# RESIDUE REVIEWS

## VOLUME 11

# RESIDUE REVIEWS

RESIDUES OF PESTICIDES AND OTHER
FOREIGN CHEMICALS IN FOODS AND FEEDS

## RÜCKSTANDS-BERICHTE

RÜCKSTÄNDE VON PESTICIDEN UND ANDEREN
FREMDSTOFFEN IN NAHRUNGS- UND FUTTERMITTELN

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# Preface

That residues of pesticide and other "foreign" chemicals in foodstuffs are of concern to everyone everywhere is amply attested by the reception accorded previous volumes of "Residue Reviews" and by the gratifying enthusiasm, sincerity, and efforts shown by all the individuals from whom manuscripts have been solicited. Despite much propaganda to the contrary, there can never be any serious question that pest-control chemicals and food-additive chemicals are essential to adequate food production, manufacture, marketing, and storage, yet without continuing surveillance and intelligent control some of those that persist in our foodstuffs could at times conceivably endanger the public health. Ensuring safety-in-use of these many chemicals is a dynamic challenge, for established ones are continually being displaced by newly developed ones more acceptable to food technologists, pharmacologists, toxicologists, and changing pest-control requirements in progressive food-producing economies.

These matters are also of genuine concern to increasing numbers of governmental agencies and legislative bodies around the world, for some of these chemicals have resulted in a few mishaps from improper use. Adequate safety-in-use evaluations of any of these chemicals persisting into our foodstuffs are not simple matters, and they incorporate the considered judgments of many individuals highly trained in a variety of complex biological, chemical, food technological, medical, pharmacological, and toxicological disciplines.

It is hoped that "Residue Reviews" will continue to serve as an integrating factor both in focusing attention upon those many residue matters requiring further attention and in collating for variously trained readers present knowledge in specific important areas of residue and related endeavors; no other single publication attempts to serve these broad purposes. The contents of this and previous volumes of "Residue Reviews" illustrate these objectives. Since manuscripts are published in the order in which they are received in final form, it may seem that some important aspects of residue analytical chemistry, biochemistry, human and animal medicine, legislation, pharmacology, physiology, regulation, and toxicology are being neglected; to the contrary, these apparent omissions are recognized, and some pertinent manuscripts are in preparation. However, the field is so large and the interests in it are so varied that the editor and the Advisory Board earnestly solicit suggestions of topics and authors to help make this international book-series even more useful and informative.

"Residue Reviews" attempts to provide concise, critical reviews of timely advances, philosophy, and significant areas of accomplished or needed endeavor in the total field of residues of these chemicals in foods, in

feeds, and in transformed food products. These reviews are either general or specific, but properly they may lie in the domains of analytical chemistry and its methodology, biochemistry, human and animal medicine, legislation, pharmacology, physiology, regulation, and toxicology; certain affairs in the realm of food technology concerned specifically with pesticide and other food-additive problems are also appropriate subject matter. The justification for the preparation of any review for this book-series is that it deals with some aspect of the many real problems arising from the presence of residues of "foreign" chemicals in foodstuffs. Thus, manuscripts may encompass those matters, in any country, which are involved in allowing pesticide and other plant-protecting chemicals to be used safely in producing, storing, and shipping crops. Added plant or animal pest-control chemicals or their metabolites that may persist into meat and other edible animal products (milk and milk products, eggs, etc.) are also residues and are within this scope. The so-called food additives (substances deliberately added to foods for flavor, odor, appearance, etc., as well as those inadvertently added during manufacture, packaging, distribution, storage, etc.) are also considered suitable review material.

Manuscripts are normally contributed by invitation, and may be in English, French, or German. Preliminary communication with the editor is necessary before volunteered reviews are submitted in manuscript form.

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September 10, 1965

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# The application of thin-layer chromatographic techniques to the analysis of pesticide residues

By

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With 11 figures

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## I. Introduction

The introduction of paper chromatography by CONSDEN *et al.* (1944) as an extension of the partition chromatographic technique of MARTIN and SYNGE (1941) was followed by a very rapid expansion of the use of these

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procedures. The advantages of paper chromatography in particular, e.g., small sample size, excellent resolution, and short times involved, over earlier separatory methods were so great that the accompanying disadvantages tended to be overlooked. While being admirably suitable for amino acids and hydrophilic compounds in general, many difficulties arose when lipophilic compounds were studied. These were only partially overcome by reversed-phase paper chromatography and although the classical adsorption chromatography introduced by Tswett (1906 a and b) worked very well for materials of this nature, no general analytical technique was available.

The use by Izmailov and Shraiber (1938) of a thin (two mm.) layer of powdered adsorbent, such as calcium oxide, magnesium oxide, or alumina, to obtain chromatographic zones from a single drop of a solution of a drug by dropwise application of a mobile solvent, passed almost unremarked. Crowe's (1941) use of adsorbent layers contained in the indentations of a spot-test plate or on a sloping petri dish also attracted little attention. The description by Williams (1946) of circular chromatograms produced on layers of adsorbent maintained between two parallel glass plates, one of which was pierced to allow the entrance of both sample and developing solvent, however, appears to have borne more fruit. Mottier and Potterat (1952 a) used similar "discs" of alumina for the chromatographic examination of oil-soluble dyestuffs. Later (Mottier and Potterat 1955, Mottier 1958) they introduced a spreading technique for the preparation of layers of alumina on glass plates which could be developed by either ascending or descending chromatography. Such loose-layers are very readily prepared and rapidly developed.

Thin-layer chromatography as it is recognised today can be said to have begun with the work of Meinhard and Hall (1949) who also followed Williams (1946) but who used starch as a binding agent in order to endow the adsorbent layers with some mechanical stability. Application of this method to the preparation of "chromatostrips" (0.5×5.25 inches) by Kirchner *et al.* (1951) demonstrated the usefulness of thin-layer chromatography for the separation of terpenoid compounds. They employed either starch or gypsum as binding agent and found the strips were easier to prepare and handle than the silica-impregnated filter paper they had previously used (Kirchner and Keller 1950). Kirchner and Miller (1953) also introduced the use of "chromatoplates" of larger size and these were used by Reitsama (1954) in a study of essential oils. The thin-layer chromatography of pesticide residues also commenced at this time with the determination by Kirchner *et al.* (1954) of biphenyl in citrus fruits following a cleanup separation on starch-bound silica gel chromatostrips.

With the publication of the series of papers by Stahl (1956, 1958 a and b, 1959 a and b) the uses of thin-layer chromatography became more widely recognised and readily applied. The commercial availability of the materials and apparatus described by Stahl (1959 b) for the production of gypsum-bound layers of silica gel, alumina, and kieselguhr was a big factor in promoting this growth. Today thin-layer chromatography is regarded as a necessary adjunct to all fields of analytical chemistry, not solely for its

diagnostic properties but also in the purification of precious specimens, for cleanup purposes before the application of other analytical methods of determination, in the study of rates of reaction, etc. The number of publications describing various aspects of thin-layer chromatography has risen exponentially over the past few years while five books on the subject have appeared within two years (BOBBITT 1963, HASHIMOTO 1962, RANDERATH 1962, STAHL 1962, TRUTER 1963). Application of the technique to pesticide residue analysis has lagged behind usage in other fields somewhat, but the appearance of about 20 publications on this aspect in 1964 as against only two in 1960 gives an indication of what may be yet to come.

## II. Procedures

### *a) Preparation of chromatoplates*

**1. Layering apparatus and techniques.** — Thin-layer chromatography depends for its success on the ability to prepare reproducibly uniform layers of the desired thickness of a suitable adsorbent upon the chosen supporting plate. While glass carrier plates are by far the most popular for this purpose, aluminium (SNYDER 1963), stainless steel (CONNORS and BOAK 1964), and plastic materials (SQUIBB 1963 a) have also been used for special purposes. The dimensions of the carrier plates are in general governed by the type of spreading apparatus to be used; 20×20 cm. and 10×20 cm. are popular sizes for use with commercial applicators but any convenient size may be suitable for hand layering.

The apparatus commercially available may be readily divided into two classes: 1) moving spreader, stationary plate, and 2) stationary spreader, moving plate. All methods depend upon placing on the carrier plate a slurry of the adsorbent in water, alcohol, or some other volatile liquid and smoothing it out to form a layer of the required thickness by some suitable means. The "moving speader" type of apparatus is of wide versatility and is easily used by unskilled hands to prepare satisfactory chromatoplates. The apparatus designed by STAHL (1959 b) and manufactured by Desaga G.m.b.H., Heidelberg [available from Camlab (Glass) Ltd., Cambridge] is universally used for the preparation of layers up to 2 mm. thick, and some 2000 sq. cm. in area can be layered in one operation. This apparatus has already been described in an earlier paper in this series by CONKIN (1964) and a very detailed account of its use for the preparation of chromatoplates for pesticide residue analysis has been given by KOVACS (1963). One slight disadvantage of this apparatus is that the thickness of the carrier plates to be sequentially layered must be very uniform if smooth operation is to be achieved. Variations in surface level as the spreader is drawn across the plates causes "jumping" to occur with consequent irregularity of the layer thickness. An apparatus based on a similar principle is available from Shandon Scientific Co. Ltd., London, which endeavours to overcome this drawback. The template on which the carrier plates are laid consists of an

inflated plastic bag which presses them against two rails, thus ensuring that the uppermost surfaces are level (SAS 1964). BADINGS (1964) has described an applicator which may be employed with plates of differing widths.

Apparatus belonging to the "stationary spreader" category is in general less expensive than the forms described above. The original apparatus described by MILLER and KIRCHNER (1954) belongs to this group but the best known design is probably that of MUTTER and HOFSTETTER as adapted by WOLLISH *et al.* (1961). This is manufactured by Camag A. G., Muttenz, Switzerland and is available from Griffin and George Ltd., Middlesex. Similar forms of this type of apparatus have been decribed by SAS (1964). With each apparatus the layers are spread by pushing the carrier plates slowly and smoothly beneath a hopper containing the slurry. The hopper is constructed of two fixed sides, the separation of which decides the width of the plate, into which are slotted two adjustable metal plates. The nearmost of these is free to ride upon the carrier plate surface and thus forms an effective seal; the front plate may be set to a given height above the carrier plate, usually determined with the aid of feeler gauges, and thus smooths out the slurry as the carrier plates are pushed through. The length of plate which can be spread may be varied at will and slight variations in plate thickness are immaterial. If many plates are to be prepared, the assistance of a second operator may be useful to remove the layered plates as they emerge from the apparatus.

Many simpler and inexpensive ways of preparing layered plates have been described, the majority being based on "moving spreader" techniques. For occasional use glass rods with suitable spacers either mounted on the rod (ČERNÝ *et al.* 1961, HERMANEK *et al.* 1961) or on the plate (DUNCAN 1962, LEES and DE MURIA 1962) may be quite suitable. Simple spreaders have also been described by BARBIER *et al.* (1959), MACHATA (1960), and VAN DAM and MAAS (1964). With certain binder-free adsorbents, layers have been prepared (BHANDARI *et al.* 1962) by making the slurry with ethyl alcohol or ethyl acetate, pouring just sufficient to obtain the desired thickness onto the plate and distributing it evenly by careful tilting before allowing the plate to dry in a horizontal position. The use of ribbed or riffled glass has been described by GAMP *et al.* (1962); window glass of this type is readily available with three sizes of rib, 1/2 inch, 1 inch, and 2 inches. Plates are prepared simply by pouring the slurry onto the plate and drawing a rule across the raised ribs of the glass thus smoothing out the layer in the intervening channels. The thickness of the layer is, of course, decided by the depth of the channel. HANSBURY *et al.* (1963) have given instructions for the preparation of such riffled plates under conditions whereby the width, depth, and cross-section of the channels may be controlled. Similar plates are also commercially available [Camlab (Glass) Ltd., Cambridge]. For pesticide residue analysis plates of this type have proved more valuable for the simple, rapid preparation of loose-layer chromatoplates (ABBOTT *et al.* 1965 c and d). The "glass rod with spacers" technique is also suitable for preparing loose-layers as described by DAVIDEK and BLATTNA (1962) and EDWARDS (1964); a simple method was also described by MOTTIER and POTTERAT (1955).

A dipping procedure is especially suitable for obtaining layers on microscope slides or lantern slides which are useful for quick spot tests or preliminary tests of many mobile solvents. PEIFER (1962) prepared slurries of silica gel, containing gypsum as binder, in chloroform or a 2+1 chloroform : methanol mixture. The slides were dipped into this slurry in pairs, back to back, the thickness of the layer coating the dipped slides depending upon the consistency of the dispersion. After evaporation of the solvent the layered slides were exposed to steam to render the binding agent effective and ensure good adhesion. This technique is unsuitable for larger plates. Dilute slurries of adsorbent have been sprayed onto the carrier plates (REITSAMA 1954, METCHE *et al.* 1963, BEKERSKY 1963), layer thickness being decided by the number of "passes" made by the spray gun across the plate. These systems have the advantage of readily allowing the use of plates of various sizes and thicknesses indiscriminately. Smooth layers are obtained in this way but the point-to-point variation in thickness is liable to be greater than that given by a correctly prepared plate made by using a moving spreader.

Whichever method of layering the plate is used, utmost cleanliness of the surface to be coated is essential, a trace of grease being sufficient to ruin the uniformity of the layer. The plates should be washed in hot water containing either soap or a non-ionic detergent and rinsed well with deionised water. Immediately before coating with the adsorbent it is advisable to wipe over the surface with a lintless tissue moistened with ethanol in order to remove any inadvertent fingerprint or deposited material. Cleanliness of the spreading apparatus is also required: the presence of a little dried adsorbent on the spreading edge can cause havoc to the layer surface.

**2. Adsorbents and binding agents.** — Any adsorbent which is used for columnar separations can be adapted for use as a thin layer or "open-column". KIRCHNER *et al.* (1951) investigated layers of silica gel, alumina, magnesium oxide, calcium hydroxide, dicalcium phosphate, bentonite, calcium and magnesium carbonates, Florisil, Filtrol, talc, and starch, usually with the aid of five percent of starch or 20 percent of plaster of Paris added as a binder. Of the many possible materials, silica gel and alumina have since proved to be of widest applicability, with appreciable usage of kieselguhr and cellulose also. All of these materials are available from several manufacturers in various forms prepared especially for thin-layer chromatography. The adsorbents silica gel *G*, alumina *G*, and kieselguhr *G* "nach STAHL" available from Merck A. G., Darmstadt, were the first to appear and contributed greatly to the spread of thin-layer chromatographic techniques; the terminal "G" signifies the inclusion of gypsum (13 to 15 percent) as a binder. Adsorbent materials are now available with and without binders, and with and without additional built-in fluorescent compounds as indicators. The proportions of adsorbent to water required to produce a suitable slurry for layering vary a great deal from product to product; for silica gel *G* and alumina *G*, one part of adsorbent to two parts of water is used, while for some binder-free adsorbents almost equal proportions are advised. When trying any new adsorbent the manufacturer's recommended

proportions should be first tried and then adapted in the light of experience to suit the layering technique in use.

Starch and gypsum remain the two most widely used binding agents. Starch-bound plates give the greatest resistance to abrasion, so much so that the surface is suitable for writing on in pencil; gypsum, however, is usually the binder of choice for organic microanalysis, including pesticide residue determinations, since it does not limit the selection of visualization reagents as does starch. Using gypsum, care must be taken over timing the layering process since the mixture is only sufficiently fluid for up to four minutes. Polyvinyl alcohol has also been suggested as a binder (Onoe 1952) but it has not been adopted to any extent, while carboxymethylcellulose has recently been recommended (Obreiter and Stowe 1964). By careful selection and grading of the particle size of the adsorbent it has been possible to prepare layers which adhere well without the addition of any binding agents (Woelm, Eschwege; Macherey, Nagel & Co., Düren); frosted glass surfaces aid this occurrence (Kabara *et al.* 1964).

Other adsorbents of more recent introduction include dextrangel, DEAE-Sephadex, and acetylated cellulose (Determann *et al.* 1963), microcrystalline cellulose (Wolfrom *et al.* 1964), ion-exchange materials (Randerath 1961), polyamides (Davidek and Davídková 1961), and basic zinc carbonate (Badings 1964). Finely ground porous glass has been found to be preferable to silica gel *G* or alumina *G* for some separations (Kramer *et al.* 1964). The choice of stationary phases for thin-layer chromatography has been discussed by Schorn (1964) and reviewed by Mangold (1961) and Russell (1963).

For pesticide residue analysis, silica gel *G* has been most often chosen, with some use of alumina *G*. Mixtures of these two materials with kieselguhr *G* have been found very useful in adjusting the adsorbent properties of the layer in order to obtain the degree of separation and $R_f$ value required (Bancher *et al.* 1963, Prey *et al.* 1963, Abbott *et al.* 1964 a, Abbott *et al.* 1965 b). Modified silica gel layers have also been prepared by using solutions of buffers for slurry preparation (Stahl 1959 a, Petrowitz 1962, Braithwaite 1963, Teichert *et al.* 1960 a and b). On acidic layers the $R_f$ values of acids are increased relative to those given on layers of neutral material, while on a basic chromatoplate these compounds will be strongly held near to the point of application; the reverse is of course true of basic compounds. Alteration of layer conditions in this way sometimes makes possible separations which are difficult to achieve on unbuffered surfaces. Similar results may be obtained by including acids or bases in the developing solvent when using neutral chromatoplates; in this way layering processes are simplified since the inclusion of buffer materials may affect the stability or setting time of the slurry.

**3. Reactivation of the layer.** — Following the spreading of the adsorbent slurry over the carrier plates, these are usually left in a horizontal position at ambient temperature until the surface takes on a matt appearance (ten to 20 minutes). The manner and degree of reactivation then required will depend upon the adsorbent and the purpose for which the chromatoplates are required. The one important fact is that a definite procedure must be

chosen and adhered to if consistent results are to be obtained. KELEMEN and PATAKI (1963) found that chromatoplates which had been allowed to dry in air overnight at room temperature yielded more consistent $R_f$ values than those which had been activated by heating at 110° C. or 120° C. for 30 minutes, although the latter plates showed higher adsorptive activities. Layers which have been allowed to dry slowly probably possess a more uniform structure and thickness; oven-activation of overnight-dried plates probably offers the best combination of consistency and activation.

It has been shown (DHONT and DE ROOY 1961) that during the heating of silica gel at 110° C. the activity at first falls, being at a minimum after about 15 minutes, and then rises to a maximum value at about 30 minutes, remaining constant thereafter. Alumina requires higher temperatures in order to reach its maximum adsorptive power; BÄUMLER and RIPPSTEIN (1961) heated for four hours at 200° to 220° C. to obtain an activity equivalent to Brockman II. At these temperatures, however, the binding power of gypsum tends to fail, dehydration to the hemihydrate beginning at about 120° C. while formation of the anhydrous salt occurs over the range 150° to 200° C. For these reasons it is preferable to activate at 120° C. when using alumina *G* although consistency of activation is not as good as that given by silica gel *G* at this temperature. For maximum activity of alumina chromatoplates, binder-free material should be employed together with heat treatment for at least four hours at 250° C.

Since the activity of the layer is dependent upon its moisture content, the reactivated chromatoplate must be protected from water vapour and other readily adsorbed vapours. They may be stored in a desiccator over silica gel, or Brockman grade I alumina where appropriate. Alternatively, storage in an oven at the temperature of activation until required, followed by cooling under desiccant conditions prior to spotting, may be preferred. In either case plates should be used within 36 hours of preparation for consistent results.

### *b) Preparation and application of sample extract*

**1. Extraction and cleanup.** — Thin-layer chromatographic procedures are, in general, much more tolerant of coextracted materials than are paper or gas-liquid chromatographic systems. The intrinsic cleanup properties of the layer materials most frequently employed, silica gel and alumina, often render minimal the amount of purification of the extract required. MORLEY and CHIBA (1964) have proposed a method for the direct thin-layer chromatography of organochlorine pesticide residues in some plant extracts without prior cleanup, samples showing possible pesticide content then being further examined by gas-liquid chromatography. This system simplifies the procedure greatly, eliminates some possible sources of pesticidal loss and extraneous contamination, and allows the rapid screening of a large number of samples. Unfortunately its application is rather limited although the introduction of an acetonitrile-hexane partition procedure as a cleanup improved the chromatoplate background and allowed the method to be used for a wider range of compounds. A similarly direct procedure has been

proposed for the detection of carbaryl (Sevin)[1] and 1-naphthol residues at the 0.02 p.p.m. level in apples and lettuce (CHIBA and MORLEY 1964).

For samples of vegetable origin the extraction and cleanup procedure of GOODWIN *et al.* (1961) is often suitable when organochlorine pesticides are under examination. Extraction with acetone followed by partition into hexane, after adding sodium sulphate solution, yields a solution sufficiently clean for gas-liquid or paper chromatography and ideally suited for application to a thin-layer chromatoplate. When examining samples of waxy vegetables or ripe fruit in this way, the extracts are not suitable for the former two techniques but may still be used for thin-layer chromatography. Organophosphorus pesticide residues may be suitably extracted from vegetable tissue with a mixture of ethyl methyl ketone and hexane (3 + 2 v/v) (EGAN *et al.* 1964 and ABBOTT *et al.* 1965 b). Dinitrophenol herbicides have similarly been extracted with 3 + 2 ethyl methyl ketone : ether (ABBOTT and THOMSON 1964 b).

The extraction and cleanup procedure used by LAWS and WEBLEY (1961) for organophosphorus pesticides is also suitable for thin-layer chromatographic purposes and partially classifies the compounds; a somewhat similar procedure was employed by BLINN (1963). Carbamates and their metabolites have been extracted from samples of animal and vegetable origin by means of a chloroform-acetonitrile procedure, Florisil columns being used for cleanup (DOROUGH and CASIDA 1964).

For the efficient extraction of pesticides from fatty materials a solvent such as hexane is essential. This, of course, also dissolves a considerable amount of the fat and although thin-layer chromatography is fairly tolerant of vegetable coextractives, fatty or waxy materials may affect the observed $R_f$ value of the chromatographed pesticide; usually a reduction of this value is noticed. Organochlorine pesticide residues after extraction from animal produce have been cleaned up for gas-liquid chromatographic examination by means of a dimethylformamide (DMF) partition process, followed by passage through an alumina or magnesia column (DE FAUBERT MAUNDER *et al.* 1964 a). A similar cleanup process is suitable for thin-layer chromatographic purposes but the columnar cleanup stage may usually be omitted. Similar partition processes using acetonitrile (JONES and RIDDICK 1952) and dimethylsulphoxide (HAENNI *et al.* 1962) have also been used for this purpose.

FISCHER and KLINGELHÖLLER (1961 a and b) showed an original approach to the extraction and cleanup problem. They extracted their samples with alcoholic potash, thereby saponifying both the fatty materials present and the organophosphorus pesticides under examination. The latter were then identified by the thin-layer chromatographic patterns given by the fragments, up to four spots being observed from some compounds.

In general it is the total load of material that is to be applied to the chromatoplate at one point that governs the thickness of the layer required for efficient chromatographic cleanup. For clean extracts the usual layer thickness of about 250 $\mu$ is adequate; for preparative and cleanup purposes

[1] See Table XII for the chemical identification of pesticides mentioned in text.

500 $\mu$ or one mm. thickness is more usual as will be described later. The amount of previous cleanup required may therefore be governed, in part, by the availability of layering apparatus capable of producing layers of various thickness.

When leaves which had been sprayed with Colep were washed with benzene to remove residues, the amount of natural material simultaneously removed by this simple process was sufficient to reduce the $R_f$ values on chromatoplates by 0.05 to 0.1 units (CONKIN 1964). Possible interferences of this kind by such coextractives may be countered in two ways. The addition of a dyestuff of similar migratory properties can introduce an internal standard by means of which corrected $R_f$ values may be calculated, it being assumed that the pesticide and the dye are held back proportionately by the interfering materials. Butter yellow, Sudan red, and Sudan yellow have been found to be suitable marker dyestuffs for use with pesticidal compounds, particularly the organochlorine class. DHONT and DE ROOY (1961) quote results in terms of an "$R_B$" value where this figure represents the ratio of the distances travelled by the compound and by Butter yellow. The "$R_B$" value was found to be less variable than the more usual $R_f$ value. The alternative possibility requires that the extract be divided into two equal portions, to one of which is added a known specimen of the suspected pesticide. Following development and visualization in an appropriate manner the presence of single spot in the "loaded" sample tends to confirm the identity of the compound, while the appearance of two spots shows their dissimilarity.

**2. Application of the sample extract.** — In order to obtain accurate and reproducible thin-layer chromatograms a few simple rules must be observed during the application of the sample extract to the adsorbent layer. The nature of the solvent used for the final solution of the extract to be applied to the plate is very important. In order to ensure that the size of the spots shall be as compact as possible, the chosen solvent should be of as low a polarity as is consistent with good solubility of the pesticide. This is particularly important in the case of very dilute solutions when it may be necessary to apply the extract repeatedly to one spot in order to ensure sufficient material for clear visualization. Such "over-spotting" is liable to induce radial chromatography at the origin where polar solvents are involved. The solvent should also be readily volatile yet not of so low a boiling point that standard solutions cannot be maintained in constant concentration or that evaporation, with consequent deposition of the dissolved material, occurs while within the applicator. Solvents with boiling points in the range 40° to 60° C. are usually preferred whenever possible.

The sample solution may be applied to the layer surface by gently touching it with a filled calibrated capillary or micropipette or by means of a microsyringe of suitable capacity; volumes of the order of one to five microlitres ($\mu$l.) per spot are preferred. To aid in the accurate alignment of a series of spots across the chromatoplate, a suitably drilled template, usually constructed of a transparent plastic material, is often used as a guide and to protect the surface of the layer. The line of spots so applied should be about 15 to 20 mm. from one edge of the carrier plate and

parallel to it; individual spots being at least one cm. apart. The line should also be perpendicular to the direction of spreading to avoid possible unevenness in thickness across the layer. Care must be taken not to penetrate the layer with the tip of the applicator or irregular shaped spots are liable to result on developing the chromatogram (TRUTER 1963). In order to maintain the desired compactness ($<$ 0.5 cm.) of the origin spots, MANGOLD (1961) advised the use of a stream of nitrogen to ensure rapid evaporation of the solvent from the plate, while MILLER and KIRCHNER (1954) employed a low-temperature hotplate for the same purpose.

RITTER and MEYER (1962) have mechanised the operation of spotting the samples onto the layer. They designed a syringe which traverses the chromatoplate, applying controlled doses of the solution as required. MORGAN (1962) produced a multiple capillary device for the simultaneous application of many solutions to a single chromatoplate. As alternative modes of spotting, METZ (1962) has used a system of elution from a filter paper triangle, while TATE and BISHOP (1962) described the use of a wire loop such as is employed for bacteriological purposes.

Although the application of circular spots is most often recommended, HONEGGER (1962 a) and WAGNER *et al.* (1961) advocated that the sample should be applied as a thin band, about one cm. long. Techniques of this nature are especially suitable for extracts of pesticide residues which have not undergone a very thorough cleanup process and in which the ratio of coextractives to pesticide is rather high. The simplest way to do this is to use a calibrated micro-syringe (50 $\mu$l. to one ml. capacity) and to depress the plunger gently and evenly whilst moving the tip of the needle across the plate and just above its surface. Care must be taken to avoid cutting into the layer and thus forming a barrier to the developing mobile solvent. Sample extracts may be applied to loose-layer chromatoplates in any of the above mentioned ways. Any gross "holing" of the layer may be remedied by the careful addition of a little of the adsorbent (TAYLOR and FISHWICK 1964).

While sample extracts are usually applied to a prepared, re-activated chromatoplate immediately before development with the chosen mobile phase, in certain cases it is possible, and indeed advisable, to give a further heat treatment after the sample has been applied to the plate. In this way the maximum activity, and thus the greatest cleanup capacity, of the adsorbent is ensured. A procedure of this type has been described for the cleanup of dinoseb extracts (ABBOTT and THOMSON 1964 b).

The load of material which may be successfully applied to the chromatoplate is limited by the layer thickness on one hand and by the sensitivity of the visualization system on the other. Generally speaking, amounts of pesticide in the range 0.5 to ten micrograms ($\mu$g.) may be chromatographed on 250 $\mu$. thick layers to give clearly defined regular spots with little or no streaking. Where larger amounts are encountered it is advisable to dilute the extract further and to repeat the chromatogram. Variable $R_f$ values have been observed where overloading of the adsorptive capacity of the layer has occurred (BRENNER and NIEDERWIESER 1960). In the case of purely preparative thin-layer chromatography, however, this factor is frequently

less important than in diagnostic work and large loads may then be safely used.

### *c) Development techniques*

Thin-layer chromatoplates are normally developed by means of an ascending mobile solvent, the plate being held in an approximately vertical position. Descending-solvent chromatographic techniques are reserved for the separation of substances of low $R_f$ values, extended runs being obtained by allowing excess solvent to drip from the bottom of the chromatoplate (STANLEY and VANNIER 1957). Horizontal development may also be used but since a wick of some sort is required to maintain the supply of the mobile solvent, this mode is somewhat difficult to apply. STAHL (1958 b) has described an apparatus for radial chromatography by horizontal development, a procedure which may prove useful in giving better resolution of materials which are incompletely resolved by the more usual linear chromatographic procedure. The apparatus of BRENNER and NIEDERWIESER (1961) was designed for continuous-flow horizontal development. A similar apparatus is available commercially as the *B-N* Chamber (Desaga G.m.b.H., Heidelberg) which is also suitable for descending development. Near-horizontal development is of course essential for loose-layer chromatoplates, the maximum allowable inclination being about 15° to 20°.

There are many factors governing the successful development of a thin-layer chromatoplate. To ensure even running of the solvent front it is advisable to remove adsorbent from both side edges of the plate, leaving about five mm. of clear carrier plate. If many samples are spotted closely across the plate it may also be necessary to rule channels between each pair of adjacent spots in order to ensure that no cross contamination can occur should a sample contain an unduly large amount of pesticide.

The mobile phase chosen for the development will depend upon the nature of the separation required, and also upon the activity of the adsorbent material. Separation systems which have been used for pesticidal compounds are described in section III of this review. When dealing with a new or unknown compound, preliminary tests should be made using an active adsorbent together with a non-polar solvent such as hexane. Successive small proportions of a polar solvent may then be added as necessary to obtain the required $R_f$ value or degree of resolution if a mixture is being studied. Microscope slides bearing layers produced by a spray technique are very suitable for tests of this nature.

Development by the ascending-solvent technique is obtained by placing the prepared chromatoplate, bearing the sample spots, into a tank containing the chosen solvent in such a way that the origin line is parallel to the solvent surface and about one cm. above it, i.e., a depth of immersion of about 0.5 to one cm. is obtained. The tank should then be sealed by some suitable means, a well-fitting, ground glass cover being frequently employed. Development is then usually allowed to proceed at ambient temperature until the solvent front has traversed a fixed distance or has taken a certain time. It is frequently recommended that the tank should be lined with filter

paper dipping into the mobile solvent and should be left to stand for at least one hour before inserting the chromatoplate. Lining the tank in this way serves to ensure that the solvent and its vapour quickly reach equilibrium within the confines of the chamber, a state which assists in maintaining a level solvent front across the layer (STAHL 1959 b). When unlined tanks are used, a longer period of equilibration is necessary but similar results are eventually obtained. MANGOLD (1961) has observed that chromatography in lined tanks requires a more polar mobile solvent than that needed to obtain similar separation in unlined tanks and also that separations may not be as sharp. Similar results have been observed with pesticidal compounds as is illustrated in Tables I and II. Where several

Table I. *$R_f$ values of some organochlorine pesticides in tanks of different sizes. Silica gel G chromatoplates developed in hexane for 30 minutes*

| Pesticide | $R_f \times 100$ | | | | | |
|---|---|---|---|---|---|---|
| | A[a] | | B[b] | | C[c] | |
| | Unlined | Lined | Unlined | Lined | Unlined | Lined |
| *p,p'*-DDE ..... | 59 | 45 | 65 | 45 | 73 | 41 |
| *p,p'*-DDT . . . | 39 | 31 | 43 | 31 | 52 | 28 |
| Endosulfan *A* . | 15 | 8 | 30 | 8 | 34 | 7 |
| Heptachlor. . . | 58 | 41 | 64 | 4 | 76 | 40 |
| Methoxychlor . | 3 | 2 | 8 | 2 | 12 | 2 |
| ,*p'*-TDE . . . | 22 | 16 | 24 | 17 | 29 | 14 |
| Distance travelled by solvent front (cm.) . . . . | 15.3 | 14.5 | 12.7 | 14.9 | 10.7 | 15.1 |

[a] Tank 21×21×6 cm.
[b] Tank 22×21×9.5 cm.
[c] Tank 28×26.5×21 cm.

Table II. *$R_f$ values of some organophosphorus pesticides in tanks of different sizes. Silica gel G chromatoplates developed in 9+1 hexane: acetone for 40 minutes* (ABBOTT *et al.* 1965 b)

| Pesticide | $R_f \times 100$ | | | | | |
|---|---|---|---|---|---|---|
| | A[a] | | *B*[b] | | C[c] | |
| | Unlined | Lined | Unlined | Lined | Unlined | Lined |
| Azinphos methyl | 10 | 10 | 14 | 12 | 15 | 14 |
| Carbophenothion | 60 | 57 | 71 | 59 | 82 | 66 |
| Malathion . . . | 22 | 24 | 27 | 25 | 35 | 33 |
| Parathion . . . | 40 | 38 | 43 | 39 | 58 | 47 |
| Phorate . . . . | 66 | 61 | 76 | 61 | 87 | 67 |
| Phosphamidon . | 37 | 37 | 42 | 38 | 56 | 49 |
| Distance travelled by solvent front (cm.) . . . . | 13.7 | 15.3 | 12.4 | 13.6 | 10.0 | 13.2 |

[a] Tank 21×21×6 cm.
[b] Tank 22×21×9.5 cm.
[c] Tank 28×26.5×21 cm.

plates are to be developed simultaneously, BRENNER and NIEDERWIESER (1960) have suggested that it is necessary to fasten a filter paper to the back of each plate. In order to maintain equilibrium conditions all plates must be inserted into or removed from the development chamber at one time.

The time required for the full development of the chromatogram depends on several factors. It is proportional to the distance to be travelled by the solvent front which in turn is dependent upon the thickness of the layer, the atmosphere in the tank, the solvent used, and the angle at which the plate is held. The rate of migration of the solvent front tends to be inversely proportional to its distance from the surface of the mobile phase. BANCHER *et al.* (1963) found that an ascent of six cm. could be achieved within six to ten minutes which was sufficient to obtain useful separations. An ascent of ten cm. has been generally chosen (STAHL 1958 a, WALDI *et al.* 1961, KOVACS 1963) as giving good resolution within an acceptably short time. It is sometimes recommended that a line be scored across the layer at a distance of ten cm. from the origin in order to stop development proceeding further; some spotting templates are provided with markers for the purpose (MANGOLD 1961). Unfortunately, although the solvent front is prevented from moving forward, development of the chromatogram can still proceed as has been illustrated by BRENNER and NIEDERWIESER (1960). Therefore the plate must be removed from the tank immediately the solvent front touches this mark, an occurrence which it is not always possible to observe accurately. For this reason it is probably preferable to develop the chromatogram for a fixed time rather than distance. Stopping the development after 30 or 45 minutes, as the case may be, ensures that a constant volume of mobile solvent passes over a given point on the chromatoplate, assuming that such possible variables as particle size, solvent-vapour equilibrium, temperature, tank volume, etc., are maintained constant. Some layer adsorbents and viscous mobile solvents are comparatively slow running but a maximum time of development of about two hours should be set or diffusion effects are likely to offset other advantages given by the thin-layer procedure.

An often-overlooked factor in thin-layer chromatography is that of the volume of the development chamber. Tanks supplied with the various types of commercial spreading apparatus are usually only slightly larger than the standard 20×20 cm. carrier plate and about five to ten cm. wide. Paper chromatographic development chambers are usually rather larger than this and since they are already available in most pesticide residue analytical laboratories, their conversion to thin-layer use has frequently occurred. Whichever size of tank is chosen it must be closely adhered to for all accurate comparative work. Provided that known standard pesticides are spotted on to every plate and that confirmation of identity is all that is required, small variations in tank volume will be relatively unimportant. The extent of the effects which have been observed on changing the volume of the development chamber was illustrated by Tables I and II which showed the $R_f$ values given by organochlorine and organophosphorus pesticides in lined and unlined tanks of different dimensions. Differences in the distance travelled by the solvent front in a fixed time was also given.

The effect of tank size is seen to be greater on the $R_f$ values of the organophosphorus pesticides, which were developed in a two-component mobile solvent, than on the organochlorine compounds developed with hexane alone. This difference is presumably due to the fact that since acetone is appreciably more volatile than hexane there is a greater proportional acetone content in the vapour phase than in the solvent phase; it is also possible that an equilibrated concentration gradient is set up. Since the more polar acetone induces higher $R_f$ values than does hexane, its higher concentration toward the top of the chromatoplate tends to increase the migration rate of those compounds with high $R_f$ values. The more polar compounds, which have lower $R_f$ values are less effected by this partial vapour pressure effect. Similar variations in $R_f$ value have been observed when several plates, separated by varying distances, were developed together in a large tank. From these observations it is probable that the use of a large, unlined chamber is preferable for pesticide residue analytical separations where a high $R_f$ value is required in order to ensure good cleanup from coextractives. However, where a well cleaned-up extract is available and constancy of $R_f$ value is prefered for confirmation of identity then lined tanks should be used, their volume being less critical.

The benefits of the uses of very narrow development chambers have been advocated by several workers (JÄNCHEN 1961, WASICKY 1963, BRENNER and NIEDERWIESER 1961, STAHL 1962). These are so constructed that the chromatoplate itself forms one wall of the tank, three-sided spacing pieces of various materials about one to three mm. thick being used to separate it from an unlayered carrier plate of similar size. On placing the "sandwich" so formed into a trough containing the mobile solvent, the tank is completed. Owing to the very small volume so enclosed, development proceeds very rapidly and resolution is excellent, in some cases band formation occurs rather than the more normal circular spots. Such a system has been successfully used for the separation of chlorinated phenoxy-acid herbicides on layers spread by hand on window glass (ABBOTT *et al.* 1964 a).

The humidity of the atmosphere within the development chamber has been found to have a profound influence on the separation of some 2,4-dinitrophenylhydrazones (BADINGS 1964). In the absence of water vapour excellent resolution of six compounds was obtained, while all substances migrated with the solvent front when atmospheric moisture was not excluded. In order to obtain such satisfactory separations it was necessary to devise a tank through which dry nitrogen could be circulated prior to allowing the mobile solvent to contact the chromatoplate. This apparatus was similarly found useful for preventing oxidation effects during the chromatography of methyl esters of some unsaturated fatty acids. GEISS and SCHLITT (1963) have also shown the variation of $R_f$ value of polyphenyl hydrocarbons which could be induced by large changes in the relative humidity of the development chamber atmosphere.

Differing views have been expressed in the literature as to the extent of the effect of variation in the temperature at which development proceeds upon the $R_f$ values observed on thin-layer chromatoplates. STAHL *et al.* (1956), studying essential oils on silica gel chromatoplates developed in a

hexane-acetic acid mixture, found no alteration in running time on changing the temperature from 20° to either 4° or 28° C. The effects of insecure closure of the tank and variation in the depth of immersion of the layer in the mobile solvent were found to be more important. BRENNER *et al.* (1962) similarly found that raising the temperature of development from 18° to 38° C. had virtually no effect on the $R_f$ values of a number of amino acids developed with a phenol-water mixture, although the reproducibility of these values became poorer.

On the other hand MULLER and HONERLAGEN (1960), in their study of the thin-layer chromatography of cinchona bark alkaloids developed with a mixture of kerosine-diethylamine-acetone as mobile solvent, found that the observed $R_f$ values were strongly temperature dependent; they advised the use of a controlled 25° C. as being most convenient and suitable for their purpose. Similar effects were described by HARTHON (1961), who

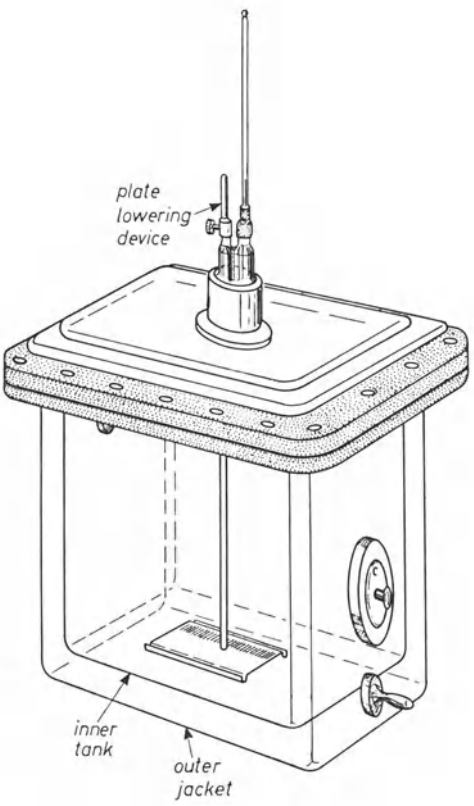



Fig. 1. Developing tank for low temperature thin-layer chromatography (STAHL 1964 b)

advised the inclusion of a reference compound on each chromatoplate to act as a check against variations in $R_f$ value due to temperature fluctuation. Use of the effect of temperature on $R_f$ value has been made by MALINS and

MANGOLD (1960) in their separation of palmitic and oleic acids on silica gel chromatoplates developed at 4° to 6° C., these compounds being unresolved at normal ambient temperatures. STAHL (1964 a) has recently devised a chamber for isothermal operation at temperatures ranging from −50° to +50° C. (Fig. 1). Development at low temperatures allows the use of low boiling solvents as mobile phase and also renders possible the chromatographic separation of compounds that are appreciably volatile at room temperatures. His results indicate that the effect of variation of temperature on $R_f$ value is more dependent on the nature of the mobile solvent than upon the material under investigation.

The effects of varying the temperature of development upon the $R_f$ values of organochlorine (ABBOTT *et al.* 1964 b) and organophosphorus (ABBOTT *et al.* 1965 b) pesticides have been determined and are illustrated in Figures 2 and 3, respectively. It is clear that the $R_f$ values of all of these

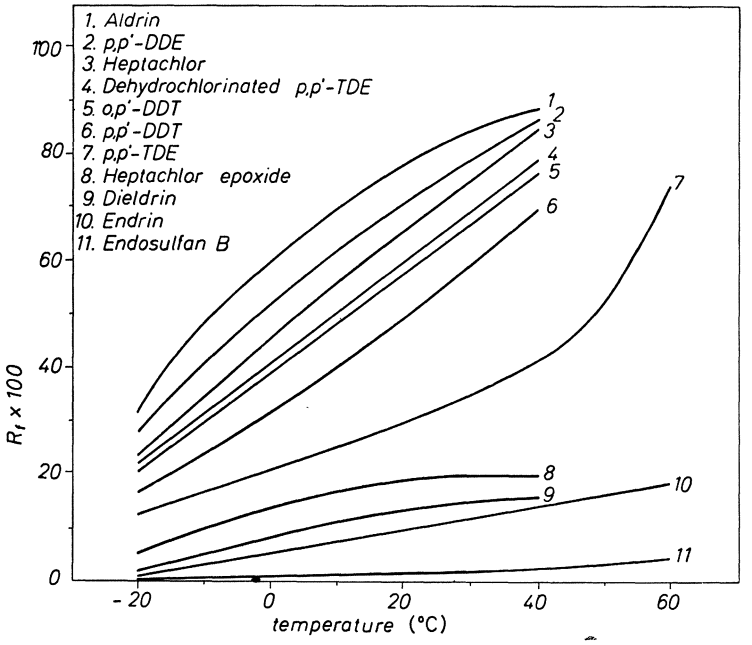



Fig. 2. Temperature effect on $R_f$ values for thin-layer chromatography (ABBOTT *et al.* 1964 b)

compounds were temperature dependent to some extent though differences are observed between the two classes of pesticide. The organochlorine compounds were developed with hexane on silica gel chromatoplates. Compounds with $R_f$ values above 0.40 at room temperature show the greatest temperature dependence. Endrin and endosulfan *A* showed little variation at high temperatures but a decided drop in $R_f$ below 0° C. A mobile solvent of 9+1 hexane : acetone, was used to develop the silica gel chromatoplates bearing the organophosphorus compounds; in each case a steady rise in $R_f$ with increasing temperature was observed. For practical purposes develop-

ment at 0° C. has some uses. Development is more rapid and in consequence more compact spots are obtained, thus improving the resolution observed between compounds of close $R_f$ values. Phorate, carbophenothion, and

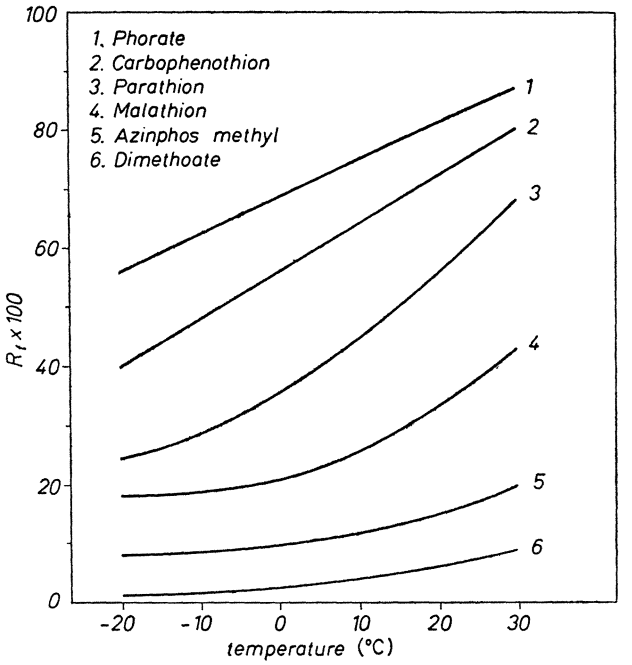



Fig. 3. Temperature effect on $R_f$ values of organophosphorus pesticides (Abbott *et al.* 1965 b)

parathion are more widely separated when chromatographed at 0° C. than at 20° C.; the reverse, however, is true of malathion, azinphos methyl, and dimethoate in the system under examination.

The procedures described above relate in general to a single linear development in one direction. Thin-layer chromatography is also suitable for two-dimensional separations, either by means of different mobile solvents in directions perpendicular to one another (Stahl 1960, Kohler *et al.* 1964) or by a combination of normal solvent development with an electrophoretic separation (Honegger 1961). Multiple or step development techniques may also be applied, either by repeated application of the same solvent (Henkel and Ebing 1964) or by sequential use of different solvents (Stahl and Kaltenbach 1961, Mangold and Kammereck 1961). Gradient elution techniques, whereby the polarity of the mobile solvent is continually changed while the development proceeds, have also been used, suitable apparatus having been described by Determann *et al.* (1962), and Rybicka (1962). A review of some uses of these techniques has been given by Russell (1963).

### *d) Visualization of pesticides*

Since very few pesticidal compounds are either coloured or fluorescent, the application of a visualization reagent is usually required before the developed chromatogram may be observed. Most reagents suitable for paper chromatographic indication purposes may similarly be applied to thin-layer chromatograms. Additionally, corrosive reagents and elevated temperatures may be used where necessary. Visualization agents fall readily into two classes: general or universal reagents and specific or semi-specific indicators. The reagent is usually applied in the form of a spray; for the best results the droplet size must be very small and also uniform, the reagent must be evenly applied, and only the minimum quantity required to produce the desired effect should be used. In order to retain the compactness of the located spot, and hence conserve sensitivity, the compound sought should not be soluble in the solvent used for the spray and should not form soluble complexes with the reagent. It is usual to remove as much as possible of the mobile solvent before spraying the developed chromatoplate with the visualization reagent. However, it is preferable to spray loose-layer chromatograms while they are still wet with mobile phase which acts as a binding agent and prevents the dispersal of the layer materials by the mechanical force of the spray. Some visualization agents may be incorporated in the layer-mix when spreading the plates; fluorescent compounds in particular may be used in this way. Sometimes, however, such "built-in" indicators interfere with the normal development of the chromatogram.

