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TOXICANT DISTRIBUTION

Determining the Distribution of Organic Insecticides in Powder Formulations

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The location and the quantitative distribution of the toxicant in a powder formulation of an organic insecticide, with an insoluble carrier or diluent, may influence greatly the effectiveness of the formulation. Results indicate that the deliquescence method is capable of determining this distribution. Exposure of the formulation to the vapor of an organic solvent causes deliquescence of the toxicant particles, and the resulting droplets could be observed under the microscope and used as an index to the location and quantity of toxicant originally present.

DUST AND WETTABLE POWDER FORMULATIONS of organic insecticides may vary considerably in effectiveness, with the nature of the carrier or diluent, and with the technique used to combine the components (5). Much of this variation arises from differences in the distribution of the toxicant within the mixture and in the nature and extent of its association with the carrier. Most of the evidence on this point is indirect. For example, formulations prepared by mechanical grinding have been shown, in field experiments (7), to be markedly inferior to impregnated formulations in which the toxicant would be expected to be more uniformly distributed throughout the bulk, as well as on the surface of the individual particles. Harrison (3), using the air permeation method, compared the average particle sizes of different formulations with the average sizes of the particles left after extraction of the toxicant. A closer association of the components in some formulations than in

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others was indicated and laboratory tests on insects correlated well with these results. Work along these lines has been greatly handicapped by the lack of a satisfactory method for the direct observation of the distribution of the toxicant in a finished formulation.

Some information can be obtained by a careful microscopic examination of the formulation. Glass (2) observed free droplets of parathion when a wettable powder was mixed with water, and found that powdered materials which would eliminate these free droplets greatly reduced injury to susceptible apple foliage. Methods based on differences in optical properties between carrier and toxicant, such as refractive index or behavior in polarized light, give some information, but are seriously limited by the heterogeneous nature of most carriers and diluents. They are almost useless where the components are closely associated and, particularly, where a nonvolatile solvent for the toxicant is also present.

The most clear-cut difference between toxicant and carrier is that of solubility. Most of the diluents and carriers are

inorganic minerals, and the few organic carriers, such as tobacco stems and walnut shell flour, have a relatively low content of material soluble in organic solvents, while most toxicants are extremely soluble in certain organic solvents. Exposure of a formulation to the vapor of a suitable solvent should, therefore, cause deliquescence of the toxicant but little or no deliquescence of the other constituents. Preliminary experiments showed that the phenomenon could be controlled so as to show, under the microscope, the location and relative quantity of toxicant either as separate particles or associated with particles of carrier, by producing liquid droplets at the location of each portion of toxicant. A procedure for this purpose was worked out and some tests made to determine the extent of the information which the method could furnish.

Procedure

Two microscope slides are cleaned and, if desired, coated with Dri-Film or other solvent-resisting coating. A

representative sample of the powder formulation to be examined is deposited on one of the slides, thinly enough to minimize coalescence of droplets formed later on neighboring particles, and with a minimum of aggregation, overlapping of particles, and segregation of particles of different sizes. A small dust tower, in which complete settling is permitted, is satisfactory for the purpose. A hole about 0.25 inch in diameter is punched in the center of a piece of filter paper of approximately the same dimensions as the slide. This filter paper must be thick enough to avoid contact of the upper slide with the formulation particles and the droplets formed later. The paper is laid carefully on the treated slide and covered with the second slide, and the two slides are bound together with strips of gummed tape. The preparation must be handled carefully to avoid movement of the powder.

The deposit of powder in the small chamber formed by the two slides and the opening in the filter paper can then be examined microscopically before further treatment. Depending upon the information sought, the particles in one or more fields or in the whole area can be counted, measured, or photographed. A mechanical stage aids greatly where it is desirable to examine the same field again after deliquescence.

The solvent or solution suitable for producing deliquescence is then dropped on the exposed edges of the filter paper until it is uniformly wet. A few minutes are required for deliquescence of all of the particles and for the resulting droplets to reach equilibrium with the solvent vapor in the chamber. During this period, some information can be obtained on the location of extremely small particles of toxicant on the slide or on different parts of the surfaces of individual diluent or carrier particles by noting the appearance of minute droplets of liquid before they have had time to grow sufficiently to spread and to coalesce with neighboring droplets.

