

# IN THE LABORATORY

## Preparation of Standard Films of DDT Crystals for Toxicity Studies

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The preparation of dry films of crystalline insecticides is a frequent necessity among investigators concerned with studies of toxicity and mode of action of toxicants. With the advent of the complex organic compounds which have been introduced to the field, the problem of preparing standard films of dry crystalline material which will remain uniform from day to day has become important.

Various techniques have been used in the preparation of such films with DDT; however, any method which involves solution and subsequent precipitation of the compound results in the deposition of supercooled droplets of material and the slow growth of crystals. This leads



FIG. 1. Typical dispersions of DDT/mm<sup>2</sup>: A—25% DDT by weight, B—50% DDT by weight.

to a constant change in the nature of the film deposited and size of the crystals. When dry DDT crystals are dispersed in a dust tower without a diluent, the tendency toward clumping makes it difficult to achieve a uniform film.

To obviate these difficulties a very simple technique has been devised. It consists of formulating the dry crystals, which have been sorted to the desired size range, by mixing them with soluble starch. The dust with the starch carrier is blown into a settling tower with an air stream, and samples are collected on glass plates which have been treated with a very thin film of Mayer's albumin. The plates are prepared in the same manner as are slides for mounting histological sections. The starch can be removed quantitatively from the films by washing in running water. Dispersions of the insecticide can be controlled by varying the per cent-by-weight composition of the dust formulation. The photomicrographs shown in Fig. 1 illustrate the crystal dispersion per square millimeter achieved with 25% and

50% formulations prepared according to this technique. The tendency for the crystals to agglomerate in formulations containing more than 50% DDT limits the preparation of films with a higher number of crystals per unit area.

This method is satisfactory for the preparation of DDT films and can be applied to any other insecticide which is insoluble in water, which can be prepared in a stable crystalline form, and which exhibits the property of supercooling when precipitated from solution.

## Simple Preparation of Transparent Scales<sup>1</sup>

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The Beckman infrared spectrophotometer records values of transmitted energy directly onto a strip chart. The calculation of percentage transmission involves comparison of incident and transmitted energy at many wave lengths and becomes tedious when individual energy values must be read from two separate charts.

Willis and Philpotts (2) suggest the use of transparent scales which can be placed directly on the recorded chart and from which a direct reading of the percentage transmission can be taken. The two records can be placed side by side on the chart by rewinding after the initial run. Their scales were made by a photographic process from a drawn master copy, with subsequent reversal onto a transparent film of the desired size. The process requires photographic equipment for handling large-sized film, and care must be taken to avoid distortion during the process.

In a recent article Neuberger (1) suggested a technique for making lantern slides from cellulose acetate "Permafilm (dull)."<sup>2</sup> This technique can be modified slightly for preparing transparent scales and graphs of any size or type. The "Permafilm," cut slightly oversize, is mounted carefully on a sheet of clear Cellophane and smoothed to remove air bubbles. The desired chart or scale is drawn directly on the dull surface of the "Permafilm" in India ink. A cleaner line can be drawn if the surface is scoured gently with an eraser or a wet piece of cleansing tissue or cloth. The completely dry drawing is then covered with a sheet of "Permafilm" for protection, and the edges are trimmed.

The resultant sandwich is flexible, quite transparent, has a low reading error, slides easily, and is quickly made without special equipment. It does not seem particularly

<sup>1</sup> This work was part of a research program supported by a Research Corporation Grant-in-Aid.

<sup>2</sup> Denoyer-Geppert Company, 5235 Ravenswood Avenue, Chicago 40, Illinois.

subject to elongation or other distortion. This type of scale should find many useful applications.

#### References

1. NEUBERGER, HANS. *Science*, 1948, 107, 23.
2. WILLIS, H. A., and PHILPOTTS, A. R. *Trans. Faraday Soc.*, 1945, 41, 187.

## Continuous Recording of Body Temperatures of Mice

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A record of the body temperature of experimental animals often yields significant information. The usual techniques of thermometry require frequent observations in order that a temperature change of short duration may not be missed. A technique which we have developed for the continuous recording of subcutaneous temperature in mice, using a sensitive recording thermocouple, is described below.<sup>1</sup>

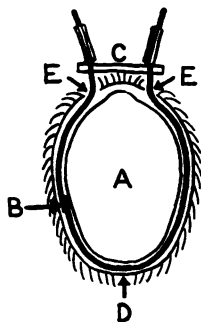


FIG. 1. Cross-section of mouse illustrating location of thermocouple: (A) body of mouse, (B) junction of thermocouple, (C) fiber collar, (D) incision over sternum, (E) incisions over scapulae.

With the mouse under ether anesthesia, three small longitudinal incisions are made: one over the sternum and two posteriorly over the midscapular regions. The thermocouple wire is passed into one of the posterior incisions and manipulated through the subcutaneous tissue to the anterior chest region and around to the other side so that it exits through the second posterior incision. The three incisions are closed by sutures, the thermocouple being left in the subcutaneous tissue of the anterior thoracic region with one wire passing beneath the skin around to the posterior thoracic region of each side (Fig. 1).

The wires are passed through a fiber yoke which is attached to the fur on the back of the animal with collodion. This serves to keep the wire leads from contact with each other. Small cylindrical glass beads, approximately  $\frac{1}{4}$ " long, are threaded onto each wire for a distance of about 5", thus providing adequate insulation for the wires and preventing tangling and breaking of the leads when the

<sup>1</sup>Thirty-gauge iron Constantan enameled wires, silver soldered to form a thermocouple junction, were connected to a Leeds & Northrup Company Speedomax recorder.

animal moves about. The beads are prevented from separating by knotting the wire after the last bead has been threaded (Fig. 2).

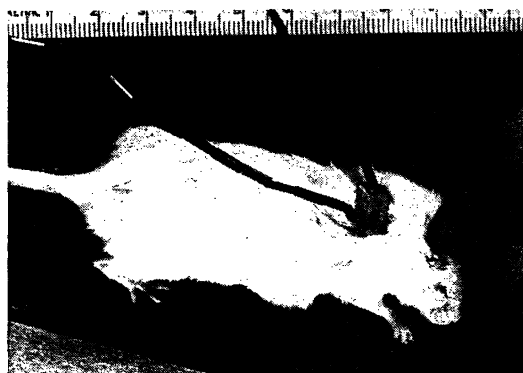


FIG. 2. Lateral view of mouse with thermocouple in place.

The animal is placed in a mason jar of suitable size with a wire mesh insert in the screw-top ring. The lead wires are led through short pieces of rubber tubing secured in this mesh and connected to the terminals of the recording device (Fig. 3). By the use of a Speedomax



FIG. 3. Method of housing mouse when connected to recording thermocouple.

multiple-point recorder it is possible to record the temperatures of as many as 10 animals simultaneously.

This method provides a means for the accurate recording of body temperatures of mice continuously over