Sulphuric acid may be used either alone (Fiori and Marigo 1958) or in combination with other compounds, such as nitric acid (Kirchner *et al.* 1951), sodium dichromate (Brown and Benjamin 1964), potassium permanganate (Ertel and Horner 1962), or vanillin (Stahl 1958 b). Following such acid sprays it is usual to heat the treated plate to 100° to 200° C., various colour changes frequently being observed (Mangold 1961) which aid in the identification of the compounds under examination. Beroza (1963) sprayed with sulphuric acid containing either chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulphonate) or furfural during a study of pyrethroids synergists. Although it is not usual to use starch-bound chromatoplates when sulphuric acid is included in the spray reagent, Yamamura and Niwaguchi (1962) made use of such a combination when investigating the separation of aldrin, dieldrin, endrin, and endosulfan. Alkaline potassium permanganate was found to be preferable to sulphuric acid for the location of pesticides of the aldrin-dieldrin type (Petrowitz 1961 a).

Other reagents of general applicability include antimony trichloride or pentachloride (Stahl 1958 a), iodine (Malins and Mangold 1960), and various fluorescent compounds, the latter being frequently used together with a bromine vapour treatment. In the pesticide field the above-mentioned antimony chlorides have been used to locate pyrethroids (Stahl 1960) and chlorophenoxyacid esters (Henkel and Ebing 1964). Iodine vapour has been used as a general visualization indicator by Conkin (1964); the appearance of the brown spots is reversible and the located compound is

unaffected by the reagent, a useful attribute if the material is required for further study. WALKER and BEROZA (1963) list the limits of detection for 62 pesticides and associated compounds located by exposure to iodine vapour for five minutes; the values quoted range from 0.1 to five $\mu$g. with an average of about 1.5 $\mu$g. Iodine vapour has also been used by STAMMBACH *et al.* (1963) for phenkapton and its commercial impurities, by MARCO and JAWORSKI (1964) for Colep metabolites, by CHIBA and MORLEY (1964) in a study of carbaryl, and by KATZ (1962) for locating DDE and dichlorobenzophenone.

Fluorescent compounds have enjoyed wider popularity among pesticide residue analysts, the spots usually being located and marked by observing the quenching effect of the pesticide on the background fluorescence when viewed under ultraviolet light. KIRCHNER *et al.* (1954) used zinc-cadmium sulphide as a built in phosphor to indicate the presence of biphenyl on their chromatostrips. Fluorescein, either built-in or spray-applied, has been used by SPICKETT (1957) for pyrethroids and by SALO *et al.* (1962) for organophosphorus pesticides, exposure to bromine vapour showing the yellow spots on a red background. WALKER and BEROZA (1963) added silver nitrate to this fluorescein-bromine combination and give limits of detection ranging from 0.5 to ten $\mu$g. for 59 pesticidal compounds; for the organochlorine compounds seven minutes irradiation with ultraviolet light was also required. Dichlorofluorescein has been found useful for organophosphorus pesticides (ABBOTT *et al.* 1965 b). Some esters of MCPA and mecoprop have been located by means of rhodamine *B* (HENKEL and EBING 1964).

Among the more specific visualization agents two have been widely used. Palladous chloride has been found preferable for organophosphorus compounds by BÄUMLER and RIPPSTEIN (1961), BLINN (1963 and 1964), and STELLER and CURRY (1964). Silver nitrate, with ultraviolet irradiation, has been most popular for chlorine-containing pesticides. The "chromogenic reagent" of MITCHELL (1958), i.e., silver nitrate with 2-phenoxyethanol, has been universally used for visualizing paper chromatograms and has generally been adopted (KOVACS 1963, WALKER and BEROZA 1963) for similar purposes on thin-layers. On silica gel or alumina plates, however, it is not very satisfactory because dark backgrounds are obtained which limit its sensitivity. A simple 0.5 percent ethanolic solution of silver nitrate has been found to be more satisfactory (ABBOTT *et al.* 1964 a). Other variations on this theme include ammoniacal silver nitrate (YAMAMURA *et al.* 1962, MORLEY and CHIBA 1964), ethanolamine or potassium hydroxide with silver nitrate (PETROWITZ 1961 a and b), silver nitrate-formaldehyde-potassium hydroxide (SALO *et al.* 1961), and silver nitrate-nitric acid (HENKEL and EBING 1964). ABBOTT *et al.* (1964 b) have described spray reagents consisting of a combination of silver nitrate with one of several pH indicator compounds. Bromophenol blue gave yellow spots on a blue background with organochlorine pesticides without irradiation with ultraviolet light. Bromocresol green also showed promise but required irradiation and further heating for the best results. Plates containing built-in silver nitrate are suitable for the detection of organochlorine pesticides although a rather "grainy" background may be obtained. For organophosphorus compounds,

however, such plates are quite unsuitable as considerable streaking of the pesticide occurs. One benefit given by the use of silver nitrate-ultraviolet irradiation as an indicator system is that since only a very small amount (< ten percent) of the pesticide is affected by the irradiation, the bulk may be extracted for examination by other means (ABBOTT *et al.* 1965 b).

KATZ (1964) has recently used a zinc chloride-diphenylamine reagent for the indication of DDT, kelthane, methoxychlor, captan, and toxaphene, each compound giving a different coloration. DOROUGH and CASIDA (1964), in a study of the metabolites of carbaryl, used 2,6-dibromo-*p*-benzoquinone-4-chlorimine, a reagent which has also been used by BRAITHWAITE (1963) to detect 0.1 to 0.2 $\mu$g. of a number of organic phosphorothioates and phosphorodithioates on silica gel plates prepared at pH four. Other reagents which have been suggested include sodium azide-iodine (FISCHER and KLINGELHÖLLER 1961 a and b) for organophosphorus hydrolysis products and potassium iodoplatinate for organochlorine pesticides (BÄUMLER and RIPPSTEIN 1961) and triazine herbicides (HENKEL 1964). Dragendorff's reagent has also been used for the triazines (HENKEL and EBING 1964) with a sensitivity of one to three $\mu$g. MARCO and JAWORSKI (1964) sprayed with sodium hydroxide to indicate *p*-nitrophenol derived from the hydrolysis of Colep. DNOC and dinoseb have been found to be self-indicating as yellow spots on silica gel-kieselguhr chromatoplates (ABBOTT *et al.* 1964 a, ABBOTT and THOMSON 1964 b).

ABBOTT *et al.* (1965 b) have studied the properties of a number of indicator compounds, belonging in the main to the azo and triphenylmethane classes of dyestuff, when applied as visualization agents for pesticidal compounds. Brilliant green (colour index No. 42 040) showed very useful reactions and good sensitivity. With the aid of this material it is possible to locate organochlorine, organophosphorus, and triazine compounds and to distinguish between them. On spraying the silica gel chromatoplate with an 0.5 percent solution of brilliant green in acetone, organochlorine pesticides are observed as pale yellow spots on a green background and may be readily marked. On placing the sprayed plate into an atmosphere of bromine vapour (the plate must be still damp with acetone) the green background and the spots of organochlorine pesticide disappear but triazine herbicides are located as semi-transient green spots on white and organophosphorus pesticides appear as permanent dark green or yellow spots.

Among the specific visualization methods mention must be made of the use of esterase inhibition procedures for the detection of organophosphorus compounds and their metabolites. The sensitivity of this procedure varies very markedly with the compound studied, for example the anticholinesterase activity of phorate oxygen analogue sulphone is 1,000 times that of the parent phorate and its limit of detection is correspondingly lower. Methods used on paper chromatograms (GETZ and FRIEDMAN 1963, MCKINLEY and JOHAL 1963) have by transfer been similarly applied to thin-layer separations (BUNYAN 1964). The developed paper or plate is briefly exposed to bromine vapour and placed in contact with a sheet of filter paper which has been impregnated with liver homogenate or out-dated

human plasma. After incubation for 10 to 20 minutes at 35° to 40° C. the paper is removed and sprayed with acetylcholine bromide and bromothymol blue or bromophenol blue. After five to ten minutes the transferred spots appear in blue on the yellow background. Using techniques of this nature sensitivities down to about ten nanograms (ng.) have been obtained from developed thin-layer chromatograms. FLANAGAN and SUCKLING (1964) have used liver extract as the source of esterase and an ester of a substituted umbelliferone as substrate to give indication of organophosphorus pesticides on the silica gel chromatoplate itself.

### *e) Documentation of chromatograms*

As thin-layer chromatography increased in importance and widened in scope, the need for a documentation system became recognised. The number and variety of suggested systems implies that this is not an easy matter. This is one field in which paper chromatography usually scores over thin-layer procedures; the filter papers are readily marked, should the spots be transient, and can be stored almost indefinitely for future reference as required. The fragile nature of the layer of material, however, and the relative expense of carrier plates makes prolonged storage of thin-layer chromatoplates impractical and uneconomic. Published methods for preserving or recording thin-layer chromatograms can be divided into four main classes: (1) preservation of the layer, (2) simple tracing processes, (3) photographs or photocopies, and (4) direct documentation onto sensitised papers.

MEINHARD and HALL (1949) preserved their chromatograms by applying a length of transparent adhesive tape to the layer in such a way that the two ends were free for notebook mounting while the layer surface bearing the located spots adhered to the tape. Complete chromatoplates were also protected by covering them with a film of transparent material. For the fixation and preservation of loose-layer chromatograms, MOTTIER and POTTERAT (1952 b and 1955) employed impregnation with paraffin wax or cetyl alcohol at 104° C.; spraying with collodion or polyvinylacetate was found to be successful only in fixing the surface of the layers. For bound-layers on plastic plates, SQUIBB (1963 a) used a spray of "Tuffilm No. 543" which gave the surface an acetate-film finish and enabled the plates to be sliced into strips for radioactivity counting purposes. LICHTENBERGER (1962) found that a spray of a polyvinylpropionate solution could be used to fix the layer material together in such a way that the entire chromatogram could be peeled off the plate or floated off after a short immersion in water. Similar sprays are available commercially ("Neatan", E. Merck and Co.), but in our hands only limited success has been achieved, fragmentation readily occurring.

The use of tracing paper or cloth (REITSAMA 1954, BENNETT and HEFTMANN 1962) to document chromatograms has the benefit of simplicity but manual processes of this nature can only yield an approximate representation and the rather soft nature of the layer surface makes the procedure somewhat indecisive. Similarly a copy-drawing (STAHL 1958 a) of the plate, while adequate for the reproduction of the observed colours of spots and

their relative position, is unlikely to yield an accurate document in terms of $R_f$ value, size of spots, etc.

Photography, in black and white or in colour, has been widely used for the production of copies or slides for projection (REITSAMA 1954, STAHL 1958 a, HANSBURY *et al.* 1962). BROWN and BENJAMIN (1964) recommend illuminating the chromatogram by transmitted as well as by reflected light in order to obtain the clearest representation by photographic means. Such methods, however, require expensive apparatus and experienced workers if true records are required; the time involved in processing the developed film and preparing prints is comparatively long and the process is wasteful if only one or two copies are required at a time. Since some visualization agents rapidly fade, the camera must always be available when required and therefore cannot be put to other uses.

Commercial automatic copying machines have also been brought into use for documentation purposes. GETZ and LAWSON (1961) found the "Photorapid" diffuse transfer process suitable and HILTON and HALL (1962) made use of a "Xerox 914" office copier to obtain positive replicas of thin-layer chromatograms in about 30 seconds. Once again these machines are costly and can hardly be justified for the purpose of copying chromatograms alone, although where they are available they may be put to good use.

The direct use of photosensitive papers provides a rapid and inexpensive way of documentation. GORDON (1958) described a method for recording ultraviolet-absorbing spots on paper chromatograms by ferric ferricyanide blueprint paper, development with water being required. Photographic contact paper was similarly used by ABELSON (1960) for thin-layer plates. Activated zinc oxide papers were used by SPRENGER (1964) together with a triboluminescent compound, but darkroom facilities are essential. The use of diazo dry process papers, as suggested by EISENBERG (1962), offered a simple method. A combination of photoflood illumination with pre-ringing of the developed spots (ABBOTT *et al.* 1964 b) has reduced the elapsed time required from over ten minutes to less than one minute. In view of its simplicity and wide applicability the procedure is described here in some detail.

Diazo or ammonia process papers are, in general, insensitive to normal levels of illumination and can therefore be freely handled under ordinary laboratory lighting conditions. Papers of various degrees of sensitivity are available, the fastest papers giving the best contrast. When intense light falls on the paper, the diazo-compound is destroyed and can therefore no longer couple with the built-in coupling agent on exposure to ammonia vapour. Where the light has not fallen, coupling occurs and a coloration quickly appears: blue, black, and red papers are widely available. "Ammonax" 8. *M.* 13 Positive Diazo Paper (Hall-Harding Ltd., London) is a very sensitive blue paper and "Densblack" Ammonia Process Paper, Type 45, 9x (E. Mason and Sons, Ltd., Colchester) is a black paper giving very good contrast and definition. Either positive or negative copies can be obtained on these papers and the process is equally suitable for copying paper chromatograms, graphs, typescripts, etc., although longer exposures are required.

To copy a chromatoplate, either circumscribe the spots with a soft-lead pencil or scrape them completely from the plate. Similarly mark the origin points and the solvent front; any other information may also be inscribed on

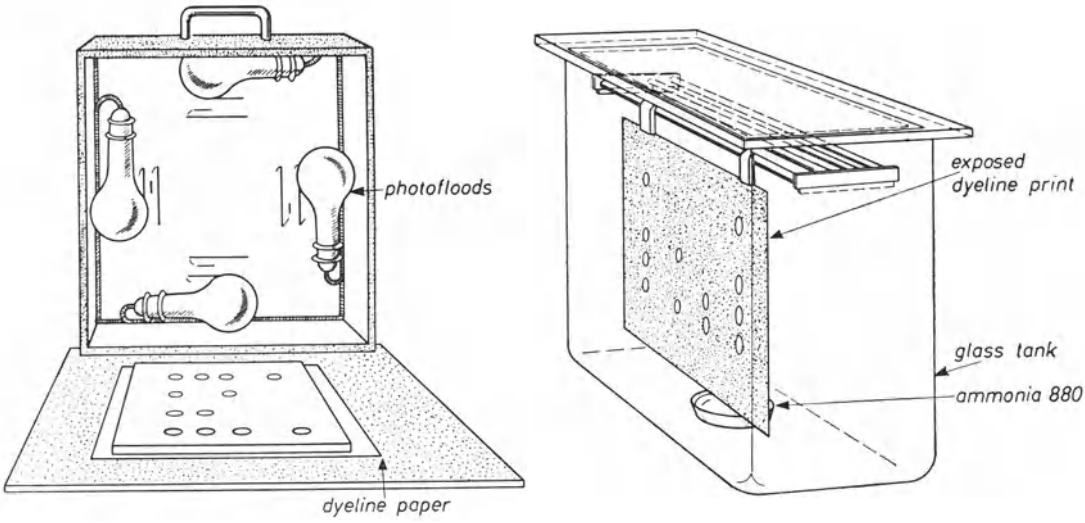



Fig. 4. Documentation process (Abbott *et al.* 1964 b)

the layer, writing letters backwards and from right to left. Remove excised material by gentle blowing and place the marked plate face downward on the sensitised surface of a sheet of the diazo-paper; 10×8 in. is a convenient size when using 20×20 cm. chromatoplates. Illuminate from above with four 275 watt photoflood lamps mounted in a reflector (25×25×20 cm.) placed centrally over the plate. After exposing for from five to 15 seconds, depending on the speed of the paper, remove the light source and suspend the paper in an atmosphere of ammonia (Fig. 4). The marked spots appear almost immediately as white rings or spots on a coloured background. The copies so obtained are permanent, readily filed for later inspection and may be inscribed as desired.

### *f) Quantitative evaluation of chromatograms*

The techniques which have been applied to the quantitative evaluation of thin-layer chromatograms fall readily into two classes. That which has the largest following among pesticide residue analysts comprises those procedures in which the chromatographed compound is separated from the layer adsorbent before the application of standard microchemical analytical methods such as spectrophotometry, gas-liquid chromatography, etc. The alternate technique involves the relationships which exist between the weight of compound contained in the located spot and its size, density of coloration (visual or photometric), or radioactivity.

The very small amounts (micrograms) of material usually applied to a thin-layer plate preclude the use of simple weighing of the eluted compound unless many chromatoplates are developed and the products combined (PURDY and TRUTER 1963). Spectrophotometry in the visible and ultraviolet regions of the spectrum can readily be carried out with the weights of material (zero to ten $\mu$g.) which may be eluted from a single spot on a chromatoplate. KIRCHNER *et al.* (1954) and STANLEY *et al.* (1957) used ultraviolet spectrophotometry to determine biphenyl eluted from silica gel; DETERS (1962) has similarly determined traces of pentachlorophenol. With these techniques some interference may be observed from extraneous material contained in the adsorbent layer. Pre-extraction with methanol has been found (GÄNSHIRT and MORIANZ 1960) to reduce this interference but the preparation of a blank extract of an adjacent area of the layer was still necessary for full correction; other errors were introduced by incomplete elution from the adsorbent. Dimethoate and its oxygen analogue have been determined in vegetable tissue by STELLER and CURRY (1964) who digested the eluted pesticides with a nitric-perchloric acid mixture and evaluated the liberated phosphorus by a molybdenum blue spectrophotometric procedure. Dinoseb has been determined spectrophotometrically as its sodium salt after a cleanup on wedge-layer plates (ABBOTT and THOMSON 1964 b).

Infrared spectrometry has also been used for the quantitative determination of organophosphorus pesticides (ABBOTT *et al.* 1965 b) as well as simultaneously providing conclusive evidence of the identity of the separated pesticide. Gas-liquid chromatography provides a very convenient method for the determination and further cleanup of the eluted pesticide spots. The use of electron-capture detection for both organochlorine and organophosphorus compounds (DE FAUBERT MAUNDER *et al.* 1964 b, EGAN *et al.* 1964) gives very great sensitivity. The use of a silver nitrate/ultraviolet irradiation procedure for the visualization of the chromatogram leaves the bulk of the pesticide unaffected in each spot since only the surface of the layer comes under the influence of the irradiation. Thus elution of the pesticide from the treated chromatoplate with dichloromethane gives ample material for an approximately quantitative evaluation. If insufficient material is present to give a visible spot with the visualization reagent used, then elution of that area of the layer suspected of containing the pesticide may give sufficient material for further examination by gas-liquid chromatography.

Biological assay, using *Drosophila melanogaster,* has been used by SALO *et al.* (1962) to determine some organochlorine and organophosphorus pesticides separated on silica gel containing built-in fluorescein as indicator. MARCO and JAWORSKI (1964) utilized $C^{14}$-labelled Colep for the determination of residues of the pesticide and its various metabolites by radiometry. A suitable scanner for thin-layer chromatography has been described by WILDE (1964).

A useful description of the sequence of techniques involved in the routine quantitative analysis of materials which have been separated by thin-layer chromatography has been given by MILLETT *et al.* (1964). Application of the sample, removal of the layer material bearing the

located compound, and the elution of the latter from the adsorbent are detailed; the accuracy and precision (one to two percent) of the techniques are also discussed.

The second class of methods used for the quantitative evaluation of thin-layer chromatograms follows more closely those techniques which have been well established in the field of paper chromatography (FISHER *et al.* 1948 and 1949, FOWLER 1951). Spot density measurements have been used by PRIVETT and BLANK (1961) in the determination of component triglycerides. The areas under the densitometer curves were found to be directly proportional to the amount of sample applied for the saturated triglycerides and the glyceryl residues of the unsaturated glycerides, spots being located by charring with 50 percent sulphuric acid. However, the area given varied with the type of structure of the compound. SQUIBB (1963 b) made densitometric measurements on amino acids located with ninhydrin and found a coefficient of variation of $\pm 6.9$ percent. Reflectance densitometry as used for the paper chromatographic determination of herbicides (ABBOTT *et al.* 1964 a) has been found to be less successful with thin-layer chromatograms of similar compounds, largely owing to the fragile nature of the layer surface and the difficulty in obtaining a sufficiently clean and uniform background with the silver nitrate reagent used.

Methods based upon the measurement of spot area avoid the difficulties associated with elution of the material from the adsorbent and the possibilities of further pollution of the purified sample. SEHER (1960 and 1961) simultaneously chromatographed samples of the unknown and a series of standards applied to the chromatoplate in equal volumes of solution. After rendering the developed chromatogram visible, the areas occupied by the standard sample spots were determined and plotted against the corresponding weight of material. Reference of the area of the unknown sample spot to this curve gave a measure of the material present. PURDY and TRUTER (1962 a) showed that the square root of the area of the spot is a linear function of the logarithm of the weight of the material it contains. Statistical evaluation (PURDY and TRUTER 1962 b) showed that this relationship was preferable to those of area against logarithm of the weight or logarithm of the area against logarithm of the weight. Planimetric means were used by AURENGE *et al.* (1963) to determine spot areas: graphs of area squared against weight of material were linear. They also traced the chromatograms, cut out the traced spots, and weighed them in order to determine the area of the spot more accurately, the weight per square centimetre of the tracing paper being known.

Methods of this type do not appear to have been used on any scale in pesticide residue analysis. KOVACS (1963) mentions that the size and intensity of the spot gives an estimate of the quantity present and ABBOTT *et al.* (1964 b) found a silver nitrate spray suitable for the semiquantitative estimation of organochlorine pesticides by visual intensity comparisons. Triazine herbicides extracted from soil and water have recently been quantitatively determined by a thin-layer chromatographic method. Sample and standards were applied in the same volume of solvent. The areas of the spots were determined by carefully scraping the marked area from the

plate, preparing a copy by the diazo-paper process described earlier, and measuring this area on the copy by a "counting-squares" technique. Measuring the areas in this way was found to be easier than attempts to use the

Table III. *Accuracy of quantitative analytical methods* (PURDY and TRUTER 1964)

| Method | Mean coefficient of variation in percent | No. of observations |
|---|---|---|
| Spectrophotometric | | |
| Correcting for adsorbent impurities. | 5.3 | 200 |
| Via calibrated extinction coefficients | 2.3 | 60 |
| Optical densitometric. . . . . | 6.9 | 7 |
| Spot-area | | |
| Adsorption . . . . . | 3.1 | 600 |
| Partition . . . . . | 3.6 | 982 |

chromatoplate directly; the excised material could also be extracted with solvent in order to obtain sufficient material for confirmation of identity by gas-liquid chromatographic means (BENFIELD and CHILWELL 1964). The graphs of the square root of the spot area against the logarithm of the weight of material contained in the spot were linear over the range one to ten $\mu$g. for each of the eight triazines examined. The method as a whole gave recoveries of about 90 percent of the herbicides added to samples of soil and water at the 0.1 part-per-million level.

The simplicity of the area-measurement quantitative methods and the accuracy they can give (Table III) should recommend their wider adoption for residue analyses. Elution methods, however, do possess one useful advantage over spot-area procedures. Where incomplete resolution is obtained the application of other methods, such as spectrophotometry or gas-liquid chromatography, to the eluted material may yield quantitative estimates that could not otherwise be obtained.

### *g) Special applications*

**1. Preparative-layer chromatography.** — The use of chromatographic techniques for the preparation of pure specimens of organic compounds has until comparatively recently been confined to columnar separations. The introduction of gas-liquid chromatography on a preparative scale has been invaluable in many cases but its use is limited to compounds that are stable at the temperatures required to ensure their volatility. Thin-layer chromatoplates offer an excellent means of preparing pure specimens of substances available in limited quantity. Where larger samples are required the usual 250 $\mu$.-thick layer is inadequate as its acceptable load is limited. Increasing this thickness to one mm. or more assists considerably (HONEGGER 1962 b) but many 20×20 cm. chromatoplates must be developed to obtain reasonable quantities, acceptable loads being five to 25 mg. per one mm. thickness of adsorbent. Equipment has now been described (HALPAAP 1963) which employs carrier plates one m. long by 20 cm. wide on which layers up to four mm. thick can be spread, either by a moving spreader of increased

capacity or by "casting" the layer *in situ* within a framework. Apparatus using carrier plates 40×20 cm. has been advocated by STAHL (1964 a). In practice two-mm. thick layers have been found to possess optimum properties in respect of ease of preparation, adhesion of the layer, and uniformity of resolution. Samples are applied as a streak along one edge of the chromatoplate and up to five such plates may be developed at one time in a stainless-steel tank; multiple runs are frequently necessary to obtain suitable resolution. Fluorescent indicators are used as visualization agents since they do not affect the separated compounds. The observed bands are marked under ultraviolet illumination, scraped from the carrier plate and extracted with a suitable solvent to recover the purified specimen. In this way very pure materials can be obtained, up to one g. per chromatoplate being a practical amount, provided that good resolution from associated impurities is obtained.

When using thin-layer chromatoplates for pesticide preparative purposes on a microscale, with a view to subsequent electron-capture gas-chromatographic examination, it is preferable to pre-extract the adsorbent with ether to remove interfering impurities before spreading the layer. Several solvents may be used to elute the pesticide from the layer adsorbent after developing the chromatogram. Table IV lists recoveries of organochlorine

Table IV. *Recovery of organochlorine pesticides from chromatoplates* (HARRISON 1965)

| Pesticide | Layer | Percent recovery | | | | |
|---|---|---|---|---|---|---|
| | | Hexane | Acetone | Ethyl acetate | Dichloro-methane | Chloroform |
| Gamma-BHC. . | Silica gel *G* | 82 | 72 | 64 | 51 | 61 |
| | Alumina *G* | 77 | 65 | 64 | 43 | 57 |
| *p,p'*-DDT . . . | Silica gel *G* | 96 | 89 | 89 | 80 | 89 |
| | Alumina *G* | 87 | 75 | 83 | 80 | 84 |
| Dieldrin . . . . | Silica gel *G* | 94 | 97 | 91 | 88 | 92 |
| | Alumina *G* | 88 | 79 | 84 | 79 | 82 |
| Endrin . . . . | Silica gel *G* | 94 | 98 | 93 | 88 | 94 |
| | Alumina *G* | 88 | 80 | 85 | 79 | 84 |
| Heptachlor | Silica gel *G* | 92 | 89 | 84 | 74 | 80 |
| epoxide . . . | Alumina *G* | 84 | 72 | 76 | 67 | 75 |
| *p,p'*-TDE . . . | Silica gel *G* | 99 | 89 | 89 | 86 | 93 |
| | Alumina *G* | 87 | 75 | 82 | 80 | 83 |

pesticides from silica gel and alumina chromatoplates, determinations being carried out by gas-liquid chromatography. In general, hexane is the solvent of choice; low results for gamma-BHC shown by dichloromethane and chloroform were induced by the need to evaporate these solvents away completely before electron-capture detection could be employed, some co-evaporative losses occuring. For the elution of organophosphorus pesticides dichloromethane has been found preferable, recoveries ranging from 70 to 100 percent at levels from ten to 80 $\mu$g. (ABBOTT *et al.* 1965 b).

**2. Wedge-layer chromatography.** — The advantages of the greater acceptable load of thick preparative chromatoplates and the higher $R_f$ values and greater resolution of thin (100 $\mu$) layers have been combined in

the properties of wedge-layer chromatoplates which were designed for pesticide residue cleanup purposes (ABBOTT and THOMSON 1964 a). In this development the cross section of the spread layer is not uniform but is

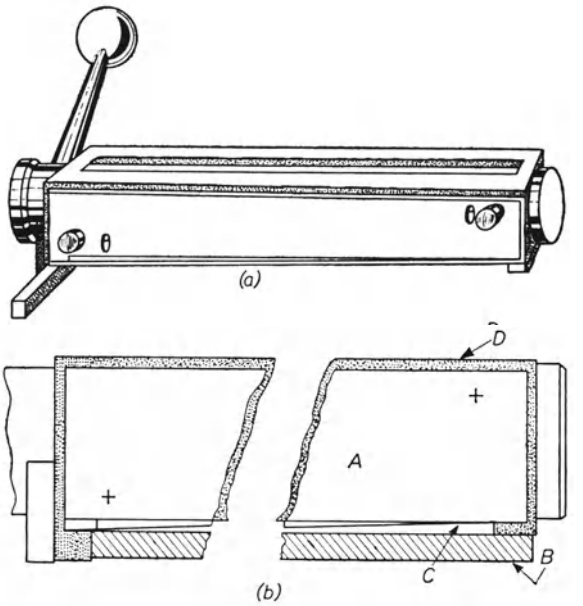



Fig. 5. Wedge-layer spreader (ABBOTT & THOMSON 1964 b): (a) Perspex plate attached to Desaga spreader; (b) *A*, perspex plate; *B*, glass carrier plate; *C*, wedge-layer; and *D*, spreader

wedge-shaped, tapering from two mm. to less than 100 $\mu$. Such layers are spread by simple adaption of commercial layering apparatus. For the Desaga spreader a perspex trapezium, 210 mm. long with vertical sides of 40 and 38 mm., replaces the normal calibrated applicator plates (Fig. 5). Other models of spreader are more simply adapted by adjusting the smoothing edge to feeler gauges of different thickness at either end, a sloping edge being thus ensured.

In use the sample extract is applied as a streak parallel to the edge of the chromatoplate at which the layer is thickest and about two cm. from it. After a further period of activation to ensure maximum cleanup the chromatogram is developed by the ascending-solvent technique in the usual way. The use of this technique in the determination of traces of dinoseb has been described (ABBOTT and THOMSON 1964 b); the procedure has also been shown to be suitable for organochlorine and organophosphorus pesticides whose $R_f$ values on silica gel are normally at least 0.50 when developed with a fairly non-polar solvent. This system is particularly of value where the ratio of coextractives to pesticide residue is high.

Wedge-layer chromatoplates composed of silica gel *G*, alumina *G*, and kieselguhr *G* and mixtures of any two of these materials have been prepared and examined for pesticide residue analysis. Mixtures of silica gel with either

alumina or kieselguhr were prone to cracking across the wedge, though this was without any marked effect upon their chromatographic properties. Alumina and kieselguhr appeared to be of closer physical properties and

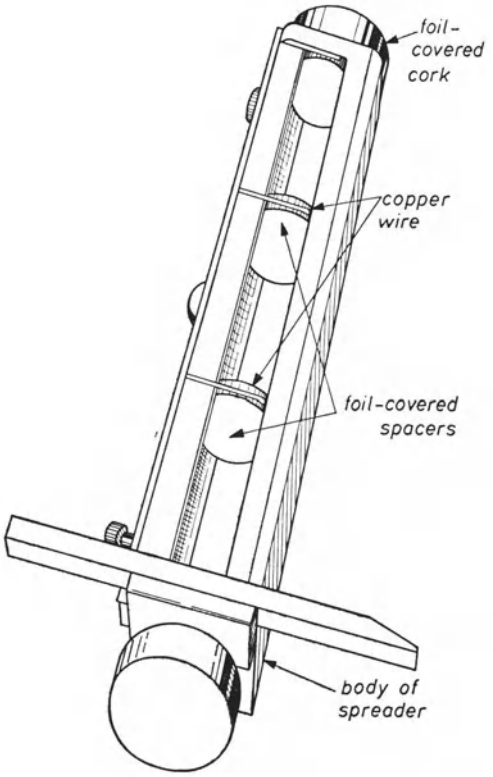



Fig. 6. Multi-band thin-layer chromatography: Underside view of Desaga spreader showing spacers (Abbott & Thomson 1965)

mixtures of these materials did not show this cracking; they also showed useful adsorptive characteristics.

**3. Multi-band chromatography.** — The use of multi-band or panel-layered chromatoplates has proved very useful both for diagnostic and for cleanup purposes (Abbott and Thomson 1965). By inserting close-fitting partitions of suitable materials, e.g., PTFE, cork, aluminium, etc., into the body of the spreading apparatus (Fig. 6) it is possible to prepare chromatoplates composed of two, three, or four parallel panels of different adsorbents. Suitable positioning of the partitions enables panels of various widths to be layered on appropriate areas of the carrier plate. The fluid mixes of adsorbent are prepared in the usual way and are poured simultaneously into the required compartment, the assistance of a second operator being required if more than two panels are to be layered. In this way up to five chromatoplates each 20×20 cm. may be prepared at one time, each

bearing four different adsorbent materials, thus giving sufficient material for the investigation of 20 adsorbent-solvent systems.

Panel-layered plates can obviously be developed in two distinct ways. By spotting the sample and a known standard onto each of the panels and developing vertically up the bands, a useful diagnostic system is apparent. In this way it is possible to compare $R_f$ values on several adsorbents developed in one mobile solvent under identical conditions of time, temperature, solvent-vapour equilibrium, etc. Including a known dyestuff with

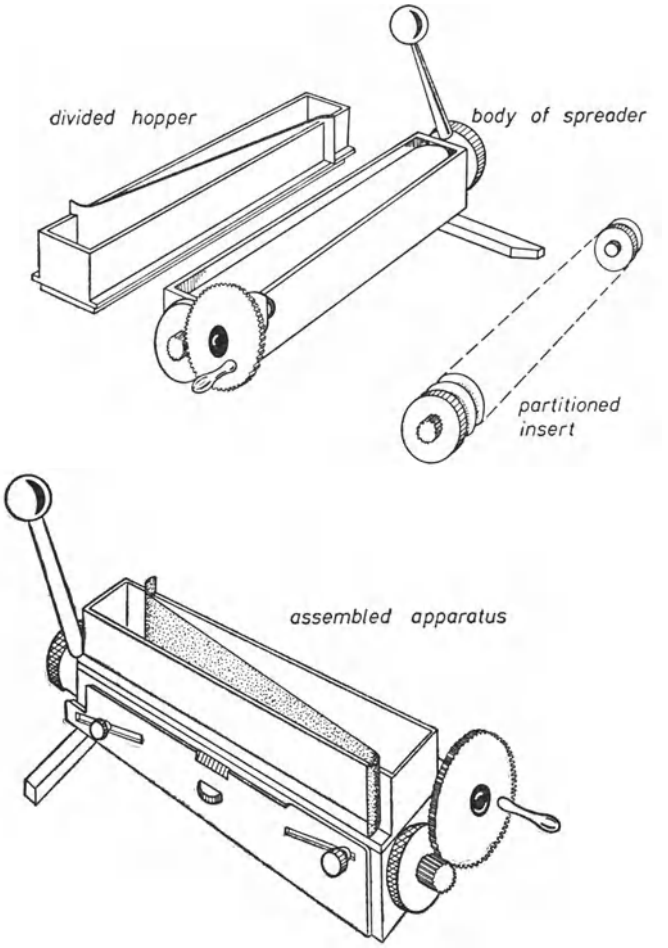



Fig. 7. Gradient-layer spreader (Stahl 1964 b)

both sample and standard as a reference material makes it possible to correct $R_f$ values for any hold-up due to coextractives. Under these conditions relationships between the corrected $R_f$ values on, say, three

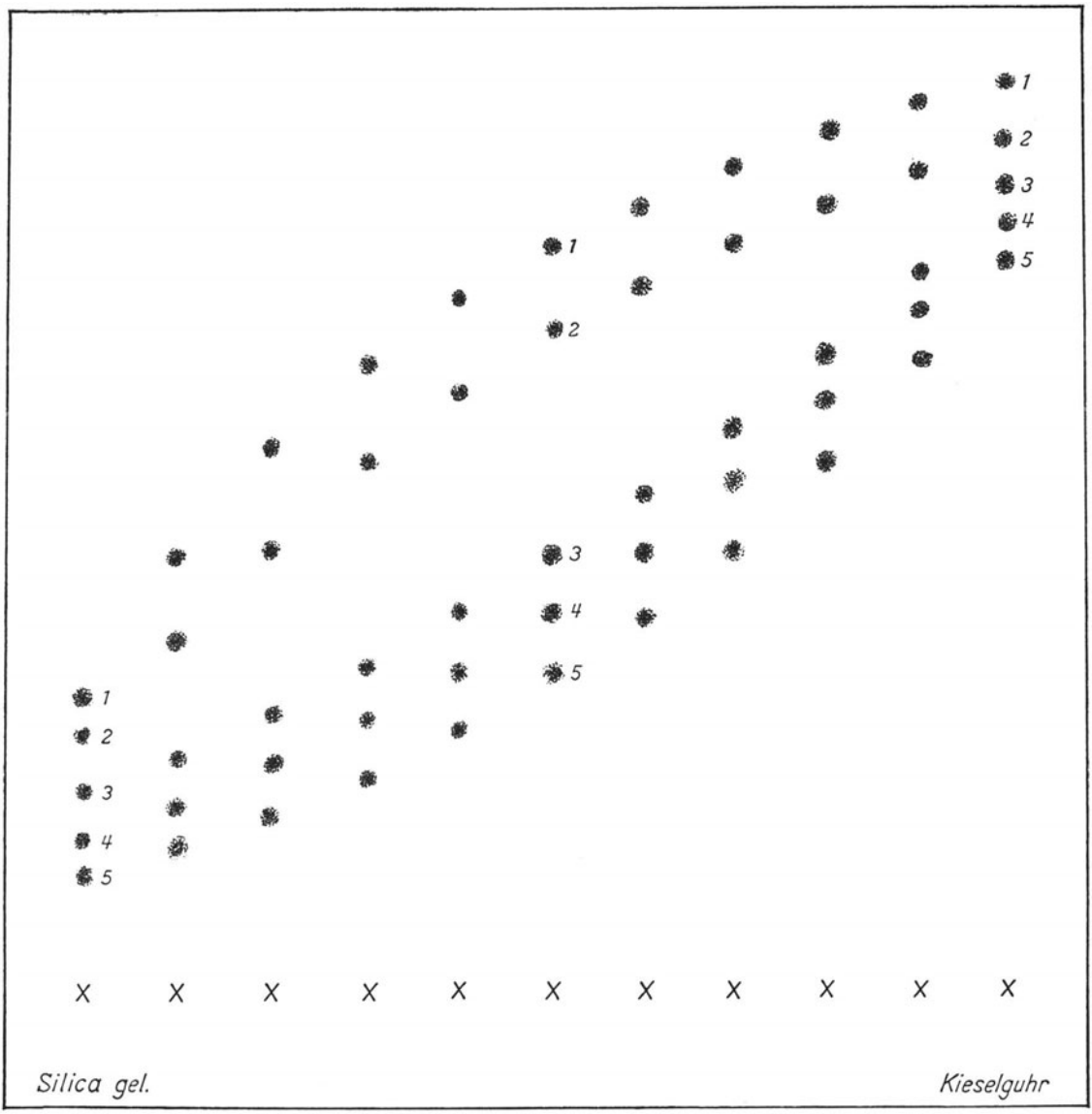



Fig. 8. Representation of gradient-layer chromatoplate. Mobile solvent-cyclohexane : benzene : acetic acid : liquid paraffin in the ratios 20 : 3 : 2 : 1.
1: MCPB; 2: 2,4-DB; 3: MCPA; 4: 2,4,5-T; and 5: Dalapon

different adsorbents may be taken as indicative of the identity of the unknown compound, this being more positive than using one adsorbent only.

The second manner of use, i.e., development across the panels, adds to the usefulness of these chromatoplates. Sequential elution from a highly adsorptive layer on to a less active panel enhances the general resolution of compounds and is valuable for cleanup purposes. An opposite effect is observed when developing from an adsorbent of low activity to a more adsorptive material. This similarly has uses in limiting the likely position of unknown compounds on the chromatoplate, a tight band being formed at the junction of the two bands. Thus by suitable choice of the composition and position of the individual panels, separations can be enhanced or condensed at will.

A combination of these two modes of operation also has useful properties. By choice of a suitable mobile solvent it is possible to separate the pesticides from coextractives by development along a silica gel panel. By turning the plate through 90° and developing in a more polar solvent on to an alumina panel, mixtures of pesticides can be separated in a clean state.

**4. Gradient-layer chromatography.** — A novel variant of the "moving-spreader" layer applicator has been introduced by STAHL (1964 b). With this apparatus (Fig. 7) it is possible to prepare layers of uniform thickness which are graded from pure adsorbent *A* at one side to pure adsorbent *B* at the other, intermediate zones consisting of varying mixtures of the two materials with a one: a one composition in the centre. By developing the chromatoplate from reagent *A* to reagent *B*, separations may be enhanced or minimised according to which adsorbent is the more active. In this way its action is somewhat similar to a bipartite multi-band plate prepared as described above, although it shows considerably less versatility in that only two adsorbents are used. However, its main use would appear to be as a research tool in investigating the retention characteristics of mixtures of two adsorbents in varying ratio. By applying a series of spots of the material under examination across the plate from reagent *A* to reagent *B* and developing in such a way that each spot migrates along a lane of constant composition, chromatoplates similar to that illustrated in Figure 8 may be obtained. By examination of such chromatograms a suitable admixture may be chosen which will effect the desired separation.

## III. Separations of pesticides

The procedures and techniques described in the foregoing pages are now widely applied to the problems of pesticide residue analysis. Thin-layer chromatography has proved to be particularly useful in this field, being admirably suited to the conditions usually prevailing, i.e., the need to find, identify, and determine a very small amount of an organic compound in the presence of large quantities of natural materials. For this purpose it is necessary to be able to separate the pesticide from coextractives and from other similar compounds, identification being essential for toxicity consider-

ations. The rate of growth of the application of thin-layer chromatography to pesticides and their residues is shown by the fact that of the 60-odd references to this aspect of the subject given in this review only ten were published prior to 1962 and one-half refer to dates after 1963. The following pages review such separations as have been reported for organochlorine, organophosphorus, herbicidal, and miscellaneous pesticidal compounds. Unless otherwise stated, chromatoplates used were 250 $\mu$ thick and prepared by standard techniques.

### *a) Organochlorine pesticides*

YAMAMURA and NIWAGUCHI (1960 and 1962) studied the separation of aldrin, dieldrin, endrin, and endosulfan on starch-bound silica gel chromatoplates developed with several mixtures of cyclohexane or hexane with acetone. A mobile solvent consisting of 9+1 cyclohexane: acetone was found to be most suitable for separating pure specimens of these compounds but a slightly lower proportion of acetone (92+8) was preferred when technical products were examined, tailing then being less pronounced. Gypsum-bound silica gel activated for one hour at 70° to 80° C. was later used (YAMAMURA *et al.* 1962) in a similar way to resolve aldrin, dieldrin, and endrin.

Using silica gel chromatoplates, BÄUMLER and RIPPSTEIN (1961) were unable to find a suitable mobile solvent for the separation of organochlorine pesticides; however, using binder-free alumina activated at 200° to 220° C. for four hours and developed with hexane for 45 minutes, excellent resolution was obtained of aldrin ($R_f$ 0.78 to 0.82), DDT (0.59 to 0.62), perthane (0.48 to 0.50), gamma-BHC (0.39 to 0.41), dieldrin (0.17 to 0.19) and methoxychlor (0.10 to 0.12).

Descriptions of the qualitative and quantitative examination of several organochlorine compounds have been given by PETROWITZ (1961 a and b), 1962). Silica gel G chromatoplates, each 17.5×4.5 cm. and hand-layered with four g. of absorbent, were used throughout. Several mobile solvents were investigated of which cyclohexane and petroleum ether (50° to 70° C.) were of the greatest general utility, chloroform and 95+5 benzene : methanol, both giving little separation of the isomers of BHC. Cyclohexane also served to separate aldrin and dieldrin ($R_f$ 0.58 and 0.57) from endrin and isodrin ($R_f$ 0.48 and 0.48) but did not resolve these pairs of compounds; in the same solvent the $R_f$ values of alpha-BHC, gamma-BHC and delta-BHC were 0.22, 0.17, and 0.07, respectively. Using petroleum ether (50° to 70° C.) as mobile solvent spot-area quantitative measurements were made with gamma-BHC and DDT, linear relationships being given between the area and the logarithm of the weight in the range five to 100 $\mu$g. Such pesticides could be extracted from treated wood and determined in the concentrated extract (PETROWITZ 1961 b). The use of silica gel chromatoplates which had been rendered acidic by the inclusion of an acid such as boric, oxalic, citric, etc., was also studied by PETROWITZ (1962). Development of these chromatoplates with hexane gave slightly better resolution

of the BHC-isomers; benzene and chloroform were of little use for this purpose but did give clear separation from DDT.

SALO *et al.* (1962) developed silica gel *G* chromatoplates with hexane, rising ten cm. in 20 minutes, to separate DDT and gamma-BHC before applying a biological assay using *Drosophila melanogaster;* under these conditions aldrin and dieldrin did not interfere.

Two comprehensive publications on the thin-layer chromatography of organochlorine pesticides appeared almost simultaneously. That by WALKER and BEROZA (1963) also dealt with organophosphorus compounds while the work of KOVACS (1963) was solely concerned with chlorine-containing substances. The former paper has been reviewed by CONKIN (1964) and will not be further examined here except to comment that of the 19 solvent systems discussed only those based on hexane showed any useful separatory properties.

KOVACS (1963) proposed a method for the detection and estimation of residues of several pesticides and their metabolites in extracts of various food products. Prewashed alumina *G* or silica gel *G* chromatoplates were developed with *n*-heptane; for certain separations which this solvent could not effect, such as dieldrin — endrin or heptachlor epoxide — gamma-BHC, the addition of one or two percent of acetone is required. The method was applied to such foodstuffs as vegetables, dairy products, meat, fruit, grain, etc., the extraction procedure of MILLS *et al.* (1963) being used. Determinations were carried out by visual comparisons with known standards developed alongside, and were compared with results given by a microcoulometric gas-liquid chromatographic procedure. The over-all agreement between the two systems was very good considering the low residues sought and in several cases compounds could be detected on the chromatoplates which were not detected on the gas chromatograph; the method appeared to be about ten times more sensitive than similar paper chromatographic systems.

MORLEY and CHIBA (1964) have described a method for the determination of organochlorine pesticides residues in vegetable produce without prior cleanup. Silica gel chromatoplates developed with hexane were used, standards for visual comparison being also spotted onto the plate. In this way a large number of samples could be screened quickly, those samples showing the presence of any appreciable quantity of pesticide being examined further. For this purpose the chromatoplates were divided into two sections, sample extracts being spotted onto both. After development one sample spot was protected from the visualization agent during spraying. The area of adsorbent on the unsprayed portion corresponding to the portion of the pesticide indicated on the treated side of the chromatogram was then scraped from the carrier plate, extracted with a suitable solvent and this extract was examined gas-chromatographically.

It was found that although gas chromatography using an electron-capture detector is intrinsically more sensitive than thin-layer chromatography, in practice a similar order of sensitivity is often observed since the equivalent of 0.5 to five g. of a sample can be spotted onto the plate, this being far greater than the acceptable injection loading of a gas chromato-

graphic column. Also, whereas for gas-liquid chromatography a cleanup of some nature is generally required to obtain reproducible results, thin-layer chromatography can frequently be applied to the crude extract.