The same fields examined before the addition of the solvent are now available for a second examination. The particles of toxicant originally present are each represented by a droplet of solution, allowing for occasional coalescence. The droplets can be counted and their relation to the original particles of formulation noted. The undissolved particles of diluents or other insoluble constituents are still visible. Measurement of the droplet sizes can also furnish an estimate of the relative quantities of toxicant present in the different locations. If observations are made over a long period, it may be necessary to add more solvent to the filter paper, periodically, to maintain a constant vapor concentration in the observation chamber.

Check samples containing all of the constituents of the formulation, except the toxicant, should be examined for comparison. In case of serious interference by these constituents, adjustments can be made in the solvent.

Microscope and Lighting

An ordinary compound microscope was used for the observations. A phase microscope gave outstandingly clear definition of the outlines of the droplets and the included particles, but gave no information which could not be obtained with the simpler equipment. A low magnification was used for determining the over-all distribution of the toxicant because large fields could be examined and a judgment of the quantity of toxicant to be considered significant was made easier. A higher magnification was used for measuring the particles and droplets and observing details of the distribution of very small quantities of toxicant.

The droplets showed up best with light reflected from a plane mirror, without a substage condenser, for the low magnifications, and with a condenser with a small diaphragm opening for the higher. A water cell or other device for eliminating most of the heat from the light source was essential to prevent condensation on the upper slide and erratic droplet development.

Solvents for Droplet Development

DDT solvents with a wide range of volatilities were tested, and all produced deliquescence, but the highly volatile ones required too frequent application to the slide and those of low volatility were too slow in action. Xylene was the most convenient of the solvents tried, and was used in all the work reported.

Pure solvents were unsuitable for the purpose because of high blanks and condensation on the slide, and particularly because there was no definite limit to the growth of the droplets of toxicant solution. These effects were eliminated by the use of nonvolatile solutes to reduce the vapor pressure of the xylene. Saturated solutions of solutes of different solubilities permitted the reproducible development of droplets of any desired size range, which could be maintained in a stable equilibrium, almost indefinitely. The α-isomer of benzene hexachloride proved a very satisfactory solute and was used for most of the tests reported.

Other solvents or solutes might be better for different toxicants, different particle size ranges, or different toxicant concentrations. Interference by other soluble constituents of the formulation, such as surface-active agents, might be eliminated by the use of selective solvents and by maintaining their vapor pressures below those of saturated solutions of the interfering substances.

Development of droplets started around the edges of the observation opening and progressed gradually inward. With the materials and conditions used, full development of all droplets required about 5 minutes. No trouble was experienced from crystallization of the solute in the observation opening when excessive quantities of solution were avoided.

Experimental Formulations

The conditions necessary for the development of stable droplets of a suitable size range were worked out with commercial DDT dust formulations. For checking on the more quantitative aspects of the phenomenon, however, the distribution of toxicant of the commercial formulations was too uncertain, and the range of particle size was too wide for use as known standards.

To furnish closely controlled standard diluents and carriers, a sample of ignited aluminum oxide (General Chemical Co., Code 1236) was fractionated with standard sieves to give a graded series of samples of satisfactory uniformity. While these samples were coarser than most actual insecticide diluents, the observations made on them are valid for the finer materials if allowance is made, where necessary, for the difference in specific surface.

The standard carriers were impregnated by dropping, slowly, a measured volume of a standard solution of pure p,p'-DDT in benzene into a weighed sample of the carrier, and mixing thoroughly with a spatula during the addition of the solution and until the solvent was evaporated. At the levels used, the deliquescence test showed that nearly 100% of the particles received some toxicant, and that the quantity of toxicant per particle was reasonably uniform.

