounds may lead to commercially feasible substituted-vinyl phosphate insecticides.

*O,O*-Dimethyl 1-carbomethoxy-1-propen-2-yl phosphate (Compound OS-2046, Shell Development Co., Denver, Colo.) (*38*) has shown promise as a short residual systemic insecticide. Its high biological activity was first noted by Corey and others (*11*), and excellent systemic, contact, and fumigant insecticidal properties were demonstrated. Translocation studies reported by the same workers (*10*) indicated that the toxicant entered the plant to the full extent in the early minutes after application and was rapidly translocated throughout the plant. They proposed that the significant reduction in residues within 24 hours following application was a function of the volatility of the compound.

The studies reported here utilized bioassay, antiesterase, radiotracer, and chromatographic determinations to evaluate further the residual properties of compound 2046.