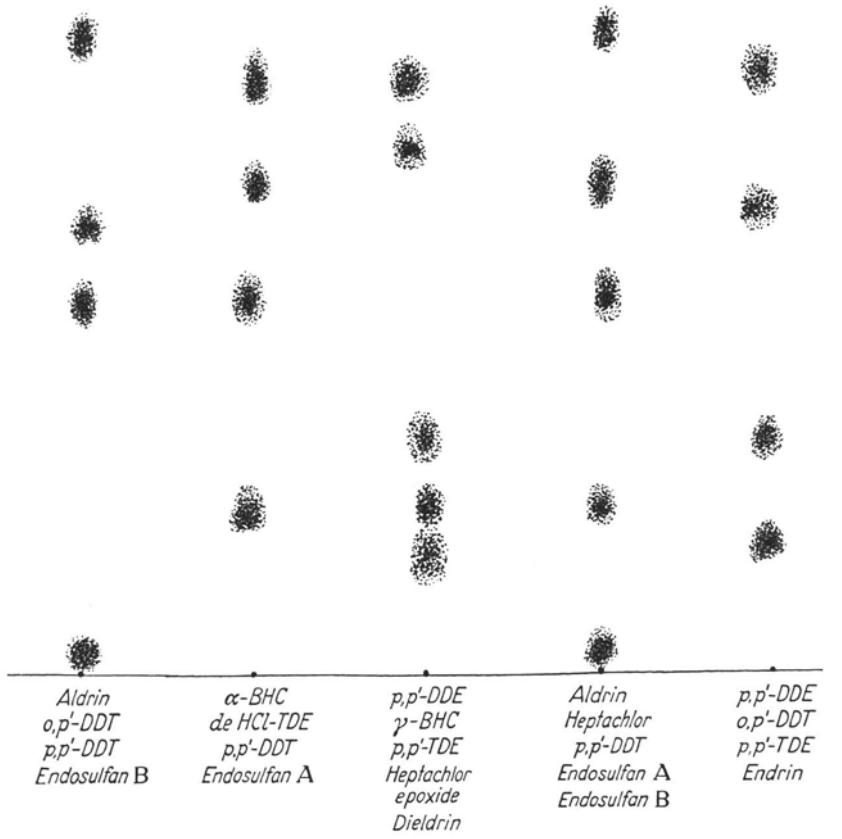



Fig. 9. Thin-layer chromatographic separation of chlorinated pesticides: silica gel plate, developed in hexane

A similar technique was employed by SCHMIT *et al.* (1963) to confirm the identity of DDT, aldrin, lipids, and pigments in extracts of tomatoes and tomato products. Silica gel chromatoplates, 8×2 inches, were developed with hexane or a mixture consisting of 90+10+1 hexane : ether : glacial acetic acid; the former solvent resolved the pesticides while the latter system differentiated between neutral lipids and a pigment. The inclusion of silver nitrate in the absorbent layer was found to have an effect upon $R_f$ value; similar results were obtained using alumina as absorbent.

Several separatory systems of a somewhat different nature have been investigated by ABBOTT *et al.* (1964 b). The bulk of the work was carried out on silica gel chromatoplates (Fig. 9) with some use of alumina and silica gel-alumina mixtures. Preliminary studies had shown that kieselguhr was an unsatisfactory adsorbent in that the majority of the pesticides either migrated with the solvent front or else streaked badly. In general the mobile solvents used were one of three types: (1) light hydrocarbon solvents, (2) light hydrocarbon solvent together with liquid paraffin or silicone oil, and (3) solvents similar to those in category (2) with the addition of a proportion of dioxane. Table V lists the $R_f$ values obtained when using a selection of the most promising of these mobile solvents. As can be seen from this table, by choice of the appropriate thin-layer chromatographic system, it is possible to separate all of the pesticidal compounds examined

Table V. *$R_f$ values of some organochlorine pesticides and allied compounds (tank size 28×26.5×21 cm.; 15 cm. development)* (ABBOTT *et al.* 1964 b)

| Compound | System number[a] and $R_f$ values | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Aldrin | 0.95 | 0.70 | 0.82 | 0.67 | 0.64 | 0.69 | 0.58 | 0.98 |
| Alpha-BHC | 0.87 | 0.34 | 0.63 | 0.52 | 0.28 | 0.43 | — | 0.69 |
| Gamma-BHC | 0.78 | 0.21 | 0.55 | 0.46 | 0.18 | 0.37 | — | 0.58 |
| *p,p'*-DDE | 0.95 | 0.65 | 0.78 | 0.65 | 0.57 | 0.62 | 0.74 | 0.98 |
| *o,p'*-DDT | 0.89 | 0.50 | 0.73 | 0.59 | 0.46 | 0.58 | 0.50 | 0.90 |
| *p,p'*-DDT | 0.89 | 0.42 | 0.69 | 0.57 | 0.39 | 0.54 | 0.52 | 0.91 |
| deHCl *p,p'*-TDE | 0.93 | 0.53 | 0.75 | 0.49 | 0.53 | 0.62 | 0.67 | 0.98 |
| Dichlorobenzophenone | 0.31 | 0.14 | 0.55 | 0.59 | 0.27 | 0.48 | — | — |
| Dieldrin | 0.37 | 0.12 | 0.52 | 0.65 | 0.48 | 0.48 | 0.30 | 0.58 |
| Endosulfan *A* | 0.65 | 0.17 | 0.64 | 0.58 | 0.35 | 0.52 | — | — |
| Endosulfan *B* | 0.04 | 0.02 | 0.09 | 0.12 | — | — | — | — |
| Endrin | 0.51 | 0.13 | 0.61 | 0.49 | 0.26 | 0.52 | — | — |
| Heptachlor | 0.95 | 0.58 | 0.78 | 0.65 | 0.53 | 0.62 | 0.48 | 0.98 |
| Heptachlor epoxide | 0.49 | 0.17 | 0.57 | 0.39 | — | — | — | — |
| Methoxychlor | — | — | — | — | 0.10 | 0.36 | 0.28 | — |
| *p,p'*-TDE | 0.71 | 0.25 | 0.57 | 0.52 | 0.26 | 0.46 | 0.67 | 0.77 |

[a] Key to systems:

| *System no.* | *Chromatoplate* | | *Mobile solvent* |
|---|---|---|---|
| 1 | Alumina | | Hexane |
| 2 | Silica gel | | Hexane |
| 3 | Alumina | 94+5+1 | Petroleum ether (40° to 60° C.) : liquid paraffin : dioxane |
| 4 | Silica gel | 94+5+1 | Petroleum ether (40° to 60° C.) : liquid paraffin : dioxane |
| 5 | Silica gel | 4+1 | Petroleum ether (40° to 60° C.) : liquid paraffin |
| 6 | Silica gel | 7+2+1 | Cyclohexane : liquid paraffin : dioxane |
| 7 | Silica gel | 9+9+2 | Cyclohexane : benzene : liquid paraffin |
| 8 | 1+1 Silica gel : alumina | 92+8 | Cyclohexane : silicone oil |

one from another; the resolution and identification of the components of mixtures of residual unknown pesticides is thus practicable. These separatory systems have been successfully applied to extracts of vegetable and

animal tissue which have been cleaned up by the *N,N*-dimethylformamide partition process of DE FAUBERT MAUNDER *et al.* (1964 b). Located pesticide spots have been scraped from the carrier plate, extracted with hexane or dichloromethane and further identified by both gas-liquid chromatography and infrared spectroscopy.

For the preparation of pure specimens of residual organochlorine pesticides from fatty samples the use of loose-layer chromatography on alumina is preferred to the systems described above. TAYLOR and FISHWICK (1964) resolved pesticide extracts into two parts by alumina developed with hexane before gas chromatographic examination; in this way aldrin, DDE, and DDT could be separated from TDE, gamma-BHC, heptachlor epoxide, endrin, and dieldrin. Aldrin and DDE could thus be distinguished from heptachlor epoxide and dieldrin which have similar retention times on the silicone gas chromatographic column used.

By replacing the analytical gas chromatograph with a semi-preparative instrument, it is possible to collect sufficient material for infrared identification, the preliminary loose-layer chromatography serving both as a cleanup and a partial separation stage. For this purpose several adsorbents and mobile solvent mixtures have been investigated by ABBOTT *et al.* (1965 c), $R_f$ values obtained being given in Table VI. The loose-layer chromatoplates were prepared by using ribbed-glass as described earlier; development was

Table VI. *$R_f$ values of organochlorine pesticides in some loose-layer chromatographic systems*

| Compound | System number[a] and $R_f$ value | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| *p,p'*-DDE | 0.55 | 0.60 | 0.53 | 0.58 | 0.66 | 0.78 | 0.87 |
| *p,p'*-DDT | 0.23 | 0.31 | 0.38 | 0.56 | 0.37 | 0.60 | 0.64 |
| Dieldrin | 0.05 | 0.07 | 0.28 | 0.37 | 0.22 | 0.52 | 0.55 |
| Endosulfan *B* | 0.00 | 0.03 | 0.06 | 0.20 | 0.21 | — | — |
| Endrin | 0.07 | 0.10 | 0.30 | 0.38 | 0.34 | — | — |
| Heptachlor epoxide | 0.06 | 0.14 | 0.16 | 0.37 | 0.28 | 0.55 | 0.60 |
| Methoxychlor | 0.03 | 0.05 | 0.18 | 0.25 | 0.24 | — | — |
| *p,p'*-TDE | 0.12 | 0.15 | 0.25 | 0.33 | 0.23 | 0.48 | 0.52 |

[a] Key to systems:

| *System no.* | *Adsorbent* | *Mobile solvent* |
|---|---|---|
| 1 | Alumina | Hexane |
| 2 | Alumina | 98+2 Hexane : acetone |
| 3 | Alumina | 95+5 Hexane : acetone |
| 4 | Alumina | 9+1 Hexane : acetone |
| 5 | Silica gel (acid washed) | 9+1 Hexane : acetone |
| 6 | 9+1 Alumina : magnesia | 9+1 Hexane : acetone |
| 7 | 3+1 Alumina : magnesia | 9+1 Hexane : acetone |

very rapid, *ca.* five minutes for ten cm. rise of the solvent front. When applied to the detection and determination of pesticide residues in butter, margarine, and lard, the development of alumina layers with 9+1 hexane :

acetone proved to be the most satisfactory system for final cleanup; for samples of codliver oil, however, the inclusion of 25 percent of magnesia in the adsorbent was advisable. Recoveries of added pesticides at the 0.5 p.p.m. level were generally better than 75 percent from these samples. Excellent infrared spectra have been obtained of dieldrin extracted from mutton fat and cleaned up by this loose-layer procedure, followed by gas-liquid chromatography on an Apiezon column in a semi-preparative instrument from which 95 percent of the emergent pesticide could be collected, only five percent passing through a flame-ionisation detector for quantitative measurements.

### *b) Organophosphorus pesticides*

While paper chromatographic procedures have been found to be generally suitable for the separation of organochlorine pesticides (MITCHELL 1958, EVANS 1962) they are less applicable to the more hydrophilic organophosphorus compounds, although several systems have been proposed (MITCHELL 1960, GETZ 1962). Thin-layer chromatography, owing to its greater versatility, has proved more useful and indeed more attention appears to have been given to its application to organophosphorus compounds than to any other of the main groups of pesticides.

FISCHER and KLINGELHÖLLER (1961 a and b) used both paper and thin-layer chromatography to identify residues of a number of organophosphorus pesticides extracted from animal tissue by hot ethanolic potash. The degradation products given by each compound gave a different spot pattern; for example, malathion yielded four spots of $R_f$ 0.32, 0.44, 0.58, and 0.66 on silica gel *G* developed with 20 + 80 + 3 methanol : dichloromethane: ten percent ammonia solution. The separation of seven pesticides on silica gel chromatoplates with 4 + 1 hexane : acetone as mobile solvent was reported by BÄUMLER and RIPPSTEIN (1961), although parathion and demeton-methyl were incompletely resolved by this system. PETSCHIK and STEGER (1962) preferred starch-bound alumina plates developed with 10 + 1 *n*-heptane : acetone for the separation of some aliphatic esters of thiophosphoric acid; for this purpose activation at 120° C. was essential, drying at 100° C. being insufficient. In association with the use of bioassays for pesticide residue determinations, SALO *et al.* (1962) identified parathion, demeton-methyl, and malathion by chromatography on silica gel *G* developed with toluene for 20 minutes. Parathion-methyl was indistinguishable from parathion by this means.

UCHIYAMA and OKUI (1962) studied the thin-layer chromatography of a number of organophosphorus pesticides on silica gel developed with 4 + 1 hexane : acetone. Residues of demeton-methyl in soybean oil and thiometon in tea leaves were identified in this way. Fenitrothion was isolated from olive oil with almost 100 percent recovery by eluting the observed pesticide from the developed chromatoplate with aqueous ethanol.

Thin-layer chromatography has been used by several workers in studies of the purity of samples of technical organophosphorus pesticides. KOVÁČ (1963) examined fenitrothion on layers of silica developed with petroleum

ether (60° to 80° C.) containing a little acetone (1.4 percent v/v); in this way four components of the technical product were isolated and identified. Phenkapton was similarly examined by STAMMBACH *et al.* (1963) using silica gel chromatoplates developed with either cyclohexane or 19 + 1 carbon tetrachloride : benzene. Impurities present were detected and determined semi-quantitatively by these means. Phosphorothioates of the demeton-*S*-methyl type have been studied by WOGGON *et al.* using a five-component mobile solvent composed of 40 + 16 + 16 + 20 + 9 toluene : *i*-propanol : methanol : acetonitrile : water, and air-dried chromatoplates prepared from silica gel with either water-glass or gypsum (33 percent) as binders. The separation of azinphos-ethyl and azinphos-methyl from one another and from other interfering benzo-triazines has been performed by KATZ and LEMPERT (1964) on silica gel *G* developed with 4 + 1 hexane : acetone.

The cleanup properties of thin-layer chromatoplates were used by BLINN (1964) to obtain specimens of phorate and its metabolites for infrared identification. The sulphoxides and sulphones of phorate and its oxygen analogue are resolved by developing silica gel chromatoplates prepared with a pH 6.0

Table VII. *$R_f$ values of some organophosphorus pesticides*

| Pesticide | System number[a] and $R_f$ value | | | | | | |
|---|---|---|---|---|---|---|---|
| | WALKER and BEROZA (1963) | | | | | BUNYAN (1964) | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Azinphos-methyl | 0.63 | 0.65 | 0.33 | 0.54 | 0.10 | 0.40 | 0.53 |
| Dimethoate | 0.13 | 0.47 | 0.02 | 0.08 | 0.00 | 0.03 | 0.10 |
| Ethion | 0.87 | 0.85 | 0.71 | 0.77 | 0.43 | 0.62 | 0.76 |
| | — | — | — | — | 0.54 | — | — |
| Malathion | 0.73 | 0.77 | 0.46 | 0.63 | 0.20 | 0.45 | 0.64 |
| Mevinphos | 0.17 | 0.57 | 0.03 | 0.09 | — | 0.10 | — |
| | 0.27 | 0.63 | — | 0.16 | — | — | — |
| Parathion | 0.84 | 0.86 | 0.65 | 0.71 | 0.31 | — | — |
| Parathion-methyl | 0.81 | 0.84 | 0.59 | 0.67 | 0.21 | 0.43 | 0.68 |
| Phenkapton | — | — | — | — | — | 0.56 | 0.75 |
| Phorate | 0.86 | 0.89 | 0.68 | 0.75 | 0.50 | 0.69 | 0.73 |
| Phosphamidon | 0.09 | 0.48 | 0.00 | 0.05 | 0.00 | 0.10 | — |
| Schradan | 0.02 | — | 0.00 | 0.00 | 0.00 | — | — |

[a] Key to systems:

| *System no.* | *Adsorbent* | *Mobile solvent (all 9+1 mixtures)* |
|---|---|---|
| 1 | Silica gel *G* | Chloroform : ethyl acetate |
| 2 | Silica gel *G* | Chloroform : acetic acid |
| 3 | Silica gel *G* | Benzene : ethyl acetate |
| 4 | Silica gel *G* | Benzene : acetone |
| 5 | Silica gel *G* | Hexane : acetone |
| 6 | Silica gel *H* | Benzene : acetone |
| 7 | Alumina (Woelm) | Benzene : acetone |

buffer solution and air-dried, with either chloroform containing 1.75 percent of methanol or 45 + 40 + 15 toluene : acetonitrile : nitromethane. Oxidation products of ethion, azinphos-methyl, carbophenothion, and disyston were

also studied by these systems and could be differentiated from the analogous products of phorate. Residues of these materials were extracted from potatoes, subjected to a thin-layer chromatographic separation, and then identified by infrared examination of the spots eluted from the chromatogram. Similar procedures were used in a qualitative and quantitative study of the ability of twelve oxidants to convert phorate to its oxygen-analogue sulphone.

A comprehensive survey of the thin-layer chromatography of pesticides was carried out by WALKER and BEROZA (1963), who included many organophosphorus compounds among the 62 materials examined. Of the 19 solvent systems they describe, those based on benzene or chloroform show the most promise for general use. Table VII shows a selection of the quoted $R_f$ values for several compounds using five of the more useful mobile solvents. One of these, 9 + 1 benzene : acetone, was used by BUNYAN (1964) with chromatoplates prepared with silica gel *H* and alumina (binder free, Woelm); $R_f$ values obtained are also given in Table VII.

In the review by CONKIN (1964) separation of Colep from its major metabolites is described, seven mobile solvents being applied to silica gel chromatoplates. A reversed-phase technique is advised for the separation of Colep, EPN, parathion-methyl, parathion, *p*-nitrophenol, and phenol; silica gel layers inpregnated with either a mineral oil or, preferably, silicone fluid are developed with 1 + 1 + 2 ethanol : acetone : water for two hours, the phenols being used as reference materials. Colep and its metabolites have also been investigated by MARCO and JAWORSKI (1964) on silica gel *G* using an ammoniacal methanolic chloroform solution as the mobile phase. Four other solvent mixtures were also used to observe the chromatographic behaviour of unknown metabolites obtained by feeding Colep labelled with $C^{14}$ to rats.

STELLER and CURRY (1964) used thin-layer chromatography on air-dried, 500-$\mu$. thick layers of 1 + 1 silica gel *G* : silica gel *HF*, developed with 3 + 1 acetone : chloroform, to separate dimethoate and its oxygen analogue extracted from apples, alfalfa, and green tomatoes. Quantitative measurements were made by total phosphorus determinations made on pesticides eluted from the adsorbent with dilute nitric acid.

The use of thin-layer and gas-liquid chromatography in the detection, determination, and infrared identification of organophosphorus pesticide residues extracted from samples of vegetable tissue has been described by ABBOTT *et al.* (1965 b). Thin-layer chromatography is used both as a preliminary tentative identification procedure and for cleanup purposes prior to the gas chromatographic preparation of a pure specimen for confirmation of identity by infrared spectroscopy. For this purpose, 500 $\mu$. thick layers of silica gel *G* are developed with 9 + 1 hexane : acetone for 40 minutes, resolved pesticide spots being eluted from the adsorbent with dichloromethane. Other chromatographic systems are recommended for further confirmatory purposes should there be insufficient material for positive infrared examination. Better resolution of the pesticides was obtained with a mobile solvent composed of 19 + 1 hexane : acetone but cleanup was not so good. The use of mixtures of equal parts of kieselguhr *G* with either silica

gel G or alumina G gave chromatoplates of useful properties. These were developed with four different mobile solvents; $R_f$ values obtained by use of some of these systems are illustrated in Table VIII. It is apparent that

Table VIII. *$R_f$ values of some organophosphorus pesticides (developed for 40 minutes in chambers 28×26.5×21 cm.)* (ABBOTT *et al.* 1965 b)

| Pesticide | System number[a] and $R_f$ value | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Azinphos-methyl | 0.05 | 0.14 | 0.24 | 0.00 | 0.27 | 0.00 | 0.03 | 0.58 | 0.00 |
| Carbophenothion | 0.52 | 0.66 | 0.80 | 0.45 | 0.96 | 0.36 | 0.39 | 0.92 | 0.27 |
| Chlorthion . . . | 0.16 | 0.27 | 0.52 | 0.11 | 0.52 | 0.10 | 0.12 | 0.81 | 0.09 |
| Dichlorvos. . . | 0.04 | 0.10 | 0.17 | 0.03 | 0.23 | 0.00 | 0.00 | 0.03 | 0.00 |
| Dimethoate . . | 0.02 | 0.04 | 0.07 | 0.00 | 0.02 | 0.00 | 0.00 | 0.12 | 0.00 |
| Ethion . . . . | 0.60 | 0.82 | 0.88 | 0.38 | 0.96 | 0.50 | 0.57 | — | 0.39 |
| | 0.40 | 0.60 | 0.74 | 0.16 | 0.90 | 0.15 | 0.17 | 0.90 | 0.13 |
| Fenchlorphos. . | 0.50 | 0.68 | 0.81 | 0.60 | 0.96 | 0.64 | 0.68 | 0.93 | 0.45 |
| Malathion . . . | 0.12 | 0.29 | 0.45 | 0.00 | 0.57 | 0.00 | 0.02 | 0.81 | 0.00 |
| Parathion . . . | 0.37 | 0.62 | 0.72 | 0.14 | 0.80 | 0.11 | 0.18 | 0.90 | 0.95 |
| Phenkapton . . | 0.54 | 0.71 | 0.84 | 0.43 | 0.95 | 0.43 | 0.38 | 0.90 | 0.30 |
| Phorate . . . . | 0.60 | 0.75 | 0.84 | 0.33 | 0.96 | 0.39 | 0.44 | 0.92 | 0.32 |
| Phosphamidon . | 0.22 | 0.44 | 0.65 | 0.02 | 0.00 | 0.00 | 0.00 | 0.78 | 0.00 |
| Thiometon. . . | 0.46 | 0.61 | 0.66 | 0.18 | 0.90 | 0.23 | 0.26 | 0.90 | 0.00 |

[a] Key to systems:

| *System no.* | *Adsorbent* | *Mobile solvent* |
|---|---|---|
| 1 | Silica gel | 19+1 Hexane : acetone |
| 2 | Silica gel | 9+1 Hexane : acetone |
| 3 | Silica gel | 6+1 Hexane : acetone |
| 4 | 1+1 Kieselguhr : silica gel | Petroleum ether (40° to 60° C.) |
| 5 | 1+1 Kieselguhr : silica gel | 9+1 Cyclohexane : acetone |
| 6 | 1+1 Kieselguhr : alumina | Cyclohexane |
| 7 | 1+1 Kieselguhr : alumina | Petroleum ether (40° to 60° C.) |
| 8 | 1+1 Kieselguhr : alumina | 9+1 Cyclohexane : acetone |
| 9 | 1+1 Kieselguhr : alumina | 20+1 Hexane : liquid paraffin |

these mixed composition chromatoplates can effect separations that were not achieved on silica gel alone, e.g., carbophenothion from fenchlorphos and parathion from thiometon.

### *c) Herbicides and growth-regulating compounds*

Methods for the detection and determination of chlorophenoxy acid herbicides in soil and water samples have been required for percolation and run-off studies. Compounds of this type were readily extracted by ether from acidified waters or obtained by Soxhlet extraction of soils followed by a partition cleanup. Paper chromatographic determination using reflectance densitometry was used for quantitative measurement (ABBOTT *et al.* 1964 a). However, paper chromatographic methods proved to be unsuitable for the resolution of MCPB and 2,4-DB or MCPA and 2,4,5-T. Thin-layer chromatography proved much more successful as a tool for the qualitative identification of chlorophenoxy acid herbicides. The mobile solvent used for

the paper chromatography of these compounds, 20+3+2+1 cyclohexane : benzene : glacial acetic acid : liquid paraffin, was satisfactory when used with chromatoplates composed of mixtures of silica gel *G* and kieselguhr *G*. Table IX shows the $R_f$ values observed for several herbicidal compounds

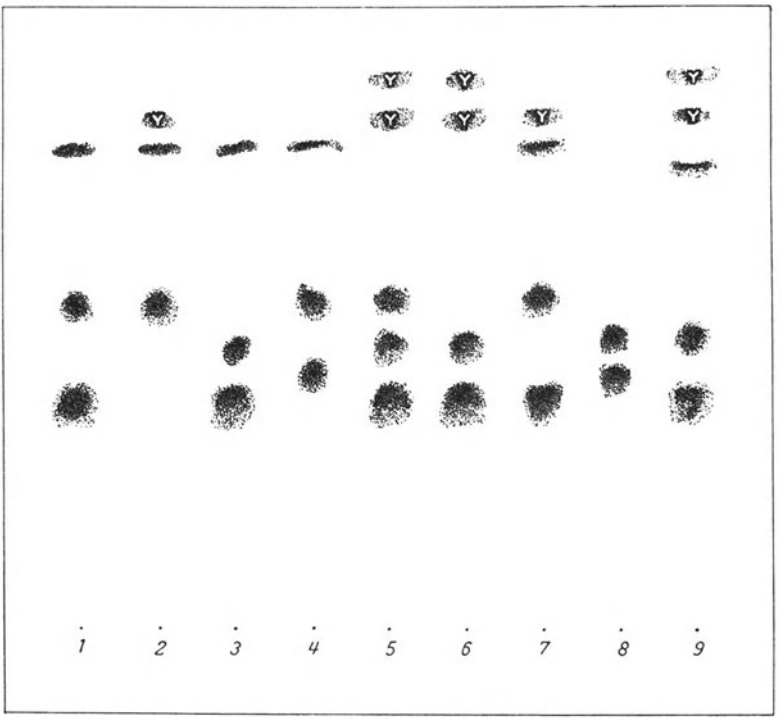



Fig. 10. Appearance of chromatogram on 60 : 40 kieselguhr *G*/silica gel *G*:

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|
| MCPB | DNOC | MCPB | MCPB | Dinoseb | Dinoseb | DNOC | 2,4,5-T | Dinoseb |
| MCPA | MCPB | 2,4,5-T | MCPA | DNOC | DNOC | MCPB | 2,4-D | DNOC |
| Dalapon | MCPA | Dalapon | 2,4-D | MCPA | 2,4,5-T | MCPA | | 2,4-DB |
| | | | | 2,4,5-T | Dalapon | Dalapon | | 2,4,5-T |
| | | | | Dalapon | | | | Dalapon |

Dinoseb and DNOC yellow spots, *Y* (Abbott *et al.* 1964 a)

Table IX. *$R_f$ values of some chlorinated herbicides on mixed silica gel G: kieselguhr G chromatoplates developed in 20+3+2+1 cyclohexane : benzene : glacial acetic acid : liquid paraffin (tank size 28×26.5×21 cm., unlined)*

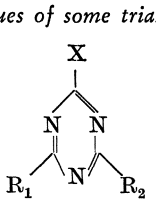
| Herbicide | $R_f$ value in percent kieselguhr *G* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0% | 10% | 30% | 40% | 50% | 60% | 80% | 100% |
| MCPB | 0.18 | 0.32 | 0.52 | 0.61 | 0.67 | 0.75 | 0.84 | 0.90 |
| 2,4-DB | 0.17 | 0.26 | 0.44 | 0.52 | 0.60 | 0.66 | 0.76 | 0.86 |
| MCPA | 0.14 | 0.17 | 0.26 | 0.32 | 0.37 | 0.45 | 0.62 | 0.82 |
| 2,4,5-T | 0.13 | 0.15 | 0.20 | 0.25 | 0.31 | 0.37 | 0.55 | 0.80 |
| 2,4-D | 0.12 | 0.14 | 0.18 | 0.21 | 0.26 | 0.32 | 0.50 | 0.80 |
| Dalapon | 0.10 | 0.12 | 0.17 | 0.20 | 0.25 | 0.30 | 0.47 | 0.76 |

on chromatoplates of this nature; a mixure of 40 + 60 silica gel : kieselguhr was found to be most suitable. Figure 10 illustrates the separations given by this system which was also found to be suitable for resolving dinoseb and DNOC.

The separation of six esters of MCPA and MCPP has been reported by HENKEL and EBING (1964). Silica gel *G* chromatoplates were developed by a two-step process with 5 + 1 cyclohexane : diisopropyl ether as mobile solvent. The chromatoplates were prepared by hand, using two g. of adsorbent per 10×20 cm. plate, and were allowed to dry in air.

In the same publication the separation of prometryne, propazine, atrazine, prometon, simazine, and atraton is illustrated. A two-step development with 3 + 2 chloroform : diisopropyl ether was used with hand-poured silica gel chromatoplates. Later work by HENKEL (1964) suggests two solvent systems for the resolution of mixtures of compounds of the triazine class. Chloroform and nitromethane are used in 1 + 1 admixture for the methoxy and methylthio-substituted compounds, and 5 + 1 for the chlorinated triazines. Table X shows the structure of the various materials examined

Table X. *Structure and $R_f$ values of some triazine herbicides* (HENKEL 1964)




| Herbicide | X | $R_1$ | $R_2$ | $R_f$ [a] |
|---|---|---|---|---|
| Prometon . . . | $OCH_3$ | NH-*i*-$C_3H_7$ | NH-*i*-$C_3H_7$ | 0.42 |
| Atraton . . . . | $OCH_3$ | NH-*i*-$C_3H_7$ | NH-$C_2H_5$ | 0.34 |
| Simeton . . . . | $OCH_3$ | NH-$C_2H_5$ | NH-$C_2H_5$ | 0.26 |
| Prometryne . . | $SCH_3$ | NH-*i*-$C_3H_7$ | NH-*i*-$C_3H_7$ | 0.68 |
| Ametryne . . . | $SCH_3$ | NH-*i*-$C_3H_7$ | NH-$C_2H_5$ | 0.59 |
| Simetryne . . . | $SCH_3$ | NH-$C_2H_5$ | NH-$C_2H_5$ | 0.50 |
| | | | | $R_f$ [b] |
| Chlorazine . . . | Cl | N-$(C_2H_5)_2$ | N-$(C_2H_5)_2$ | 0.80 |
| Ipazine . . . . | Cl | N-$(C_2H_5)_2$ | NH-*i*-$C_3H_7$ | 0.66 |
| Trietazine . . . | Cl | N-$(C_2H_5)_2$ | NH-$C_2H_5$ | 0.60 |
| Propazine . . . | Cl | NH-*i*-$C_3H_7$ | NH-*i*-$C_3H_7$ | 0.48 |
| Atrazine . . . . | Cl | NH-*i*-$C_3H_7$ | NH-$C_2H_5$ | 0.37 |
| Simazine . . . | Cl | NH-$C_2H_5$ | NH-$C_2H_5$ | 0.28 |

[a] 1+1 Chloroform: nitromethane, ten-cm. rise, 15 minutes.
[b] 5+1 Chloroform: nitromethane, ten-cm. rise, 15 minutes.

and also lists their $R_f$ values in the appropriate system. STAMMBACH *et al.* (1964) have used thin-layer chromatography together with gas-liquid chromatography in a study of the synthesis and purity of atraton, atrazine, and prometryne.

Quantitative thin-layer chromatographic procedures have recently been applied to the determination of triazine herbicides in soil and water (ABBOTT *et al.* 1965 a). The compounds are readily extracted from ammoniacal

solution by dichloromethane; a preliminary extraction with ether removes them from soil. The concentrated dichloromethane extract was spotted on to a silica gel G chromatoplate which was developed with 9 + 1 chloroform : acetone for 35 minutes. Determinations were made by measuring the spot areas and referring to standard graphs of square root of area against log of weight. Several separatory systems were also investigated, $R_f$ values being illustrated in Figure 11.

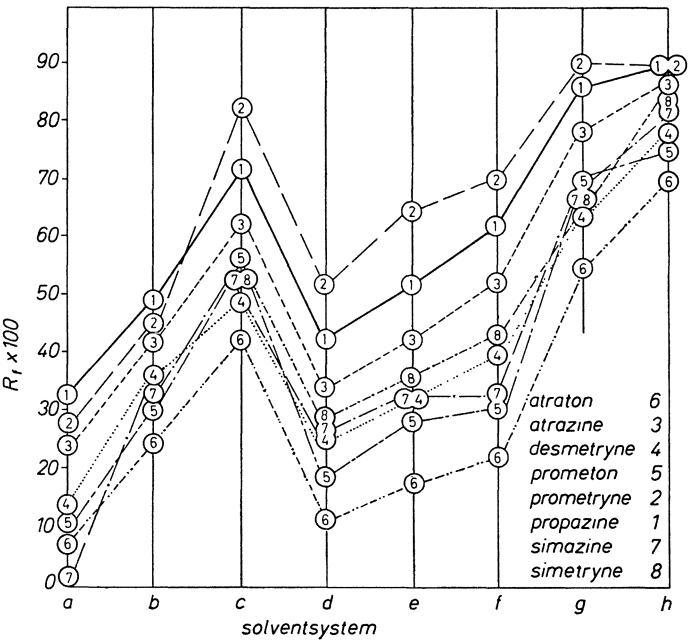



Fig. 11. Thin-layer chromatographic separation of triazine herbicides (ABBOTT *et al.* 1965 a). Solvent systems: *A*, hexane + 10 percent acetone; *B*, hexane + 20 percent acetone; *C*, chloroform + 10 percent acetone; D, nitromethane + 50 percent DCM; *E*, nitromethane + 50 percent chloroform; *F*, nitromethane + 50 percent carbon tetrachloride; *G* chloroform + 7.5 percent acetone (gel-guhr plate); and *H*, trichlorethylene + 50 percent dioxan

Growth-regulating compounds other than the triazines and chlorophenoxy acids are not well represented in the literature of thin-layer chromatography. HENKEL (1964) has investigated the separation of some substituted phenylalkyl ureas. Silica gel G chromatoplates developed with 1 + 1 chloroform : nitromethane gave poor resolution of linuron, neburon, and monolinuron ($R_f$ values 0.79, 0.77, and 0.73, respectively), but separated these from fenuron (0.31), monuron (0.41), and diuron (0.53). The separation of seven gibberellins on silica gel has been described by SEMBDNER *et al.* (1963). Several mixtures of chloroform, ethyl acetate, and acetic acid were used as mobile solvent, $R_f$ values being related to gibberellin $A_3$ as a standard. BACHE (1964) used a thin-layer procedure to determine amiben in tomatoes. Extracts were cleaned up by a partition process and spotted onto silica gel G chromatoplates which had been allowed to dry in

air overnight. Standard amiben was spotted alongside the sample in amounts of 0.1 and 0.2 $\mu$g. After development with 5 + 1 benzene : acetic acid the amiben ($R_f$ value 0.44) was rendered visible by spraying with sodium nitrite and *N*-1-naphthyl-ethylenediamine, quantitative determination being made by a visual comparison of the density of the magenta spots so obtained.

### *d) Miscellaneous pesticidal compounds*

Probably the earliest application of thin-layer chromatography to pesticide residue analysis was the determination of biphenyl, used as a fungistat on citrus fruits, by KIRCHNER *et al.* (1954). Starch-bound silica gel chromatostrips were developed with petroleum ether (30° to 60° C.) for seven minutes, the solvent front migrating about five cm. in this time. Observed biphenyl spots ($R_f$ value 0.45) were clearly separated both from citrus oil hydrocarbons ($R_f$ 0.95) and from the oxygenated components which remain at the origin. Elution with 95 percent ethanol yielded biphenyl in a purity suitable for quantitative estimation by ultraviolet spectrophotometry.

Pyrethroid compounds also attracted early attention. SPICKETT (1957) used chromatoplates prepared from 4 + 1 silica gel and plaster of Paris, developed with 4 + 1 *n*-hexane : ethyl acetate, to separate pyrethrum extract into two groups of $R_f$ value 0.42 and 0.21. This system was then transferred to columnar use for preparative purposes, seven g. of purified pyrethrum extract being chromatographed on 500 g. of silica gel. In an examination of the inactivation of pyrethroid compounds by exposure to light and air, STAHL (1960) made use of a "separation-reaction-separation" technique. Silica gel chromatoplates were developed with 3 + 1 hexane : ethyl acetate, then exposed to ultraviolet light and finally developed again in the same solvent in a direction perpendicular to that first used. Each of the main components of pyrethrum was converted in part to oxidised compounds of lower $R_f$ values and of lesser insecticidal activity as measured by biological tests with *Aedes aegyptii* and *Drosophila melanogaster*. Table XI shows the $R_f$ values observed for these compounds and also for allethrin and some synergestic compounds; three indicator compounds were also spotted on to the plates to observe changes in the activity of the absorbent between the successive developments. BEROZA (1963) made use of fourteen mobile solvents in attempts to resolve nine commercially available pyrethrin synergists. A mixture of 39 + 1 benzene : acetone gave the best separations on layers of silica gel *G*. Loose-layer chromatography on alumina, developed with 4 + 1 hexane : ethyl acetate, has been used to separate pyrethrin I and cinerin I ($R_f$ 0.60) from pyrethrin II and cinerin II ($R_f$ 0.30). The separated components were then eluted for subsequent gas-liquid chromatographic confirmation of identity (ABBOTT *et al.* 1965 d). Similar separation was obtained on silica gel *G* chromatoplates developed with 9 + 1 hexane : acetone.

Thin-layer chromatography on silica gel has proved to be a most effective technique for the resolution of carbaryl and its metabolites (DOROUGH and CASIDA 1964). Analytical chromatoplates were prepared with a thickness of 0.3 mm. while preparative layers were one mm. thick.

Development was carried out with 4+1 ether : hexane, followed on occasion by a successive development in a perpendicular direction with 4+1 methylene chloride : acetonitrile. Two-dimensional development with the former mobile solvent has shown that some decomposition occurred during

Table XI. *$R_f$ values of some pyrethroid compounds. Silica-gel chromatoplates developed two-dimensionally with 3+1 hexane: ethyl acetate* (STAHL 1960)

| Compound | $R_f$ value | |
|---|---|---|
| | First development | Second development |
| Pyrethrin I | 0.50 | 0.60 |
| II | 0.30 | 0.41 |
| Cinerin I | 0.57 | 0.72 |
| II | 0.35 | 0.49 |
| Pyrethrin I-peroxide | — | 0.23 |
| II-peroxide | — | 0.11 |
| Cinerin I-peroxide | — | 0.37 (0.23) |
| II-peroxide | — | 0.17 |
| "Lumipyrethrin" | — | 0.0 |
| Allethrin | 0.48 | — |
| Piperonyl butoxide | 0.35 | — |
| Bucarpolate | 0.23 | — |
| S 421 | 0.67 | — |
| Butter yellow | 0.42 | 0.53 |
| Indophenol | 0.35 | 0.45 |
| Sudan red *G* | 0.27 | 0.36 |

chromatographic examination. Carbaryl labelled with $C^{14}$ was used for part of these studies, spots being indicated by autoradiograms of the chromatograms. Of the eight metabolites indicated on the chromatograms, four were identified and possible structures have been proposed for the remainder. As a rapid screening procedure for the determination of carbaryl and 1-naphthol residues, CHIBA and MORLEY (1964) have used silica gel chromatoplates developed with 19+1 benzene : acetone for 35 to 40 minutes (ten cm. distance). Comparison of spot density with standards developed alongside the sample was used semi-quantitatively over the range one to ten p.p.m. on apples, lettuce, and tomato extracts, no cleanup stages being needed.

Traces of pentachlorophenol, used as a fungicide and wood preservative, have also been determined by means of thin-layer chromatographic procedures. PETROWITZ (1961 a and b, 1962) has examined several systems, including silica gel chromatoplates containing buffering acids such as boric, oxalic, citric, etc.; development with benzene or chloroform gave $R_f$ values ranging from 0.53 to 0.74. Use of hexane as mobile solvent yielded $R_f$ values around 0.07 but gave clear distinction from DDT and alpha- and gamma-BHC. DETERS (1962) prepared silica gel chromatoplates containing oxalic acid by using an $N/20$ solution to prepare the slurry instead of water. On such layers developed with chloroform the $R_f$ value of pentachlorophenol was very dependent on the amount of coextracted materials but was about 0.50. Qualitatively five $\mu$g. could be detected; for quantitative evaluation by ultraviolet spectrophotometry quantities about 120 $\mu$g. were preferred.

An excellent example of the way in which thin-layer chromatography fits into the armoury of pesticide residue analysts, together with partition cleanup, columnar separation, gas chromatography, infrared spectroscopy, etc., has been given by BLINN and GUNTHER (1963) who used silica gel plates developed with benzene containing 3.5 percent of ethyl acetate to distinguish between Aramite and some related acaricides.

Table XII. *Common and chemical names of pesticides mentioned in text*

| Common name | Chemical name |
|---|---|
| Aldrin | 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo,exo*-5,8-dimethanonaphthalene |
| Allethrin | 2-allyl-3-methylcyclopent-2-en-4-ol-1-onyl chrysanthemate |
| Ametryne | 2-methylmercapto-4-ethylamino-6-isopropylamino-*s*-triazine |
| Amiben | 3-amino-2,5-dichlorobenzoic acid |
| Aramite | 2-(*p*-tert butylphenoxy)-1-methylethyl-2-chloroethyl sulfite |
| Atraton | 2-methoxy-4-ethylamino-6-isopropylamino-*s*-triazine |
| Atrazine | 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine |
| Azinphos-ethyl | *S*-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-ylmethyl) *O,O*-diethyl phosphorodithioate |
| Azinphos-methyl | *S*-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-ylmethyl) *O,O*-dimethyl phosphorodithioate |
| BHC (isomers) | various geometric isomers of 1,2,3,4,5,6-hexachlorocyclohexane |
| Captan | *N*-trichloromethylmercapto-4-cyclohexane-1,2-dicarboximide |
| Carbaryl | 1-naphthyl-*N*-methylcarbamate |
| Carbophenothion | *S*-(*p*-chlorophenylthiomethyl) *O,O*-diethyl phosphorodithioate |
| Chlorazine | 2-chloro-4,6-bis(diethylamino)-*s*-triazine |
| Chlorthion | *O*-(3-chloro-4-nitrophenyl) *O,O*-dimethyl phosphorothioate |
| Colep | *O*-(*p*-nitrophenyl)-*O*-phenyl methylphosphonothionate |
| 2,4-D | 2,4-dichlorophenoxyacetic acid |
| 2,4-DB | α-(2,4-dichlorophenoxy)butyric acid |
| DDE | 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene |
| o,p′-DDT | 1,1,1-trichloro-2-*o*-chlorophenyl-2-*p*-chlorophenylethane |
| p,p′-DDT | 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane |
| Demeton-*S*-methyl | *O,O*-dimethyl *S*-2-(ethylthio)ethyl phosphorothioate |
| Desmetryne | 2-isopropylamino-4-methylamino-6-methylthio-*s*-triazine |
| Dichlorvos | *O,O*-dimethyl-2,2-dichlorovinyl phosphate |
| Dieldrin | 1,2,3,4,10,10-hexachloro-*exo*-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo,exo*-5,8-dimethanonaphthalene |
| Dimethoate | *O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate |
| Dinoseb | 2,4-dinitro-6-sec-butylphenol |
| Disyston | *O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate |
| Diuron | 3-(3,4-dichlorophenyl)-1,1-dimethylurea |
| DNOC | 2,4-dinitro-6-methylphenol |
| Endosulfan | 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide |
| Endrin | 1,2,3,4,10,10-hexachloro-*exo*-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo,endo*-5,8-dimethanonaphthalene |
| EPN | *O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothioate |
| Ethion | *O,O,O′,O′*-tetraethyl *S,S′*-methylene bisphosphorodithioate |
| Fenchlorphos | *O,O*-dimethyl *O*-2,4,5-trichlorophenyl phosphorothioate |
| Fenitrothion | *O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate |
| Fenuron | *N,N*-dimethyl-*N′*-phenylurea |
| Heptachlor | 1,4,5,6,7,8,8-heptachloro-3a,4,5,5a-tetrahydro-4,7-*endo*-methanoindene |
| Heptachlor epoxide | 1,4,5,6,7,8,8-heptachloro-2,3-epoxy-2,3,3a,7a-tetrahydro-4,7-methanoindene |

| Common name | Chemical name |
|---|---|
| Ipazine | 2-chloro-4-diethylamino-6-isopropylamino-*s*-triazine |
| Isodrin | 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*exo*-*exo*-5,8-dimethanonaphthalene |
| Kelthane | 4,4′-dichloro-α-(trichloromethyl)benzhydrol |
| Linuron | *N*′-(3,4-dichlorophenyl)-*N*-methoxy-*N*-methylurea |
| Malathion | *O*,*O*-dimethyl *S*-(1,2-dicarbethoxyethyl) phosphorodithioate |
| MCPA | 4-chloro-2-methylphenoxyacetic acid |
| MCPB | α-(4-chloro-2-methylphenoxy)butyric acid |
| Mecoprop | α-(4-chloro-2-methylphenoxy)propionic acid |
| Methoxychlor | 1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane |
| Mevinphos | dimethyl-2-methoxycarbonyl-1-methylvinyl phosphate |
| Monolinuron | *N*′-(4-chlorophenyl)-*N*-methoxy-*N*-methylurea |
| Monuron | *N*′-(4-chlorophenyl)-*N*,*N*-dimethylurea |
| Neburon | *N*-butyl-*N*′-(3,4-dichlorophenyl)-*N*-methylurea |
| Parathion | *O*,*O*-diethyl *O*-*p*-nitrophenyl phosphorothioate |
| Parathion-methyl | *O*,*O*-dimethyl *O*-*p*-nitrophenyl phosphorothioate |
| Perthane | 2,2-dichloro-1,1-bis(*p*-ethylphenyl)ethane |
| Phenkapton | *O*,*O*-diethyl *S*-(2,5-dichlorophenylthiomethyl) phosphorodithioate |
| Phorate | *O*,*O*-diethyl *S*-(ethylthio)methyl phosphorodithioate |
| Phosphamidon | dimethyl diethylamido-1-chlorocrotonyl(2) phosphate |
| Prometon | 2-methoxy-4,6-bis(isopropylamino)-*s*-triazine |
| Prometryne | 2-methylmercapto-4,6-bis(isopropylamino)-*s*-triazine |
| Propazine | 2-chloro-4,6-bis(isopropylamino)-*s*-triazine |
| Schradan | bis-*N*,*N*,*N*′,*N*′-tetramethylphosphorodiamidic anhydride |
| Simazine | 2-chloro-4,6-bis(ethylamino)-*s*-triazine |
| Simeton | 2-methoxy-4,6-bis(ethylamino)-*s*-triazine |
| Simetryne | 2-methylmercapto-4,6-bis(ethylamino)-*s*-triazine |
| 2,4,5-T | 2,4,5-trichlorophenoxyacetic acid |
| TDE | 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethane |
| Thiometon | *S*-[2-(ethylthio)ethyl] *O*,*O*-dimethyl phosphorodithioate |
| Toxaphene | mixture of isomers of octachlorocamphene (67 to 69 percent chlorine) |
| Trietazine | 2-chloro-4-diethylamino-6-ethylamino-*s*-triazine |

## Summary

From the foregoing account it is apparent that thin-layer chromatography has rapidly developed into a precise technique of considerable use to the pesticide residue analyst. The growth of this usage is illustrated by the fact that more than 75 percent of the literature on this subject has appeared since the end of 1962.

The inexpensiveness of the apparatus essentially required and the simplicity, speed, and versatility of the technique have combined to render its usage almost indispensible in many fields. Thin-layer chromatography has been successfully applied to the diagnostic, quantitative, and preparative studies of pesticides and their residues in animal and vegetable tissue. It has in many ways replaced the more cumbersome and limited paper chromatographic techniques previously employed, particularly for compounds of a somewhat polar nature.

The reproducibility of the excellent separations of organochlorine and organophosphorus insecticides and of herbicides and other miscellaneous compounds which can be achieved on thin-layer chromatoplates have been shown to be dependent on the strict control of several parameters. Apart

from the obvious need for standard procedures for the preparation and activation of the layers, other controlling factors such as size of development chamber, the temperature, time, and angle of development, and the nature of the solvent-vapour equilibrium within the tank must all be closely replicated if accurate comparisons with previous experiments are to be made.

Various reagents which have been proposed for the visualization of the developed chromatogram are discussed, as is the documentation of the finished product. Techniques which have been used for the quantitative evaluation of the chromatograms are also described.

In general, thin-layer chromatography appears to have been merely used as an analytical tool rather than studied as a technique in its own right. The recent advances in this procedure as exemplified by wedge-layer, gradient-layer, and multi-band spreading devices, the large-scale preparative apparatus, and temperature controlled development tanks may lead to a futher widening of thin-layer chromatography concepts.