Proportion of Active Particles

The proportion of the particles which carry a substantial quantity of toxicant and can therefore be considered insecticidally active, is an important factor in evaluating a formulation. Hutzel and Howard (4) observed that the number of active particles of rotenone dusts may be more important than the quantity of toxicant per particle. To test the reliability of the deliquescence method for giving this information, a uniform standard formulation was made up by impregnating aluminum oxide, of particle diameter between 105 and 149 microns, with approximately 6% of its weight of DDT by the method previously described. Portions of this standard formulation were diluted with

different proportions of untreated alumina of the same size range, and each such sample was well mixed with a spatula.

The slides, prepared from each sample, were examined under a sufficiently low magnification so that 200 to 400 particles were present in a single field. The particles present before the vapor treatment were mapped on cross-section paper with the aid of an ocular grid. Without moving the slide, the preparation was then exposed to the vapor from a saturated solution of the α-isomer of benzene hexachloride in xylene. The droplets formed by deliquescence were marked in on the map. To limit the positive designation to those particles carrying significant quantities of toxicant, only those particles were included which were surrounded by a clear-cut droplet. The percentage of active particles was then calculated from counts made on the map (Table I).

The negligible response of the untreated diluent and the 98% response of the undiluted formulation were considered good evidence that the method would give a true measure of the active particles. Each of the diluted formulations showed a higher content of active particles than would be expected from simple mixing. Such behavior is in agreement with the reasonable assumption that mechanical mixing would cause a transfer of toxicant from the impregnated particles to some of the untreated particles.

Some care must be taken in the observations on formulations containing a very wide range of particle sizes or a low concentration of toxicant, particularly when the vapor pressure of the developing solvent is maintained at a level well below saturation. The larger particles may give droplets which are less apparent than those around the smaller. Consequently, the larger particles must be examined more carefully than the smaller before they are classified as active or inactive.

Quantity of Toxicant per Particle

In the previous test the particles were classified into two groups by a discretionary criterion of the quantity of

toxicant to be considered significant. It is often of equal interest to estimate the relative quantity of toxicant per particle and the quantitative distribution of toxicant among the particles of different sizes.

Because the vapor pressure of the solvent is held constant, each droplet will grow until it reaches a constant concentration. The relative volumes of the droplets can therefore be taken as a direct measure of the relative weights of the portions of toxicant originally present, if sufficient time is allowed for equilibrium to be reached. If the surface of the slide is uniform, each droplet will spread to the same extent and its volume can be calculated from its diameter and focal length by the method used for aerosol droplets (6). In the simplest case, where no insoluble material is present, the distribution of toxicant is then directly proportional to that of the droplet volumes.

If a carrier or other insoluble material is present in association with the toxicant, the situation is more complex. The apparent volume of the droplet will be that of the liquid formed by deliquescence plus that of the insoluble included particle. Assuming sufficient liquid to cover the particle and to form a droplet which is essentially a spherical segment, the measured diameter of the droplet on the slide is designated by D, and assuming the particle to be spherical, its diameter is designated by d. Assuming the distortion of the composite droplet (particle plus the sheath of deliquesced liquid) at rest on the slide to be similar to the distortion of a droplet consisting only of liquid, then the measured diameter, D, of the composite droplet can be related to the true diameter, x (of the undistorted composite droplet) and x is proportional to D, or $x = kD$, where k is a proportionality constant or "spreading factor." k can be calculated from the measured focal length of droplets containing no diluent (6). The volume of the composite droplet is then $V_x = \frac{\pi}{6} k^3 D^3$. The volume of the particle is $V_p = \frac{\pi}{6} d^3$. The volume of the liquid alone in the droplet is $V_l = V_x - V_p = \frac{\pi}{6}(k^3 D^3 - d^3)$. If the concentration of the toxicant in the droplet is determined for the solvent, solute, and temperature used, and the density of the carrier is known, the weight concentration of toxicant on any desired particle can be calculated from V_l and V_p.

It is also of particular interest to consider the variation of the quantities V_p and V_l, which are direct measures of the toxicant distribution, with the quantities D and d, which can be measured directly by microscopic means.