For the separation of pesticides and their metabolites there seem to be as many proposed combinations of adsorbent and mobile solvent as there are pesticide residue analysts. Certain broad generalisations may be drawn, however, and the use of silica gel or alumina chromatoplates together with a non-polar mobile solvent, such as hexane, to which additions of polar solvents may be made as required, would give a good starting point for any investigation.

Although it has been shown to possess many useful attributes, thin-layer chromatography can by no means provide the whole answer to every problem. However, when its evidence is taken into consideration with that given by other chromatographic or identifying procedures, then the final decision is that much more certain.

## Résumé *

Des mémoires précédents il apparaît que la chromatographie en couche mince s'est rapidement transformée en une technique précise de grand intérêt pour l'analyse des résidus de pesticides. L'extension de son emploi est illustrée par le fait que plus de 75 pour cent des mémoires sur ce sujet ont été écrits depuis fin 1962.

La modicité du prix de l'appareillage et la simplicité, la vitesse et la souplesse de la technique s'allient pour rendre son usage presque indispensable dans bien des domaînes. La chromatographie en couche mince a été appliquée avec succès à l'analyse qualitative, quantitative et préparative des résidus dans les tissus animaux et végétaux. Elle a remplacé bien souvent les techniques limitées et plus laborieuses de la chromatographie sur papier précédemment utilisées, particulièrement pour les composés quelque peu polaires.

La reproductibilité des excellentes séparations des pesticides organo-chlorés et organo-phosphorés, des herbicides et des autres composés divers qui peuvent être obtenues sur les plaques de couches minces dépend du controle

* Traduit par R. MESTRES.

rigoureux de plusieurs paramètres. En dehors de la nécessité évidente de protocoles standardisés pour la préparation et l'activation des couches, d'autres facteurs tels que la taille de la chambre de développement, la température, la durée et l'angle de développement ainsi que la nature de l'équilibre liquide-vapeur à l'intérieur de la cuve doivent être étroitement reproduits pour autoriser des comparaisons exactes entre les expériences précédentes.

La discussion intéresse divers réactifs proposés pour la révélation des chromatogrammes ainsi que leur documentation. Les techniques qui ont été utilisées pour l'évaluation quantitative des chromatogrammes sont aussi décrites.

En général la chromatographie en couche mince semble avoir été plutôt utilisée comme moyen analytique qu'étudiée comme une technique en elle même. Les progrès récents de ce procédé comme en témoignent les dispositifs d'étalement en coin, en gradient et en bandes multiples, les appareils pour chromatographie préparative et les cuves à température controlée peuvent conduire à un élargissement des concepts de la chromatographie en couche mince.

Pour la séparation des pesticides et de leurs métabolites il semble y avoir autant de combinaisons d'adsorbants et de phases mobiles que de chimistes de résidus de pesticides. Des conclusions générales peuvent cependant être tirées et l'emploi de plaques de silica gel ou d'alumine avec un solvant mobile non polaire comme l'hexane auquel des solvants polaires peuvent être ajoutés selon le cas, donneront un bon point de départ à toute recherche. Bien que l'on ait montré que la chromatographie en couche mince possède de nombreuses qualités utiles, elle ne peut en aucune façon résoudre complétement tous les problèmes. Cependant lorsque ses résultats sont comparés à ceux donnés par d'autres procédés chromatographiques ou d'identification, il est plus facile d'énoncer la décision finale.

## Zusammenfassung *

Aus der vorliegenden Zusammenstellung wird deutlich, daß sich die Dünnschichtchromatographie rasch zu einer präzisen, breit anwendbaren Technik für den Analytiker von Pestizid-Rückständen entwickelt hat. Wie sehr ihre Verwendung zunahm, erhellt allein aus der Tatsache, daß mehr als 75% der einschlägigen Literatur seit Ende 1962 erschienen sind.

Die hauptsächlich benötigten Geräte sind nicht teurer, und da auch die Technik einfach, schnell und vielseitig ist, wurde ihr Einsatz auf vielen Gebieten nahezu unentbehrlich. Die Dünnschichtchromatographie ist mit Erfolg verwendet worden für diagnostische, quantitative und präparative Untersuchungen von Pestiziden und ihrer Rückstände in tierischen und pflanzlichen Geweben. In mancher Hinsicht hat sie die früher benutzten, mühsameren und in der Anwendung engeren papierchromatographischen Verfahren ersetzt, besonders für Verbindungen mit polarerer Natur.

Die Reproduzierbarkeit der hervorragenden Auftrennungen, die sich mit

* Übersetzt von H. Frehse.

Organochlor- und Organophosphor-Pestiziden, Herbiziden und verschiedensten anderen Verbindungen auf Dünnschichtplatten erzielen lassen, hängt von der strikten Beachtung verschiedener Parameter ab. Neben der selbstverständlichen Standardisierung bei der Herstellung und Aktivierung der Schichten müssen auch die anderen entscheidenden Faktoren (Größe der Entwicklungskammer, Temperatur, Zeit und Winkel bei der Entwicklung, Art des Lösungsmittel/Dampf-Gleichgewichts in der Kammer) streng eingehalten werden, wenn genaue Vergleiche mit vorausgegangenen Versuchen angestellt werden sollen.

Zahlreiche zum Sichtbarmachen der chromatographierten Substanzen vorgeschlagene Reagenzien werden diskutiert, ebenso die Dokumentation fertiger Chromatogramme. Des weiteren werden Verfahren zur quantitativen Auswertung von Chromatogrammen beschrieben.

Im allgemeinen scheint die Dünnschichtchromatographie fast ausschließlich als Mittel zur Analyse eingesetzt worden zu sein, vielmehr jedenfalls als sie als Technik selbst untersucht worden ist. Die neueren Entwicklungen, wie z. B. Keilschichten (wedge layers), Gradienten-Schichten (gradient layers) und Mehrschichtenauftraggeräte (multi-band spreading devices) sowie präparative Apparaturen für größere Ansätze und temperaturregulierte Entwicklungskammern, können künftig zu einer noch breiteren Anwendung der Dünnschichtchromatographie führen.

Für die Auftrennung von Pestiziden und ihrer Metaboliten sind offenbar ebensoviele Kombinationen von Adsorbentien und mobilen Phasen beschrieben worden wie es Rückstandsanalytiker gibt. Gewisse breite Verallgemeinerungen sind jedoch möglich; z. B. bietet die Verwendung von Platten mit Silikagel- oder Aluminiumoxid-Schichten mit unpolaren Laufmitteln wie Hexan, erforderlichenfalls unter Zusätzen von polaren Lösungsmitteln, einen guten Ansatzpunkt für Untersuchungen aller Art.

Trotz ihrer vielen nützlichen Eigenschaften kann die Dünnschichtchromatographie keinesfalls eine vollständige Antwort auf jede Problemstellung geben. Wenn die Aussagen, die sie zu machen gestattet, jedoch in Zusammenhang mit den Befunden aus anderen chromatographischen oder für Identifizierungen geeigneten Verfahren gesehen werden, dann fällt die endgültige Entscheidung um ein Wesentliches gesicherter aus.

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# Rogor (dimethoate) residues in food crops

By

P. DE PIETRI-TONELLI *, B. BAZZI **, and R. SANTI **

With 4 figures

## Contents



## I. Introduction

Owing to its high insecticidal activity, systemic properties and relatively low toxicity to mammals, Rogor [*O,O*-dimethyl *S*-(*N*-methyl carbamoylmethyl)phosphorodithioate], also known as dimethoate in the USA

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and in other countries and as Fosfamide in the Soviet Union, has found a progressively increasing use for the control of several species of phytophagous insects and mites injurious to a great number of cultivated plants. Its spectrum of activity and properties are consistently documented by a wide literature which has been recently reviewed by DE PIETRI-TONELLI (1965).

Since the discovery of Rogor many researches on its residues have been undertaken but we may say that they cover only a limited number of the most important botanical species injured by pests which are effectively controlled by the use of this insecticide. Even if much work is still to be done, the studies which have been already carried out have started to investigate the main aspects of the problem of Rogor residues and namely a) quantitative determination of the insecticide and of its degradation products and metabolites, b) qualitative research on the chemical composition of its metabolites originating in the vegetal tissues, c) the pattern of distribution of the insecticide and of its metabolites in the vegetal organs, d) the toxicological effects of its residues upon the health of human beings, domestic animals, and wildlife. This paper gives a rather comprehensive (but certainly incomplete) picture of the present knowledge on Rogor residues, restricted to food crops and vegetal food products. After a review of the analytical methods developed by several workers, information is summarized on the residue degradation and persistence curves of the insecticide in food crops and data are reported for harvest residues in fruits and vegetables.

## II. Research methods

### *a) Physico-chemical procedures*

Two procedures for the determination of Rogor residues in cherries were worked out by SANTI and BAZZI (1956). The first technique, via colorimetry, is based on the extraction of Rogor from the fruits, by means of chloroform fortified with a small amount of acetonitrile, followed by hydrolysis of the active ingredient with hydrochloric acid after evaporation of the solvent. Methylamine hydrochloride thus formed reacts with ninhydrin to give formaldehyde. The latter is determined by means of chromotropic acid. Possible metabolites having in common with Rogor the methylamidic function are estimated at the same time. With the second technique, the chloroform extracts of Rogor and of its $P=O$ metabolite are cleaned up by repeated extractions, isolated by paper-chromatography, and detected via phosphorus with the molybdic reagent adopted by HANES and ISHERWOOD (1949). The sensitivity of the two procedures is 0.2 and 0.6 p.p.m., respectively. DE PIETRI-TONELLI *et al.* (1959) used the above-mentioned chromatographic technique for the determination of Rogor residues in peaches. In order to detect quantities of the insecticide as low as 0.3 microgram they employed the more sensitive reagent 2,6-dibromo-*N*-

chloro-*p*-quinoneimine (MENN 1957) in addition to the HANES and ISHERWOOD reagent, and the paper was sprayed with a 50 percent aqueous solution of propylene glycol.

A method based on extraction with chloroform, paper chromatography, and chromatic detection of Rogor and of its $P=O$ analog was developed by SANTI and DE PIETRI-TONELLI (1959 b) for the analysis of broad beans.

VASSILIOU *et al.* (1959) worked out a procedure for olives, also based on the colorimetric determination of phosphorus, which was later extended by VASSILIOU *et al.* (1961) to other fruits such as apples and pears. The plant material was extracted either with 40 percent aqueous acetone or with benzene, whereas the oxidation of the insecticide was accomplished by means of perchloric acid or 30 percent ammonium persulphate solution.

Following the methods suggested for olive oil by BAZZI *et al.* (1956 and 1958) and for olives by VASSILIOU *et al.* (1959), PEREIRA RAMOS and MENDES COSTA (1960) analysed Rogor in fresh olives and in the same fruits processed with sodium hydroxide.

A procedure for the quantitative estimation of Rogor residues on peeled tomatoes was presented by ALESSANDRINI *et al.* (1959).

CHILWELL and BEECHMAN (1960) described a method for the analysis of fresh vegetable and fruit crops which was also extended, with modification of the extraction procedure, to crops of high solid content such as dried hops, tobacco, and tea. According to this technique the plant tissues were macerated with water acidified to pH 4.0 with acetic acid and, after neutralization, the aqueous extracts were extracted with chloroform. The extracted insecticide was purified by microdistillation as reported by OTTER (1956), HEATH *et al.* (1956), and BAZZI (1957) and colorimetrically determined via phosphorus as indicated by BEREMBLUM and CHAIN (1938) with modification by HEATH *et al.* (1956). The method is sensitive to 0.1 p.p.m. and detects all the phosphorus compounds which can be extracted by chloroform from water und which can be distillated under the reported conditions. It does not permit, therefore, the separation of Rogor from its $P=O$ derivative. This goal may be attained, however, by following the technique adopted by BAZZI *et al.* (1964) for the selective determination of the same compounds previously separated from Cidial[1]. This technique, similar to that reported by EICHENBERGER and GAY (1960), is founded on the different water/chloroform partition coefficient between the two products.

Two procedures for the determination of Rogor in olives, via phosphorus, were worked out by BAZZI (1960) who also used the isotope dilution technique to prove the efficiency of one of them. The methods are applicable, using one or the other, depending on the ratio between the amounts of oily residues and of active ingredient in the extracts. This ratio was found to be dependent upon the ripeness of the drupes, the date of treatment, and the rate of application of the insecticide. Both techniques give good recovery of Rogor but only a partial recovery of the oxygenated analog. The sensitivity is 0.4 p.p.m. and 0.05 p.p.m., respectively. During the preliminary operations, in both procedures conditions were realized to

[1] Cidial is the ethyl ester of O,O-dimethyldithiophosphoryl-1-phenylacetic acid.

allow the utilization of the same extracts also for chromatographic and infrared spectrophotometric researches and for bioassays.

EICHENBERGER and GAY (1960) published a method for the semiquantitative determination, via paper-chromatography, of the residues of several systemic organophosphorus insecticides, including Rogor. The procedure, used for the analysis of Rogor in sugar beets, was divided into the following steps: extraction of the vegetal material, cleanup, transfer onto Whatman paper, chromatography with developing aqueous systems (BUSH 1952), detection with potassium iodoplatinate reagent or with blue tetrazolin and identification of Rogor and of its $P=O$ derivative according to their $R_f$ values.

SAMPAOLO (1961), partially following the technique reported by BAZZI *et al.* (1956, 1957, and 1960), gave a method for olives, based on the colorimetric detection of phosphorus, which permitted a quantitative estimation of Rogor together with its $P=O$ metabolite and also a qualitative chromatographic detection of the latter compound.

LAWS and WEBLEY (1961) developed a procedure for the extraction of several insecticides from cabbages and other treated vegetable crops. The insecticides were divided into two groups, one of which included the light petroleum-soluble products and the other the water-soluble products. Rogor was included in the second group. The cleanup was accomplished by means of chromatography on graded alumina or on active carbon, whereas the final determination of the active ingredient was carried out via spectrophotometric evaluation of phosphorus as the molybdenum-blue complex.

BÄUMLER and RIPPSTEIN (1961) described the separation of Rogor and of other organophosphorus insecticides by means of thin-layer chromatography and indicated a new chromatic reaction (palladium-II chloride) which was demonstrated to possess higher sensitivity than other chromogenic agents such as bromine, fluorescein, and potassium iodoplatinate. This technique can be successfully adopted also when vegetal extractives are present.

SANTI and GIACOMELLI (1962) investigated the metabolic fate of $P^{32}$-Rogor in olives for oil and in eating olives by means of radiochromatographic procedures which were also employed for the analysis of cherries (SANTI 1961), peaches (SANTI 1961), and sugar beets and fodder beets (SANTI *et al.* 1962). The procedures are based on the extraction of Rogor and of its $P=O$ derivative with chloroform ("chloroform solubles"), further extraction with water to separate the "water solubles" from "chloroform solubles". The $P^{32}$-content of the substances in the above indicated fractions and of the water and chloroform insoluble substances are estimated radiometrically and also by the following techniques: partition chromatography with a silica-gel column to separate Rogor from ist oxygenated analog by a method described by TSUYUKI *et al.* (1955) and by DAUTERMAN *et al.* (1959 and 1960) and adapted for olives; paper-chromatography and paper-electrophoresis, with autoradiographic detection of $P^{32}$-containing compounds, to check the components of the radioactive fractions partitioned with a silica-gel column; and ion-exchange separation of the water-soluble

hydrolysis products on Dowex 1-X8 and elution and partition in groups with solvents having an increasing gradient of acidity (DAUTERMAN *et al.* 1959, PLAPP and CASIDA 1958). The above summarized techniques were applied to ascertain the effect of oil yielding processes on the amount and composition of residues in oil and the influence of industrial processing of eating-olives with sodium hydroxide (as reported by ORPHANIDIS *et al.* 1958) on residue levels.

GEORGE *et al.* (1962) developed a method for the determination of Rogor residues in dry lima beans, green beans, cabbages, turnip greens, tomatoes, and other products. According to this procedure the insecticide is submitted to alkaline hydrolysis and the resultant methylamine reacts with 1-chloro-2,4-dinitrobenzene to give a colored compound. The optical density follows Beer's law between five and 100 micrograms, which is the established working interval. A method also based on the hydrolysis of Rogor and methylamine determination was previously described (BAZZI *et al.* 1956, SANTI and BAZZI 1956).

GIANG and SCHECHTER (1963) reported a procedure for cabbages, tomatoes, spinach, and apples founded on hydrolysis of Rogor with alkali to yield thioglycolic acid. The latter is determined following the original FOLIN's uric acid method (FOLIN 1930 and 1934, STEEL 1958) using sodium phospho-18-tungstate as chromogenic reagent. The method determines both Rogor and its $P=O$ derivative but does not distinguish between them nor does it determine any of the metabolites or degradation products of the two compounds. The sensitivity limit, working with 200 g. of treated plant sample, is about 0.05 p.p.m.

Two paper chromatographic procedures for the identification of several organophosphorus insecticides, including Rogor, in plant extracts cleaned up on a cellulose/charcoal column were described by MACRAE and MCKINLEY (1963). Both procedures were applied for the analysis of apples, lettuce, cabbages, and orange extracts. The detection of Rogor on the paper was by means of the ferric chloride/salicyl sulfonic acid reagent (MACRAE and MCKINLEY 1961) or with the iodoplatinate reagent (EICHENBERGER and GAY 1960). Thin-layer chromatography with different solvent systems and detection reagents was used by WALKER and BEROZA (1963) for the quantitative estimation of 62 pesticides including Rogor and its $P=O$ derivative; their procedure offers many solvent systems as a solution to the interference of vegetal extractives.

For the analysis of Rogor in carrots, STOBWASSER (1963) adopted the procedure by SUTER *et al.* (1955) for Diazinon [2] based on the hydrolysis of the active ingredient followed by determination of the evolved hydrogen sulfide. He replaced petroleum ether, however, with benzene as extraction solvent.

A method for gas chromatographic separation and detection of submicrogram quantities of Rogor and of several organophosphorus pesticides has been described by EGAN *et al.* (1964). A gas chromatographic procedure,

[2] Diazinon is O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate.

by electron affinity detection, was reported also by VAN MIDDELEM and WAITES (1964), who determined Rogor on snap bean extracts and compared the gas chromatographic method with a dinitrochlorobenzene colorimetric method derived from original procedure by GEORGE (1962). The adopted gas chromatographic technique does not efficiently separate the $P=O$ derivative from its parent compound, however.

ENOS and FREAR (1964), who previously (ENOS and FREAR 1962) reported a semiquantitative method for the analysis of Rogor in milk, adapted the procedure originally described for Diazinon by SUTER *et al.* (1955) for the determination of Rogor in alfalfa, apples, grapes, and cherries. From the principle on which the method is based (i.e., extraction of the insecticide from a hexane solution with hydrobromic acid, hydrolysis, and estimation of the evolved hydrogen sulfide as methylene blue) it appears that the interference with compounds which yield hydrogen sulfide on hydrolysis cannot be avoided. An improved procedure for residue determination of organophosphorus pesticides, including Rogor, in several crops (alfalfa, apples, orange peel, and sugar beets) based on flask combustion, according to SCHÖNIGER (1960), and colorimetric measurement via phosphorus (STELLER and CURRY, 1964), was described by BLINN (1964) after ST. JOHN *et al.* (1960) had developed a technique for Rogor residues in hemlock by oxygen flask combustion. The recovery in BLINN's method is about 90 percent and the sensitivity about 0.1 p.p.m.

Another technique via phosphorus and including a preliminary separation by means of thin-layer chromatography, was indicated by STELLER and CURRY (1964) who tested their method on apples, green tomatoes, and alfalfa. According to them the residues of Rogor and of its $P=O$ analog are extracted from macerated vegetable tissues and the extract, partitioned with solvents and concentrated, is spotted on a thin-layer chromatographic plate. After development, the areas corresponding to the two compounds are scraped and eluted. In the eluates Rogor and its $P=O$ analog are determined via phosphorus. The sensitivity is about 0.06 p.p.m.

### *b) Biochemical procedures*

A paper chromatographic procedure based on COOK's serum cholinesterase inhibition spot test (COOK 1955) was described by GETZ and FRIEDMAN (1963) who determined the residues of several pesticides, including Rogor, on food products. The active ingredients were extracted and cleaned up as indicated by GETZ (1962), separated by paper chromatography according to MITCHELL (1957), and finally detected and quantitatively estimated by means of appropriate standards, on the basis of the inhibition spots produced on the paper sprayed first with an enzyme-indicator solution and then with the substrate solution (acetylcholine bromide). Pesticides which were not direct inhibitors (as Rogor) required bromination in order to produce inhibition spots.

MCKINLEY and JOHAL (1963) improved a previously developed enzyme inhibition technique (MCKINLEY and READ 1962) for the determination of micrograms of Rogor and of several organophosphorus pesticides separated

by paper chromatography. This technique (which implies the conversion of Rogor and of some other products to their oxygen analogs) was employed for the analysis of extracts of apples, lettuce, strawberries, and peas cleaned up as indicated by COFFIN and MCKINLEY (1963).

An enzymatic procedure for the determination of residues of phosphoric esters, which may also be applied to Rogor, was reported by LIPPARINI and SANDI (1964). It is founded on the measure of the size of the inhibited areas on agar-agar medium fortified with cholinesterase.

### *c) Bioassay*

As is well known from the wide literature on this subject and from the extensive reviews given by SUN (1957), HOSKINS (1957), NAGASAWA (1959), and by HOSKINS and CRAIG (1962) the main features of bioassay, which can be conducted directly on homogenized plant tissues or on extracts, are simplicity, adaptability, and high sensitivity.

**1. Homogenate technique.** — The technique based on the exposure of insects to macerated and homogenized plant tissues offers the advantage of the short time necessary to carry out the analysis, because the long lasting processes of extraction and removal of plant extractives (cleanup) are completely eliminated. It gives, however, disadvantages represented by the lack of specifity, as the amount of the insecticide originally applied is determined together with that of its possible degradation products and metabolites exerting toxic effects to the test insects.

Adults of *Drosophila melanogaster* Meig. appear to be a most suitable test organism for the determination of Rogor residues because they are particularly susceptible to the original insecticide and to its $P=O$ metabolite [*O,O*-dimethyl *S*-(*N*-methyl carbamoylmethyl) phosphorothioate] which generally occurs in plant material (SANTI and DE PIETRI-TONELLI 1959 a and b).

The technique of preparing the samples and exposing the insects to homogenized plant tissues for the determination of Rogor residues was described in detail by DE PIETRI-TONELLI (1956 a), and by DE PIETRI-TONELLI and BARONTINI (1960 a). The sensitivity of the method ranges between 0.1 and 0.3 p.p.m. and reaches even 0.05 p.p.m., depending on the species and organs of the plants or the stage of ripeness of fruits. The highest sensitivity can be achieved by adding a water solution of honey to macerated leaves or to unripe fruits and a more accurate reproducibility of results is obtained by adding agar-agar to very juicy fruits. The method cannot, however, be adopted for the analysis of some vegetal parts and products, as, for example, olive fruits, olive oil, and peach leaves, which, owing to their natural content of particular substances, have been proved to exert masking or lethal effects to the test insects.

By means of this method cherries were analyzed by DE PIETRI-TONELLI (1956 b) and by SANTI and DE PIETRI-TONELLI (1959 b), apricots and peaches by DE PIETRI-TONELLI *et al.* (1959), tangerines by DE PIETRI-TONELLI and BARONTINI (1960), apples and pears by DE PIETRI-TONELLI

and BARONTINI (1958), and artichoke and lettuce by DE PIETRI-TONELLI and BARONTINI (1959).

**2. Extracts technique.** — The procedure based on the exposure of insects to evaporated extracts implies the extraction of plant tissues with properly chosen solvents and, in some cases, the more or less complete removal of the extraneous plant extractives (cleanup). It presents therefore the same disadvantage (long time for preparation of extracts) and advantage (possibility of separation of original insecticide from its metabolites and degradation products) of other physico-chemical analytical methods from which it differs only by the substitution of physicochemical means of detection with the mortality of insects.

Several test organisms are commonly adopted, namely, adults of *D. melanogaster* (for the residual film technique), adults of *Musca domestica* L. (for topical application), and mosquito larvae (for the dipping technique), but for the determination of Rogor residues the highly susceptible adults of *D. melanogaster* are generally preferred.

The sensitivity of the extract procedure depends on the possibility to submit to the extraction process, with high recoveries, a large quantity of vegetal material to be analyzed. When the operation is properly conducted, residual amounts of the insecticide can be detected even to as low as 0.01 p.p.m.

The method was applied by BAZZI *et al.* (1956) for the assay of Rogor in olive oil and in this case the insecticide and its $P=O$ derivative were extracted first with 50 percent ethanol and then with benzene. DE PIETRI-TONELLI *et al.* (1959) bioassayed Rogor and its $P=O$ metabolite in cherries previously extracted with a mixture of chloroform and acetonitrile (9 : 1), whereas SANTI and DE PIETRI-TONELLI (1959 b) analyzed the same fruits by using chloroform alone, which extracted both the insecticide and its $P=O$ metabolite. The same solvent was used by BAZZI *et al.* (1960) for both unripe and ripe olives but he adopted two different cleanup techniques. For the bioassay of apple leaves, TEW and SILLIBOURNE (1964) employed a method which they had developed before (TEW and SILLIBOURNE 1961) and extracted the plant material with dry methylene chloride.

Among others, MAIER-BODE (1963) bioassayed Rogor in treated strawberries and gooseberries but he did not state whether he adopted the homogenate or the extract procedure.

### *d) Autoradiography*

Autoradiography represents a very efficient tool for the investigation of the pattern of distribution of insecticides and particularly of systemic compounds in vegetal organs and tissues. Obviously it provides no information regarding the differential location of the original insecticide and of its possible metabolites labeled with the same radioactive element, but the identification of the organs and tissues which were reached by the radioactive material, at several time intervals from treatment, appears very useful and enables further analytical research.

In the case of Rogor, autoradiographic investigations were carried out by using samples of the insecticide tagged with $P^{32}$ (specific radioactivity between 0.47 and 2.06 mc/mM). The entire organs (leaves) or macrosections of fruits, roots or tubers, prepared by freezing the specimens according to particular procedures (DE PIETRI-TONELLI and BARONTINI 1960 a, b, c, and 1961) were placed in contact (by apposition) with photographic emulsions (Kodirex X-ray or Ferrania Simplex) and kept at —6 °C. for the whole exposure period to prevent vegetal juices from spreading out over the specimens.

Working with systemic insecticides, histoautoradiography would certainly be of considerable help, as demonstrated by TIETZ (1954), who attempted to investigate the cellular distribution of $P^{32}$-labeled Systox [3] in pear leaves. However, it is our opinion, after many attempts to localize $P^{32}$-Rogor in several plant microsections, by using either the same method described by TIETZ (1952 and 1954) or the conventional wet mounting stripping technique, that contact with aqueous solutions and with processing fluids allows microquantities of the insecticide and of its even more water-soluble metabolites to diffuse out of the specimen or to change their original distribution. More realistic knowledge of the cellular localization of organic insecticides in plant tissues is therefore to be expected by adopting the "dry" mounting technique recently developed by FITZGERALD *et al.* (1961 a and b) when a more satisfying method of sectioning vegetal specimens in the cryostat will be available.

## III. Residue degradation and persistence curves of Rogor and of its metabolites

### *a) Fruit crops*

**1. Cherries.** — On cherries Rogor is mainly applied for the control of *Rhagoletis cerasi* L. and aphids.

From the first research on the use of this insecticide against the newly hatched larvae of *R. cerasi*, DE PIETRI-TONELLI (1956 b) employed the homogenate method for the bioassay of fruits picked from plants very accurately sprayed (at full coverage), or dusted, with Rogor at the time when cherries had started to change their color. The results in Table I show that, from both the experimented formulations, the active ingredient penetrates a few hours after treatment, through the epicarp into the pulp of the fruits where it undergoes a progressive degradation. According to the corresponding curves obtained from the data plotted on semi-logarithmic paper, the rate of disappearance of residues having insecticidal activity can be expressed by a half-life of about five days.

Further bioassays were carried out by SANTI and DE PIETRI-TONELLI (1959 b) who separately homogenized and extracted (with chloroform) the fruits harvested, at very close time intervals, from plants sprayed with

[3] Systox is a mixture of *O,O*-diethyl *O* (and *S*)-2-(ethylthio)ethyl phosphorothioates.

Rogor (at high concentration) when the cherries had just started to change their color. The results reported in Table II demonstrate a good agreement between the data obtained with both methods and indicate moreover that the concentration of the insecticidal substance in the fruits significantly

Table I. *Cherries. Bioassay of homogenized fruits picked from trees sprayed or dusted with two experimental formulations of Rogor* (DE PIETRI-TONELLI 1956 b)

| Days between treatment[a] and sampling | Concentration (p. p. m.) of Rogor equivalents | | | |
|---|---|---|---|---|
| | Rogor 0.024% (liquid) | | Rogor 3% (dust) | |
| | Unwashed fruits without stones | Pulp | Unwashed fruits without stones | Pulp |
| 5/12 | 11.2 ± 0.0 | 7.4 ± 0.3 | 7.0 ± 0.7 | 4.2 ± 0.2 |
| 1 | 5.0 ± 0.5 | — | 4.0 ± 0.3 | 3.1 ± 0.1 |
| 5 | 3.9 ± 0.3 | 3.3 ± 0.2 | 3.9 ± 0.1 | 2.6 ± 0.2 |
| 12 | 1.5 ± 0.1 | 1.5 ± 0.0 | 1.3 ± 0.0 | 1.1 ± 0.3 |

[a] Treated June 17, 1955.

increased in the first days after treatment and then regularly diminished. The increase of concentration has to be possibly attributed to systemic migration of the insecticide from leaves and twigs into the fruits. The same phenomenon was observed by BACHMANN (1957) on cherries from plants

Table II. *Cherries. Bioassay of homogenized or extracted fruits from a tree sprayed with 0.05 percent Rogor (Rogor 20 L[a])* (SANTI and DE PIETRI-TONELLI 1959 b)

| Days between treatment[b] and sampling | Concentration (p.p.m.) of Rogor equivalents in washed fruits without stones | |
|---|---|---|
| | Homogenate method | Extracts method |
| 1/6 | 5.6 | 5.7 |
| 1 | 6.6 | 7.4 |
| 2 | 11.4 | — |
| 3 | 6.5 | — |
| 5 | 6.5 | 5.3 |
| 7 | 4.6 | — |
| 9 | 4.5 | 3.0 |
| 12 | 3.9 | — |
| 13 | 3.8 | 4.3 |
| 16 | 2.8 | 3.1 |
| 22 | 1.5 | — |

[a] Commercial formulation produced by Montecatini.
[b] Treated June 5, 1957.

sprayed with Posphamidon [4], and the same fact was proved by DE PIETRI-TONELLI and BARONTINI (1961) on untreated olives picked from partially sprayed olive trees.

[4] Phosphamidon is O,O-dimethyl O-(2-chloro-2-(*N*-diethylcarbamoyl)-1-methyl-vinyl) phosphate.

Analytical determinations and research on the metabolic fate of Rogor in cherries were also conducted by SANTI (1961), who sprayed a tree with $P^{32}$-labeled Rogor at the dosage recommended for the control of *R. cerasi*. The fruits, collected at several time intervals after spraying, were extracted with chloroform and water and the partitioned extracts determined by different techniques as reported in section II. The results (Table III) show

Table III. *Cherries. Radiochromatographic analysis of fruits from a tree sprayed with $P^{32}$-labeled Rogor 0.02 percent, formulated as Rogor 20 L*[a] (SANTI 1961)

| Days between treatment[b] and sampling | Concentration (p. p. m.) of Rogor equivalents in unwashed fruits without stones | | | | | |
|---|---|---|---|---|---|---|
| | $CHCl_3$ solubles | | | $CHCl_3$ insolubles | | Total |
| | Total | Rogor | $P=O$ derivative | $H_2O$ solubles | $H_2O$ insolubles | |
| 1/12 | 1.85 | 1.85 | 0.00 | 0.01 | 0.01 | 1.87 |
| 2 | 0.95 | 0.88 | 0.07 | 0.15 | 0.07 | 1.17 |
| 7 | 0.31 | 0.21 | 0.10 | 0.34 | 0.06 | 0.71 |
| 21 | 0.06 | 0.01 | 0.05 | 0.50 | 0.01 | 0.57 |

[a] Commercial formulation produced by Montecatini.
[b] Treated May 8, 1961.

that the concentration of Rogor in the fruits rapidly decreases (half-life about one day) and that the insecticide (Table IV) penetrates very quickly from the epicarp into the pulp. In the fruits Rogor originates several phosphorus-containing metabolites (Table III), namely: the $P=O$ deri-

Table IV. *Cherries. Radiochromatographic analysis. Partition of Rogor between the outside and inside of the fruits at several time intervals after spraying* (see Table III) (SANTI 1961)

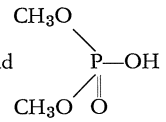
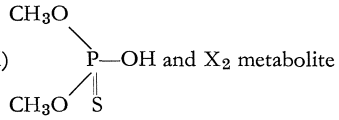
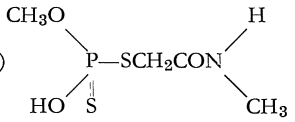
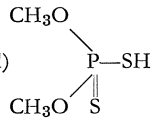
| Days between treatment[a] and sampling | Concentration (p. p. m.) of Rogor | | | Rogor inside the fruits % |
|---|---|---|---|---|
| | Total | Outside the fruits | Inside the fruits | |
| 1/12 | 1.85 | 1.04 | 0.81 | 43.78 |
| 2 | 0.88 | 0.11 | 0.77 | 87.50 |
| 7 | 0.21 | 0.02 | 0.19 | 90.47 |
| 21 | 0.01 | 0.00 | 0.01 | 100 |

[a] Treated May 8, 1961.

vative, whose concentration reaches a maximum after about seven days; some water soluble metabolites, whose concentration steadly raises even after 21 days; and some chloroform and water insoluble metabolites, which appear to occur at the highest concentration after about two days. Eight substances were separated from the chloroform insolubles in the fruits, and five were chemically detected and quantitatively estimated, as reported in Table V.

In addition to the above, other analyses were carried out by ENOS and FREAR (1964) who adopted a colorimetric technique for the determination

Table V. *Cherries. Radiochromatographic analysis. Chemical composition and concentrations of some water soluble metabolites of Rogor in the fruits* (see Table IV) (SANTI 1961)

| Metabolites exchanged (*A*) and not exanged (*B*) on Dowex 1−x8 | Concentration (p.p.m.) of Rogor equivalents in the fruits | |
|---|---|---|
| | After 7 days | After 21 days |
| (*A*) $H_3PO_4$ and  | 0.200 | 0.044 |
| (*A*) $X_1$ metabolite | 0.048 | 0.024 |
| (*A*)  | 0.056 | 0.088 |
| (*A*)  | 0.017 | 0.002 |
| (*A*)  | traces | 0.001 |
| (*B*) $X_3$ metabolite | 0.022 | 0.321 |

of Rogor in cherries picked from trees sprayed with "2 lb. 25 percent W.P.". The following concentrations of Rogor equivalents were found one, seven, and ten days after spraying: 3.6 p.p.m., 1.5 p.p.m., and 0.8 p.p.m. From those data a corresponding half-life of about 5 days can be calculated.

Because when cherries are treated the grass and the vegetable crops under the trees can be contamined by spray drift or run-off, MAIER-BODE (1963) bioassayed strawberries and gooseberries under cherry trees sprayed with a liquid formulation of Rogor. As shown in Table VI, the residue

Table VI. *Vegetable crops under cherry trees. Bioassay of strawberries and gooseberries under cherries sprayed with Rogor 0.04 percent (Perfektion* [a]*)* (MAIER-BODE 1963)

| Days between treatment [b] and sampling | Concentration (p.p.m.) of Rogor equivalents | |
|---|---|---|
| | Strawberries | Gooseberries |
| 0 | 1.0 | 0.8 |
| 9 | 0.9 | 1.0 |
| 12 | < 0.4 | < 0.4 |
| 15 | < 0.4 | < 0.4 |

[a] Commercial formulation produced by Badische Anilin & Soda Fabrik AG.
[b] Treated June 29, 1962.

disappearance curve data are rather irregular. The residues persist in fact about at the initial level for the first nine days and then drop below 0.4 p.p.m., which is the sensitivity level of the adopted bioassay method.

**2. Apricots.** — Rogor is used on apricots for the control of *Ceratitis capitata* Wied. and aphids. Bioassays of apricots were reported by DE PIETRI-TONELLI *et al.* (1959), who determined the amount of insecticide in the fruits collected from trees sprayed with Rogor at two dosage rates when the pulp of apricots, still in the preripe stage, was beginning to soften. The data in Table VII indicate for both concentrations a substantially similar

Table VII. *Apricots. Bioassay of homogenized fruits picked from trees sprayed with two dilutions of Rogor (Rogor 20 L* [a]*)* (DE PIETRI-TONELLI *et al.* 1959)

| Days between treatment [b] and sampling | Concentration (p.p.m.) of Rogor equivalents in unwashed fruits without stones | |
|---|---|---|
| | Rogor 0.005% | Rogor 0.015% |
| 4 | 1.2 | 4.6 |
| 6 | 0.7 | 1.5 |
| 13 | 0.35 | 0.5 |
| 20 | 0.25 | 0.3 |

[a] Commercial formulation produced by Montecatini.
[b] Treated June 5, 1958.

rate of disappearance of residues having insecticidal activity. The half-life is approximately seven days.

**3. Peaches.** — As on apricots, the principal use of Rogor on peaches is directed to the control of *Ceratitis capitata* Wied. and aphids.

Extensive bioassays were conducted by DE PIETRI-TONELLI *et al.* (1959) in order to establish the disappearance curves of the insecticide in the fruit and to investigate their relation with the date of treatment of different peach varieties, with the concentration of the product applied to the plants, and with the growth of fruits. The analytical results obtained from peach varieties all treated when fruits started to change color of peel are reported in Table VIII. From the half-life values, calculated by plotting the data on semi-logarithmic paper, it appears that the concentration of residues having insecticidal activity decreases at higher rates in the varieties which, in the same locality, were treated in June than in those sprayed in September or October, the half-life values being in fact about four-five days for the former and about nine-twelve days for the latter. It was furthermore demonstrated that the weight of the pulp of the fruits increases more rapidly in the early than in the late varieties and that the different dosages of Rogor applied to the plants do not appreciably affect the slope of the persistence curves of residues. It may therefore be concluded, at least for plants in the same climatic area, that date of treatment of fruits at the same stage of ripeness, and thus of the same variety, exerts a significant influence on the rate of disappearance of Rogor residues.

In addition to above, SANTI (1961) used radiochromatographic techniques for the analysis of peaches picked from a plant sprayed, when the

Table VIII. *Peaches. Bioassay of homogenized fruits picked from different varieties of plants sprayed with Rogor (Rogor 20 L [a]) at several dosages and dates of treatment* (DE PIETRI-TONELLI *et al.* 1959)

| Peach varieties | Date of treatment and localities | Sprayed dosages, % active ingredient | Time between treatment and sampling | | Concentration (p.p.m.) of Rogor equivalents in unwashed fruits without stones |
|---|---|---|---|---|---|
| | | | Hours | Days | |
| Alexander . . . | June 25, 1958 Signa (Firenze) | 0.015 | 1 | — | 3.0 |
| | | | | 1 | 1.7 |
| | | | | 2 | 1.5 |
| | | | | 5 | 1.4 |
| | | | | 7 | 1.3 |
| | | | | 12 | 0.6 |
| Alexander | June 25, 1958 Signa (Firenze) | 0.03 | 1 | — | 5.5 |
| | | | | 2 | 3.8 |
| | | | | 5 | 2.0 |
| | | | | 7 | 1.8 |
| | | | | 12 | 1.1 |
| Elberta . . . . . | July 16, 1958 Signa (Firenze) | 0.03 | 1 | — | 6.6 |
| | | | | 1 | 6.0 |
| | | | | 2 | 5.5 |
| | | | | 5 | 4.0 |
| | | | | 14 | 1.5 |
| | | | | 19 | 0.7 |
| | | | | 23 | 0.5 |
| Hale . . . . . | July 28, 1958 Giarre (Catania) | 0.02 | | 4 | 1.2 |
| | | | | 10 | 0.8 |
| | | | | 20 | 0.4 |
| | | | | 28 | 0.2 |
| Hale . . . . . | July 28, 1958 Giarre (Catania) | 0.02 | | 4 | 2.3 |
| | | | | 10 | 1.2 |
| | | | | 20 | 0.6 |
| | | | | 28 | 0.4 |
| Hale . . . . . | July 28, 1958 [b] Giarre (Catania) | 0.02 | | 4 | 1.1 |
| | | | | 9 | 0.7 |
| | Aug. 6, 1958 [c] Giarre (Catania) | 0.02 | | 11 | 0.8 |
| | | | | 20 | 0.6 |
| Toschina di Settembre . . | Sept. 4, 1958 Signa (Firenze) | 0.04 | 1 | | 5.2 |
| | | | | 1 | 4.0 |
| | | | | 4 | 3.7 |
| | | | | 7 | 2.4 |
| | | | | 11 | 2.2 |
| | | | | 15 | 2.0 |
| | | | | 20 | 1.3 |
| | | | | 25 | 1.0 |
| | | | | 29 | 0.6 |
| | | | | 33 | 0.5 |
| Toschina di Ottobre | Sept. 25, 1958 [b] Signa (Firenze) | 0.015 | 1 | | 2.2 |
| | | | | 1 | 1.7 |
| | | | | 4 | 1.1 |
| | | | | 8 | 0.7 |
| | | | | 12 | 0.6 |
| | Oct. 7, 1958 [c] Signa (Firenze) | 0.015 | 5 | | 0.92 |
| | | | | 6 | 0.51 |
| | | | | 13 | 0.48 |
| | | | | 20 | 0.37 |
| Toschina di Ottobre | Sept. 25, 1958 Signa (Firenze) | 0.03 | 1 | | 4.2 |
| | | | | 1 | 3.0 |
| | | | | 4 | 2.2 |
| | | | | 8 | 1.3 |
| | | | | 12 | 1.0 |
| | | | | 18 | 0.6 |
| | | | | 25 | 0.5 |
| | | | | 32 | 0.3 |

[a] Commercial formulation produced by Montecatini.
[b] First treatment.
[c] Second treatment.

fruits started to change their color, with $P^{32}$-labeled Rogor at a dosage higher than that recommended for the control of *C. capitata*. The results (Table IX) indicate that the concentration of Rogor in the peaches treated

Table IX. *Peaches. Radiochromatographic analysis of the fruits from a tree sprayed with $P^{32}$-labeled Rogor 0.06 percent formulated as Rogor 20 L* [a] (SANTI 1961)

| Days between treatment [b] and sampling | Concentration (p.p.m.) of Rogor equivalents in unwashed fruits without stones | | | | | |
|---|---|---|---|---|---|---|
| | $CHCl_3$ solubles | | | $CHCl_3$ insolubles | | Total |
| | Total | Rogor | $P=O$ derivative | $H_2O$ solubles | $H_2O$ insolubles | |
| 1/12 | 3.33 | 3.33 | 0.00 | 0.022 | 0.022 | 3.384 |
| 3 | 2.29 | 2.23 | 0.06 | 0.274 | 0.022 | 2.586 |
| 10 | 0.90 | 0.83 | 0.07 | 0.266 | traces | 1.666 |
| 18 | 1.15 | 1.10 | 0.05 | 0.670 | 0.09 | 1.910 |

[a] Commercial formulation produced by Montecatini.
[b] Treated July 7, 1961.

in July diminishes at a rate expressed by a half-life of seven-eight days and that the insecticide (Table X) penetrates from the epicarp into the pulp, but

Table X. *Peaches. Radiochromatographic analysis. Partition of Rogor between the outside and inside of the fruits at several time intervals after spraying* (see Table IX) (SANTI 1961)

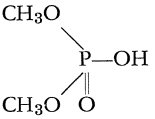
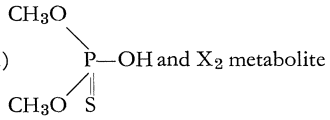
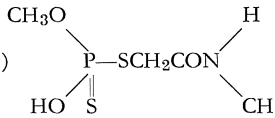
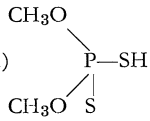
| Days between treatment [a] and sampling | Concentration (p.p.m.) of Rogor | | | Rogor inside the fruits % |
|---|---|---|---|---|
| | Total | Outside the fruits | Inside the fruits | |
| 1/12 | 3.33 | 2.37 | 0.96 | 28.82 |
| 3 | 2.23 | 1.15 | 1.08 | 48.43 |
| 10 | 0.83 | 0.30 | 0.58 | 63.85 |
| 18 | 1.10 | 0.12 | 0.98 | 89.09 |

[a] Treated July 7, 1961.

more slowly than was observed for cherries (Table IV). The $P=O$ metabolite occurs in a very limited amount (Table IX) and reaches the maximum after about ten days. Whereas the concentration of the water-soluble phosphorus containing metabolites progressively increases, the chloroform- and water-insoluble derivatives appear to decrease rather slowly. By means of partition chromatography of the water solubles (Table XI), five metabolites were separated and chemically detected among eight substances whose presence was established. The eight compounds occurring outside the fruits and in the pulp were also quantitatively determined ten and 18 days after spraying.

After a preliminary research by DE PIETRI-TONELLI *et al.* (1959) carried out by means of bioassays, DE PIETRI-TONELLI and BARONTINI (1961) investigated the distribution of Rogor in peaches sprayed with 0.005 percent $P^{32}$-labeled Rogor. From the autoradiograms of macrosections of the fruits

Table XI. *Peaches. Radiochromatographic analysis. Chemical composition and concentration of some water soluble metabolites of Rogor in the fruits* (see Table X) (SANTI 1961)

| Metabolites exchanged (*A*) and not exchanged (*B*) on Dowex 1−x8 | Concentration (p.p.m.) of Rogor equivalents | | | |
|---|---|---|---|---|
| | Outside the fruits | | Inside the fruits | |
| | After 10 days | After 18 days | After 10 days | After 18 days |
| (*A*) $H_3PO_4$ and  | 0.052 | 0.074 | 0.012 | 0.042 |
| (*A*) $X_1$ metabolite | 0.01 | 0.011 | 0.012 | 0.042 |
| (*A*)  | 0.005 | 0.006 | 0.07 | 0.16 |
| (*A*)  | 0.002 | 0.003 | 0.007 | traces |
| (*A*)  | 0.001 | 0.003 | traces | traces |
| (*B*) $X_3$ metabolite | 0.004 | 0.005 | 0.089 | 0.325 |

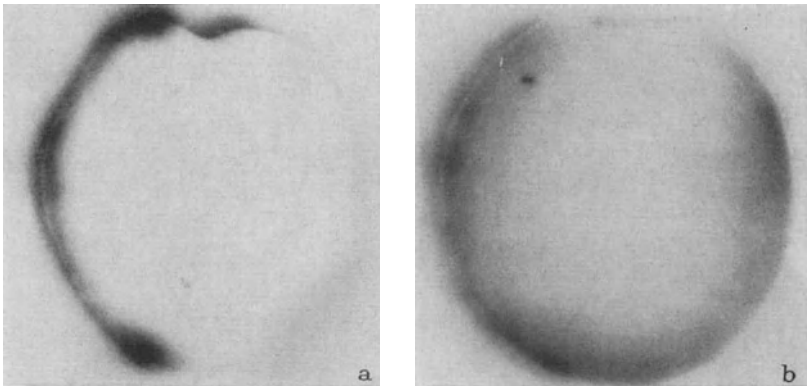



Fig. 1. Autoradiograms of longitudinal macrosections of peaches one day (*a*) and nine days (*b*) after the plants were sprayed with $P^{32}$-Rogor. From DE PIETRI-TONELLI and BARONTINI (1961)

harvested at several time intervals, it is clearly shown (Fig. 1) that the insecticide and its radiophosphorus containing metabolites gradually and centripetally diffuse in the whole pulp, but they reach a lower concentration

in the portions of the pulp underlaying those parts of the epicarp which remain incompletely treated when the product was applied to the plant.

**4. Olives.** — For about ten years Rogor has been widely used in the Mediterranean area for the defence of olives from the attacks of *Dacus oleae* Gmel. and *Prays oleellus* F. (ALESSANDRINI 1962).

The degradation and persistence of residues of the insecticide were investigated in olives for oil and also in eating olives both fresh and processed with sodium hydroxide.

On olives for oil analytical determinations were carried out by BAZZI *et al.* (1960) who sprayed on olive trees 0.06 percent Rogor (the maximum dosage recommended for the control of *D. oleae*) and studied the influence

Table XII. *Olives for oil. Colorimetric analysis and bioassay of the extracts of drupes picked from trees sprayed with Rogor 0.06 percent (Rogor 20 L [a]) at several dates* (BAZI *et al.* 1960)

| Date of treatment | Variety | Days between treatment and sampling | Concentration (p.p.m.) of Rogor equivalents in unwashed fruits without stones | |
|---|---|---|---|---|
| | | | Colorimetric analysis | Bioassay |
| Aug. 5, 1959 | Frantoio | 1 | 10.3 | — |
| | | 5 | 3.4 | — |
| | | 19 | 0.6 | — |
| | | 40 | 0.06 | — |
| Aug. 24, 1959 | Frantoio | 1 | 9.9 | — |
| | | 9 | 1.7 | — |
| | | 32 | 0.12 | — |
| Sept. 29, 1958 | Leccino | 1 | 14.9 | 15.1 |
| | | 3 | 12.7 | 11.4 |
| | | 7 | 9.1 | 6.5 |
| | | 14 | 4.6 | 4.0 |
| | | 22 | 2.9 | 2.1 |
| | | 45 | 0.9 | 0.9 |
| Oct. 14, 1958 | Frantoio | 1 | 14.5 | — |
| | | 38 | 3.9 | — |
| | | 47 | 2.9 | 2.2 |
| | | 55 | 2.1 | 2.0 |
| | | 63 | — | 1.3 |
| | | 91 | 0.92 | 0.83 |
| Nov. 26, 1958 | Frantoio | 1 | 14.1 | 13.9 |
| | | 13 | 8.6 | 8.9 |
| | | 20 | 7.5 | 7.5 |
| | | 48 | 4.8 | 4.3 |
| | | 58 | 3.5 | 3.0 |

[a] Commercial formulation produced by Montecatini.

of the date of treatment (from August to November) upon the rate of disappearance of residues. For this purpose the fruits were extracted with chloroform, in two different ways according to the ripeness of the drupes, and the extracts were bioassayed and colorimetrically estimated. The results obtained by means of both procedures appear to be in good agreement (Table XII) and indicate that there is a tremendous variation in the rate of

disappearance of Rogor residues related to the date of treatment. A clear evidence of this relation is provided by the different slope of the curves of Figure 2 a, where the concentration of Rogor equivalents is plotted against the time after treatment on semi-logarithmic paper. The half-life values, ranging from three-four days when the insecticide is applied in August, to about 23 days when it is applied in November, show that the persistence of residues largely increases the later in the season the olive trees were sprayed. A similar relation between the rate of disappearance of residues and the date of treatment was demonstrated by BAZZI *et al.* (1960) to occur also for parathion [5] (Fig. 2 b) whose half-life values in olives appear however to be far higher than those of Rogor. This would represent a practical advantage in the use of parathion for the control of *D. oleae* if the larvae were not less susceptible to this insecticide than to Rogor, as demonstrated by the same workers.

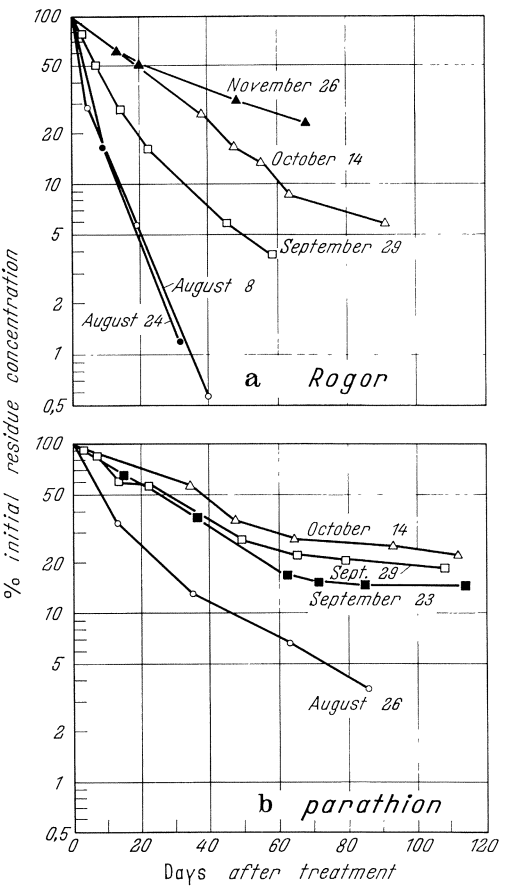



Fig. 2. Percentage residue disappearance in olive fruits picked from plants treated at several dates with Rogor and with parathion at the same dosage, i. e. 0.06 percent. From BAZZI *et al.* (1960)

Further studies on olives for oil were undertaken by SANTI and GIACOMELLI (1962) who adopted radiochromatographic techniques for the analysis of the drupes picked from trees sprayed with $P^{32}$-labeled Rogor at three times of the year (i. e., July, September, and October). The results, which were also briefly reported by ALESSANDRINI (1960) and by ALESSANDRINI and SAMPAOLO (1961) and later reviewed by ALESSANDRINI (1962), indicate (Table XIII) that the concentration of Rogor in the drupes progressively diminishes, that the amount of $P=O$ derivative rises to a maximum and then decreases, and that the concentration of water solubles

[5] O,O-diethyl-O-*p*-nitrophenyl phosphorothioate.

Table XIII. *Olives for oil. Radiochromatographic analysis of the fruits from an olive tree sprayed with $P^{32}$-labeled Rogor, formulated as Rogor 20 L* [a] (SANTI and GIACOMELLI 1962)

| Date of treatment | Dosage applied % | Days between treatment and sampling | Concentration (p.p.m.) of Rogor equivalents in unwashed fruits with the stones | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | $CHCl_3$ solubles | | | $CHCl_3$ insolubles | | Total |
| | | | Total | Rogor | $P=O$ derivative | $H_2O$ solubles | $H_2O$ insolubles | |
| July 8, 1960 | $P^{32}$-Rogor | 0 | 16.87 | 16.87 | 0.00 | — | — | 16.87 |
| | 0.01 | 4 | 5.85 | 5.31 | 0.54 | 1.85 | 0.74 | 8.44 |
| | + | 12 | 1.77 | — | — | 3.11 | 0.96 | 5.84 |
| | Rogor | 17 | 1.40 | — | — | 3.55 | 1.63 | 6.58 |
| | 0.06 | 23 | 1.33 | — | — | 4.66 | 2.16 | 8.15 |
| | | 45 | 0.51 | — | — | 5.92 | 1.63 | 8.06 |
| Sept. 15, 1960 | $P^{32}$-Rogor | 0 | 11.91 | 11.91 | 0.00 | — | — | 11.91 |
| | 0.06 | 4 | 4.96 | 4.20 | 0.76 | 1.48 | 0.30 | 6.74 |
| | | 11 | 3.03 | 2.00 | 1.03 | 3.99 | 0.66 | 7.68 |
| | | 18 | 1.70 | 0.70 | 1.00 | 3.55 | 0.81 | 6.06 |
| | | 25 | 1.74 | — | — | 2.88 | 0.74 | 4.29 |
| | | 28 | 0.59 | 0.18 | 0.41 | 3.15 | 0.62 | 4.36 |
| | | 35 | 0.74 | — | — | 3.10 | 0.54 | 4.58 |
| Oct. 13, 1960 | $P^{32}$-Rogor | 0 | 8.21 | 8.21 | — | — | — | 8.21 |
| | 0.06 | 4 | 8.36 | 7.72 | 0.60 | 0.52 | 0.14 | 9.02 |
| | | 11 | 4.14 | 3.40 | 0.74 | 1.33 | 0.22 | 5.69 |
| | | 20 | 2.95 | 1.90 | 1.07 | 2.44 | 0.59 | 6.00 |
| | | 35 | 1.41 | 0.73 | 0.68 | 2.07 | 0.59 | 4.07 |
| | | 45 | 0.59 | 0.07 | 0.52 | 0.92 | 0.29 | 2.15 |

[a] Commercial formulation produced by Montecatini.

steadly increases whereas the chloroform and water insolubles begin to reduce about five weeks after treatment. The analysis of oil, aqueous phase, and olive husks obtained, in the process of oil-yielding, from olives treated with $P^{32}$-Rogor in October (i.e., at the time when the rate of degradation

Table XIV. *Olive oil. Radiochromatographic analysis of oil, aqueous phase, and olive husks obtained from olives treated with $P^{32}$-Rogor 0.06 percent formulated as Rogor 20 L* [a] (SANTI and GIACOMELLI 1962)

| Days between treatment [b] and sampling | Concentration (p.p.m.) of Rogor and of its $P=O$ derivative | | | | |
|---|---|---|---|---|---|
| | Oil [c] | Aqueous phase | | Husks | |
| | Rogor | Rogor | $P=O$ derivative | Rogor | $P=O$ derivative |
| 7 | 1.80 | 4.15 | 0.66 | 5.88 | 0.32 |
| 14 | 0.90 | 2.26 | 1,21 | 3.36 | 0.49 |
| 25 | 0.40 | 0.56 | 1.23 | 1.05 | 0.33 |
| 35 | 0.22 | 0.29 | 0.91 | 0.75 | 0.29 |
| 45 | 0.10 | 0.14 | 0.62 | — | — |

[a] Commercial formulation produced by Montecatini.
[b] Treated October 13, 1960.
[c] In all samples the amount of $P = O$ derivative was 0.00 p.p.m.

of the insecticide was demonstrated to be very low), confirmed (Table XIV) the results achieved by PELLEGRINI *et al.* (1958) and moreover supported the evidence that in the oil produced by olives picked even seven days only after treatment, the $P=O$ metabolite is absent or below 0.01 p.p.m. and that after 14 days the concentration of Rogor in the oil is below one p.p.m. and gradually decreases in the following days.

On fresh eating olives, SANTI and GIACOMELLI (1962) determined, by means of radiochromatography, the changes in the concentrations of Rogor, of its $P=O$ metabolite, and of its chloroform insoluble derivatives. Bearing in mind the different ratio of surface-to-mass of the fruits belonging to eating olive varieties and the influence of this factor on the initial concentration of the insecticide in the fruits, the results reported in Table XV can

Table XV. *Eating olives. Radiochromatographic analysis of fresh unprocessed fruits picked from an olive tree sprayed with $P^{32}$-Rogor 0.02 percent formulated as Rogor 20 L* [a] (SANTI and GIACOMELLI 1962)

| Days between treatment [b] and sampling | Concentration (p.p.m.) of Rogor equivalents in unwashed fruits with the stones | | | | | |
|---|---|---|---|---|---|---|
| | $CHCl_3$ solubles | | | $CHCl_3$ insolubles | | Total |
| | Total | Rogor | $P=O$ derivative | $H_2O$ solubles | $H_2O$ insolubles | |
| 0 | 2.59 | 2.59 | — | — | — | 2.59 |
| 4 | 2.81 | 2.48 | 0.33 | — | — | 3.40 |
| 11 | 1.63 | 1.04 | 0.59 | 1.04 | 0.22 | 2.89 |
| 22 | 0.81 | 0.32 | 0.49 | 1.11 | 0.15 | 2.07 |
| 30 | 0.59 | 0.16 | 0.43 | 0.96 | 0.30 | 1.85 |
| 44 | 0.37 | — | — | — | — | 1.55 |

[a] Commercial formulation produced by Montecatini.
[b] Treated October 17, 1960.

be considered very similar to those obtained on the olives for oil (Table XIII). A definite effect on residue levels in eating olives, however, is exerted by the usual industrial processing to which the harvested fruits have to be submitted in order to be utilized for human consumption. In fact the data in Table XVI clearly show that after the olives have been soaked for

Table XVI. *Processed edible olives. Radiochromatographic analysis of olives picked 30 days after spraying with $P^{32}$-Rogor and soaked in water (five days) after 24-hour treatment with 1.8 percent sodium hydroxide solution* (SANTI and GIACOMELLI 1962)

| Concentration of $P^{32}$-Rogor sprayed on trees [a] % | Concentration (p.p.m.) of Rogor equivalents in the olives | | | | $P^{32}$ left in processed olives referred to total $P^{32}$ before processing % |
|---|---|---|---|---|---|
| | Before treatment with NaOH | After dipping in 1.8% NaOH | After dipping in water | | |
| | Rogor | Rogor | $CHCl_3$ solubles | $CHCl_3$ insolubles | |
| 0.06 | 6.91 | 1.26 | 0.02 | 0.13 | 2.16 |
| 0.04 | 5.01 | 0.85 | 0.00 | 0.10 | 1.99 |
| 0.02 | 1.74 | 0.18 | 0.00 | 0.107 | 0.98 |

[a] Treated October 17, 1960.

24 hours in 1.8 percent sodium hydroxide solution and then transferred to water for five days, only a negligible percentage of chloroform soluble $P^{32}$-containing substances (i.e., Rogor and its $P=O$ derivative) are still found in the edible drupes.

The distribution of Rogor and of its phosphorus-containing metabolites in the olive fruits was studied by DE PIETRI-TONELLI and BARONTINI (1961), who sprayed an olive tree with 0.06 percent $P^{32}$-Rogor and prepared autoradiograms of fruit macrosections. In the case of unripe drupes, having a still very soft stone, they were freehand cut into one-two millimeter thick slices but in the case of almost ripe fruits, having a very hard stone, they were chilled to $-15°$ C. and milled by means of a milling machine to their longitudinal or transversal halves, or the stone was carefully taken out and milled and the pulp with its epicarp was separately freehand sectioned.

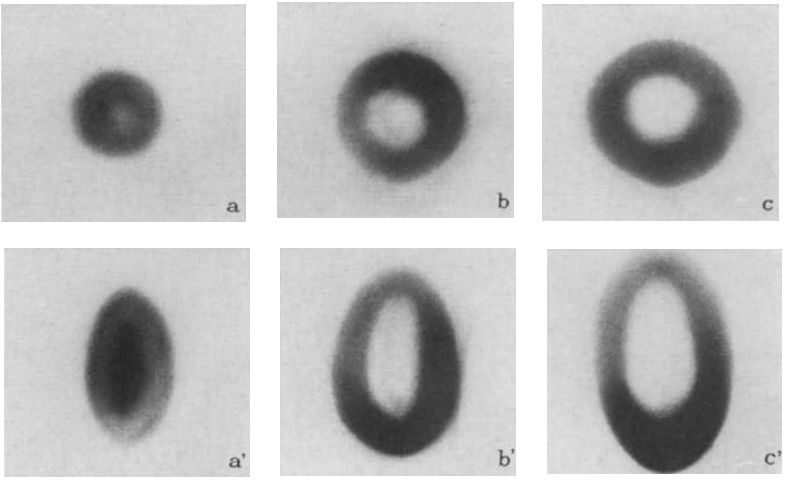



Fig. 3. Autoradiograms of transversal (*a*, *b*, *c*) and longitudinal (*a'*, *b'*, *c'*) macrosections of $P^{32}$-Rogor treated olive fruits. *a*, *a'* = unripe olives for oil 12 days after treatment; *b*, *b'* = ripe olives for oil 15 days after treatment; *c*, *c'* = ripe eating-olives 22 days after treatment. From DE PIETRI-TONELLI and BARONTINI (1961)

The autoradiograms (Fig. 3 a, a', b, b') indicate that in the olives for oil still completely unripe, Rogor penetrates from the epicarp into the pulp and diffuses, together with its phosphorus-containing metabolites, into the stone and into the seed, whereas in the almost ripe fruits the radioactive substances appear to be concentrated in the pulp and only traces of radioactivity are detectable inside the stone in correspondence to the central vascular bundles along one side of the seed. This supports the evidence that the insecticide penetrates into the olives not only through the epicarp but also systemically through the peduncle as had been demonstrated by DE PIETRI-TONELLI and BARONTINI (1961) by treating an olive branch with some fruits temporarily enclosed in polythene bags.

In eating olives (Fig. 3 c, c'), which have a larger size and a far thicker pulp, the distribution of Rogor and of its phosphorus containing metabolites is very similar to that observed in olives for oil.

**5. Tangerines.** — Rogor is widely applied on citrus against armored and soft scales, Diptera (mainly *Ceratitis capitata* Wied.), Thysanoptera, and Aleurodidae, and for the control of mites (Tetranychidae), especially *Tetranychus urticae* Koch. The degradation and persistence of Rogor residues in citrus fruits was investigated by DE PIETRI-TONELLI and BARONTINI (1960 c), who sprayed tangerine plants with 0.03 percent and 0.06 percent Rogor when the fruits started to change color from green to yellow, i.e., at the time required for control of *C. capitata.* Peel and pulp were separately bioassayed. The results (Table XVII) show that most of the insecticide is

Table XVII. *Tangerines. Bioassay of homogenized fruits picked from trees sprayed with two dilutions of Rogor (Rogor 20 L*[a]*)* (DE PIETRI-TONELLI and BARONTINI 1960 c)

| Days between treatment[b] and sampling | Concentration (p.p.m.) of Rogor equivalents | | | |
|---|---|---|---|---|
| | Rogor 0.03% | | Rogor 0.06% | |
| | Peel | Pulp | Peel | Pulp |
| 3 | 20.0 | < 0.1 | 26.0 | < 0.1 |
| 10 | 14.8 | < 0.1 | 19.6 | 0.18 |
| 20 | 5.0 | < 0.1 | 7.0 | 0.15 |
| 31 | 3.2 | < 0.1 | 5.2 | 0.14 |
| 39 | 3.0 | < 0.1 | 4.0 | 0.13 |
| 48 | 2.1 | < 0.1 | 3.0 | 0.10 |

[a] Commercial formulation produced by Montecatini.
[b] Treated December 2, 1959.

localized in the peel where it undergoes a process of degradation. Independently from the dosage of insecticide applied to the plants, the half-life of the insecticidal substances in the peel is 13—14 days. In the pulp of the fruits sprayed with 0.03 percent Rogor the residues are absent or less than 0.1 p.p.m., whereas in the pulp of those treated at 0.06 percent, very limited amounts of insecticide occur which, after ten days, start to decrease at an extremely slow rate (half-life more than 45 days).

**6. Grapefruit.** — On grapefruits Rogor was demonstrated to give good control of *Aonidiella aurantii* (Mask.) and to prevent the autbreak of *Coccus hesperidum* L. Grapefruits picked from plants sprayed by WOOD

Table XVIII. *Grapefruit. Colorimetric (?) analysis of grapefruit picked from plants sprayed with 0.1 percent Rogor (Rogor 20 W. P.*[a]*)* (WOOD 1964)

| Days between treatment[b] and sampling | Concentration (p.p.m.) of Rogor equivalents | | |
|---|---|---|---|
| | Pulp | Peel | Whole fruit |
| 7 | 0.2 | 5.3 | 1.9 |
| 56 | 0.6 | 3.9 | 1.5 |
| 92[c] | 0.7 | 2.8 | 1.2 |

[a] Commercial formulation produced by Montecatini.
[b] Treated September 28, 1960.
[c] Fruit mature.

(1964) with 0.1 percent Rogor were sent by air for analysis, from the citrus grove in Cyprus to England. The analytical results obtained by CHILWELL and BEECHAM, who (see section II) developed a colorimetric method (CHILWELL and BEECHAM 1960), indicate (Table XVIII) that the concentration of Rogor in the peel is higher than in the pulp and it diminishes very slowly. In the pulp, however, the residue level, even if it constantly remains below one p.p.m., steadily rises until the fruits become ripe (90 days after spraying). It is evident, therefore, that if the total amount of residue determined in the pulp has to be referred to Rogor, the behaviour of the insecticide on grapefruits significantly differs from that observed on tangerines (Table XVII).

**7. Apples.** — The most important use of Rogor on apples relates to the control of aphids, *Carpocapsa pomonella* L. and mites (Tetranychidae).

Table XIX. *Apples. Bioassay of homogenized fruits picked from trees sprayed with 0.05 percent Rogor (Rogor 20 L[a])* (DE PIETRI-TONELLI and BARONTINI 1958)

| Days between treatment[b] and sampling | Concentration (p.p.m.) of Rogor equivalents | | |
|---|---|---|---|
| | Unwashed fruits without core | Washed fruits without core | Core |
| 1 | 10.2 ± 0.25 | 7.2 ± 0.25 | — |
| 3 | 6.5 ± 0.10 | 4.6 ± 0.35 | — |
| 7 | 3.7 ± 0.22 | 3.3 ± 0.30 | — |
| 15 | 3.1 ± 0.30 | 3.0 ± 0.35 | 2.4 ± 0.3 |
| 25 | 1.0 ± 0.09 | 1.0 ± 0.10 | 1.0 ± 0.08 |
| 35 | 0.5 ± 0.05 | 0.5 ± 0.06 | 0.5 ± 0.05 |

[a] Commercial formulation produced by Montecatini.
[b] Treated September 5, 1957.

DE PIETRI-TONELLI and BARONTINI (1958) considered the problem of Rogor residues in the apples after the last application against *C. pomonella.* The fruit samples, picked at several time intervals after the insecticide had been

Table XX. *Apples. Colorimetric analysis of fruits picked from trees sprayed with Rogor at three rates of application* (ENOS and FREAR 1964)

| Days between last treatment and sampling | Concentration (p.p.m.) of Rogor equivalents | | |
|---|---|---|---|
| | 1 pt./100 gal. | 2 pt./100 gal. | 3 pt./100 gal. |
| 0 | 2.0 | 3.8 | 5.8 |
| 3 | 1.1 | 3.8 | 3.7 |
| 8 | 0.9 | 1.9 | 2.6 |
| 15 | 0.8 | 0.9 | 1.4 |
| 22 | 0.3 | 0.6 | — |
| 42 | 0.4 | 0.6 | 1.0 |
| 55 | 0.3 | 0.4 | 0.5 |

applied in September at the recommended dosage (0.06 percent), were divided into two subsamples: one was rinsed with water containing a wetting agent, the other was left unwashed but in both cases the core of

the fruits was taken out. It appears from the results of the bioassays, conducted by following the homogenate technique (Table XIX), that the insecticide penetrates from the epicarp into the pulp and that 15 days after treatment only a negligible percentage of it still remains outside the fruits and can be rinsed out. After penetration, the insecticide gradually diffuses into the whole pulp and also into the core and becomes metabolized at the same time, the half-life being approximately eight days.

Further data were reported by ENOS and FREAR (1964) who carried out colorimetric analyses of apples from trees sprayed with Rogor at dosages of one, two, and three pints/100 gallons. Their data, in Table XX, show that the initial concentration of the insecticide in the fruits is proportional to the dosage of Rogor applied to the plants and that the rate of disappearance of residues is very low (half-life 15—16 days).

**8. Grapes.** — Several important species of mites (Tetranychidae) injurious to grape are effectively controlled by means of Rogor. Results of colorimetric analyses of grapes sprayed in the field with Rogor "2 lb. 25% W.P." were obtained by ENOS and FREAR (1964). Their data (Table XXI) indicate that the application of the insecticide, at the above-

Table XXI. *Grapes. Colorimetric analysis of grapes harvested from plants sprayed with Rogor (two pounds of a 25 percent wettable powder)* (ENOS and FREAR 1964)

| Days between last treatment and sampling | Concentration (p.p.m.) of Rogor equivalents |
|---|---|
| 1 | 6.8 |
| 8 | 5.0 |
| 15 | 3.1 |
| 22 | 1.2 |
| 29 | 0.7 |
| 37 | 0.3 |
| 50 | 0.2 |

mentioned dosage, produces high residues (6.8 p.p.m.) one day after treatment but they drop progressively to lower levels at a rate corresponding to a half-life of eight-nine days. Concentrations of residues below one p.p.m. are in fact attained in less than 30 days following application.

### *b) Vegetable crops*

**1. Sugar beets.** — Rogor controls some of the most injurious insects damaging sugar beets, namely aphids (*Aphis fabae* Scop., *Myzodes persicae* (Sulz.), Jassidae, and Diptera (*Pegomia hyoscyami* Panz.).

Researches on Rogor residues in sugar beets were undertaken by SANTI *et al.* (1962). They employed a radiochromatographic technique for the analysis of the roots of plants whose foliage was sprayed with 0.04 percent $P^{32}$-Rogor. The first treatment was applied 15 days after the emergence of the seedlings and the second 21 days later. After the first application, the roots were analyzed only once, owing to their very small size. Notwithstanding the leaves were sprayed with a relatively high dosage, only very

low amounts of Rogor and its phosphorus containing metabolites were found in the roots as demonstrated by the data in Table XXII. The concentration of Rogor and of its $P=O$ derivative reaches a maximum a few

Table XXII. *Sugar beets. Radiochromatographic analysis of the roots of beets sprayed with $P^{32}$-labeled Rogor 0.04 percent formulated as Rogor 20 L*[a] (SANTI *et al.* 1962)

| Date of treatment | Days between last treatment and sampling | Concentration (p.p.m.) of Rogor equivalents in the roots | | | |
|---|---|---|---|---|---|
| | | $CHCl_3$ solubles (Rogor and its $P=O$ derivative) | $CHCl_3$ insolubles | | Total |
| | | | Water solubles | Water insolubles | |
| April 27, 1961[b] | 21 | 0.05 | 0.24 | — | 0.29 |
| May 18, 1961 | 1/4 | 0.69 | 0.34 | — | 1.03 |
| | 5 | 0.25 | 0.47 | — | 0.72 |
| | 13 | 0.06 | 0.49 | — | 0.55 |
| | 21 | 0.06 | 0.16 | 0.43 | 0.65 |
| | 29 | 0.04 | 0.10 | 0.27 | 0.41 |

[a] Commercial formulation produced by Montecatini.
[b] Fifteen days after the emergence of seedlings.

hours after the leaves had received the second spray, and then it diminshes at a rate corresponding to a half-life of six-seven days, whereas the water-soluble and chloroform-insoluble metabolites occur at higher concentration and their curve of disappearance has a peak 10—15 days after application.

The autoradiograms of longitudinal root macrosections of the same plants show (Fig. 4 *a*) that, 30 days after treatment, $P^{32}$-Rogor and its radioactive metabolites are distributed in the whole root and they appear particularly localized in the conducting tissues.

**2. Cotton.** — On cotton Rogor is recommended for the control of aphids, Jassidae, Thysanoptera, and mites (Tetranychidae). Considering the

Table XXIII. *Cotton. Radiometric analysis of the seeds of cotton plants cultivated in the laboratory and sprayed with $P^{32}$-labeled Rogor 0.02 percent, formulated as Rogor 20 L*[a] (DE PIETRI-TONELLI and BARONTINI 1961)

| Days between treatment[b] and sampling | Concentration (p.p.m.) of Rogor equivalents | |
|---|---|---|
| | $CHCl_3$ solubles | |
| | Delinted seeds | Lint |
| 1 | 0.37 | 0.74 |
| 3 | 0.11 | 0.44 |
| 6 | 0.09 | 0.27 |
| 12 | 0.06 | 0.18 |
| 26 | 0.07 | 0.10 |

[a] Commercial formulation produced by Montecatini.
[b] The plants were sprayed when the bolls were nearly ripe.

importance of cotton seeds as a source for oil production, DE PIETRI-TONELLI and BARONTINI (1961) carried out radiometric determinations of the chloroform-soluble extractives (i.e., Rogor and its $P=O$ derivative) in the seeds of potted cotton plants reared in the laboratory under the following conditions: temperature 24—25° C., humidity 70—80 percent,

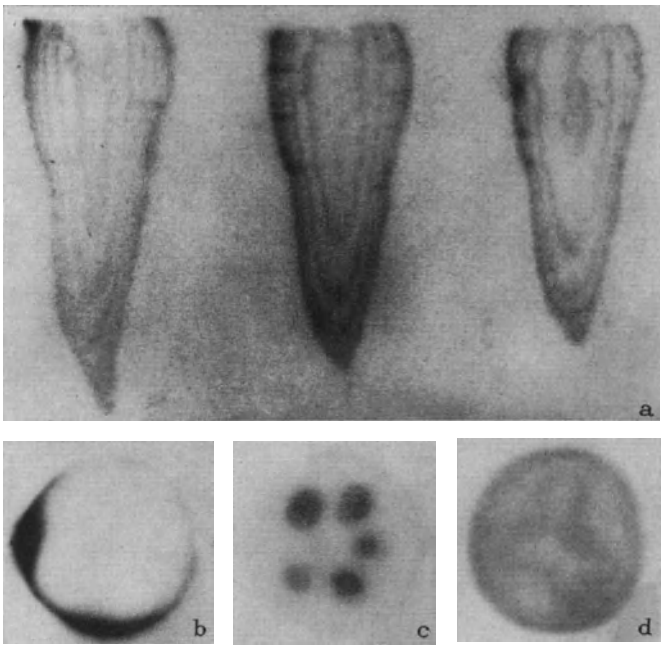



Fig. 4. Autoradiograms of longitudinal (*a*) and transversal (*b*, *c*, *d*) macrosections of sugar beet roots, cotton bolls, and potato tubers from plants sprayed with $P^{32}$-Rogor: *a*=sugar beet roots 32 days after treatment, from SANTI *et al.* (1961); *b*, *c* = cotton bolls one day and 24 days after treatment, from DE PIETRI-TONELLI and BARONTINI (1961); *d* = potato tubers 14 days after treatment. From DE PIETRI-TONELLI and BARONTINI (1961)

light 300—400 foot-candles, photoperiodism 17 hours. The plants were sprayed with $P^{32}$-Rogor 0.02 percent when the bolls were nearly ripe. The analytical data (Table XXIII), obtained until 26 days after treatment (i.e., when most of the bolls were completely open), show that systemically translocated Rogor and its $P=O$ derivative occur inside the bolls both in the delinted seeds and in the lint. Their concentration is low (below one p.p.m.) one day after application and diminishes in the following days but after 12 and after 26 days it remains about at the same level, possibly owing to the antagonistic effect of degradation and systemic translocation. The autoradiograms of the same treated bolls, taken at several time intervals (Fig. 4 *b*, *c*) confirm the localization of $P^{32}$-containing substances first in the boll walls, then in the central vascular bundles, and finally in the seeds.

**3. Potatoes.** — Aphids are the most important insects on potatoes controlled by Rogor. Preliminary investigations on penetration and residues

of Rogor in potato tubers were undertaken by DE PIETRI-TONELLI and BARONTINI (1961). They applied $P^{32}$-Rogor 0.06 percent to the foliage of potato plants in the fields and radiometrically analyzed the tubers at two time intervals after treatment. The results indicated that the total amount of $P^{32}$-containing substances increased in the tubers from 1.48 p.p.m. (after four days) to 4.44 p.p.m. (after 14 days) but the concentration of Rogor and of its $P=O$ derivative (chloroform solubles) was extremely low (0.015 p.p.m.) four days after spraying and dropped to less than 0.01 p.p.m. after ten days. Furthermore, autoradiograms of tuber macrosections showed that 14 days after treatment the radioactive substances were distributed in the whole organ and were more concentrated in the cortical parenchyma and in the conducting tissues (Fig. 4 *d*).

**4. Snap beans.** — On snap beans Rogor adequately controls aphids, Thysanoptera, and mites (Tetranychidae). VAN MIDDELEM and WAITES (1964) analyzed snap beans treated with the insecticide at three dosage rates and compared colorimetric and gas chromatographic methods. The results, reported in Table XXIV, show excellent agreement between the

Table XXIV. *Snap beans. Gas chromatographic and colorimetric analysis of snap beans sprayed with Rogor (Cygon 2 E 25 percent* [a]*) at three dosage rates* (VAN MIDDELEM and WAITES 1964)

| Days between last treatment [b] and sampling | Concentration (p.p.m.) of Rogor equivalents | | | | | |
|---|---|---|---|---|---|---|
| | Gas chromatographic analysis | | | Colorimetric analysis | | |
| | 0.25 lb.[c]/acre | 0.50 lb.[c]/acre | 1.0 lb.[c]/acre | 0.25 lb.[c]/acre | 0.50 lb.[c]/acre | 1.0 lb.[c]/acre |
| 1 | 1.74 | 3.86 | 7.85 | 1.65 | 3.94 | 7.21 |
| 3 | 1.14 | 2.76 | 6.79 | 1.23 | 2.69 | 6.37 |
| 7 | 0.87 | 1.65 | 4.20 | 0.81 | 1.74 | 4.48 |
| 14 | 0.39 | 0.79 | 2.36 | 0.42 | 0.96 | 2.66 |

[a] Commercial formulation produced by the American Cyanamid Co.
[b] Two applications applied 7 days apart.
[c] Active ingredient.

two procedures and demonstrate that Rogor residues, occurring in amounts which are proportional to the dosage applied to the plants, disappear at the same rate. For all three dosages the half-life values are approximately six days.

**5. Carrots.** — On carrots Rogor is mainly employed for the control of *Psila rosae* Fabr. STOBWASSER (1963) carried out colorimetric analyses on two varieties of carrots sprayed with the insecticide at two dosages. The analytical data in Table XXV give evidence that residues in the roots are very low a few weeks after the last treatment and they slowly diminish thereafter so that they are below the analysis level (0.03 p.p.m.) about 200 days after the last application. The residues are, however, felt to be higher than these because the analytical method would not detect any possible amounts of $P=O$ derivative.

Table XXV. *Carrots. Colorimetric analysis of carrots roots from plants treated with Rogor (Perfektion [a]) at two dosage rates* (STOBWASSER 1963)

| Date of treatment | Days between treatment and sampling | Variety | Concentration (p.p.m.) of Rogor equivalents | |
|---|---|---|---|---|
| | | | 0.1% | 0.3% |
| June 13, 1962 [b] | 26 | Pariser Markt | 0.05 | — |
| July 9, 1962 [c] | 15 | | 0.55 | — |
| | 30 | | 0.35 | — |
| Aug. 10, 1962 [c] | 37 | Nantaise | 0.1 | 0.25 |
| | 82 | | 0.08 | 0.15 |
| | 122 | | 0.03 | 0.06 |
| | 199 [d] | | <0.03 | <0.03 |
| | 222 [d] | | — | <0.03 |

[a] Commercial formulation produced by Badische Anilin & Soda Fabrik A.G.
[b] First treatment.
[c] Second treatment.
[d] Carrots harvested October 31 and stored.

## IV. Harvest residues

As on many other insecticides, extensive analytical work has been carried out in the last ten years on Rogor residues occurring in the edible portion of treated plants in order to investigate the health hazards to the consumer and to obtain reliable information for the assessment of safety intervals.

In Tables XXVI and XXVII are summarized two series of data regarding Rogor residues in fruit and vegetable crops, respectively. Olive fruits have been excluded as on this crop the quantitative estimation of residues was so widely conducted in many countries of the Mediterranean area and specially in Italy (as documented, for example, by ALESSANDRINI 1962) and in Greece that the results should be separately reported and discussed. Even if the data in Tables XXVI and XXVII cover only a part of the cultivated plants on which Rogor is recommended, they offer a rather broad and significant picture on harvest residues of this insecticide because they were obtained by several workers, adopting various analytical methods on plants cultivated in the fields and in greenhouses in different parts of the world, and therefore in different climatic conditions, and treated at several dates by spraying (at low and high volume), dusting, and also by soil application and seed drench.

It may be noted that the residues are most frequently below one p.p.m. Only in a few cases do they exceed that level if fruits or vegetables had been harvested at a very short time interval after treatment (between four and eight days) and if the plants had been sprayed with exceptionally high concentration of Rogor and with more than one application a few days apart.

Table XXVI. *Summary of results for Rogor harvest residues on fruit crops*

| Crop | Application | | Month and year of treatment | Days between last treatment and analysis | Rogor residues p.p.m. | Country | References |
|---|---|---|---|---|---|---|---|
| | Concentration of spray (active ingredient) | Rate | | | | | |
| Apples | 0.06% | to "run-off" | Sept. 1955 | 35 | 0.5 | Italy | DE PIETRI-TONELLI and BARONTINI (1958) |
| | 6.4 oz./100 gal. | 50 gal./acre | July 1958 | 7 | 0.4 | U. K. | CHILWELL and BEECHAM (1960) |
| | 6.4 oz./100 gal. | 300 gal./acre | July 1958 | 8 | 1.1 | U. K. | *idem* |
| | 6.4 oz./100 gal. | 250 gal./acre | July 1958 | 6 | 0.3 | U. K. | *idem* |
| | 6.4 oz./100 gal. | 250 gal./acre | July 1958 | 7 | 0.6 | U. K. | *idem* |
| | 0.036% | to "run-off" | Jan. 1958 | 9 | 0.3 | S. Africa | *idem* |
| | 0.036% | to "run-off" | Jan. 1958 | 14 | 0.2 | S. Africa | *idem* |
| | 0.036% | to "run-off" | Jan. 1958 | 21 | 0.3 | S. Africa | *idem* |
| | 0.036% | to "run-off" | Jan. 1958 | 31 | 0.1 | S. Africa | *idem* |
| | 0.04% | 12 pints/tree | Jan. 1959[a] | 7 | 1.2 | Australia | *idem* |
| | 12.8 oz./100 gal. | to "run-off" | April 1959 | 10 | 0.9 | N. Zealand | *idem* |
| | 0.018% | to "run-off" | Sept. 1957 | 4 | 0.5 | Switzerland | *idem* |
| | 0.018% | to "run-off" | Sept. 1957 | 10 | 0.2 | Switzerland | *idem* |
| Apricots | 0.05% | to "run-off" | June 1958 | 20 | 0.25 | Italy | DE PIETRI-TONELLI *et al.* (1959) |
| | 0.15% | to "run-off" | June 1958 | 20 | 0.3 | Italy | *idem* |
| Black currants | 8 oz./100 gal. | 100 gal./acre | July 1958 | 7 | 0.1 | U. K. | CHILWELL and BEECHAM (1960) |
| | 8 oz./100 gal. | 100 gal./acre | July 1958 | 14 | 0.4 | U. K. | *idem* |
| Cherries | 3% dust | — | June 1955 | 28 | 0.4 | Italy | DE PIETRI-TONELLI (1956b) |
| | 0.006% | to "run-off" | June 1955 | 28 | <0.3 | Italy | *idem* |
| | 0.012% | to "run-off" | June 1955 | 28 | 0.3 | Italy | *idem* |
| | 0.024% | to "run-off" | June 1955 | 28 | 0.4 | Italy | *idem* |
| | 0.02% | to "run-off" | June 1956 | 15 | 0.3—0.5 | Italy | DE PIETRI-TONELLI *et al.* (1957) |
| | 0.2% | low volume | June 1956 | 15 | 0.4—0.7 | Italy | *idem* |
| | 0.02% | to "run-off" | May 1961 | 21 | 0.06 | Italy | SANTI (1961) |
| | 2 lb. | — | — | 10 | 0.8 | U. S. A. | ENOS and FREAR (1964) |

Table XXVI. (continued)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Grapefruit (peeled) | 0.133% | 3 l./tree | Oct. 1958 | 14 | 0.1 | U. K. | CHILWELL and BEECHAM (1960) |
| | 0.133% | 3 l./tree | Oct. 1958 | 21 | 0.1 | U. K. | *idem* |
| | 0.1% | full-cover | Sept. 1960 | 92 | 0.7 | Cyprus | WOOD (1964) |
| | 0.1% | full-cover | Sept. 1960 | 105 | 1.4 | Cyprus | *idem* |
| Grapes | 0.018% | to "run-off" | Sept. 1957 | 4 | 1.6 | Switzerland | CHILWELL and BEECHAM (1960) |
| | 0.018% | to "run-off" | Sept. 1957 | 10 | 0.7 | Switzerland | *idem* |
| | 0.018% | to "run-off" | Sept. 1957 | 20 | 0.3 | Switzerland | *idem* |
| | 0.5 lb. | — | — | 50 | 0.2 | U. S. A. | ENOS and FREAR (1964) |
| Figs (dried) | 0.15% | full cover | July 1962 | 113 | < 0.1 | Purtugal | BAZZI (1962) |
| | 0.15% | full cover | Aug. 1962[b] | 94 | < 0.1 | Purtugal | *idem* |
| | 0.3% | full cover | July 1962 | 112 | < 0.1 | Purtugal | *idem* |
| Lemons (peeled) | 0.133% | 40 l./tree | Oct. 1958 | 14 | 0.4 | U. K. | CHILWELL and BEECHAM (1960) |
| | 0.133% | 40 l./tree | Oct. 1958 | 21 | 0.1 | U. K. | *idem* |
| Nectarines | 0.04% | 12 pt./tree | Jan. 1959 | 7 | 1.2 | U. K. | *idem* |
| Oranges (pecled) | 0.2% | 25—30 l./tree | July 1958 | 14 | 0.7 | Cyprus | CHILWELL and BEECHAM (1960) |
| | 0.2% | 25—30 l./tree | July 1958 | 21 | 0.7 | Cyprus | *idem* |
| | 0.1% | 25—30 l./tree | July 1958 | 14 | 0.3 | Cyprus | *idem* |
| | 0.1% | 25—30 l./tree | July 1958 | 21 | 0.5 | Cyprus | *idem* |
| | 0.133% | 30 l./tree | Jan. 1959 | 14 | 0.6 | Cyprus | *idem* |
| | 0.133% | 30 l./tree | Jan. 1959 | 21 | 0.7 | Cyprus | *idem* |
| | 0.2% | 3 l./tree | April 1958 | 18 | 0.1 | Kenya | *idem* |
| | 0.014% | 1.7 l./tree | Jan. 1959 | 16 | 0.3 | Canary Isl. | *idem* |
| | 0.1% | 2 gal./tree | Jan. 1959[c] | 14 | 0.6 | Australia | *idem* |
| | 0.1% | full cover | Sept. 1960 | 131 | 0.3 | Cyprus | WOOD (1964) |
| | 0.1% | full cover | Sept. 1960 | 155 | 0.3 | Cyprus | *idem* |
| Peaches | 0.015% | to "run-off" | June 1958 | 12 | 0.6 | Italy | DE PIETRI-TONELLI *et al.* (1959) |
| | 0.03% | to "run-off" | June 1958 | 12 | 1.1 | Italy | *idem* |
| | 0.03% | to "run-off" | July 1958 | 23 | 0.5 | Italy | *idem* |
| | 0.02% | to "run-off" | Aug. 1958[d] | 20 | 0.6 | Italy | *idem* |

Table XXVI. (continued)

| Crop | Application | | Month and year of treatment | Days between last treatment and analysis | Rogor residue p.p.m. | Country | References |
|---|---|---|---|---|---|---|---|
| | Concentration of spray (active ingredient) | Rate | | | | | |
| | 0.04% | to "run-off" | July 1958 | 24 | 0.4 | Italy | *idem* |
| | 0.02% | to "run-off" | July 1958 | 24 | 0.2 | Italy | *idem* |
| | 0.04% | to "run-off" | Sept. 1958 | 33 | 0.5 | Italy | *idem* |
| | 0.015% | to "run-off" | Oct. 1958 | 20 | 0.37 | Italy | *idem* |
| | 0.03% | to "run-off" | Sept. 1958 | 32 | 0.3 | Italy | *idem* |
| | 0.053% | to "run-off" | Jan. 1958 | 8 | 1.1 | S. Africa | CHILWELL and BEECHAM (1960) |
| | 0.053% | to "run-off" | Jan. 1958 | 20 | 0.4 | S. Africa | *idem* |
| | 0.053% | to "run-off" | Jan. 1958 | 30 | 0.1 | S. Africa | *idem* |
| | 0.04% | 12 pt./tree | Jan. 1959 | 7 | 2.0 | Australia | *idem* |
| Pears | 0.025% | to "run-off" | July 1958 | 24 | 0.22 | Italy | DE PIETRI-TONELLI and BARONTINI (1958) |
| | 0.05% | to "run-off" | July 1958 | 24 | 0.32 | Italy | *idem* |
| | 0.05% | to "run-off" | July 1958 | 24 | 0.4 | Italy | *idem* |
| | 0.1% | to "run-off" | July 1958 | 24 | 2.3 | Italy | *idem* |
| | 0.018% | to "run-off" | Jan. 1958 | 3 | 0.2 | S. Africa | CHILWELL and BEECHAM (1960) |
| | 0.018% | to "run-off" | Jan. 1958 | 8 | 0.4 | S. Africa | *idem* |
| | 0.018% | to "run-off" | Jan. 1958 | 13 | 0.1 | S. Africa | *idem* |
| | 0.018% | to "run-off" | Jan. 1958 | 20 | 0.3 | S. Africa | *idem* |
| | 0.04% | to "run-off" | July 1960 | 15 | 0.1 | Greece | BAZZI (1963) |
| | 3% (dust) | — | July 1960 | 15 | $< 0.1$ | Greece | *idem* |
| Plums | 0.018% | to "run-off" | Jan. 1958 | 3 | 0.2 | S. Africa | *idem* |
| Strawberries | 12.6 oz./100 gal. | 100 gal./acre | June 1957 | 20 | 0.2 | U. K. | *idem* |
| | 6.4 oz./100 gal. | 100 gal./acre | July 1958 | 7 | 0.3 | U. K. | *idem* |
| Tangerines | 0.03% | to "run-off" | Dec. 1959 | 48 | $< 0.1$ | Italy | DE PIETRI-TONELLI and BARONTINI (1960 c) |
| | 0.06% | to "run-off" | Dec. 1959 | 48 | 0.1 | Italy | *idem* |

[a] Three sprays at seven-day interval.
[b] Two sprays at 23-day interval.
[c] Two sprays at one-month interval.
[d] Two sprays at nine-day interval.