From the equations given,

$$\frac{V_l}{V_p} = \frac{k^3 D^3}{d^3} - 1$$

therefore, $\quad \frac{D}{d} = \frac{1}{k}\sqrt[3]{\frac{V_l}{V_p} + 1} \quad (1)$

The ratio D/d and its variation with d can therefore be used to indicate the distribution of toxicant on the different particles by weight or volume.

The simplest but least probable distribution of a toxicant on a carrier of a wide range of particle size is the association with each particle of a weight or volume of toxicant directly proportional to its own weight or volume. V_l/V_p would then be constant, and therefore D/d would also be constant. Microscopic examination of such a formulation after deliquescence would show each droplet having a radius directly proportional to that of the included carrier particle.

A more likely distribution is the association with each particle of a weight of toxicant directly proportional to the surface area of the particle. Then the ratio V_l/V_p would be proportional to the specific surface, or inversely proportional to the diameter of the particle, with $\frac{V_l}{V_p} = \frac{q}{d}$, where q is a proportionality constant including the geometrical constants, the concentration of the toxicant solution droplets, and the over-all toxicant load. Substituting in Equation 1,

$$\frac{D}{d} = \frac{1}{k}\sqrt[3]{\frac{q}{d} + 1} \quad (2)$$

From Equation 2, when the particles are large enough so that q/d is very small, D/d will change very slowly with d, and will approach a limiting value of $1/k$ as d increases indefinitely. As the particles become smaller, q/d becomes more important and D/d will increase more and more rapidly with decreasing d, approaching proportionality with $1/\sqrt[3]{d}$. The particle size d corresponding to a given D/d ratio would be increased by an increase in either the toxicant load or the dilution. Such a formulation would show the larger particles with a low and relatively constant ratio of droplet to particle diameter, but the smaller particles included in droplets of increasing relative diameter.

A third type of distribution, somewhat related to the second, is the association, with each carrier particle, of a layer of toxicant of constant thickness, independent of the size of the particle. This distribution would be practically identical with the second when the particles were large and the layers thin, but would deviate considerably when the thickness of the layer reached the same order of magnitude as the diameter of the particle.

If t equals the thickness of the toxicant layer, which perfectly circumscribes the diluent particle with a diameter of d,

Table I. Response to Xylene Vapor of Mixtures of DDT-Impregnated and Untreated Aluminum Oxide

Wt. % of Impregnated Formulation	Expected No. % of Impregnated Particles	No. % of Deliquescent Particles
100	100	98
50	48	54
25	24	35
12.5	12	18
0	0	0.3

then the volume of the toxicant is equal to

$$V_t = \frac{\pi}{6}\left[(d + 2t)^3 - d^3\right]$$

The volume of the toxicant, V_t, may be expressed in terms of the volume of the liquid V_l by using c as the volume concentration of the toxicant in the droplets under the conditions used, so $c = V_t/V_l$. This volume may be expressed as

$$V_l = \frac{\pi}{6}\left[(d + 2t)^3 - d^3\right]\frac{1}{c}$$

or $V_l = \frac{\pi}{6} \times \frac{1}{c}(6d^2t - 12dt^2 - 8t^3)$.

Substituting for V_l (with $V_p = \frac{\pi}{6}d^3$) in Equation 1

$$\frac{D}{d} = \frac{1}{k}\sqrt[3]{\frac{1}{c}\frac{6d^2t - 12dt^2 - 8t^3}{d^3} + 1} \quad (3)$$

It is apparent from Equation 3 that the value of D/d with k and c constant is fixed by the ratio of t/d rather than the absolute value of either t or d. If $m = t/d$, Equation 3 can be expressed in the form:

$$\frac{D}{d} = \frac{1}{k}\sqrt[3]{\frac{1}{c}(6m^2 - 12m^2 - 8m^3) + 1}$$

(3A)

For a given t, m becomes very small as d becomes large, and D/d approaches a limiting value of $1/k$ as in Equation 2. However, as d decreases, m increases, the term $8m^3$ becomes the governing factor, and D/d approaches proportionality with $1/d$. Thus the droplet diameter D with very small particles would be practically independent of the carrier particle size. This effect follows from the type of distribution, the limit of which would be a sphere of pure toxicant of diameter $2t$. The preparation after deliquescence would show the larger particles with a low and comparatively constant ratio of droplet to particle diameters, like that of the second type of distribution but the very small particles with a ratio increasing so rapidly with decreasing particle size that the droplets would approach a constant diameter independent of that of the particles.