Table XXVII. *Summary of results for Rogor hervest residues on vegetable crops*

| Crop | Application | | Month and year of treatment | Days between last treatment and analysis | Rogor residue p.p.m. | Country | References |
|---|---|---|---|---|---|---|---|
| | Concentration of spray (active ingredient) | Rate | | | | | |
| Artichoke | 0.03% | to "run-off" | May 1959 | 10 | 0.15 | Italy | DE PIETRI-TONELLI and BARONTINI (1959) |
| | 0.05% | to "run-off" | May 1960 | 7 | 1.1 | Italy | BAZZI (1963) |
| Beets (roots) | 16 oz./100 gal. | 100 gal./acre | Aug. 1958[a] | 7 | 0.9 | U. K. | CHILWELL and BEECHAM (1960) |
| Broad beans | 9.6 oz./100 gal. | 100 gal./acre | July 1958 | 7 | 0.5 | U. K. | *idem* |
| Brussels sprouts | 25.6 oz./100 gal. | 100 gal./acre | Aug. 1957 | 12 | 1.1 | U. K. | *idem* |
| | 25.6 oz./100 gal. | 100 gal./acre | Aug. 1957 | 19 | 0.3 | U. K. | *idem* |
| | 24.6 oz./100 gal. | 100 gal./acre | Sept. 1957 | 8 | 0.7 | U. K. | *idem* |
| | 25.6 oz./100 gal. | 100 gal./acre | Sept. 1957 | 15 | 0.1 | U. K. | *idem* |
| | 25.6 oz./100 gal. | 100 gal./acre | Nov. 1957 | 7 | 0.5 | U. K. | *idem* |
| | 6.4 oz./100 gal. | 100 gal./acre | Oct. 1958 | 7 | 0.2 | U. K. | *idem* |
| | 6.4 oz./100 gal. | 100 gal./acre | Oct. 1958 | 14 | 0.2 | U. K. | *idem* |
| Cabbage | 6.4 oz./100 gal. | 100 gal./acre | Aug. 1958 | 7 | 0.1 | U. K. | *idem* |
| (summer) | 6.4 oz./100 gal. | 100 gal./acre | Aug. 1958 | 14 | 0.7 | U. K. | *idem* |
| | 6.4 oz./100 gal. | 100 gal./acre | Aug. 1958 | 21 | 0.2 | U. K. | *idem* |
| Cabbage (autumn) | 6.4 oz./100 gal. | 100 gal./acre | Nov. 1958 | 7 | 0.3 | U. K. | *idem* |
| Carrots | 16 oz./100 gal. | 100 gal./acre | June 1958 | 14 | 0.2 | U. K. | *idem* |
| | 0.1% | — | July 1962[b] | 30 | 0.35 | Germany | STOBWASSER (1963) |
| | 0.1% | — | Aug. 1962[a] | 199 | $< 0.03$ | Germany | *idem* |
| | 0.3% | — | Aug. 1962[a] | 199 | $< 0.03$ | Germany | *idem* |
| Cauliflower | 6.4 oz./100 gal. | 100 gal./acre | Aug. 1958 | 14 | 0.5 | U. K. | CHILWELL and BEECHAM (1960) |
| | 6.4 oz./100 gal. | 100 gal./acre | Sept. 1958 | 7 | 1.3 | U. K. | *idem* |
| Cucumber | 8 oz./100 gal. | to "run off" | July 1958 | 6 | 0.3 | U. K. | *idem* |
| (greenhouse) | — | 4 oz./acre | July 1958[c] | 4 | 0.6 | U. K. | *idem* |
| | — | 4 oz./acre | July 1958[c] | 12 | 0.8 | U. K. | *idem* |
| | — | 4 oz./acre | July 1958[c] | 18 | 0.3 | U. K. | *idem* |
| | — | 4 oz./acre | July 1958[c] | 24 | 0.1 | U. K. | *idem* |
| Dwarf beans | 16 oz./100 gal. | 100 gal./acre | Aug. 1958[a] | 7 | 0.4 | U. K. | *idem* |

Table XXVII. (continued)

| Crop | Application | | Month and year of treatment | Days between last treatment and analysis | Rogor residue p.p.m. | Country | References |
|---|---|---|---|---|---|---|---|
| | Concentration of spray (active ingredient) | Rate | | | | | |
| Kaffir corn | 8 oz./morgen | — | Feb. 1959 | 17 | 0.1 | U. K. | CHILWELL and BEECHAM (1960) |
| | 8 oz./morgen | — | Feb. 1959 | 21 | 0.2 | U. K. | *idem* |
| | 12 oz./morgen | — | Feb. 1959 | 17 | 0.1 | U. K. | *idem* |
| | 12 oz./morgen | — | Feb. 1959 | 21 | 0.1 | U. K. | *idem* |
| | 15 oz./morgen | — | Feb. 1959 | 17 | 0.1 | U. K. | *idem* |
| | 15 oz./morgen | — | Feb. 1959 | 21 | 0.2 | U. K. | *idem* |
| Kale | 8 oz./100 gal. | 100 gal./acre | Aug. 1958 | 13 | 0.3 | U. K. | *idem* |
| Lettuce | 0.03% | to "run-off" | Mar. 1959 | 10 | 0.1 | France | DE PIETRI-TONELLI and BARONTINI (1959) |
| | 0.26% | to "run-off" | Nov. 1959 | 58 | < 0.1 | France | BAZZI (1963) |
| | 6.4 oz./100 gal. | 50 gal./acre | June 1957 | 13 | 0.1 | U. K. | CHILWELL and BEECHAM (1960) |
| | 6.4 oz./100 gal. | 50 gal./acre | July 1958 | 7 | 0.3 | U. K. | *idem* |
| | 2.0 oz./100 gal. | 200 gal./acre | May 1959 | 7 | 0.1 | U. K. | *idem* |
| Lettuce (greenhouse) | 2.0 oz./100 gal. | 200 gal./acre | May 1959 | 7 | 0.9 | U. K. | CHILWELL and BEECHAM (1960) |
| Onions | 16.0 oz./100 gal. | 100 gal./acre | June 1958 [b] | 14 | 0.2 | U. K. | *idem* |
| Peas (shelled) | 6.4 oz./100 gal. | 100 gal./acre | July 1958 | 20 | 0.1 | U. K. | *idem* |
| | 6.4 oz./100 gal. | 100 gal./acre | July 1958 | 7 | 0.3 | U. K. | *idem* |
| | 6.4 oz./100 gal. | 100 gal./acre | July 1958 | 21 | 0.5 | U. K. | *idem* |
| Potatoes | 16 oz./100 gal. | 100 gal./acre | June 1958 [b] | 14 | 0.1 | U. K. | *idem* |
| | 6 oz./100 gal. | 130 gal./acre | Jan. 1959 | 14 | 0.1 | S. Africa | *idem* |
| | — | — | April 1958 [d] | 150 | < 0.1 | U. K. | *idem* |
| | 12 oz./100 gal. | 100 gal./acre | Sept. 1958 | 25 | 0.1 | Canada | *idem* |
| | 12 oz./100 gal. | 100 gal./acre | Sept. 1958 | 55 | 0.1 | Canada | *idem* |
| | 1% | — | Sept. 1958 [d] | 126 | 0.1 | Canada | *idem* |
| | 0.06% | to "run-off" | Aug. 1960 | 14 | < 0.01 | Italy | DE PIETRI-TONELLI and BARONTINI (1961) |
| Runner beans | 16 oz./100 gal. | 100 gal./acre | July 1958 [a] | 7 | 0.7 | U. K. | CHILWELL and BEECHAM (1960) |

Table XXVII. (continued)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Snap beans | 1 lb./100 gal. | 100 gal./acre[a] | — | 14 | 2.36—2.66 | U. S. A. | VAN MIDDELEM and WAITES (1964) |
| | 0.5 lb./100 gal. | 100 gal./acre[a] | — | 14 | 0.79—0.96 | U. S. A. | |
| | 0.25 lb./100 gal. | 10 gal./acre[a] | — | 14 | 0.39—0.42 | U. S. A. | |
| Sugar beets | 0.02% | — | June 1959[b] | 31 | < 0.05 | Switzerland | EICHENBERGER and GAY (1960) |
| | 0.04% | to "run-off" | May 1961 | 29 | 0.04 | Italy | SANTI *et al.* (1961) |
| | 12.8 oz./100 gal. | high volume | June 1957 | 100 | < 0.1 | U. K. | CHILWELL and BEECHAM (1960) |
| | 38.4 oz./100 gal. | low volume | June 1957 | 100 | 0.2 | U. K. | *idem* |
| | 6.4 oz./100 gal. | high volume | July 1957 | 77 | < 0.1 | U. K. | *idem* |
| | 3.2 oz./100 gal. | high volume | July 1957 | 77 | < 0.1 | U. K. | *idem* |
| | 51.2 oz./100 gal. | low volume | July 1957 | 48 | < 0.1 | U. K. | *idem* |
| | 38.4 oz./100 gal. | low volume | June 1957 | 100 | 0.2 | U. K. | *idem* |
| | 6.4 oz./100 gal. | high volume | June 1958 | 131 | 0.2 | U. K. | *idem* |
| Tomatoes | 6.4 oz./100 gal. | to "run-off" | Aug. 1958 | 7 | 0.4 | U. K. | *idem* |
| (greenhouse) | — | 4.3 oz./acre | July 1958[c] | 5 | 0.4 | U. K. | *idem* |
| | — | 4.3 oz./acre | July 1958[a,c] | 7 | 0.6 | U. K. | *idem* |
| Turnips | 16 oz./100 gal. | 100 gal./acre[a] | June 1958 | 14 | 0.4 | U. K. | *idem* |
| Wheat | 1 lb./100 gal. | 30 gal./acre | Sept. 1958 | 58 | 0.2 | Canada | *idem* |
| | 5% (dust) | — | Sept. 1958[d] | 114 | 0.1 | Canada | *idem* |

[a] Two applications.
[b] Three applications.
[c] Soil application.
[d] Seed treatment.

## Summary

This paper presents first a review of methods developed by several workers for the qualitative detection and quantitative estimation of Rogor and of its metabolites in treated plants. Several physico-chemical procedures are thus reported based on colorimetry, column, paper and thin-layer chromatography, paper electrophoresis, gas chromatography, and radiometry. Then a brief account is given of bioassay techniques used for the same purpose and applied directly on homogenized plant tissues or on the extracts. Autoradiographic procedures are also mentioned which were adapted for the investigation of the pattern of distribution of $P^{32}$-Rogor and of its radioactive metabolites in macrosections of plant organs. The residue degradation and persistence curves of Rogor and its metabolic fate in plants are then considered and data are reported regarding several treated fruit crops (cherries, apricots, peaches, olives, tangerines, grapefruit, apples, and grapes) and vegetable crops (sugar beets, cotton, potatoes, snap beans, and carrots). The findings support the evidence that the concentration of Rogor and of its $P=O$ metabolite in plant organs decreases at a variable rate depending, among other factors, upon the botanical species and variety, rate of growth, morphological structure, and location of the organs in the plant and on the date of treatment.

Finally a summary is presented of harvest residues determined by various methods on 35 fruit and vegetable crops treated with Rogor in different ways and under different conditions.

## Résumé*

Cet article constitue en premier lieu une mise au point des méthodes établies par divers chercheurs pour la détection et la détermination quantitative du Rogor et de ses métabolites dans les végétaux traités. Plusieurs techniques physico-chimiques, basées sur la colorimétrie, la chromatographie sur colonne, sur papier et en phase gazeuse et la radiométrie sont ainsi présentées. Sont décrites ensuite de façon sommaire les techniques biologiques utilisées dans le même but et appliquées directement aux tissus végétaux homogénéisés ou à leurs extraits. Sont également mentionnées les méthodes auto-radiographiques adaptées à l'étude de la répartition du Rogor marqué au $P^{32}$ et de ses métabolites radioactifs dans les coupes macroscopiques d'organes végétaux. Sont ensuite considérées les courbes de dégradation et de persistance du Rogor, ainsi que sa destinée métabolique et des données sont présentées en ce qui concerne une série de fruits (cerises, abricots, pêches, olives, mandarines, pamplemousses, pommes et raisins) et de cultures (betterave, sucriére, coton, pomme-de-terre, haricots et carottes). Les résultats obtenus montrent que les concentrations du Rogor et de son métabolite $P=O$ dans les tissus végétaux décroissent à une vitesse variable dépendant, entre-autres facteurs, de l'espèce et de la variété botanique, de la vitesse de croissance, de la structure morphologique, de la localisation des organes et de la date du traitement.

* Traduit par R. TRUHAUT.

Finalement est donné un tableau résumé des taux de résidus à la récolte déterminés par diverses méthodes sur 35 cultures fruitières ou légumières traitées par le Rogor selon des méthodes diverses et dans différentes conditions.

## Zusammenfassung*

Diese Arbeit bringt zuerst eine Übersicht über die Methoden, welche verschiedene Autoren für den qualitativen Nachweis und die quantitative Bestimmung von Rogor und dessen Metaboliten in damit behandelten Pflanzen entwickelt haben. In ihr wird über die verschiedenen physikalisch-chemischen Verfahren berichtet, die auf Colorimetrie, Säulen-, Papier- und Dünnschichtchromatographie, ferner auf Papierelektrophorese, Gaschromatographie und Radiometrie beruhen. Dann folgt eine kurze Mitteilung über Testverfahren, die dem gleichen Zwecke dienen und direkt an homogenisierten Pflanzenmaterialien oder an Extrakten angewendet werden. Auch autoradiographische Verfahren finden Erwähnung, die auf die Untersuchung des Verteilungsschemas von $P^{32}$-Rogor und dessen radioaktiven Metaboliten in Makroschnitten von Pflanzenorganen ausgerichtet waren. Weiterhin werden die Kurven, welche Abbau und Haltbarkeit der Rückstände von Rogor und den Verlauf des Abbaus in Pflanzen wiedergeben, besprochen und Zahlenwerte mitgeteilt, die von zahlreichen behandelten Frucht- und Gemüsekulturen stammen: Kirschen, Aprikosen, Pfirsiche, Oliven, Orangen, Grapefruit, Äpfel und Weintrauben bzw. Zuckerrüben, Baumwolle, Kartoffeln, Bohnen und Karotten. Die Ergebnisse sind ein weiterer Beweis dafür, daß die Konzentration von Rogor und dessen $P=O$-Metaboliten in Pflanzenorganen mit verschiedener Geschwindigkeit abnimmt, die, neben anderen Faktoren, von der botanischen Art und Varietät, von der Wachstumsgeschwindigkeit, der morphologischen Struktur, von der Lage der Organe in der Pflanze und vom Zeitpunkt der Behandlung abhängt.

Zuletzt wird eine Zusammenfassung über Einzelrückstände gegeben, die mittels verschiedener Methoden in 35 Frucht- und Gemüsekulturen bestimmt wurden, welche in verschiedener Anwendungsart und unter unterschiedlichen Bedingungen mit Rogor behandelt worden waren.

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* Übersetzt von O. R. KLIMMER.

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# The Pesticides Safety Precautions Scheme*

By
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## Contents



## I. Introduction

Eleven years ago two important events occurred in respect to the safe use of pesticides used in agriculture and food storage in Great Britain. Both arose from the activities of an official working party set up in July 1950 and given three tasks. Its first was:

"To make recommendations for the promotion of the safety of workers in the agricultural use of substances which are toxic or harmful to human beings;..."

* An account of the current safety arrangements for pesticides used in agriculture and food storage in Great Britain based in part on an Invitation Address to the First International Convention of the National Pest Control Association, New York, October, 1964.

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The Government took this course in response to public fears expressed following the deaths of agricultural workers who had sprayed dinitro-ortho-cresol (DNOC) over long hours in hot weather, wearing little or none of the recommended protective clothing. The working party completed its task expeditiously and reported six months later (ANONYMOUS 1951). The Government acted equally promptly on the main recommendations (see section II).

The working party's second task was:

> "To investigate the possible risks from the use in agriculture of toxic substances on the agricultural product and the stored product, and, if protective measures appear desirable, to make recommendations as to their form to the Ministers concerned."

This proved to be a much more difficult task. Little was known about the extent of use of pesticides on home produced foodstuffs or about residues actually present on them or on imported produce. Methods of analysis for detecting and determining the residues were mainly lacking or inadequate and the pharmacological significance of those residues found was unknown. The working party took just two years to complete this investigation and its second report appeared in October 1953 (ANONYMOUS 1953).

The third and final task of the (ZUCKERMAN) Working Party on Precautionary Measures against Toxic Chemicals Used in Agriculture, was:

> "To investigate the possible risks to the natural flora and fauna of the countryside from the use in agriculture of toxic substances, including the possible harmful effects for agriculture and fisheries, and to make recommendations."

The decision to ask the working party to undertake this review arose from a series of incidents culminating in widespread deaths among wildlife from acute organophosphorus poisoning in the autumn of 1952.

The working party started this task in May, 1953 but again was greatly handicapped by a lack of data. So little was known about wild bird, mammal, and insect populations and normal fluctuations within those populations that the working party was unable to assess the effects of pesticides on populations as distinct from individuals. In its third report, issued in June, 1955 (ANONYMOUS 1955) the working party was able to make only general recommendations. Nevertheless, it insisted that risks to wildlife should receive close attention by the standing committee that in its second report it had recommended should be established.

MARTIN (1963) has already briefly described some outcomes of the working party's recommendations. The purpose of this paper is to enlarge on his account and to bring it up-to-date.

The second report contained two important recommendations. Firstly, a scheme should be established to look after the safety aspect of pesticides. It should be voluntary, but the report made it clear that should it fail to work legislation should follow. All manufacturers would be expected to observe the scheme, participation in which would be free. Secondly, the Government should set up a standing committee to advise it on all risks arising from the use of pesticides in food production and storage.

To implement the first recommendation, members of the three trade associations representing the majority of pesticide manufacturers in Great

Britain, namely the *Association of British Chemical Manufacturers* (ABCM), the *Association of British Manufacturers of Agricultural Chemicals* (ABMAC) [1] and the *Industrial Pest Control Association* (IPCA) met with officials to draw up a list of rules which industry would observe and officials would administer. In this way the voluntary Notification of Pesticides Scheme was devised and, after two years trial, was formally introduced early in 1957.

The purpose of the scheme was — and still is — "to safeguard human beings (whether they be users, consumers of treated produce, or other members of the public), livestock, domestic animals, and wildlife against risks from pesticides." The acceptance of its obligation to observe fully the scheme is contained in ABMAC's published "Code of Practice for the safe use of Agricultural Pesticides in Great Britain."

## II. Operator protection legislation

The recommendation that the scheme should be voluntary was, at first sight, unexpected to many people. In its first report, the working party had come out strongly in favour of there being legislation designed to protect the agricultural worker when using products containing the more toxic pesticides, and this advice had been acted upon promptly. The Agriculture (Poisonous Substances) Act, passed in 1952, has been entirely successful. Prior to its enactment there had been eight deaths of agricultural workers using pesticides. Since it was passed there has been only one such death and that from a pesticide now no longer used. The accident situation is no less impressive. Out of a current average 12,000 non-fatal accidents on farms in England and Wales each year, about five are attributable to pesticides.

The provisions of this act do not extend to self-employed persons or to members of the general public, but are restricted to employees of farmers, growers, and contractors. Under the terms of the act, the *Minister of Agriculture, Fisheries and Food* and the *Secretary of State for Scotland* are authorised to make regulations (subject to Parliamentary approval) which may be amended or revoked as necessary. Before doing so they are required to consult with representative organisations of the industries concerned, although they are not obliged to accept the advice proffered.

Regulations made under the act are designed to take into account the fact that one method of using a pesticide may be inherently more dangerous than another. Thus, other factors being equal, granular application is safer than spraying which in turn is less hazardous than aerosol application in glasshouses. Currently the regulations schedule 17 different operations and list the types of protective clothing which must be worn according to the pesticide being used. At present, of the 190 or so pesticides commercially available in Great Britain, about 30 are specified in the regulations.

[1] At that time known as the *Association of British Insecticide Manufacturers* (ABIM).

The regulations impose obligations on employers, who must provide the prescribed protective clothing and make certain that workers wear it, as well as on employees. Other provisions specify the maximum number of hours workers may be employed to carry out scheduled operations over a specified time and the minimum age at which they may use regulated pesticides; they require the maintenance of protective clothing, the provision of washing facilities for workers, the notification of sickness, the training and supervision of workers carrying out scheduled operations, the provision of drinking water and vessels, and the keeping of a register of all scheduled operations carried out on ground crops or in glasshouses over certain acreages.

A *Safety Inspectorate* enforces the act and any regulations made under it. These inspectors have rights of entry onto land, can enforce the production of certain documents, take statements and also samples of pesticide products for independent analysis, all with a view to possible prosecution. They give advice and assistance in connection with the precautions to be taken under the regulations. They are also empowered to grant a certificate of exemption from some or all of the provisions of the regulations if they are satisfied either that the worker can be protected adequately by other means or that the provisions are unnecessary under the proposed conditions of use. Such a certificate is granted only on specific conditions which are binding on both employer and worker.

A range of official advisory leaflets on safety have been published and are revised periodically. They are aimed at different types of users and, apart from giving general safety advice, endeavour to explain in simple language the requirements of the regulations. A set of notes has also been published for the guidance of medical practitioners who may have to attend poisoning cases arising from the use of regulated pesticides. These notes are the responsibility of the *Ministry of Health* and are kept up to date with the help of the *National Poisons Information Centre* in London.

The operator in the field of food storage practice is also protected, but by rather older legislation. Under the Hydrogen Cyanide (Fumigation) Act 1937, for instance, strict conditions are imposed when this substance is used for fumigating ships and buildings. Additionally, official advisory leaflets on methyl bromide, ethylene oxide, and the liquid fumigants carbon tetrachloride, ethylene dichloride, and ethylene dibromide have been published whilst codes of conduct are now being considered jointly by industry and Government departments for the use of these more toxic fumigants in different situations.

All these regulations controlling the use of the more toxic pesticides are closely linked with the Poisons and Pharmacy Act 1933 and regulations made thereunder which control their sale and apply in general to all toxic chemicals. They control such aspects as, who may sell them, how they must be stored prior to sale, and to whom they may be sold. They also specify certain labelling requirements, colouring of the product, and packaging.

Why then, did the working party in its second report favour a voluntary scheme in the first place? This decision was based to a large extent

on some 11 years experience with another voluntary scheme, concerned with the official approval of pesticide products for efficiency.

## III. Approval (efficiency) scheme

This scheme, which first appeared in 1942 as the Crop Protection Products Approval Scheme, was inaugurated after quite a long period of informal discussions between industry and Government departments, in the course of which the opinion was developed that a voluntary scheme would be preferable to legislation. The purpose of the approval scheme was to enable users to select, and advisers to recommend, efficient and appropriate crop protection products and to discourage the use of unsatisfactory products. Manufacturers were able, at their discretion and on payment of a fee, to obtain approval for the efficiency of their products. They were required to supply all the data in support of their claims for efficiency and to submit the label of the product for approval. Approval having been given, the label was required to carry an approval mark (currently like a large capital *A* supporting a crown) and no change could be made in the label contents without further agreement. This scheme continued virtually unchanged until 1960 when it was reconstituted under the title of the Agricultural Chemicals Approval Scheme. It still concerns itself only with efficiency and it still applies only to pesticide products used in agriculture, horticulture, and the home garden. It sets higher standards for approval yet enables approval to be given at an earlier stage in the development of a pesticide. But, approval can be given to a product containing a new pesticide only after the latter has been cleared under the safety scheme (section IV).

Early each year two official publications appear, one listing approved products for farmers and growers, and the other of products for use in the garden. The 1965 lists (ANONYMOUS 1965 a and b) contain over 100 pesticides formulated as nearly 800 products.

## IV. Safety schemes

Returning to the Notification Scheme, whilst it placed firmly on manufacturers the obligation to notify [2] their proposals to market products containing new pesticides or new uses or formulations of existing ones and to provide all the data in support of their safety claim, it also allowed them a measure of discretion as to whether to notify or not. If a manufacturer judged that his product would offer no hazard he was not obliged to notify. If in doubt he frequently notified or at least obtained informal guidance.

Having established the procedure to be followed, representatives of industry and Government departments next jointly produced guides on

[2] "To notify" is to inform the appropriate Government department in writing.

the manner of presenting the notification [3] and on the toxicological and residue data required in support. Later, guides on the information required on wildlife risks, on labelling, and on carcinogenicity testing were added.

These guides were issued as appendices to the scheme. They were deliberately drafted in broad terms, intended to guide and not to direct the manufacturer on the sort of information required. When a rather specialised piece of information was called for, such as the neurotoxicity testing of organophosphorus compounds, the effectiveness of cholinesterase reactivators, testing for toxicity to fish, or field observations on hazards to wild birds, detailed advice was issued in the form of a working document.

Detailed advice has also been issued on the presentation of the scientific data on the pesticide and its formulated product(s), and on residue data as well as on the labelling of products for various "fields" of use.

By the early 1960's it was clear that the scheme needed an overhaul to make it meet present day requirements more effectively. On industry's initiative, revision began and finally it re-emerged in May 1964 as the Pesticides Safety Precautions Scheme, with seven appendices and nine working documents. A further two working documents on labelling have been issued and more are being prepared.

Possibly the most important change was that the discretion enjoyed by manufacturers under the older scheme had, to all intents and purposes, disappeared — at industry's request, it should be noted — so that nowadays everything has to be notified other than quite minor changes in formulation. The scheme now requires manufacturers to put the common or other recognised name of the pesticide on the label of the product. It also makes clear the fact that a manufacturer cannot obtain efficiency approval for his product if it contains a new pesticide until he has obtained safety clearance for that pesticide. He still pays for approval, which is still truly voluntary; in practice he has little option but to comply with the safety scheme, which is still free.

The scope of the Pesticides Safety Precautions Scheme is a little broader than that of the Agricultural Chemicals Approval Scheme in that it applies not only to pesticides used in agriculture, horticulture, and the home garden but also to those used in food storage practice and against bird and land pests including rodents.

Certain veterinary products that may be purchased and used by the farmer are covered by the broadly parallel Veterinary Products Safety Precautions Scheme established early in 1964.

## V. The advisory committee and its subcommittees

How are the safety schemes administered? Here it is appropriate to give details about the standing committee set up to advise the Government on all risks arising from the use of pesticides in food production and storage — the second recommendation in the second report as mentioned

[3] A "notification" is a manufacturer's written proposal set out as required under the scheme.

earlier. A committee was established early in 1954 and one its first acts was to form a scientific subcommittee, to advise it on the scientific aspects of safe use. Early in 1964 its veterinary subcommittee was formed, to advise it on veterinary matters.

When first formed, the standing committee was named the Advisory Committee on Poisonous Substances Used in Agriculture and Food Storage (see section XV for its current title). Although set up by a number of Government departments, it was primarily responsible to the *Minister of Agriculture, Fisheries and Food* and the *Secretary of State for Scotland.* Its present members are scientific and administrative representatives from those Government departments and research councils which are concerned with the use of pesticides, namely the *Ministeries of Agriculture, Fisheries and Food,* and of *Health* and their Scottish equivalents, the *Ministry of Labour,* the *Ministry of Technology* and the *Department of Education and Science,* the latter being responsible for the *Agricultural, Medical and National Environment Research Councils.* In addition, apart from the chairman it has six members, mainly from the universities, who are entirely independent of government. The membership of the scientific subcommittee is of scientists also from Government departments and research councils, each appointed for his specialist-knowledge of at least one aspect of pesticides, the whole chosen to give full coverage of the subject.

The membership of the veterinary subcommittee is selected in the same way. Both have strong medical and toxicological representation with chemists, and veterinarians and, in the case of the scientific subcommittee, entomologists, plant pathologists, agriculturists, and experts on stored products and wildlife.

There are no trade interests on the advisory committee or its subcommittees.

The scientific subcommittee has a number of panels to which it can turn for specialised advice. These include the medical, analytical, wildlife, operator protection, and antibiotics panels (the last being shared with other official committees).

## VI. Notification procedure

Both the Pesticides Safety Precautions Scheme and the Veterinary Products Safety Precautions Scheme are administered by the *Ministry of Agriculture, Fisheries and Food* on behalf of the other interested Government departments. Notifications of products containing pesticides for use in agriculture, horticulture and the home garden go to the *Ministry's* Plant Pathology Laboratory; those for products used in food storage practice, as rodenticides or against other land pests and birds, go to the *Ministry's* Infestation Control Laboratory; notifications on veterinary products are dealt with at the *Ministry's* Veterinary Laboratory.

Notification under the Pesticides Safety Precautions Scheme is not expected whilst a pesticide is at the stage of laboratory or small scale trials, carried out under the control and direction of the developer. If,

however, it is to be used in trials by agricultural workers subject to regulations made under the Agriculture (Poisonous Substances) Act (section II), or if treated produce from trials is to go for human or animal consumption, the pesticide must first be notified.

The director of the respective laboratory, on receiving a notification, decides whether to recommend its clearance on the advice of selected advisers (the "quick procedure") or whether it should be processed through the committees (the "committee procedure"). Whichever course a notification takes, the final decision on clearance is made by Government departments. By the former route an answer can be given in a matter of weeks; "committee procedure" is lengthier as the notification has to go through one of the subcommittees, and usually the advisory committee as well, before a reply can be given by Government departments.

The onus is on a notifier [4] to provide all the information in support of his application. If possible the Secretariat of the appropriate subcommittee supplements that information with other published or unpublished data. If a notification goes to a subcommittee, the notifier has the right to attend the meeting at which it is considered, to make a statement, and to answer questions but not to remain whilst the subcommittee makes its decision.

Any recommendations by a subcommittee to the advisory committee are based purely on a scientific assessment of the notification. The advisory committee, in considering that advice, takes into account other factors, such as economic and administrative, before submitting it, as such or modified, to Government departments.

The greater part of the work of the advisory committee and its subcommittees consists of dealing with notifications, but they are also concerned with matters of policy and with reviewing pesticides contained in products already on the market.

## VII. Official safety recommendations

The decision by Government departments to clear a notification of a pesticide for marketing is usually made known in the form of recommendations issued as supplements to a loose-leaf booklet entitled "Chemical Compounds used in Agriculture and Food Storage in Great Britain — User and Consumer Safety — Advice of Government Departments".

These recommendations sheets normally relate to the pesticide contained in the notified product. If more than one type of formulated product containing the same pesticide is included, the recommendations sheet may give separate advice for each type. When a product containing more than one pesticide is cleared, separate recommendations sheets are usually issued for each pesticide involved.

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[4] A "notifier" is a manufacturer, importer, formulator, distributor, servicing company or anyone who prepares or approves the claims and precautions which will appear on the label of the product as used by a servicing company or as purchased by a user.

The recommendations usually appear under three headings: protection of the operator or user; protection of the consumer; and protection of others, such as workers, passers-by, children, pets, domestic animals, livestock, game, and wildlife.

### *a) Operator and wildlife safety*

The operator or user safety recommendations may state that the pesticide is regulated under one or other of the appropriate laws, may give safety advice in the form of phrases which the notifier is expected to include on the label of his product or may state that no special precautions are needed other than common sense general advice to treat all pesticides with care. Under the livestock and wildlife heading a warning of the risk is given, followed by a selection of safety phrases which, again, the notifier is expected to include on the label. These phrases will give advice such as, for example, avoiding harm to bees by not applying the pesticide when plants are flowering and by ensuring that flowering weeds are kept down in orchards; avoiding harm to livestock by keeping them away from treated areas for a specified minimum period; avoiding harm to fish by not spraying near ponds or waterways or contaminating them with concentrate, washings, or empty containers; and avoiding harm to animals, pets, and children by preventing them from having access to the product, by storing full and part-full containers tightly closed in a safe place, and by washing out empty containers thoroughly before disposing of them safely.

The phrases used will, as far as possible, be drawn from those standard phrases agreed on with industry and stated in the relevant working document (section IV). The purpose of using standard phrases or parts of standard phrases is to ensure, as far as possible, that warnings of risk and safety precautions are concisely stated, and that the same phrases will always have the same meaning when appearing on labels of different products. By keeping to a minimum the use of non-standard phrases, the effectiveness of the standard phrases is maintained.

### *b) Consumer safety*

This part of the recommendations sheet contains a varying amount of advice, determined by the inherent toxic nature of the pesticide, its persistence, and the crops on which it is cleared for use. The manufacturer is not required at present to use standard phrases for consumer safety but instead is permitted to incorporate the appropriate official recommendations with his instructions for the efficient use of his product.

The official recommendations may, as with a fairly toxic persistent systemic pesticide, specify on which edible crops it may be used, quoting a maximum dosage rate, frequency of application, and, when necessary, last date of application. With a non-systemic pesticide of relatively low toxicity and limited persistence some or all of these conditions may be dispensed with. In nearly every case, however, a minimum interval, which must elapse between last application and harvesting an edible crop, will be stipulated.

On occasions — and these are becoming more frequent — the official recommendations will also quote what may be described as a "use level" — that is, the maximum expected residue level following the use of the pesticide as recommended.

From the time the safety scheme started it was recognised that there would never be a surfeit of residue data and that the interpretation of data provided would depend to a large extent upon the suitability of the analytical methods employed in obtaining them. The first outcome was the decision to establish collaborative studies on methods of residue analysis (MILLER *et al.* 1964). The second was the decision that whilst, to support a proposal for safe use a notifier may submit toxicological and other data, including a method of residue analysis, obtained from abroad, the residue data had to be on edible crops grown and treated in this country. Supplementary residue data from abroad, however, were always acceptable for purposes of comparison.

The amount of home-produced residue data had to be sufficient to satisfy the health authorities that the level occurring on or in the harvested crop was unlikely to offer a consumer hazard. The official recommendations for safe use were made with these "use levels" (sometimes formerly referred to as "administrative levels" — see DORMAL and HURTIG 1962) in mind although the levels were not published. Government departments took this course because they considered that, whilst they were satisfied the data supplied enabled them to assess the hazard, the amount was not sufficient to justify the publication of a precise figure which, forthwith, might be regarded in some quarters as immutable and any attempt to change it later might give rise to considerable misunderstanding.

Following the publication of the report of the research study group (see section X) this policy was reviewed and it was concluded that, in future, every effort should be made to include a "use level" figure in the official recommendations. This policy is but slowly being implemented — an indication of the desire to be reasonably certain that the figure quoted satisfactorily represents the upper level likely to be found on or in crops treated in various parts of this country with the very variable climate and soil conditions experienced, and that there is a method of residue analysis able to meet the needs of any official analyst called upon to examine a foodstuff for residues of that pesticide.

With but two exceptions, such "use levels" that have been issued have no statutory backing. Although the Food and Drug Acts enable legal tolerances to be established, these powers have only been used to date to establish general tolerances for arsenic and lead (MARTIN 1963).

The safety recommendations go to the notifier for acceptance before being issued. When issued they are distributed on a wide scale both in Great Britain and abroad, where they go to over 60 countries. In Great Britain copies go to Government departments, local authorities, medical officers of health and public analysts, public libraries, hospitals, universities, agricultural and horticultural research stations, food processing firms, the farming and medical press, pesticide manufacturers and formulators (who use them as the basis for the safety advice on the label), the official

advisory services, and individuals. Distribution is free so that every user of a pesticide has the opportunity to see the official recommendations on its safe use. As a specific example of their use at home they, together with the list of approved products, form the basis of spray programmes which, a number of food processing firms insist, growers under contract should strictly follow, under pain of cancellation of contract.

## VIII. Types of clearances

Not infrequently a notification is either rejected or agreed to only in part, usually on the grounds of insufficient information. In such cases it may be re-submitted later when the missing data have been obtained. To reduce to a minimum the occasions when a notification cannot be cleared for lack of the necessary supporting information, firms are encouraged to discuss their proposals quite early with those officials concerned with the safety schemes so that they can be guided in the assembly of the necessary information.

This early consultation means that under the Pesticides Safety Precautions Scheme a pesticide may go through four stages of clearance, the first two being pre-market. A *trials clearance*, given for one year only, specifies a number of conditions to be met by the notifier before the pesticide enters the next stage of *limited clearance*, under which quite a large amount of treated foodstuff — a growing crop or stored produce — may be disposed of for consumption. A limited clearance is given for one year only and again the notifier is called upon to provide specified information before the pesticide can proceed to the next stage, of a *provisional commercial clearance*. This allows a firm to market its pesticide and, from this stage onwards, official recommendations are issued. Provisional commercial clearance, which may be given for one or more years, still requires the notifier to provide some specified information. Finally, when no further information is required the firm will be given a *commercial clearance*. Even this is not necessarily the end of the story for Government departments can, at any time, review the use of a pesticide if new information comes to hand which suggests that a review is necessary. On the other hand, not every pesticide has to go through all four stages of clearance.

Whether a notification is dealt with by the "quick" or the "committee" procedure, it is looked at from the viewpoint of all those concerned with pesticides: agriculture, food, health, labour, and wildlife. The official recommendations are made after balancing the various and often conflicting interests, although, of course, safety must be the prime consideration.

## IX. The arsenites incident

An isolated but unhappy incident in 1959 proved to be the first of several serious tests of the effectiveness of the voluntary safety arrangements.

Some years earlier an acute shortage of sulphuric acid for potato haulm destruction led to the introduction of alkali arsenites for this purpose in Great Britain. Its use was rapidly taken up by growers who found it a cheap and satisfactory substitute, despite the annual toll of deaths of cattle from eating sprayed haulm.

The incident in question arose through a grower filling his spray tank directly from a static drinking water supply. Back flow occurred and the drinking water became contaminated with arsenites. One of the six persons who drank this water subsequently died.

This incident, coupled with those annually involving cattle caused Government departments to invite industry voluntarily to withdraw the sale of alkali arsenites for use as potato haulm killers and total herbicides. The negotiations which followed concluded satisfactorily with the compromise that manufacturers were given a year in which to use up stocks "in the pipeline" after which alkali arsenites would no longer be offered for sale in Great Britain. To reinforce this voluntary decision, and at industry's request, the Government department concerned amended the Poisons Rules to make their sale for these purposes illegal.

## X. The research study group

In another direction concern had began to build up over the deaths of game and wild birds, particularly in the spring, in areas where cereal seed dressed with mixtures of organomercury fungicide and aldrin, dieldrin, or heptachlor insecticide had been sown. Protests, often vigorous, began to be made by various wildlife interests and others through the media of the press, radio, and television. There followed a full scale debate late in 1959 in the House of Commons chiefly on the scale and scope of the research currently in progress on pesticides in Great Britain. Soon afterwards the Government set up the (SANDERS) Research Study Group:

> "To study the need for further research into the effects of the use of toxic chemicals in agriculture and food storage, and to make recommendations."

In its report, issued late in 1961 (ANONYMOUS 1961 a), the group said that it was satisfied that the safety arrangements, outlined above, were working satisfactorily, and that in the field of research no important aspect was entirely neglected, but that research into certain aspects needed intensifying. The latter involved basic research [5], which is the responsibility of the research councils (mentioned earlier) and applied research, which is normally conducted by Government departments in the discharge of their obligations and advisory functions in relation to the control of pesticides. The report detailed a number of suggestions, which included selective surveys of commercially treated foodstuffs for residues, research into analytical residue methods, toxicological studies on animals and birds, and research into various aspects of the effect of pesticides on wildlife.

[5] The terms "basic" and "applied" research used here are as defined in the Report of the Committee on the Management and Control of Research and Development (ANONYMOUS 1961 b).

## XI. Basic research

The Government accepted the group's recommendations. To ensure that there was the necessary liaison between the research councils and between them and Government departments, the *Agricultural Research Council* established the (FRAZER), Research Committee on Toxic Chemicals:

> "To keep under review research done under the auspices of the Research Councils on the effects of toxic chemicals used in agriculture and food storage; and to make recommendations for future research..."

In these terms of reference "toxic chemicals" was interpreted as defined in the research study group report namely "... any pesticide, that is, insecticide, fungicide, herbicide, rodenticide, or similar chemical, used in agriculture (including horticulture) and food storage."

Its first report (ANONYMOUS 1964 a) was, in effect, a progress report following on that by the research study group. In confirmed that there were no major gaps in basic research involving pesticides, adding that although certain aspects of research had received attention there were some which still needed to be intensified. General toxicological and biological studies of birds and wildlife, the development of integrated control methods and of plant breeding for resistance to crop pests, and investigations into the more efficient use of pesticides received specific mention, as did persistence, the development of pest resistance, the evaluation of the toxicological significance of the presence of pesticide residues in human tissues, and the development of analytical residue methods.

## XII. Applied research

One particular recommendation by the research study group, promptly taken up by Government departments, was that:

> "selective surveys should be carried out of the amounts of residues occuring where pesticides have been applied in commercial practice."

These have been undertaken under the aegis of the Panel on Residues of Pesticides in Foodstuffs of the scientific subcommittee. Both home grown and imported foodstuffs have been examined and, as would be expected, attention has been concentrated on the presence of organochlorine pesticide residues in staple foodstuffs such as meat, fat, milk, butter, and potatoes. In addition, fresh fruit and vegetable crops, such as lettuce and strawberries which are eaten raw, as well as imported grain, have been examined for residues of selected organophosphorus compounds. Full reports on these surveys have yet to be published, but a summary of the results obtained for certain organochlorine pesticides is to be found in the report of the review described in section XV.

## XIII. Insecticidal seed dressings

A second outcome of the increasing evidence of bird deaths from eating dressed grain was a field investigation by the *Ministry of Agriculture, Fisheries and Food* into incidents as and when reported. The

findings from both the *Ministry's* investigations and those by wildlife interests were discussed freely at a meeting, attended also by representatives of pesticide manufacturers and merchants, held in June 1961. Similar meetings have been held annually since then.

At that meeting, agreement was reached with all the interests concerned that, from 1962, no cereal seed intended for spring sowing should be treated with aldrin, dieldrin, or heptachlor, and that these pesticides should be used on autumn sown seed only in areas where there was a real danger of attack from the wheat bulb fly. This proved indeed to be the solution, for in the four seasons which followed, the number of deaths of birds from acute poisoning through eating seed dressed with a mixture of insecticide and fungicide fell dramatically.

As with the arsenites, this agreement was also reached under the aegis of the Pesticides Safety Precautions Scheme. It, too, was a severe test of the scheme's voluntary nature, for firms were asked to take a course of action that would affect them financially to varying degree, and yet could not be enforced by law. Still, as before, it succeeded because of the responsible attitude taken by the majority of firms and users.

## XIV. The fluoroacetamide incidents

After a very brief pause, the voluntary safety arrangements were again tested by two isolated incidents involving fluoroacetamide early in 1963.

This chemical, an intermediate in the preparation of sodium fluoroacetate ("1080"), had been developed in Great Britain both as a systemic aphicide and as a rodenticide, despite its recognised high toxicity. The use of fluoroacetamide on edible crops was limited and it was scheduled under the Agriculture (Poisonous Substances) Regulations.

The first of the two incidents occurred in the South of England where a factory accident led to a quantity of the chemical contaminating soil and ditch water. Cattle which drank the latter subsequently died. The second occured in South Wales where a number of cats and dogs died after eating the flesh of a pony which had been found dead near a refuse tip. Scientific tests showed that the pony's flesh contained an organofluorine compound, probably fluoroacetamide, but despite extensive enquiries it was not possible to establish how the pony had acquired it.

The outcome of these incidents was that, by the voluntary agreement of the manufacturers, under the Pesticides Safety Precautions Scheme, fluoroacetamide was withdrawn as an insecticide and its sale as a rodenticide restricted under the Poisons Rules for use only in ships and sewers on production of a certificate signed by the Medical Officer of Health of a port or local authority.

## XV. The review of the persistent organochlorine pesticides

The problem of the acute toxic effects of cereal seed dressings having been largely overcome, wildlife interests showed increasing concern over a new phenomenon that had become apparent, not only in Great Britain

but also in other countries. This was the unusual persistence of a relatively small group of pesticides — small in number but extensive in use — namely aldrin, dieldrin, heptachlor, DDT, BHC (including lindane), endrin, endosulfan, chlordane, "Toxaphene", and "Rhothane" (TDE or DDD).

The persistent property of these pesticides has long been known and, in fact, is considered one of their most valuable features. But with the development of highly refined and sensitive analytical procedures, analysts began to find traces everywhere: in the soil, water, air, in the bodies of animals, birds, fish, crustaceans, even man. The cry went up that the whole environment was being contaminated by these pesticides through their persistence beyond the point of need.

Public opinion being aroused once again by the press, radio, and television, and not least by the publication initially in the United States of the remarkable book "Silent Spring", the House of Lords early in 1963 debated fully, thoroughly, and with some intensity the subject of pesticides and their control.

This widespread interest resulted in the advisory committee in June, 1963 being asked:

> "In the light of existing information and that now coming to hand generally to review the risks arising from the use of chlorinated hydrocarbon pesticides (more particularly those containing aldrin, dieldrin and heptachlor) in agriculture (including gardening) and food storage and to make recommendations."

The first part of its report on aldrin, dieldrin, heptachlor, DDT, and BHC was published in February, 1964 (ANONYMOUS 1964 b) and the supplement on endrin, endosulfan, chlordane, "Toxaphene", and "Rhothane" in October of that year (ANONYMOUS 1964 c).

The advisory committee's main findings on these persistent organochlorine (or chlorinated hydrocarbon) pesticides were as follows. It found no evidence of a serious immediate hazard to human beings arising from their use, and no evidence to support the charges that they were severe liver poisons or likely to cause cancer. The committee agreed, however, that the levels of the more toxic organochlorine residues found in various foodstuffs were undesirable whether or not they were proved to be harmful, and justified some measure of restriction on their use. The committee accepted that there was circumstantial evidence for the view that the decline in the populations of certain predatory birds in Great Britain was related to the residues found in such species arising from the use of aldrin, dieldrin, and heptachlor and, to some extent DDT, but concluded that there was no evidence that the populations of other bird species had been affected by pesticides. It was likely that the effect on predatory birds was through the "food chain".

The advisory committee's main concern was with the evidence of widespread contamination of the environment by some of these pesticides and considered that the time had come for steps to be taken, as a precautionary measure, to contain this contamination by placing restrictions on their use, restrictions which (to quote):

> ...", on the basis of proved hazards arising from their use considered in isolation, it might not be easy to justify."

The advisory committee accordingly recommended restrictions on the use of aldrin, dieldrin, and heptachlor which, when put into effect, would reduce the current extent of use in agriculture, food storage, and veterinary practice to roughly one-third. The greater part of this reduction would result from the withdrawal of aldrinated fertilisers, aldrin and dieldrin sheep dips, and of these two pesticides from use in the home garden. Certain uses of a minor but essential nature should still be allowed, but all should be reviewed in three years' time with a view to their discontinuance.

The advisory committee recommended that, with minor exceptions, the current uses of endrin, endosulfan, chlordane, "Toxaphene", and "Rhothane", all on a very small scale, should continue but should be reviewed in 1967. If, in the meantime, the amount used of any of these five pesticides showed a large increase, it should be reviewed immediately. The review in 1967 should apply also to DDT, the use of which was not otherwise restricted; BHC came out of the enquiry unscathed.

Before the report and its supplement were published, informal discussions were held with industry. This again was to be a severe test of the voluntary safety arrangements for, in certain cases, industry were to be asked voluntarily to cease supplying a considerable quantity of material to the British farmer. This was difficult enough but even greater could be the likely impact on countries abroad who watch pesticide trends in Great Britain and who tend to be guided by them. The negotiations were tough and prolonged but eventually the recommendations were accepted by industry, not so much because they fully agreed with them but for the sake of preserving the close relations which exist between industry and the Government through the voluntary schemes. It was mutually agreed that the proposed restrictions should apply from the beginning of 1965 except for sheep dips, which would cease to be available after the end of that year.

In its review, the advisory committee drew attention to a number of non-agricultural uses of some of these ten pesticides which could be contributing to the general contamination of the environment and for which no controlling arrangements existed at present. This resulted in the Government extending the committee's terms of reference to cover other uses and its title was changed appropriately to the (Cook) Advisory Committee on Pesticides and Other Toxic Chemicals. Because of this extension of its functions, it is now responsible to the *Secretary of State for Education and Science,* though still available to advise other ministers.

## XVI. Review of the safety arrangements for pesticides used in agriculture and food storage

Another outcome of the review of the persistent organochlorine pesticides was that the advisory committee was asked:

> "To consider and advise on any improvements and extensions of the present safety arrangements that may be desirable to provide greater protection against the hazards arising from the use of pesticides in agriculture and food storage."

In particular, the committee was asked to advise whether stricter criteria should be applied when notifications of products containing new pesticides or reviews of existing pesticides were being considered.

An invitation went to about 80 organisations which had a direct or indirect interest in pesticides, inviting their views and posing a series of questions to assist them in arriving at a considered opinion. Replies were received from nearly 50 organisations and individuals by the end of 1964, when the committee began its task.

Some believed that legislation was inevitable and that it would need to be comprehensive, governing sale more strictly than at present, controlling use, laying down tolerances for residues in foodstuffs, and protecting wildlife including bees. Others took the opposite view, believing that the present voluntary arrangements had a flexibility not enjoyed by law, thereby allowing the schemes continuously to be modified to keep up with the almost daily changes occurring in this very dynamic subject. And, of course, between these extremes came every other shade of view!

The outcome of this review will not be known for some time.

## Summary

The present arrangements in Great Britain for the safe of pesticides in agriculture and food storage are described. Reference is made to three reports issued in the early 1950's by an official working party, the recommendations of which led to the present arrangements which are part voluntary, part mandatory. Mention is also made of the older voluntary scheme for the efficiency approval of agricultural pesticide products.

The procedure for the present safety arrangements, made under the voluntary Pesticides Safety Precautions Scheme, are described, as is the part played by an official standing committee appointed to advise the Government in all risks arising from such uses.

Five incidents and events, which severely tested the voluntary nature of the arrangements at the time, are described together with the circumstances leading to the appointment of an official study group to investigate the position of research into pesticides. Also described is the consequentially established standing committee to coordinate that research.

The account concludes with a reference to the review of the safety arrangements now in progress.

## Résumé *

On décrit les dispositions actuelles prises en Grande Bretagne pour assurer la sécurité de l'emploi des pesticides pour l'agriculture et le stockage des denrées alimentaires. Référence est faite aux trois rapports publiés au début de 1950 par un groupe de travail officiel, dont les recommandations ont conduit aux dispositions actuelles, en partie librement consenties, en

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partie imposées. Il est également fait mention de l'ancienne réglementation librement consentie qui régit l'agréation des pesticides agricoles en ce qui concerne leur efficacité.

On décrit la procédure des mesures actuelles de sécurité prises à titre bénévole par le «Pesticides Safety Precautions Scheme» ainsi que le rôle joué par un comité permanent officiel, chargé d'instruire le gouvernement de tous les risques résultant de l'usage des pesticides.

Cinq incidents et cas, qui ont rigoureusement éprouvé le caractère bénévole des dispositions actuelles, sont mentionnés, avec les circonstances qui ont conduit à charger un groupe de travail officiel d'examiner l'état de la recherche en matière de pesticides. On décrit aussi le comité permanent qui fut créé ensuite, dans le but de coordonner cette recherche.

L'exposé conclut par une référence à l'examen des dispositions de sécurité actuellement en voie d'élaboration.

## Zusammenfassung *

Die gegenwärtig in Großbritannien geltenden Vorkehrungen zum sicheren Gebrauch von Schädlingsbekämpfungsmitteln in der Landwirtschaft und im Vorratsschutz werden beschrieben. Drei Berichte werden erwähnt, die anfangs der 50er Jahre von einer offiziellen Arbeitsgruppe herausgegeben wurden und zu den heutigen, teils fakultativen, teils obligatorischen Bestimmungen führten. Auch die früheren fakultativen Bestimmungen betreffend Anerkennung der Wirksamkeit von landwirtschaftlichen Schädlingsbekämpfungsmitteln werden erwähnt.

Die Einzelheiten der gegenwärtig benutzten Sicherheitsvorkehrungen, die in dem freiwilligen Pesticides Safety Precautions Scheme enthalten sind, werden beschrieben, wie es im Aufgabenbereich des offiziellen ständigen Komitees liegt, das die Regierung über alle auftretenden Risiken zu beraten hat, die im Zusammenhang mit der Anwendung von Schädlingsbekämpfungsmitteln vorkommen können.

Fünf Unfälle und Vorfälle, die die Freiwilligkeit der Sicherheitsmaßnahmen in jener Zeit auf eine harte Probe stellten, werden beschrieben, ebenso wie die Umstände, die zur Bildung einer offiziellen Studiengruppe führten, die den Stand der Forschung auf dem Gebiet der Schädlingsbekämpfung abklären muß. Auch das schließlich geschaffene ständige Komitee zur Koordinierung der Forschung wird erwähnt.

Der Rechenschaftsbericht endet mit einem Hinweis auf die zusammenfassende Darstellung der Sicherheitsmaßnahmen, die man gegenwärtig ausarbeitet.

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# Herbicide residues in soils and their phytotoxicities to crops grown in rotations

By
T. J. SHEETS* and C. I. HARRIS*

With 3 figures

## Contents



## I. Introduction

Herbicide residues in soils pose four potential problems or hazards: (1) injury to sensitive plants grown in rotations with sprayed crops, (2) accumulation of residues from application rates which exceed rates of dissipation, (3) unlawful residues in crops grown in rotations with treated crops, and (4) inhibition of beneficial soil microorganisms. The purpose of this review is to assess hazards of persistence and accumulation of herbicides in soils and to discuss phytotoxicity of herbicides to crops in rotation systems.

## II. Carry-over *versus* accumulation

In several areas of the United States arsenic levels in soils have been increased by the application, year after year, of inorganic sprays for insect control in crops. JONES and HATCH (1937) reported that one commercial apple orchard received more than 3,500 pounds of lead arsenate per acre

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over a 25-year period. Accumulation of arsenic is not too surprising and can be explained by its immobility and insolubility.

Usually apple trees grew unaffected in soil containing comparatively high levels of inorganic arsenic residues because the arsenic remained largely in the upper six to eight inches of soil (JONES and HATCH 1937), above the level of most roots of this species. However, when young peach or apricot trees were planted in old apple orchards, arsenic residues sometimes caused severe injury or death (VANDECAVEYE *et al.* 1936, JONES and HATCH 1937, BLODGETT 1941). In many such orchards, alfalfa or barley seedlings failed to develop normally and often succumbed to the toxic effects. Reclamation of old orchard soils for forage or food-crop production is difficult and slow; many remain unproductive.

After the development of selective herbicides, some of which are active in the soil for several months, research workers became concerned that phytotoxic residues might persist and injure sensitive plants rotated with sprayed crops. Also, they have been aware of the possibility of accumulation, as with arsenic in orchards, from continuous spraying of the same soil. Consequently field research has been conducted with several herbicides

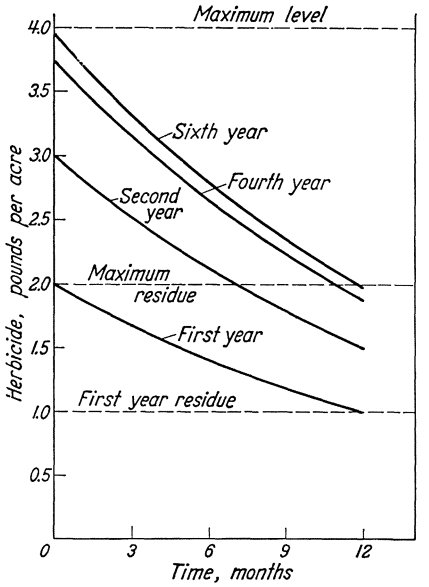


Fig. 1. Theoretical curves showing the amount of herbicide as a function of time in soil sprayed annually with two pounds per acre; assume 80 percent loss per year. Reprinted with permission from J. Agr. Food. Chem. 12, 32 (1964)

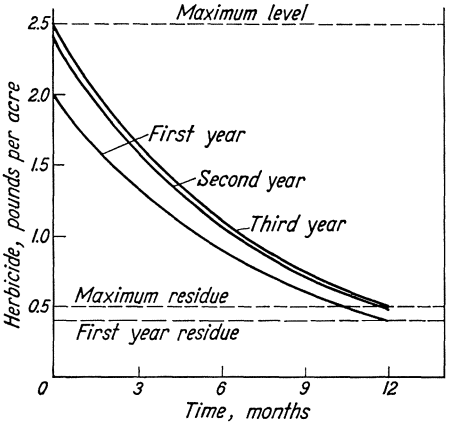


Fig. 2. Theoretical curves showing the amount of herbicide as a function of time in soil sprayed annually with two pounds per acre; assume 50 percent loss per year

used in cropping systems where some hazard existed. Short-term (one- to two-year) greenhouse and laboratory experiments have indicated the potential hazards and relative dissipation rates of new herbicides in soils.

Although such tests do not eliminate the need for more extended experiments under field conditions, they do greatly reduce their urgency.

The disappearance of many herbicides from soils is a first-order reaction (HILL *et al.* 1955, BURSCHEL and FREED 1959, BURSCHEL 1961, FREED *et al.* 1962, SHEETS 1964, and others). That is, at any given time, the rate of disappearance is proportional to the herbicidal concentration in the soil. Decomposition of some herbicides by soil microorganisms is preceded by a lag phase, but after the effective microorganisms have become adapted to utilize the herbizide in question, rapid first-order decomposition occurs. The length of the lag phase varies, but herbicides that exhibit this type of breakdown usually are not hazardous to subsequent crops. Other herbicides appear to be decomposed slowly by soil microorganisms that do not require an adaptation. Potential residues following the latter type of decomposition will be discussed more fully.

Curves in Fig. 1 and Fig. 2 show the relation between time and residue levels of slowly decomposing herbicides. These ideal curves are based on first-order rate equations; the rate constant in Fig. 1 is based on 80 percent loss per year, and the rate constant in Fig. 2 on 50 percent loss per year.

Under most climatic and edaphic conditions, the rates of disappearance of monuron, diuron, atrazine, simazine, and related herbicides[1] equal or exceed 80 percent per year. When herbicidal applications of two pounds per acre are repeated each year on the same soil and when 80 percent disappearance occurs each year, the maximum residue 12 months after application approaches 0.5 pound per acre. Therefore, residue carry-over even after continuous use is small. Field results substantiate this conclusion for monuron and diuron, at least in surface soils (HILL *et al.* 1955, RAHN and BAYNARD 1958, ARLE *et al.* 1965).

A herbicide that disappears at a rate of 50 percent per year usually is considered to be very persistent; but at this disappearance rate the residue level in soil sprayed annually with two pounds per acre would approach, and level off at, two pounds per acre 12 months after the last application (Fig. 2). Several years of continuous use would be required to accumulate the two pounds per acre residue; and elimination of one annual application would permit the residue level to drop to one pound per acre. These considerations indicate that massive accumulation of herbicides is extremely unlikely.

Herbicides are plant toxicants; but because sensitivity of plants to most herbicides varies, these chemicals are used to control undesirable species growing in association with a desirable plant. The phytotoxic effects of soil residues of herbicides insure against accumulation of herbicide residues. In contrast to those conditions under which arsenic residues accumulated in orchards, any significant accumulation or carry-over will cause injury, a sure indication of residues. Thus, in agricultural soils plowed and planted annually, even moderate accumulation of herbicides that are used for selective weed control in agronomic and horticultural crops is improbable.

[1] Chemical names of herbicides, insecticides, and chelating agents mentioned in text by common name are shown in Table III.

A situation where herbicides might accumulate unnoticed exists in deep rooted, perennial crops such as fruit trees. Here, as with arsenic accumulation from lead arsenate applications, roots of crop plants may occur mostly below the level of penetration of many water-insoluble herbicides that are strongly adsorbed to soils. Thus some accumulation may be possible in agricultural lands devoted to the production of deep-rooted perennial crops if herbicides are applied repeatedly. A continuous single-cropping system might also fail to indicate accumulation of a selective herbicide. However, since almost all chemicals used as selective herbicides in agricultural soils are ogranic chemicals, most are decomposed by soil microorganisms. Also, applications are usually infrequent and rates of application relatively low. Hence, accumulation of organic herbicides in surface soils, even where sensitive plants are not grown, seems unlikely. Considerable data support this conclusion for most herbicides in use today.

Although accumulation of selective herbicides in surface layers of agricultural soils is unlikely, herbicides which are mobile but not readily decomposed may be moved downward in soils below the root zones of certain plants (PHILLIPS 1959, DOWLER *et al.* 1963, BURNSIDE *et al.* 1963). Depth of movement would be determined by, or related to, depth of water percolation. At levels below the plow layer, organic matter is usually extremely low, pH is often different, and oxygen concentrations are often lower than in surface soils. These soil properties are known to influence the decomposition of many herbicides indirectly through their influence on soil microorganisms that decompose herbicides. Therefore, conditions at depths of two feet or more in the soil may be unfavorable for growth of microorganisms effective for decomposition of many herbicides. Research is needed on the decomposition of herbicides at different soil depths, especially herbicides with characteristics similar to 2,3,6-TBA and fenac which are readily moved downward by water and which are decomposed slowly (if at all in some soils) by microorganisms. Other herbicides such as atrazine move downward two feet or more under certain soil and climatic conditions (BURNSIDE *et al.* 1963); hence knowledge of decomposition of atrazine and related herbicides in subsoil is also desirable.

## III. Methods of application and their relation to carry-over

Herbicide applications fall into one of three categories, depending on time of planting and state of growth of plants: preplanting, preemergence, and postemergence. *Preplanting* applications are made before a crop is planted to kill existing weeds and prevent development of germinating weed seeds. Planting time is varied, depending on the herbicide, to allow sufficient dissipation to prevent crop injury. *Preemergence* applications are accomplished at planting or between planting and emergence of the crop. Most preemergence herbicides tend to remain near the soil surface and can be used selectively on moderately susceptible crops because they are absent in areas of active root growth. Many weeds germinate near the soil surface and succumb to the phytotoxic effects of herbicides. *Postemergence* appli-

Table I. *Reports of residual phytotoxicity for a number of herbicides*

| Herbicide | References | Field (F) or greenhouse (G) | Pounds per acre[a] | Residual phytotoxicity (months) |
|---|---|---|---|---|
| Amitrole | RANGEL (1959), SANDFORD and STOVELL (1960) | F | 3—18 | 1—3 |
| | NEURURER (1962) | F | 8.9 | 4—5 |
| Atrazine | SWITZER and Rauser (1960), NEURURER (1961), SWADER and FLETCHALL (1962) | F | 2—4 | 4—7 |
| | BEHRENS (1959), NEURURER (1962), HARRIS and SHEETS (1965) | F | 2—3 | 4—7 |
| | MEGGITT (1964), WISK and COLE (1965) | F | 3—8 | 12 |
| | SHEETS *et al.* (1962), SHEETS and SHAW (1963) | G | 3.2—4 | 4—8 |
| CDAA | SHEETS (1959b) | G | 80 | <2 |
| CDEC | DANIELSON *et al.* (1961) | F | 1 | 1 |
| | OTTEN *et al.* (1957) | G | 8 | <1 |
| | SHEETS (1959b) | G | 40 | 3 |
| CIPC | STEVENS and CARLSON (1951), HOLLINGSWORTH (1955), SWITZER and RAUSER (1960), DANIELSON *et al.* (1961), ROBERTS and WILSON (1962) | F | 2—15 | 1—3 |
| | OGLE and WARREN (1954), WARREN (1954) | G | 4—8 | 1—3 |
| Dalapon | SWEET *et al.* (1958), SANDFORD and STOVELL (1960) | F | 7.4—20 | 1 |
| | NEURURER (1962) | F | 20 | 3—4 |
| | WARREN (1954), KAUFMAN (1964) | G | 6—8 | 1—2 |
| Dichlobenil | SHEETS and HARRIS (1965) | G | 1 | 2—3 |
| Diphenamid | JONES *et al.* (1964), HARRIS and SHEETS (1965) | F | 3—4 | 10—12 |
| | DAVIS *et al.* (1964) | F | 3 | 3 |
| | ASHLEY and RAHN (1965) | F | 3.75 | <3 |
| Diuron | HOLLINGSWORTH (1955), WELDON and TIMMONS (1961), NEURURER (1962) | F | 3.6—4 | 5—7 |
| | HILL *et al.* (1955) | F | 1—2 | 4—8 |
| | WELDON and TIMMONS (1961) | F | 2 | 15 |
| DNBP | HARRIS (1954) | F | 8 | 6 |
| | HOLSTUN and MCWHORTER (1953) | F | 12 | <5 |
| | DETROUX (1957) | F | 0.85 | <3 |
| Endothall | COMES *et al.* (1961) | G | 6 | 1 |
| | SWITZER and RAUSER (1960) | F | 12 | 1 |
| EPTC | DANIELSON *et al.* (1961) | F | 1 | 1 |
| | SHEETS (1959b) | G | 5 | 2—3 |
| Fenac | ROBINSON (1961), HARRIS and SHEETS (1965) | F | 4—5 | 12 |
| Linuron | ZAHARCHUK (1963) | F | 4 | <4 |
| Monuron | HOLLINGSWORTH (1955), SHADBOLT *et al.* (1964) | F | 2—4 | 5—6 |
| | SWITZER and RAUSER (1960), WEBSTER (1962) | F | 4—6 | 12 |
| | LOUSTALOT *et al.* (1953), OGLE and WARREN (1954), WARREN (1954) | G | 2—5 | 1—3 |
| Neburon | DETROUX (1957) | F | 2 | <3 |
| | SWITZER and RAUSER (1960) | F | 6 | 2 |
| | SHADBOLT *et al.* (1964) | F | 2 | 9 |

[a] Rates originally reported in p.p.m. were multiplied by 2 to give pounds per acre.

Table I. (continued)

| Herbicide | References | Field (F) or greenhouse (G) | Pounds per acre[a] | Residual phytotoxicity (months) |
|---|---|---|---|---|
| NPA | OGLE and WARREN (1954) | G | 8 | 2 |
| Nore | SCHWEIZER and HOLSTUN (1965) | F | 4 | 5 |
| PCPa | LOUSTALOT and FERRER (1950a), WARREN (1954) | G | 32—60 | 1 |
| Prometryne | HARRIS and SHEETS (1965) | F | 8 | <4 |
| | SCHWEIZER and HOLSTUN (1965) | F | 2 | 5 |
| | SHEETS and SHAW (1963) | G | 6.4 | 4—6 |
| Propazine | SWITZER and RAUSER (1960) | F | 3 | 2 |
| | NEURURER (1962) | F | 1.8 | 14 |
| | SHEETS *et al.* (1962), SHEETS and SHAW (1963) | G | 6.4—25 | 6—14 |
| Simazine | FLETCHALL (1958), SWEET *et al.* (1958), BEHRENS and Thompson (1960), STROUBE and BONDARENKO (1960), SWITZER and RAUSER (1960), SWADER and FLETCHALL (1962) | F | 2—5 | 12 |
| | DETROUX (1957), AELBERS and HOMBURG (1959), NEURURER (1962) | F | 0.45—4.5 | 3—7 |
| | WEBSTER (1962) | F | 4 | 18 |
| | SHEETS *et al.* (1962), SHEETS and SHAW (1963) | G | 3.2—4.0 | 4—14 |
| TCA | RAI and HAMNER (1953), OGLE and WARREN (1954), WARREN (1954) | G | 8—60 | 1—3 |
| | ROBINSON and DUNHAM (1949), NEURURER (1962) | F | 12.5—67 | 7—12 |
| | ARAKERI and DUNHAM (1950), LOUSTALOT and FERRER (1950b) | F | 16—30 | 4 |
| Trifluralin | SCHWEIZER and HOLSTUN (1965) | F | 4 | 5 |
| 2,4-D | DEROSE (1946), KRIES (1947), BROWN and MITCHELL (1948), JORGENSEN and HAMNER (1948), HERNANDEZ and WARREN (1950), OGLE and WARREN (1954), WARREN (1954) | G | 4—40 | 1 |
| | DEROSE and NEWMAN (1947) | F | 5 | 1 |
| 2,4,5-T | WARREN (1954) | G | 4 | 1 |
| | DEROSE and NEWMAN (1947) | F | 5 | 3 |
| 2,3,6-TBA | PHILLIPS (1959), WEBSTER (1962) | F | 15—20 | 12—32 |
| | DEWEY and PFEIFFER (1959), ROBINSON (1961) | F | 3—4 | 5—12 |

[a] Rates originally reported in p.p.m. were multiplied by 2 to give pounds per acre.

cations are made after the crop has emerged from the soil; the herbicides may be applied to the soil between crop rows to control weeds that germinate during mid and late season, or they may be applied to the foliage of existing weeds. Injury to the crop is avoided by the use of selective chemicals, or by directing the spray in such a way that it covers the weeds and not the crop.

The duration of weather favorable for herbicide dissipation, subsequent to application and prior to the planting date of a sensitive crop, is im-

portant. The later the application date, the greater the chances of residues the next year. When a crop is planted in summer or fall after one that requires chemical weed control in the same season, herbicides with short residual activity must be used, or special care must be taken to be sure the crop is tolerant of possible residues.

For minimum residue carry-over, early spring applications would be the most desirable. However, some crops do not provide enough competition to control weeds late in the season, and postemergence applications during mid or late season are often necessary.

Herbicides are often applied as bands over the rows; weeds in the middle between rows are controlled by cultivation. This method reduces the herbicidal rate per acre in proportion to the width of the band sprayed and the row spacing. Band applications are employed extensively in cotton culture, and thorough cultivation after harvest is a common practice. With these practices diuron and monuron can be used as selective herbicides without major soil-residue problems.

## IV. Persistence and dissipation rates of herbicides in soils

The duration of detectable phytotoxic residues in soil has been reported for many herbicides; a number of these are listed in Table I. One must keep in mind that environmental conditions have important effects on persistence and that a report of long or short persistence of a given herbicide under one set of conditions is not sufficient for a general characterization of its persistence pattern. Also, instances of prolonged persistence are more likely to be reported than are instances of no persistence problems. Nevertheless, any herbicide which injures plants longer than three or four months after application at normal rates must be considered a potential hazard to crops grown in rotation until the tolerance of each crop for the herbicide is determined.

Most herbicides are eventually decomposed in the soil, but in some soils at least 2,3,6-TBA is very persistent (PHILLIPS 1959, DOWLER *et al.* 1963). However, DEWEY and PFEIFFER (1959) presented data which suggested inactivation of 2,3,6-TBA by soil microorganisms. Proliferation of effective microorganisms apparently did not occur, and the rate of inactivation was not directly proportional to the rate of application as with diuron, monuron, simazine, and related herbicides.

The general persistence pattern of fenac is similar to that of 2,3,6-TBA (ROBINSON 1961). Other benzoic and phenylacetic acids with different substituents on the aromatic ring are much more susceptible to decomposition. Two commercially important ones are dicamba and amiben.

Variations of persistence have also been observed within the phenoxyalkylcarboxylic acid herbicides. ALEXANDER and ALEEM (1961) studied the decomposition of several compounds within this group and found that the aromatic nucleus was more stable when it contained a halogen in a position *meta* to the phenolic hydroxyl. Length of the side chain was also involved; cleavage was rapid for acetate and caproate but not for propionate and

valerate. The herbicide 2,4-D was readily broken down, but 2,4,5-T was moderately resistant to decomposition.

The chlorinated aliphatic acids such as dalapon are readily attacked by soil microorganisms (KAUFMAN 1964) and do not generally give rise to persistence problems in soils.

Carbamates also do not cause serious persistence problems. Recently, KEARNEY and KAUFMAN (1965) isolated an enzyme from soil organisms that attacked the ester linkage of a broad spectrum of carbamates. It seems likely that this type of reaction occurs readily in the soil. Thiolcarbamates were not affected by the enzyme.

Most *s*-triazine herbicides are more resistant to decomposition than the phenoxyalkylcarboxylic acids, the chlorinated aliphatic acids, and the carbamates. Soil microorganisms in pure culture can utilize simazine and other *s*-triazines (KAUFMAN *et al.* 1963), but utilization appears to occur only very slowly in soil. SHEETS *et al.* (1962) compared the persistence of a group of *s*-triazines and found that simetone was more persistent than any of eight chloro-substituted triazines. In the same study atrazine was slightly less persistent than simazine.

The phenylureas exhibit persistence patterns in soil similar to those of the *s*-triazines. Monuron was inactivated at a slightly more rapid rate than simazine in two soils (SHEETS 1959 a). Persistence of monuron and diuron was about the same in four soils studied by WARREN (1954), but in studies by SHEETS (1958) diuron dissipated at a slower rate than monuron. Fenuron was quite rapidly inactivated in soils.

Diquat, a pyrazidiinium salt, is reported to be almost completely adsorbed to soil and unavailable to plants (STUBS 1960). The same is probably true for paraquat. Both compounds form divalent cations in solution, and strong interactions with the negatively charged soil particles are therefore to be expected.

Organic arsenical herbicides are used as foliar sprays to control young weeds. Although inorganic arsenic will eventually be released from these compounds in the soil, information on decomposition rates is lacking.

## V. Carry-over problems encountered in crop rotations

Herbicide carry-over from one crop season to another has occurred in a few instances. Sensitive crops in rotation systems have been damaged, and in some cases the stands have been completely lost.

Diuron has been used extensively as a preemergence herbicide for the control of weeds in cotton. This herbicide usually disappears more slowly from soil than such herbicides as 2,4-D and dalapon and consequently controls weeeds for several weeks. An acre usually receives about 0.14 to 0.50 pound applied as bands over the row. The low rate is recommended for medium sandy loams, and the high rate for clay loams or light clays. Diuron is not recommended as a preemergence herbicide on sands, loamy sands, heavy clays, or soils high in organic matter.

Significant residual phytotoxicity from preemergence band applications of diuron usually disappears from sprayed soils within two to four months. However, HOLLINGSWORTH (1955) reported that diuron at slightly excessive rates persisted for 5 to 5.5 months under field conditions and injured seedling oats. Most injury occurred from broadcast applications of diuron at two to four pounds per acre. The four-pound rate is greater than that recommended for weed control in cotton; broadcast applications do not usually exceed 1.5 pounds per acre (*Weed Control Handbook of Mississippi* 1961, WESTMORELAND *et al.* 1954). HOLLINGSWORTH (1955) concluded that broadcast preemergence applications of diuron at rates of 1.0 to 1.5 pounds per acre would not damage oats planted the following fall or winter if the soil was thoroughly mixed before planting oats. Band applications further reduce the hazard because the total amount of herbicide per acre is about one-third that of broadcast applications.

HILL *et al.* (1955) studied the disappearance of diuron from soil at four locations in the eastern and southern United States. They concluded that when diuron as a broadcast preemergence application at one to two pounds per acre was followed by another application 12 months after the first, phytotoxicity disappeared within four to eight months after each application.

Frequently, late season weeds emerging after dissipation of preemergence herbicides and after the last cultivation of cotton interfere with mechanical harvesting and lower the quality of the crop. Between 1955 and 1960 great interest developed in the use of diuron and monuron for late season weed control in cotton. Mid and late season applications of these herbicides at rates of one to two pounds per acre effectively control weeds; but sensitive crops planted in the fall after cotton harvest are often injured (ARLE *et al.* 1965). In the middle southern area of the United States, spring or early summer plantings of soybeans on some soils, when sprayed with one to two pounds per acre of diuron at the last cultivation of cotton during the previous season, may occasionally by subject to slight injury. The *U.S. Department of Agriculture* "Summary of Registered Agricultural Pesticide Chemical Uses" (1963) lists the following limitations for diuron and monuron applied at the last cultivation of cotton: "Do not plant crops other than cotton, corn, or grain sorghum on treated land in spring following treatment; if both preemergence and early postemergence applications have been made, reduce dosage so that total dosage for season does not exceed 3.2 lbs/A."

In an experiment conducted in California, diuron and monuron, applied in November at one and two pounds per acre as preemergence sprays for weed control in spinach, injured tomatoes seeded in April (ASHTON and SHEETS 1961). Injury often occurred at slightly lower rates of monuron than of diuron. This difference was attributed more to differences in availability to the plant than to rates of disappearance (SHEETS 1964).

Monuron as a preemergence application is recommended for weed control in asparagus, and diuron has been used experimentally for this purpose. In the moderately humid climate of Delaware, monuron was applied twice annually to asparagus at rates of 1.6 and 3.2 pounds per acre (3.2

and 6.4 pounds per acre per year, respectively) for three years to study disappearance rates (RAHN and BAYNARD 1958). At 3.2 pounds per acre per year, monuron did not persist from one year to next. At the high rate, about 0.4 to 0.6 pound per acre (about six to nine percent) persisted from one year to the next in the upper eight inches of soil.

In Washington, asparagus plots were sprayed each February or March for four successive year with four pounds per acre of monuron or for two successive years with four pounds per acre of diuron (BRUNS *et al.* 1962). The herbicides were incorporated to a depth of one to two inches immediately after application. Soil samples collected one year after the last application from depths of zero-to-one and one-to-two inches contained sufficient diuron residues to kill or severely injure seedling oats. Oat injury in soil from monuron plots was less severe than that in soil from diuron plots; but injury occurred in soil taken from all depths down to five inches, the greatest depth sampled. BRUNS *et al.* (1962) also observed injury — in some cases severe injury — to wheat planted 16 months after the last applications of monuron and diuron. They concluded that sensitive plants such as wheat and oats should not be seeded for at least two years after the last application of these herbicides for weed control in asparagus in the State of Washington.

BURNSIDE *et al.* (1963) also have shown that, in contrast to rates of disappearance in humid regions, monuron may remain active in soil for more than 12 months in regions having low rainfall and long periods of low soil temperatures. SHADBOLT *et al.* (1964) reported that under conditions of southern California phytotoxic residues from two pounds per acre of monuron disappeared within nine months. Twelve months after application, four and eight pounds per acre of monuron were not phytotoxic to cabbage, radish, and barley, and residue levels, determined colorimetrically, were below 0.3 p.p.m.

Neburon, another phenylurea, was more persistant than diuron (SHADBOLT *et al.* 1964). Phytotoxic residues from two and four pounds per acre of neburon disappeared within 12 months; but eight pounds per acre injured corn 18 months after application and approximately 0.8 p.p.m. of neburon was found by colorimetric analysis. Twenty-nine months after application, the level of neburon in soil treated originally with eight pounds per acre was below 0.3 p.p.m., and no crop injury occurred.

Linuron, a methoxy analog of diuron, exhibited shorter residual activity than monuron and should therefore not be too hazardous to sensitive crops in rotation systems (SHEETS 1964).

Atrazine and simazine are widely used to control weeds in corn. Corn exhibits tolerance to these herbicides, but several crops, notably cereals, soybeans, sugar beets, lespedeza, Ladino clover, snap beans, cotton, flax, squash, cucumber, and alfalfa, are susceptible and can be injured or killed by low concentrations of atrazine, simazine, and some related herbicides in soils.

Corn and sugar beets are often grown in rotation in the Great Plains of the United States and Great Lakes areas of Canada. Sugar beets planted the season after corn have been injured by atrazine residues. The extent

of injury in the United States is not well documented, but a survey conducted in southwestern Ontario, Canada, does document the extent and degree of injury to sugar beets in that province (FRANK 1965). In one case involving 483 acres sprayed with atrazine in 1961 and planted to sugar beets in 1962, 16 percent (78 acres) was damaged severely, 38 percent moderately to slightly, while 46 percent remained unaffected (FRANK 1965). Similar data were reported for the beet crops of 1963 and 1964. Carry-over of atrazine appeared to be correlated with time, rate, and method of atrazine application. Some of the injury to sugar beets was attributable to negligence of the applicator. When atrazine was applied preemergently, injury was less severe on land plowed during the fall after corn harvest than on land plowed the following spring; the effect of time of plowing was not evident on land that received postemergence applications (FRANK 1965). Injury developed and intensified during hot dry periods. This observation agrees with the results showing that upward translocation of simazine in plants is greater under conditions that favor rapid transpiration.

An experiment was conducted in Michigan to study the effects of soil residues of simazine and atrazine on several crops (MEGGITT 1964). Little or no simazine and atrazine residues existed one year after application of one or two pounds per acre. One year after the second of two successive annual applications of two pounds per acre, some phytotoxic residues were found in the upper eight inches of soil. In South Dakota, cereals planted the year after application of two pounds per acre of simazine or atrazine were severely injured (SPLITTSTOESSER and DERSCHEID 1962). Oats, soybeans, and forage grasses and legumes planted one year after corn treated with two pounds per acre of simazine suffered stand reduction in Missouri (FLETCHALL 1958, SWADER and FLETCHALL 1962, FINK and FLETCHALL 1963). At three locations in Nebraska, simazine and atrazine persisted for at least 16 months at rates as low as 2.5 pounds per acre, and movement to a depth of two feet occurred (BURNSIDE *et al.* 1963). Such soil persistence and movement patterns indicate that residue problems may occur when simazine and atrazine are used as selective herbicides in rotational systems involving sensitive plants.

In the midwestern United States injury to soybeans from atrazine, applied to corn the previous season, has been sporadic in extent and light-to-moderate in degree. Injury to cereals or other sensitive crops seeded in the fall after corn harvest may occur. Registration limitations prescribe that treated fields should not be planted to any crop other than corn during the season of spraying (*U.S. Department of Agriculture:* "Summary of Registered Agricultural Pesticide Chemical Uses" 1963), and one of the precautions on the label of Atrazine 80 W[2] is "Do not follow treated corn with sugar beets, tobacco, or vegetables in rotation."

Atrazine is an effective herbicide for the control of weeds during the fallow season of wheat-fallow rotations, but carry-over problems have occurred in Nebraska, Oregon, and Wyoming when this herbicide was

[2] Mention of a proprietary product in this manuscript does not imply indorsement by the *U. S. Department of Agriculture.*

applied at rates required for weed control through the fallow season (WICKS *et al.* 1964).

There have been some indications that atrazine is more persistent when applied in granular form than when applied as a spray (WILSON and COLE 1964, MEGGITT 1964, WISK and COLE 1965, ASHLEY and RAHN 1965). Some workers believe that application of sprays in which the wettable powder is utilized would eliminate most residue problems with atrazine. However, there is less than complete agreement on this point, and more data are needed for clarification.

Diphenamid was recently approved by the *U.S. Department of Agriculture* for use as a preemergence herbicide for the control of annual grasses and broadleaf weeds in tobacco, tomatoes, potatoes, and certain other crops. Residual activity of this herbicide contributes to its effectiveness for weed control; however, injury to cover crops seeded after tobacco harvest may occur (JONES *et al.* 1964). At rates necessary for weed control, diphenamid residues persisted for at least ten months in tobacco soils. An oat-plant bioassay showed that phytotoxic residues equivalent to 0.57 and 0.39 p.p.m. of diphenamid were present in two tobacco soils treated ten months earlier with five pounds per acre of the herbicide (JONES *et al.* 1964). In an experiment conducted by HARRIS and SHEETS (1965), diphenamid applied at four pounds per acre in June, 1963 injured oats severely 12 months later. These results indicate that some limitations for crops used in rotation with diphenamid-treated tobacco, tomatoes, and potatoes may be necessary.

Fenac is an effective, selective herbicide for control of *Striga asiatica* (L.) Kuntze (witchweed) in corn. Tobacco, cotton, peanuts, and soybeans are frequently used in rotations with corn in the witchweed-infested areas of North and South Carolina. Injury to these crops from fenac residues one and two years after application limits use of this herbicide for witchweed control (DOWLER *et al.* 1963).

In the past, inorganic arsenicals were used extensively on cotton and tobacco for insect control. Yields of certain crops were reduced by arsenic residues in some cotton soils (COOPER *et al.* 1931). Undocumented reports from the lower Mississippi Valley implicate arsenic residues in certain abnormalities that occur very sporadically in rice growing on old cotton land. Use of inorganic arsenical pesticides has declined rapidly during the last ten to 15 years; but recently organic arsenicals, especially DSMA, have gained wide acceptance as herbicides. Although their application rates are much lower than those of the inorganic pesticides used for insect control, the new herbicides containing arsenic may add to residues already present. There are no published reports to indicate an increase of arsenic residues from the use of the organic arsenicals, but these herbicides have been in wide use only for one year. Persistence and dissipation data are urgently needed for this group of herbicides.

Although not widely observed and perhaps of infrequent occurrence, interactions between herbicides and other pesticides might result in injury to plants growing in soils that contain a low level of residues. One interaction is known in which rice, tomato, and potato plants — normally tolerant

to rates of propanil which control seedlings weeds — are susceptible to the herbicide in the presence of carbaryl and certain organophosphate insecticides (UNGER *et al.* 1964). In another study (HACSKAYLO *et al.* 1964), phytotoxicity to cotton was greater when diuron or monuron was applied with phorate or the related O,O-diethyl *S*-2-(ethylthio)ethylphosphorodithioate than when the herbicides or the systemic insecticides were applied alone. We must be alert for other interactions between two pesticides, and for possible injury to crop plants due to altered plant susceptibility to one pesticide in the presence of residues of another.

## VI. Reducing and alleviating problems with herbicide residues in soils

The ideal selective herbicide controls undesirable plants without injury to the crop and dissipates by the time weed control is no longer needed — at least by harvest. Many common herbicides approach the ideal under optimum climatic and edaphic conditions, but variations of conditions cause variations in dissipation rates.

When phytotoxic residues persist beyond the harvest date, plowing and thorough disking will often reduce or eliminate residual phytotoxicity (HOLLINGSWORTH 1955, RAHN and BAYNARD 1958, FLETCHALL 1958, ARLE *et al.* 1965). In some cases fall plowing immediately after corn harvest, as opposed to plowing the following spring, reduced injury to sugar beets the next season from atrazine residues (FRANK 1965). Tillage operations effectively reduce the concentration of herbicides in soils because zones of high concentrations on or near the soil surface are mixed with soil of low concentrations throughout the plow layer.

One approach to the elimination of residues that are potential hazards to crops grown in rotation with treated crops has been to control the time and rate of decomposition. Although the idea is intriguing and may be sound (at least for some herbicides), little success has so far been attained in practice. CASTELFRANCO and DEUTSCH (1962) reported that inactivation of simazine was accelerated by the addition of calcium polysulfide to soil, but ZEMANEK (1964) reported that calcium polysulfide had no effect on the residual phytotoxicity of atrazine. In similar trials with simazine we have been unable to show any effect of sodium polysulfide.

DANIELSON *et al.* (1961) demonstrated that persistence of EPTC in soil varied widely with several solvents used in application, and KAUFMAN (1965) showed that sodium azide as an additive in CIPC applications extended residual toxicity, presumably by inhibition of organisms effective in the decomposition of the herbicide. Their efforts were aimed at extending residual phytotoxicity and minimizing variations in weed control efficiency, rather than at eliminating soil residues.

Use of surfactants and other spray additives to enhance phytotoxicity of herbicides to target plants might reduce the rate of herbicide required for weed control and result in less hazard from residual phytotoxicity. For postemergence mid and late season weed control in cotton, the rate of

diuron can be greatly reduced by use of a surfactant in the spray mixture (McWHORTER 1963).

Molecules of most herbicides will adsorb on activated charcoal. Finely divided activated charcoal has been investigated as an inactivating agent in soils containing simazine residues (AHRENS 1964). In greenhouse tests 0.25 p.p.m. of simazine reduced fresh weights of tomatoes 33 percent, but tomatoes grew normally in soil containing 0.5 p.p.m. of simazine and 50 p.p.m. of charcoal. In field trials 200 pounds per acre of charcoal reduced but did not eliminate injury to oats from 0.5 pound per acre of simazine. Thorough mixing of the charcoal with soil was essential for uniform injury reduction. Strawberry, cabbage, privet, and forsythia transplants were made more tolerant to simazine in the soil by dipping their moistened roots into activated charcoal (AHRENS 1965 b). In the experiments of ZEMANEK (1964), activated carbon reduced the phytotoxicity of atrazine. Promising results of protection against simazine and atrazine for cabbage, tobacco, beans, and beets have also been reported (AHRENS 1965 a).

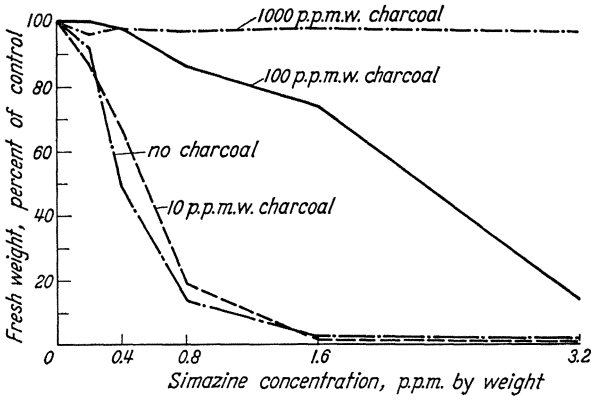


Fig. 3. Effects of activated charcoal (Norite *A*) as a soil amendment on the fresh weight of seedling oats growing in soil containing simazine

Fig. 3 shows that activated charcoal (Norite A) effectively reduced phytotoxicity of simazine in Yolo clay loam. Similar effects of activated charcoal on simetone were obtained. Between 100 and 1,000 p.p.m. of charcoal were required to eliminate phytotoxicity of simazine to seedling oats at herbicide concentrations of 0.8, 1.6, and 3.2 p.p.m. In another trial activated charcoal was effective, but large quantities of organic matter in the form of dry barnyard manure were ineffective (Table II).

The amount of activated charcoal required to eliminate phytotoxicity of simazine may rule out large-scale use. However, on limited acreages of high production potential or high-value crops, its use might be profitable.

Irrigation provides a means for reducing or eliminating residues of some herbicides (RAHN and BAYNARD 1958, WELDON and TIMMONS 1961). With frequent flood irrigation under semiarid conditions of Wyoming, phytotoxic residues from two pounds per acre of diuron disappeared from

a sandy clay loam in one season, but persisted for two seasons under infrequent irrigation (WELDON and TIMMONS 1961). Because soil microorganisms are responsible for the decomposition of most herbicides, adequate

Table II. *Contratoxication of simazine in Yolo clay by activated charcoal and barnyard manure as shown by fresh weight of oats seeded after addition of contratoxicant to the soil*

| Simazine (lb./A.) | Amount of contratoxicant added 4 weeks after simazine application (lb./A.) | Fresh weight of oats (% of control) |
|---|---|---|
| 0 | None | 100 |
| 0 | 80 charcoal | 99 |
| 0 | 200 charcoal | 99 |
| 0 | 4,000 manure | 100 |
| 0 | 10,000 manure | 104 |
| 2 | None | 1 |
| 2 | 80 charcoal | 33 |
| 2 | 200 charcoal | 60 |
| 2 | 4,000 manure | 1 |
| 2 | 10,000 manure | 1 |

soil moisture is essential for decomposition; thus timely irrigations, especially in arid climates during spring, summer, and fall months, should accelerate decomposition of most herbicides. Movement downward in the soil profile by water reduces concentration of herbicidal residues in root zones of shallow-rooted plants. Such practices, while eliminating the immediate problem of residual phytotoxicity, might, if frequent, result in deposition of readily leachable herbicides at lower depths in soils and create a different problem — one that might be more difficult to solve than ridding the root zone of phytotoxic residues by other means. Information on rates of decomposition of herbicides in subsoils is very scanty.

Rotation of effective herbicides on the same crop and rotation of crops and herbicides, as outlined by ENNIS *et al.* (1963), would lessen hazards from carry-over. In a rotation system, a herbicide or herbicide combination with short residual action could be used immediately preceding a sensitive crop. Herbicides which persist for several months would be used only when a tolerant crop succeeds their use.

Researchers have attempted to overcome effects of arsenic residues on desirable species by treating the soil with reagents which reduce uptake of arsenic by plants. THOMPSON and BATJER (1950) reported that nitrogen fertilization reduced arsenic toxicity to young peach trees. A combination of zinc sulfate and high nitrogen was even more effective and almost eliminated arsenic toxicity in their experiments. BATJER and BENSON (1958) reduced toxicity to peach trees growing in arsenic-toxic soil by soil applications of four chelating agents: ZnEDTA, ZnHEEDTA, FeEDTA, and FeDPTA.

Data of COOPER *et al.* (1931) showed that phosphate fertilization increased arsenic toxicity to cowpeas. On high phosphorus plots, iron sulphate application tended to offset the effect of arsenic on growth of the

test plant. They suggested that phosphorus, in addition to the arsenic, was immobilized by iron through complex formation. Thus, heavy applications of phosphorus complexed with iron and left more arsenic available for uptake by plants. In the presence of calcium arsenate residues, cowpeas grew better on red soils with high iron content than on gray soils with low iron.

BENSON (1953) studied the effects of phosphate fertilization and soil acidity on response of barley to arsenic. In greenhouse pot tests, yields of barley growing in arsenic-toxic soil increased when phosphate was added to cultures; response to phosphate was greatest in acid soils (pH four to

Table III. *Common and chemical names of herbicides, insecticides, and chelating agents mentioned in text*

| Common name | Chemical name |
|---|---|
| Amiben | 3-amino-2,5-dichlorobenzoic acid |
| Amitrole | 3-amino-1,2,4-triazole |
| Atrazine | 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine |
| Carbaryl | 1-naphthyl methylcarbamate |
| CDAA | 2-chloro-*N*,*N*-diallylacetamide |
| CDEC | 2-chloroallyl diethyldithiocarbamate |
| CIPC | isopropyl *N*-(3-chlorophenyl)carbamate |
| Dalapon | 2,2-dichloropropionic acid |
| Dicamba | 2-methoxy-3,6-dichlorobenzoic acid |
| Dichlobenil | 2,6-dichlorobenzonitrile |
| Diphenamid | *N*,*N*-dimethyl-2,2-diphenylacetamide |
| Diquat | 6,7-dihydrodipyrido(1,2-*a*:2′,1′-*c*)-pyrazidiinium salt |
| Diuron | 3-(3,4-dichlorophenyl)-1,1-dimethylurea |
| DNBP | 4,6-dinitro-*o*-*sec*-butylphenol |
| DSMA | disodium methanearsonate |
| Endothall | 7-oxabicyclo(2.2.1)heptane-2,3-dicarboxylic acid |
| EPTC | ethyl *N*,*N*-dipropylthiolcarbamate |
| FeDPTA | monosodium ferric diethylenetriaminepentaacetate |
| FeEDTA | disodium ferric ethylenediaminetetraacetate |
| Fenac | 2,3,6-trichlorophenylacetic acid |
| Fenuron | 3-phenyl-1,1-dimethylurea |
| Linuron | 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea |
| Monuron | 3-(*p*-chlorophenyl)-1,1-dimethylurea |
| Neburon | 1-butyl-3-(3,4-dichlorophenyl)-1-methylurea |
| Norea | 3-(hexahydro-4,7-methanoindan-5-yl)-1,1-dimethylurea |
| NPA | *N*-1-naphthylphthalamic acid |
| Paraquat | 1,1′-dimethyl-4,4′-bipyridinium salt |
| PCP | pentachlorophenol |
| Phorate | *O*,*O*-diethyl *S*-(ethylthio)methylphosphorodithioate |
| Prometryne | 2,4-bis(isopropylamino)-6-methylmercapto-*s*-triazine |
| Propanil | 3′,4′-dichloropropionanilide |
| Propazine | 2-chloro-4,6-bis(isopropylamino)-*s*-triazine |
| Simazine | 2-chloro-4,6-bis(ethylamino)-*s*-triazine |
| Simetone | 2-methoxy-4,6-bis(ethylamino)-*s*-triazine |
| TCA | trichloroacetic acid |
| Trifluralin | α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine |
| 2,4-D | 2,4-dichlorophenoxyacetic acid |
| 2,4,5-T | 2,4,5-trichlorophenoxyacetic acid |
| 2,3,6-TBA | 2,3,6-trichlorobenzoic acid |
| ZnEDTA | disodium zinc ethylenediaminetetraacetate |
| ZnHEEDTA | sodium zinc hydroxyethylethylenediaminetriacetate |

six). However, in contaminated field soils, phosphate applications were ineffective for over-coming arsenic phytotoxicity. In a recent discussion, DANIEL (1960) reported that heavy applications of phosphorus fertilizers reduced the effectiveness of certain organic arsenical herbicides used for the control of crabgrass in lawns. He explained this effect as competition between phosphorus and arsenic for uptake by plant roots. More research is needed to clarify the relations between fertilization and arsenic phytotoxicity.

## Summary

Inactivation and dissipation rates of herbicides in soils vary widely Some herbicides are inactive in the soil and have no residual phytotoxicity; others exhibit various levels of phytotoxicity and different rates of dissipation from soils.

Sensitive crops have been injured by residues of herbicides that persisted from spring or summer to the next fall and spring. Cereals planted in the fall after crops that have received summer applications of diuron, atrazine, simazine, diphenamid, and some related herbicides are most vulnerable. However, injury to soybeans, sugar beets, oats, and forage grasses and legumes has been encountered in a few instances ten to 12 months after applications of atrazine on corn. Tobacco, cotton, peanuts, and soybeans have been injured by fenac residues one and two years after application at rates used for selective weed control in corn, and diphenamid has persisted for ten to 12 months and injured seedling oats.