In actual formulations, the smaller particles are usually observed to be surrounded by comparatively larger droplets than are the larger particles, indicating that the distribution follows a pattern closer to that of the second or third types discussed rather than the first. In practice, the particle-size range is usually so wide that it is difficult to adjust the vapor pressure of the solvent so that all particles are completely covered by liquid droplets. In order to keep the droplets around the smaller particles small enough to avoid extensive coalescence, the vapor pressure must be low enough so that the larger particles are only partially enclosed. In this case, the height of the droplet is main-

Table II. Effects on Droplet Size Due to Different Levels of DDT Deposited on Aluminum Oxide of Different Particle Sizes

| | Diameter, μ | | Droplet/ |
% DDT	Mean particle	Mean droplet	Particle Ratios
2.8	84	122	1.45
	153	197	1.29
5.7	86	130	1.50
	165	252	1.54

tained constant by the included particle, so that any change in liquid volume must be accomplished in the other two dimensions. The apparent diameter of the droplet and the ratio D/d, therefore, change more rapidly with the volume than would be indicated by the calculations for the simpler case. It is not uncommon for some of the largest particles to show no surrounding droplet, but to become more transparent, owing to a ring of liquid completely underneath the particle. In qualitative observations it is important to recognize that such a phenomenon may be due, not to a preferential impregnation of the smaller particles, but to the normal geometrical effect of a uniform surface distribution. If careful quantitative calculations are to be made, droplets which do not completely enclose the associated particle cannot be used without prohibitive complication of the calculations.

As a preliminary test of the minimum size differences at which these effects might show up, two fractions of aluminum oxide, one averaging twice the diameter of the other, were each impregnated with two levels of DDT, one twice as high as the other. The diameters of approximately 200 particles of each were measured and averaged. The droplets were developed, and the droplets around the same particles measured and the diameters averaged (Table II).

The range of both size and DDT level were very small compared with those to be expected in practical formulations. The DDT levels were low enough so that the included insoluble particles were not completely enclosed by the droplets. Particles of both sizes carrying the higher level of DDT showed a measurably higher ratio of diameters than those carrying the lower level. The ratio dropped with increasing particle size at the lower DDT level as expected, but increased slightly at the higher level. The low level of DDT on the larger particles had the same calculated surface concentration as the high level on the smaller. As expected, the latter showed a higher ratio than the former. With one exception, the effects were in the direction predicted, indicating that the method could be depended upon to show up differences not greatly

exceeding 2 to 1. By the examination of larger samples and by adjusting conditions so that the droplets completely enclosed the particles, the measurement might be made considerably more sensitive and reliable.

Discussion

The deliquescence method has not as yet been tested on a wide variety of commercial formulations. The theoretical basis of the method is so well known, however, that most of the phenomena can be predicted with certainty once the necessary experimental conditions are established. The present work with arbitrary uniform formulations was designed to establish these conditions and to give a basis for estimating the order of magnitude of the differences which might easily be demonstrated. It was not considered necessary to work with a variety of toxicants to establish the feasibility of the method because good volatile solvents are available for most toxicants and the principles involved are the same.

The method is completely unsuitable in the presence of a constituent consistently more soluble than the toxicant, unless this other material is a solvent which accompanies the toxicant throughout the formulation. In this case, account must be taken of both soluble constituents in any interpretation of the results. As in any microscopic observation on a heterogeneous material, the reliability of the results depends upon care in representative sampling, and upon the size of the sample which it is practical to observe. The precision and accuracy of quantitative conclusions are limited by the difficulties in measurement of irregular particles, in obtaining droplets of uniform spread, in the establishing and maintaining of equilibrium conditions, and by the time required for detailed calculations.

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