Methods of reducing or eliminating carry-over hazards are: (1) band application of herbicides, (2) thorough plowing and disking of soils containing residues, (3) use of activated charcoal as a soil amendment, (4) use of surfactants to increase postemergence herbicidal activity at reduced rates of application, (5) timely irrigations, and (6) application of specific mineral elements.

Although low levels of phytotoxic residues have persisted from one season to the next, data from many sources indicate that accumulation of massive levels of selective, organic herbicides is unlikely. Inherent phytotoxicity provides a natural indicator for residues and a defense against accumulation of herbicides that are used for selective weed control in crops.

## Résumé *

Les vitesses d'inactivation et de disparition des herbicides dans les sols varient largement. Quelques herbicides sont inactifs dans le sol et n'ont pas de phytotoxicité résiduelle; d'autres présentent différents degrès de phytotoxicité et de vitesses de disparition des sols.

Des plantes sensibles ont été atteintes par des résidus d'herbicides qui persistaient depuis le printemps ou l'été jusqu'à l'automne et au printemps

* Traduit par R. MESTRES

suivant. Les céréales semées en automne après des récoltes pour lesquelles avaient été faits des traitements d'été de diuron, atrazine, simazine, diphénamide et d'autres herbicides semblables sont les plus vulnérables. Cependant, les dommages au soja, aux better aves à sucre, à l'avoine, aux fourrages et aux légumes ont été signalés un petit nombre de fois 10 à 12 mois après l'application d'atrazine sur le maïs. Le tabac, le coton, les noisettes et le soja ont été affectés par des résidus de fenac un ou deux ans après application aux taux normalement utilisés pour le désherbage séléctif du maïs, et la diphénamide a persisté 10 à 12 mois, détruisant les semis d'avoine.

Les méthodes pour réduire ou supprimer ces risques de transmission sont: (1) l'application en parcelles des herbicides, (2) la labour et le disquage parfait des sols contenant des résidus, (3) l'emploi de charbon actif comme amendement du sol, (4) l'emploi de surfactants pour augmenter l'activité herbicide de post-émergence et des taux d'application réduits, (5) des arrosages à bon escient et (6) l'emploi d'éléments minéraux spécifiques.

Bien que des faibles concentrations en résidus phytotoxiques aient persisté d'une saison à la suivante, les résultats de nombreuses études indiquent que l'accumulation de teneurs massives en herbicides organiques sélectifs est improbable. La phytotoxicité inhérente fournit un indicateur naturel pour les résidus et une défense contre l'accumulation des herbicides qui sont utilisés pour un controle sélectif des mauvaises herbes dans les récoltes.

## Zusammenfassung *

Die Inaktivierungs- und Abbau-Geschwindigkeit von Herbiziden im Boden ist sehr verschieden. Manche Herbizide werden im Boden inaktiviert und besitzen keine Rest-Toxicität gegenüber Pflanzen; andere hingegen zeigen einen verschiedenen Grad von Phytotoxizität und unterschiedliche Abbaugeschwindigkeit in Abhängigkeit von der Bodenart.

Empfindliche Gewächse werden durch Herbizid-Rückstände geschädigt, die vom Frühjahr oder Sommer bis zum folgenden Herbst und Frühling erhalten bleiben. Winter-Getreide, das auf Gewächse folgt, welche eine Sommerbehandlung mit Diuron, Atrazin, Simasine, Diphenamin oder einigen anderen Herbiziden erhalten haben, sind am empfindlichsten. Außerdem werden einige Beispiele von Schäden an Sojabohnen, Zuckerrüben, Hafer, Futtergräsern und Gemüsen berichtet, welche 10—12 Monate nach Atrazin-Behandlung an Mais-Vorfrucht auftraten. Tabak, Baumwolle, Erdnuß und Soja werden noch 1—2 Jahre durch Rückstände von Fenac geschädigt, welches zur selektiven Unkrautbekämpfung im Mais angewandt wurde. Diphenylamin hat nach 10—12 Monaten noch Rückstände, die Hafer-Keimlinge schädigen.

Folgende Methoden zur Verminderung bzw. Vermeidung von Rückstands-Schäden in der Fruchtfolge werden beschrieben: 1. Streifenförmige

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Anwendung von Herbiziden, 2. Pflügen und Behandlung mit Scheibeneggen der Böden, welche Rückstände enthalten, 3. Bodenverbesserung mit aktiver Kohle, 4. Zusatz von Netzmitteln, um bei verminderter Anwendungsdosis eine Steigerung der Herbizid-Aktivität beim Auflaufen zu erreichen, 5. zeitweise Bewässerung, und 6. Anwendung spezifischer Mineral-Elemente.

Obgleich geringe Mengen von phytotoxischen Rückständen von einer Jahreszeit zur nächsten im Boden erhalten bleiben, ergibt sich aus zahlreichen Angaben, daß eine Akkumulation von selektiven, organischen Herbiziden auf eine höhere Konzentration unwahrscheinlich ist.

Die verbleibende Phytotoxizität stellt einen natürlichen Indikator für Rückstände dar und ist zugleich eine Sicherung gegen die Akkumulation von Herbiziden, die zur selektiven Unkrautbekämpfung im Getreidebau verwendet werden.

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# Public health problems arising from the use of pesticides

By
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## Contents



## I. Introduction

It is important that the experts in food technology and chemistry who have contributed to the principles of safety in relation to pesticide residues on foods lend their talents and attention to the many other dimensions of pesticide safety.

If one can judge the success of a technological tool by widespread and increasing use, pesticides have enjoyed considerable success. At the same time, widespread use of any product increases the likelihood that inherent undesirable characteristics will come to light. The very success of pesticides has illustrated in bold relief a dilemma of modern technology. How can society enjoy the benefits of an extremely valuable technological tool but avoid the dangers inherent in the use of that tool?

By augmenting the production of food and fiber and by helping in the control of vector-borne disease, pesticides have made a generous contribution to the public's health. Concurrently, the use of pesticides has resulted in many public health problems, and it is to these problems that this discussion will be directed. Much of the information to be presented is based upon experience in California, where the leading industry is agriculture, where at least 20 percent of the nation's pesticides are used, and where over 40 percent of the nation's vegetable crops are grown. It is also

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the state where a substantial number of signatures were collected for an initiative which, if it had been successful in reaching the 1964 ballot, would have asked voters to consider banning the use of most pesticides in California's agriculture.

Public health pesticide problems are noted for their technical complexity, scientific controversy, and public confusion. Such problems are somewhat analogous to those generated by the use of certain drugs whose unwanted side effects are occasionally the object of widespread concern. The difference, of course, is that with pesticides literally the entire population is at risk of exposure. Furthermore, the general citizenry has been sensitized to the problems of environmental contamination by years of controversy over the possible deleterious effects to health of air pollution and radioactive fallout.

Inherent in any discussion relative to toxicology, there must be a clear understanding of the concepts of exposure and effect. We have found it most useful to think of *exposure* as either acute or long-term and to consider *effect* as either immediate or delayed. Thus, when considering the impact of synthetic organic pesticides on human health, it becomes apparent that most is known about "acute" exposures which produce "immediate" effects; less is known about "acute" exposures which produce "delayed" effects; and least is known about "long term" exposures which produce harmful effects, either immediate *or* delayed. Table I shows these exposure-effect relationships and lists several illustrations of each. Included in parentheses is a pesticide which has been reported to be capable of producing the indicated effect under conditions of the given exposure. All of these exposure-effects relationships have been demonstrated to a greater or lesser extent in man, with the exception of the longterm exposure-delayed effect relationship which has only been observed in experimental laboratory animals and in wildlife. It is quite possible that this latter relationship might never be demonstrated in man, especially where the long-term exposure is also a low level exposure as is the case with pesticide residues on food. One of the great difficulties in this regard is finding a suitable, unexposed control group in a population where pesticides are ubiquitous.

Table I. *Examples of pesticide exposure-effect relationships*

| Acute exposure | | Long-term exposure | |
|---|---|---|---|
| Immediate effects | Delayed effects | Immediate effects | Delayed effects |
| 1. Acute intoxication (parathion) | 1. Demyelination of nerve fibers and paralysis (Isopestox, BIDSTRUP *et al.* 1953) | 1. Cutaneous allergy (malathion, MILBY and EPSTEIN 1964) | 1. Neoplasia (dieldrin, aldrin, DAVIS and FITZHUGH 1962)[a] |
| 2. Local parasympathetic stimulation (tetraethylpyrophosphate, QUINBY and DOORMINK 1965) | | 2. Bone marrow damage with pancytopenia (lindane, BEST 1963) | 2. Decreased viability of pheasant offspring (DDT, HUNT 1965)[a] |

[a] Not reported in human beings to date.

Because no comprehensive surveillance program relating pesticides and human health is available, only examples of reports describing some aspect of these health problems can be selected for presentation.

## II. Morbidity

For California, morbidity (non-fatal disease) from pesticides can be only roughly estimated for young children; described more precisely for workers; and remains largely unknown for the remainder of the population. The *California Medical Association,* the *State Department of Public Health,* and the *Governor's Interdepartmental Committee on Pesticide Review* have recommended that prompt medical reporting to the health agencies of all deaths and poisonings attributed to pesticides be made mandatory. If this recommendation is implemented, California will then have a measurement of morbidity for the general population.

Table II. *Occupational diseases attributed to pesticides and other agricultural chemicals, California, 1953—1963*

| Year | All industries | Agriculture | Other industries[a] |
|---|---|---|---|
| 1963 | 1013 | 746 | 267 |
| 1962 | 827 | 545 | 282 |
| 1961 | 911 | 578 | 333 |
| 1960 | 975 | 668 | 307 |
| 1959 | 1093 | 782 | 311 |
| 1958 | 910 | 599 | 311 |
| 1957 | 749 | 434 | 315 |
| 1956 | 789 | 464 | 325 |
| 1955 | 531 | 326 | 205 |
| 1954 | 391 | 248 | 143 |
| 1953 | 377 | 227 | 150 |

[a] Includes service, construction, manufacture, government, etc. (*State of California, Department of Public Health:* Occupational disease in California attributed to pesticides and agricultural chemicals, Annual Reports).

A study of hospital emergency care of children was made by the *State of California Department of Public Health* which indicated that 3,000 California children received emergency medical or hospital treatment because of ingestion of pesticides during 1960. This number was about ten percent of the total receiving emergency care for ingestion of noxious substances.

Reports describing occupational disease are available through reports of work injury required of all physicians in California [1]. The number of

[1] Each physician who attends an injured employee and each employer of such a worker is required by Section 6407 of the *California Labor Code* to file a report with the *State Department of Industrial Relations* when disability lasts beyond the day of injury or requires medical service other than ordinary first-aid treatment. By definition, work injury includes occupational disease. Under an interagency agreement with the *State Department of Industrial Relations,* the *California State Depart-*

doctors' reports involving pesticides and other agricultural chemicals have doubled since 1954, and have ranged from 800 to 1,100 reports annually since 1958. The highest numbers were reported in 1959 and 1963 (Table II). Most of the reports come from agriculture, which has the highest occupational disease rate of any industry in California. About one-half of these 800 to 1,100 reports are classified as skin disease and about one-third as systemic poisoning (Table III). The phosphate ester pesticides, parathion

Table III. *Occupational disease reports attributed to pesticides and other agricultural chemicals for all conditions and systemic poisonings, California 1953—1963* [a]

| Total, all conditions | | Systemic poisonings | | | |
|---|---|---|---|---|---|
| | | Total | Phosphate ester pesticides | DDT, lindane, endrin, dieldrin | Other agricultural chemicals |
| Total | 8566 | 3066 | 2277 | 117 | 672 |
| 1963 | 1013 | 345 | 267 | 14 | 64 |
| 1962 | 827 | 219 | 140 | 21 | 58 |
| 1961 | 911 | 268 | 194 | 14 | 60 |
| 1960 | 975 | 368 | 283 | 7 | 78 |
| 1959 | 1093 | 499 | 407 | 10 | 82 |
| 1958 | 910 | 328 | 227 | 14 | 87 |
| 1957 | 749 | 252 | 189 | 12 | 51 |
| 1956 | 789 | 281 | 197 | 11 | 73 |
| 1955 | 531 | 183 | 126 | 4 [b] | 53 |
| 1954 | 391 | 122 | 101 | 1 [b] | 20 |
| 1953 | 377 | 201 | 146 | 9 [b] | 46 |

[a] *State of California, Department of Industrial Relations: Doctor's first report of work injury.* Statistics compiled by *State of California, Department of Public Health.*
[b] DDT only.

[O,O-diethyl O-(*p*-nitrophenyl) phosphorothioate], Phosdrin [alpha isomer of 2-carbomethoxy-1-methylvinyl dimethyl phosphate], and Thimet [phorate or O,O-diethyl *S*-(methylthio-ethyl) phosphorodithioate], account for most of the poisoning cases. There has been for the past ten years about one occupational death from pesticides reported for each 100 reports of occupational poisoning from these chemicals. For phosphate ester pesticides the rate has been one death per 200 reported poisoning cases. Data on occupational disease from pesticides and other agricultural chemicals are probably understated, because reports of occupational disease are not received from self-employed agricultural workers, a group which comprises about one-third of all agricultural workers.

In considering the impact of pesticides on the health of workers whose occupation requires their use, two assumptions were made by early planners which later proved to be false. The first of these assumptions presupposed

*ment of Public Health* through its *Bureau of Occupational Health,* reviews and analysis those doctor's reports (Doctor's First Report of Work Injury) which concern occupational disease. Reports are received only for the 80 percent of employed persons in California covered by the California Workmen's Compensation Law. Among the 20 percent excluded are federal employees, maritime workers, railroad workers in interstate commerce, and self-employed.

that farmers, spray operators, and their employees were generally prepared by knowledge, training, and equipment to handle the very difficult and responsible task of safe application of hazardous pesticides. Unfortunately, it was soon found that employers were often either uninformed themselves or reluctant to provide adequate occupational safety information because they did not want to "alarm" workers about the hazard. Moreover, pesticide salesmen often were reluctant to provide adequate safety information about their products. In short, economic incentives did not act in the direction of encouraging occupational safety in the application of pesticides because it was often less expensive to risk occupational disease than prevent it. This combination of ignorance and conflict of interest has resulted in the actual handling of pesticides being far removed from what many persons, otherwise well informed about pesticides, imagine it to be. The second assumption which proved to be false presumed that physicians were universally prepared to deal with the casualties. Health agencies at all levels of government soon realized that this was not the case. Both the realities of pesticide application and the casualties resulting thereform were upon us before prevention and treatment information was developed and disseminated.

Since workers who regularly formulate and apply agricultural chemicals are among the first to be exposed to newly introduced pesticides, their health should be the subject of intense and continuous observation. Such observation would not only be essential to the well being of the worker but would also constitute an invaluable mechanism for discovering toxic manifestations which might have been missed during the course of the animal studies carried out in conjunction with the initial evaluation of the pesticidal chemical in question.

## III. Mortality

Accidental deaths from acute pesticide poisoning are reported at about 150 a year nationally. In California from 1961 to 1963, deaths attributed to pesticides occurred at an average of about six per year (about four percent of the national total). About two-thirds of these pesticide deaths involved young children and from 1955 to 1960, pesticide deaths accounted for one-fourth of all deaths from solid and liquid poisons among children under five years old (Table IV). The average of six pesticide fatalities annually in California during the years 1961 to 1963 represents a considerable improvement over the previous six years, during which time an average of eleven deaths were reported annually (Table V). This improvement can be partially accounted for by the reduction in deaths among children from sodium arsenite weedkiller which was removed from the home market in 1961.

There has been no improvement in the number of deaths among workers exposed to pesticides (Table V). They have averaged about two annually over the past ten years. The most frequent causes were phosphate ester

pesticides and methyl bromide. Information from the investigation of occupational deaths indicate that deaths from pesticides can be missed easily if a history of exposure is overlooked and appropriate chemical tests

Table IV. *Accidental fatal poisoning from solid and liquid substance in children under five years of age, California 1955—1960* [a]

| Year | Accidental deaths from solid and liquid substances | |
|---|---|---|
| | Total | From pesticides |
| Total | 163 | 42 |
| 1960 | 27 | 4 |
| 1959 | 25 | 10 |
| 1958 | 32 | 6 |
| 1957 | 28 | 8 |
| 1956 | 35 | 11 |
| 1955 | 16 | 3 |

[a] *State of California, Department of Public Health:* Vital statistics reports, 1955—1960, and death certificates of pesticide deaths, 1955—1960.

omitted. Conversely, deaths attributed at first to pesticides have occasionally proved on more thorough investigation, to be due to other causes.

In just one of the 67 counties in Florida, there were eight accidental and five suicidal deaths from phosphate ester pesticides in 1963 alone (DAVIS 1963). In this county the *County Medical Examiner* had under-

Table V. *Accidental deaths attributed to pesticides and other agricultural chemicals, California 1951—1962* [a]

| Year | Total | Children | | | | Workers | | | | Others | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total | Arsenic | Organic phosphates | Other | Total | Organic phosphates | Methyl bromide | Other | Total | Arsenic | Other |
| Total | 121 | 73 | 42 | 12 | 19 | 27 | 11 | 6 | 10 | 21 | 10 | 11 |
| 1963 | 6 | 3 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 2 | 0 | 2 |
| 1962 | 5 | 4 | 1 | 2 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| 1961 | 6 | 3 | 2 | 0 | 1 | 3 | 2 | 0 | 1 | 0 | 0 | 0 |
| 1960 | 4 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1959 | 18 | 10 | 4 | 2 | 4 | 5 | 1 | 2 | 2 | 3 | 1 | 2 |
| 1958 | 13 | 6 | 2 | 0 | 4 | 3 | 1 | 1 | 1 | 4 | 3 | 1 |
| 1957 | 12 | 8 | 5 | 1 | 2 | 2 | 1 | 0 | 1 | 2 | 1 | 1 |
| 1956 | 18 | 11 | 9 | 1 | 1 | 4 | 0 | 2 | 2 | 3 | 2 | 1 |
| 1955 | 6 | 3 | 1 | 0 | 2 | 1 | 0 | 0 | 1 | 2 | 2 | 0 |
| 1954 | 12 | 9 | 3 | 4 | 2 | 2 | 1 | 0 | 1 | 1 | 1 | 0 |
| 1953 | 10 | 4 | 3 | 1 | 0 | 4 | 3 | 0 | 1 | 2 | 0 | 2 |
| 1952 | 6 | 5 | 4 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| 1951 | 5 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 |

[a] *State of California, Department of Agriculture:* Annual reports, Bureau of Chemistry 1951—1962. *State of California, Department of Public Health:* Death records and annual agricultural chemical reports 1951—1962.

taken a special study because it had become apparent to him that deaths from pesticides could be and were frequently missed. Eight out of the thirteen deaths would have been missed had not this special investigation been made. In previous years, two homicides involving parathion were detected in the same county because of these special investigations. Unless there is a high degree of suspicion and if a cholinesterase test ist not performed the true cause of death may not be detected. In Florida and in most other states anyone can purchase for a few dollars enough parathion to kill several thousand people. In California the highly toxic phosphate esters are sold only after a permit is obtained from the *County Agricultural Commissioner*. This procedure may account at least partially for the fact that California reports a much lower proportion of pesticide fatalities than do other states (WEST 1963).

It is of importance to note that while only about 13 percent of pesticides are sold for use outside of agriculture, about one-half of all deaths from pesticides come from pesticidal materials sold for non-agricultural use. These deaths usually occur in children following accidental ingestion of pesticides left around the home.

Here are some examples of accidental fatal pesticide poisoning in California.

*Case 1.* A 16-year-old and a 21-year-old farm laborer were hired to apply a pesticide dust to strawberries. The dust consisted of 1.5 percent Phosdrin and ten percent sulfur. The workers used knapsack dusters, starting work at 7 : 30 a. m. At noon, the 21-year-old worker became ill and remained at the side of the field in his car an vomited. After a while, he felt better and drove home. (Fortunately, he did not have an auto accident. Workers with phosphate ester poisoning are poor risks with any moving machinery.) The 16-year-old worked until 4 p.m. when he vomited and went home. At 8 p.m., he complained of weakness and giddiness and was taken to a physician's office. The boy's clothing was reported to have been covered with sulfar. The physician called the *Poison Information Center* for information about sulfur which is relatively nontoxic. The boy was sent home with a prescription. At 9 : 30 p.m., the boy became worse and was taken to the local hospital. This time, the label from the pesticide container was brought with the patient. The boy was again sent home although he was unable to walk. At 7 : 30 a.m. the boy was found moribund in his bed, still in his contaminated work clothing. He died in the ambulance en route to the hospital. Death due to phosphate ester poisoning was confirmed by postmortem cholinesterase tests.

The 21-year old worker reported the next day for a cholinesterase test which confirmed that he had been poisoned by a phosphate ester chemical. He had not worked with phosphate ester pesticides before. The 16-year-old had applied the same pesticide on one occasion for the same employer two months before when he was 15 years of age.

There were a number of errors committed in the series of events leading to this death. The permit to purchase and apply the pesticide had expired so that it was purchased and applied illegally. The highly toxic phosphate

ester was applied by hand duster, a primitive and entirely unsafe method of application. The container label was not read until after the second illness. No medical supervision was provided. No advance arrangements were made with a physician for prompt adequate care of poisoning emergency. The two workers were not instructed about hazards and precautions for using the pesticide. They were not provided with protective clothing. No medical attention was sought for the worker who quit at noon because of illness, and no medical examination was considered for the younger employee who kept on working. The victim was not told to bathe, wash his hair, and change into clean clothes after work. When the boy was taken to a physician, no one could provide information about the pesticide which the workers had applied.

On first visit, the physician released the victim as only mildly ill without ruling out serious poisoning. He should have insisted on seeing a label from the pesticide container. On second visit, the physician was furnished the label but did not follow the medical treatment recommended on it. He may have been confused by two entirely different doses of atropine prescribed. The label listed the large doses which should have been administered, but also listed the conventional dose of atropine by tablet for first aid (the practice of recommending tablets for first aid should be discontinued). The physician did not call a consultant or the *Poison Information Center* for information about the pesticide mixture listed on the label. The boy was not kept under close medical observation for 24 hours. He was not decontaminated, and no cholinesterase determination was made.

The supplier of the pesticide did not check the number of permit given by the purchaser to assure that the permit was valid. The product was also misbranded; it contained two to four times the phosdrin specified on the label.

Just about every error possible occurred, and avoidance of any one of the more serious errors could have saved the boy (WEST 1964).

*Case 2.* A young man came to work as a swamper for an agricultural aircraft operator. On the first day, he was put to work steam-cleaning and washing a cropdusting aircraft. It was reported that he was not informed of any hazard nor was he given any protective clothing or equipment. His clothing was observed to have been thoroughly wet while he was working. In the early afternoon, he complained of not feeling well. His employer gave him two atropine tablets and the swamper returned to work. No long afterwards, he was found unconscious. He was admitted to the hospital and died several hours later. Apparently, the aircraft he was cleaning had been used to make several applications of demeton {(Systox), *O,O*-diethyl *O*-[2-(ethylthio) ethyl] phosphorothioate and *O,O*-diethyl *S*-[2-(ethylthio) ethyl] phosphorothioate in a 2 : 1 ratio}. The diagnosis of phosphate ester pesticide poisoning was confirmed by postmortem cholinesterase tests.

*Case 3.* A young sprayer was found dead in the field in the tractor which had been pulling his spray-rig. He had been working alone pouring and mixing parathion concentrate into the spray-rig tank. In the process

of mixing the concentrate, the worker contaminated his gloves inside and out. He rested his gloved hands on his trousers as he pulled the rig to apply the spray. Parathion was absorbed through the skin of his hands and thighs. He began to vomit, an early symptom of parathion poisoning. He could not remove his respirator and he aspirated the vomitus and choked. The diagnosis of poisoning was confirmed by postmortem cholinesterase tests.

*Case 4.* A 28-year-old worker had no record of occupational exposure to pesticides until his employment as a sprayer by a licensed agricultural pest control operator. He began his employment by applying parathion, TEP, and Phosdrin under the direction of a more experienced sprayer. He attended one safety meeting at the company headquarters. His first job alone was assigned after three weeks of employment and was at a ranch in the adjoining county where he was to apply a mixture of Phosdrin, parathion, and TDE [1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethane] to lettuce. There is no record of his being placed under medical supervision as required by *The California Division of Industrial Safety's* "Agricultural Safety Orders". No baseline cholinesterase tests were performed, nor were the required arrangements made in advance with a physician to take care of any poisoning emergency which might arise.

He began to spray 40 acres of lettuce at the ranch about 9 : 30 p.m. He was working alone and was last seen alive at midnight by an irrigator who stated he had then sprayed half of the field. He was expected to complete his job at about 2 a.m. It was a cool, cloudy evening and he had no illumination except the headlamps of this vehicle. The ground spraying apparatus included a closed system for mixing the concentrates. However, the Phosdrin was not mixed in the enclosed system as was the parathion and TDE. The Phosdrin, a 50 percent concentrate, was poured manually from a five-gallon can into a tank on the truck and the sprayer had spilled the material on himself in the process.

The sprayer finished the job, secured his equipment on the truck, and drove 1/4 mile to the main road where he stopped the truck and began to vomit. He apparently tumbled out of the truck, landing face down in the ditch at an estimated time of 2 : 30 a.m. At 8 : 00 a.m. he was found by the owner of the ranch who described him as possibly still alive frothing at the mouth. He was pronounced dead on arrival at the hospital. Red cell, plasma, and brain cholinesterase were all close to zero postmortem, confirming the diagnosis of phosphate ester poisoning.

The area and the equipment pertinent to the incident were investigated. A wet spot smelling like the Phosdrin formulation used was found on the ground where the nurse rig had been located. Three respirators were found in the rig and the torn mate to the glove was found where the nurse rig had been. The respirators were of the proper type. There was no change of clothes on the rig. There was vomitus in the truck cab. It was not clear if any protective device, except possibly the gloves, had been worn. The sprayer was reported to have had adequate water to decontaminate himself in case of a spill but there was no evidence that he had made an attempt to do so. Erratic tractor tracks were found in the field.

This death was entirely preventable. It occurred because an improperly informed and unsupervised worker was allowed to work alone at night in an operation which required the pouring of a concentrate of one of the most acutely toxic chemicals available. Phosdrin is absorbed through the skin and takes an estimated 12 drops of 50 percent concentrate remaining on the skin to be fatal to an adult. Pouring such materials is a hazardous operation in full daylight and with the most adequate protective clothing. Without help and in the dark, decontamination would have been difficult at best. Without help and without decontamination, a spill on the clothing and skin, such as occurred here, means certain death. However, this worker could have survived if there had been someone working with him to help with decontamination and obtain prompt and adequate medical care. This death is the second in two years in California in which a spray applicator spilled a concentrated phosphate ester pesticide while he was working alone and was later found dead. The need for more closed systems for measuring concentrates is abundantly apparent, as is the need to heed the basic safety rule "Never work alone with a hazardous chemical".

In the cirumstances surrounding pesticide poisoning among workers, the operation of vehicles on the highway by poisoning victims is becoming more ominous. In this case the victim and his truck and rig were entering a well-traveled highway. In case # 1, the companion worker drove his car home while experiencing symptoms of poisoning which include dim vision.

*Cases 5, 6, 7, 8, and 9.* One four-year old found a ginger ale bottle in a neighbor's garden and drank from it. It had been used to mix sodium arsenite weed killer. A two-year-old played with an "empty" drum of parathion found in a tool shed on the ranch where he lived. Another ate a "fly cake" containing an organic phosphate, DDVP (O,O-dimethyl-2,2-dichlorovinyl phosphate). A four-year-old girl playing in a paving yard washed her orange from the faucet of a drum of arsenic weed killer. Another toddler played "mud pies" with a bottle of TEPP which he found stored in the barn. All of these children died.

*Cases 10 and 11.* In 1963 a year-old infant found lindane tablets for a vaporizer under the sink at her home. She ate four to five tablets. Several hours later, convulsions began abruptly. She died at the hospital shortly after. Five years before in the same California city, an 18-month-old boy swallowed half a lindane tablet found on the floor of the family car which was moving household belongings to a new home (JOSELIN 1958). Several hours later, there was a sudden onset of convulsions. He died about 12 hours after ingesting the tablet. Lindane vaporizers have been widely advertised and sold for home use for many years in spite of authoritative medical and public health recommendations against them. Their sale for home use is fortunately now prohibited.

Since 1957, five Californians are known to have died from aplastic anemia or related blood dyscrasias in which exposure to the chlorinated hydrocarbon pesticide, lindane (hexchlorocyclohexane, gamma isomer), has been implicated either directly or circumstantially. None of the deaths are

as yet attributed to lindane in mortality statistics. The *American Medical Association's Council on Drugs* maintains a "Registry on Blood Dyscrasias" and lists 18 reports of major blood dyscrasias in which lindane exposure was implicated (BEST 1963). Additional cases have been reported and the problem has been discussed in medical literature from many countries (SANCHEZ-MEDAL *et al.* 1963, Editorial in *The British Medical Journal* 1958, DANOPOULOS *et al.* 1953, MASTROMATTEO 1964, *Council on Pharmacy and Chemistry* 1952 and 1953, SCOTT *et al.* 1959, HUGULEY 1961, JEDLICKA *et al.* 1958, FRIBERG 1953, MARCHAND *et al.* 1956, ALBAHARY *et al.* 1957).

The California deaths which have come to the attention of the *State Health Department* include a 39-year old housewife. The interior of her home had been treated by a pest control operator every three months for several years by vaporizing lindane. On one occasion an insect was found just after treatment and the application was repeated. A heavier than usual "white film" was found over the walls and surfaces of the home. After cleaning the house thoroughly, the housewife became seriously ill and a diagnosis of aplastic anemia was made. On several occasions before her death she noted relapses after eating in a restaurant where a lindane vaporizer was operating. Three men have died from blood dyscrasias following exposure to lindane. One was 34 years old and lived in Northern California, another was 56 years old and was from Southern California. The home of the younger man had been fumigated with lindane three months before the onset of his illness. The older man had a continuously operating lindane vaporizer in his den for three months before onset of his illness. A third man of 58 years developed a sudden onset of his illness after application of lindane to his garden. An eight-year-old girl occupied for several hours a day for several years a kitchen in which a lindane vaporizer was operating. She became ill with aplastic anemia and also experienced a sudden relapse when patronizing a restaurant with a lindane vaporizer in operation.

It should be noted here that a stable or persistent chemical such as lindane vaporized into living quarters, whether by an continuously operating dispenser or at intervals by a pest control operator, produces a continuous exposure. The pesticide recirculates in the dwelling assisted by air currents and heating and ventilating equipment. The potential for long-term, continuous, and substantial exposure by inhalation does exist and it can be of magnitude greater than workers handling lindane in industry may experience.

The two best sources of decades of abundant human toxicological experience with chemicals are found in the fields of pharmacology and industrial medicine. In both arenas of human experience, lists of chemicals have been compiled which have been reported to damage adversely and sometimes permanently the developing blood cells in the bone marrow (WINTROBE 1961, BEST 1963). Apparently only a small proportion of persons exposed is seriously affected, and the degree of exposure is not necessarily related to the extent of damage to the bone marrow (OSGOOD 1953). In pharmacology it is the antibiotic, choramphenicol, which has

been the best known offender (SCHMICK *et al.* 1964, SHARP 1963). In industry, it has been the solvent, benzol (VIGLIANI 1964, WINTROBE 1961). This phenomenon is not predictable by animal experimentation. There are no laboratory tests which can prove or disprove a cause and effect relationship in the individual case. Only when large numbers of people are involved, such as there were with choramphenicol, have epidemiological studies been useful in providing statistical evidence of the association between chemical exposure and bone marrow damage.

The mechanism by which chemicals affect the bone marrow is unknown. Two theories are most often advanced. One theory is that a direct toxic effect is imposed upon the stem cells of the bone marrow and this effect is present to some degree among most persons experiencing significant exposure. However, in only a small proportion of such persons does the damage progress to the point where it becomes irreversible and hence fatal. Another theory which also attempts to explain why a small proportion of those exposed are seriously affected is that the damage is produced as the result of an allergic or immunological response. In two California lindane cases and in a report involving DDT from Mexico (SANCHEZ-MEDAL 1963) sudden exacerbations were reported following inadvertent re-exposure to the same pesticide, an observation which is most consistent with the allergic theory. However, both mechanisms and perhaps others might be involved in different cases.

It is not surprising that, as human experience with pesticides accumulates, the problem of bone marrow damage arises here as it has with therapeutic drugs and industrial chemicals. In drug manufacture and in industry, stringent controls have been placed upon chemicals where epidemiological or even circumstantial evidence indicates that a blood dyscrasia hazard may exist. However, only recently, after many years of use and many warnings from authoritative sources (*Council on Pharmacy* 1952 and 1953, *California State Board of Health* 1952) have lindane vaporizers for home use become illegal in California. Even though a cause and effect relationship between lindane exposure and blood dyscrasias has not been proved, it would seem only prudent to restrict all exposures of this chemical until research efforts can be mounted to settle the question of its relationship to bone marrow damage.

## IV. Environmental contamination

Although the use of pesticides in agriculture does not account for the largest segment of recognized pesticide mortality in California, it does account for the greatest source of contamination of the environment. Another differences is apparent in that most of the pesticides involved in environmental contamination are chlorinated hydrocarbons. Those involved in human mortality have most often been either arsenic-containing compounds or the phosphate esters.

Contamination of the environment has been reported from many sources. Chlorinated hydrocarbons have been found in most surface waters

examined (BRIENDENBACH and LICHTENBERG 1963), some well waters (KRAYBILL 1963), in the ambient air of California cities (*California Department of Public Health* 1964), in the oil of fish off the Americas, Europe, and Asia (*President's Science Advisory Committee* 1963), in the blubber of whales (WEST 1964), in duck eggs in the Upper Yukon, in most eagles and other wildbirds examined in 22 states and Canada (UDALL 1963), in oysters, in shellfish off both coasts, in clothing (*Stanford Research Institute* 1963), in bedding, in the fat of persons in America and Europe (DALE and QUINBY 1963, HOFFMAN *et al.* 1964, HAYES and DALE 1963, HUNTER 1963, DURHAM and ARMSTRONG 1961, READ *et al.* 1961, MAIER 1960, QUINBY 1965), in human milk (WEST 1964, LAUG *et al.* 1951), and on foods meant for human consumption (usually in accordance with legal tolerances, but occasionally from accidental contamination). MARTH (1965) has recently extensively reviewed this subject and lists many other examples.

Contamination may be minute or substantial. Of particular concern is contamination of water. The ability of aquatic life to hold the persistent pesticides so that each link in the food chain presents an increasing buildup of pesticide leads to the inescapable conclusion that for wildlife there is no known predictable safe level for persistent pesticides in water (HUNT and BISHOFF 1960).

The example of Clear Lake in California is often presented to illustrate the insidious phenomenon of concentration of persistent pesticides in the aquatic food chain (HUNT and BISHOFF 1960). Because the unexpected events which followed were the result of known direct applications of TDE for gnat control, a unique opportunity was presented to compare the amounts of TDE applied and its inimical effects upon bird, amphibian, and fish populations in the lake. The first of three treatments was made in 1949 with an application of one part of TDE to 70 million parts of lake water. In 1954 a second application at one part of DDD to 50 million parts of lake water was made. Six months and nine months later, many western grebes, a common waterfowl inhabitant of the lake, were found dead but no cause was discovered. The last application of one part of TDE to 50 million parts of lake water was made in 1957. Three months later many more grebes were found dead or dying. This time, a toxicological examination was carried out and 1,600 parts per million (p.p.m.) of TDE were found in the fatty tissue of the birds. Fish taken alive from the lake had from 40 to 2,500 p.p.m. of TDE in their fat. Five years after the last application, grebes showed 656 p.p.m., catfish 379 p.p.m., and bass 253 p.p.m. of TDE in fatty tissue.

Aquatic life varies with respect to the rapidity with which it can concentrate persistent pesticides. Particularly efficient is the oyster. After seven days in water at ten parts per billion (p.p.b.) of DDT, eastern oysters were analyzed and found to contain 151 p.p.m. of DDT, a 15,000 fold increase (UDALL 1963). Because fish and wildlife concentrate certain persistent pesticides in their fat, they should be brought into the pesticide food minotoring programs and subject to tolerances established for agricultural commodities.

It should be noted that as far as water for human consumption is concerned amounts of pesticides found in raw waters have been considerably below levels considered hazardous to people. Usually, before pesticide concentrations in water become harmful for people, they become lethal for fish and wildlife. Nevertheless, the need to monitor drinking water for pesticides and to establish tolerances is rapidly becoming apparent.

Contamination of the environment occasionally has produced acute human pesticide poisoning. A bale of blue jeans became contaminated from a leaky drum of Phosdrin (mevinphos, or 2-methoxycarbonyl-1-methylvinyl dimethyl phosphate) concentrate during transit by truck. Because Phosdrin is a highly toxic phosphate ester pesticide and can be absorbed through the skin, six boys who wore unwashed jeans from this bale eight months later were poisoned, two very seriously (WARREN 1963). This substantially contaminated bale had also been stored in the ventilation intake area for a large department store for several months.

In the state of Washington a small boy was poisoned almost fatally from parathion which had survived the winter snow and rain after being spilled in the driveway of his home. The boy had eaten mud pies made from contaminated soil which was found to contain one percent parathion (QUINBY 1961).

In California, during August and September of 1963, an outbreak of illness which sent 94 peach harvesters to physicians was traced to parathion residues on the foliage of the orchards in which the affected individuals worked. The cause of the outbreak was shown to be related to the rate of parathion application and not to an unusually early entry into the orchards by the harvesting crews. Information obtained revealed that, although parathion could easily be recovered from all elements of the orchard environment, it was not present in amounts sufficient to account for the observed illness. This inconsistency suggested the presence in the spray residue of a compound evolved from parathion alteration which was considerably more toxic than parathion, but identifiable by routine analytical procedures only as parathion. Paraoxan was considered a likely suspect and was postulated as a prime cause of the outbreak (MILBY *et al.* 1964).

In August 1963, two air pollution episodes involving one percent TEPP dust applied by aircraft to agricultural crops were reported from the state of Washington. In the larger episode, 15 people developed mild pulmonary symptoms and 15 cattle developed severe systemic poisoning of which two died. In the other instance, two people and one heifer were involved. The dust had been applied during a thermal inversion and remained in the air over fields and farmhouses for several hours (QUINBY and DOORMINK 1965).

## Summary

Examples of the public health problems arising as by-products of the use of pesticides have been presented and discussed. They are not necessarily representative of the total situation because there has been insuf-

ficient environmental monitoring and human surveillance to provide representative and comprehensive data. The information presented from California is limited to but does not include all of the obvious immediate effects from overexposure to pesticides which may have occured. Present methods of obtaining health data are not sufficiently sensitive to pick up reliable whatever delayed or less obvious effects may exist. Furthermore, there are many gaps in the knowledge necessary to carry out a comprehensive surveillance and monitoring system, and gaps in knowledge necessary to interpret information already available.

Nevertheless, the information presented reveals the occurrence of deaths and illnesses which were entirely preventable. Investigations into the handling and application of pesticides have demonstrated a widespread discrepancy between the technical demands of these tasks and the ability of the operators to perform them safely. These responsibilities are too often assigned to workers who do not have the necessary education and training. They are not often provided with the proper operating or protective equipment. In some cases, economic incentives have pressed toxic pesticides into general service before those who were permitted to take the responsibility for handling them were prepared to do so.

Technical experts who are concerned with the safety of pesticide residues should lend their talents an attention to all of the ramifications of pesticide use. It is not enough to be cognizant of the complex safety evaluations of chemicals which persist in food, for there are a number of other avenues through which pesticides may reach and affect people and their environment. They can have a direct bearing on the safety of food residues and upon the net value and public acceptance of pesticides as a technological tool. Other public health problems arising from pesticides have not received the same quality or quantity of research and regulatory attention as food residues. Moreover, there is an unfortunate tendency to avoid the issue by discounting some of the untoward events from pesticides as "occasional mishaps" or as due to "carelessness or misuse" and to point to other causes of injury and disease as more prevalent, as if their presence justified pesticide injury. Instead, there should be a free and intensified scientific inquiry to discover, evaluate, and solve all of the problems arising as by-products of the use of pesticides. Scientific progress requires continuous re-evaluation and correction if it is to contribute the most to human welfare.

**Résumé** *

Des exemples de problèmes de santé publique se posant comme conséquence de l'emploi des pesticides ont été présentés et discutés. Ils ne reflètent pas nécessairement la situation dans son ensemble, car les mesures de surveillance des ambiances et des sujets exposés n'étaient pas suffisantes pour permettre l'obtention d'informations complètes et absolument significatives. Les informations recueillies en Californie sont limitées aux effects immédiats indiscutables provoqués par une éventuelle surex-position aux

* Traduit par R. TRUHAUT.

pesticides, sans pour autant les englober tous. Les méthodes actuelles de rassemblement des informations épidémiologiques ne sont pas suffisamment sensibles pour révéler de façon sûre tous les effets lorsqu'ils sont moins apparents ou se manifestent après une phase de latence. En outre, il y a beaucoup de lacunes dans les connaissances nécessaires pour réaliser une surveillance et un dispositif d'alerte suffisamment étendus ainsi que dans celles indispensables pour l'interprétation des informations déjà existantes.

Cependant, les informations présentées révèlent l'existence de morts et de maladies qui auraient pû être totalement évitées par des mesures de prévention. Des enquêtes sur la manipulation et l'épandage des pesticides ont mis en évidence une nette opposition entre l'importance des demandes techniques pour ces tâches et la possibilité pour les opérateurs de les accomplir en toute sécurité. Les responsabilités sont trop souvent confiées à des travailleurs n'ayant pas l'éducation et l'entrainement nécessaires. Souvent ils ne sont pas pourvus des équipements opératoires ou protecteurs convenables. Parfois, des méthodes économiques conduisent à l'introduction hâtive de pesticides toxiques sur le marché avant que ceux ayant la permission de les manipuler soient préparés à assumer cette responsabilité.

Les experts techniques chargés des problèmes de sécurité concernant les résidus de pesticides doivent diriger leur compétence et leur attention vers toutes les ramifications de l'emploi des pesticides.

Il n'est pas suffisant d'être au courant des procédés complexes d'évaluation de la sécurité d'emploi des agents chimiques persistant dans les aliments, car nombre d'autres voies existent par lesquelles les pesticides peuvent atteindre l'homme et son milieu. Elles peuvent avoir une influence directs sur les risques liés aux résidus dans les aliments, ainsi que sur la valeur réelle et l'acceptation par le public des pesticides comme outils technologiques. D'autres problèmes de santé publique posés par les pesticides n'ont pas fait l'objet de recherches à un niveau aussi élevé, qualitativement et quantitativement, non plus que d'une attention aussi régulière que les résidus dans les aliments. De plus, il existe une tendance regrettable à éviter de tirer des conclusions, en négligeant quelques accidents malencontreux causés par les pesticides en les considérant comme «contre-temps occassionnels» ou comme des effets résultant d'une absence de précaution ou d'un emploi inadéquat et à incriminer, de préference, d'autres causes de dommage ou de maladie, comme si leur existence justifiait les effets nocifs provoqués par les pesticides. Au lieu d'une telle attitude, devraient être mises en oeuvre des enquêtes approfondiés objectives et scientifiques ayant pour but de découvrir, d'évaluer et de résoudre tous les problèmes pouvant se poser comme conséquences de l'emploi des pesticides. Le progrès scientifique exige une réévaluation et une correction continues s'il doit contribuer, au maximum, au bien être de l'homme.

## Zusammenfassung *

Es wurden Beispiele von Problemen für die Volksgesundheit, die sich als Folgen der Anwendung von Schädlingsbekämpfungsmitteln ergeben,

* Übersetzt von O. R. KLIMMER.

beschrieben und besprochen. Sie spiegeln jedoch nicht unbedingt die Gesamtsituation wider, weil der Schutz der Umgebung und die Kontrolle des Menschen nicht ausreichten, als daß man diese Daten als wirklich repräsentativ und umfassend betrachten könnte. Das aus Kalifornien stammende Material beschränkt sich zwar, umfaßt jedoch nicht alle die offensichtlichen und unmittelbaren Auswirkungen erhöhter Exposition durch Schädlingsbekämpfungsmittel, die aufgetreten sein können. Die gegenwärtigen Methoden, um Unterlagen über die Gesundheitsverhältnisse zu bekommen, sind nicht empfindlich genug, um verläßlich alles zu erfassen, was an verzögerten oder unauffälligeren Wirkungen vorhanden sein kann. Darüber hinaus bestehen viele Lücken in unserem Wissen, das Voraussetzung für den Aufbau eines umfassenden Überwachungs- und Schutzsystems und für die Interpretation bereits vorliegenden Informationsmaterials ist.

Nichtsdestoweniger deckt das vorgelegte Informationsmaterial vorgekommene Fälle von Tod und Erkrankung, die alle hätten vermieden werden können, auf. Untersuchungen über den Umgang mit und die Anwendung von Schädlingsbekämpfungsmitteln haben eine weitverbreitete Diskrepanz zwischen den technischen Anforderungen, welche diese Aufgaben stellen, und der Fähigkeit der damit Beschäftigten, sie ohne Gefährdung durchzuführen, gezeigt. Diese Verantwortung wird zu oft Arbeitern übertragen, welche die notwendige theoretische und praktische Ausbildung nicht besitzen. Sie sind auch oft nicht mit der notwendigen Arbeits- und Schutzausrüstung ausgestattet. In einigen Fällen hat wirtschaftlicher Anreiz giftigen Schädlingsbekämpfungsmitteln vorschnell zu allgemeiner Anwendung verholfen, bevor diejenigen, welche die Erlaubnis zu einer verantwortungsbewußten Anwendung dieser Stoffe hatten, dazu überhaupt in der Lage waren.

Technische Sachverständige, die sich mit der Frage der Unbedenklichkeit von Schädlingsbekämpfungsmittelrückständen beschäftigen, sollten ihr ganzes Können und ihre volle Aufmerksamkeit all den vielfältigen Gebieten der Schädlingsbekämpfungsmittelanwendung widmen. Es genügt nicht, mit den komplexen Bestimmungsmethoden der Unbedenklichkeit chemischer, in Lebensmitteln haltbarer Stoffe vertraut zu sein, denn es gibt eine ganze Anzahl anderer Kanäle, durch welche Schädlingsbekämpfungsmittel den Menschen und seine Umgebung erreichen und schädigen können. Sie können eine direkte Bedeutung für die Frage der Sicherheit von Rückständen in Lebensmitteln, ihren realen Wert und für die öffentliche Anerkennung der Schädlingsbekämpfungsmittel als technische Mittel besitzen. Andere, mit Schädlingsbekämpfungsmitteln zusammenhängende Probleme der Volksgesundheit haben nicht die gleiche quantitative und qualitative Beachtung auf dem Gebiete der Forschung und der Schutzmaßnahmen gefunden wie die Rückstände in Lebensmitteln. Weiterhin besteht eine unglückliche Tendenz den Resultaten auszuweichen, indem man unerwünschte Vorfälle, die durch Schädlingsbekämpfungsmittel zustande kamen, als „zufällige Pannen" verharmlost oder auf „fahrlässige oder falsche Anwendung" zurückführt oder darauf hinweist, daß andere Unfall- und Erkrankungsursachen bei weitem überwiegen, als ob deren Auftreten die Schädigungen durch Schädlingsbekämpfungsmittel rechtfertigen würde. Statt dessen sollte eine unabhängige und verstärkte wissenschaftliche Untersuchung durchgeführt wer-

den, um alle die sich aus der Anwendung von Schädlingsbekämpfungsmitteln nebenbei ergebenden Probleme der Schädigung zu erkennen, zu bewerten und zu lösen. Der wissenschaftliche Fortschritt erfordert eine ständige Neubewertung und Korrektur, wenn er sein Bestes zum Wohlergehen der Menschen beitragen soll.

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