Reports

Identification of Insecticides and Acaricides by Comparative Bioassay

Abstract. Toxicants in purified form can be identified by applying a base dose, consisting of the amount causing 50-percent mortality in one organism, to other susceptible organisms. This yields a mortality pattern which "fingerprints" the compound. Toxicants related by chemical structure or biological activity tend to possess similar mortality patterns.

Suitably sensitive, accurate, and rapid methods for qualitative determination of pesticides are needed. Bioassay procedures have desirable features but, almost without exception, only for quantitative determination of known toxicants. This report (*1*) outlines a bioassay method that shows promise with highly purified toxicants.

It has been suggested that limited identification of chemically related pesticides could be made by observing their rate or type of action. Sun (*2*) suggested that organophosphorous compounds could be differentiated as a class from chlorinated hydrocarbons by their faster rate of action in houseflies. Davidow and Sabatino (*3*) distinguished chlordane and related cyclodienes from unrelated chlorinated hydrocarbons by the characteristic rates of response of goldfish. Laug (*4*) could differentiate the gamma isomer from other isomers of benzene hexachloride by comparing the relative toxicity to the housefly. Burchfield and Storrs (*5*) noted differences in the photomigration response of mosquito larvae affected by different toxicants. The methods developed thus far seem to be limited to separation within a known and restricted group of toxicants, or between groups of compounds widely different in chemical composition and biological activity.

It is well known that there are large differences in the susceptibility of organisms to pesticides. This is a substantial factor in the development of the great variety of insecticides and acaricides in current use. Busvine and Barnes (*6*) showed these differences experimentally but did not utilize them for qualitative determination of toxicants. The method reported here is based on such differences, in four quite unrelated (and presumably physiologically different) organisms.

The four test organisms were the wild strain of the pomace fly, *Drosophila melanogaster* Meig.; the rusty grain beetle, *Cryptolestes ferrugineus* Stephens; a stored-products mite, *Tyrophagus putrescentiae* Schrank; and the brine shrimp *Artemia salina* Leach. *Drosophila* has been widely used by a number of investigators for quantitative determination of at least 13 pesticides. *Cryptolestes* has not been used previously for bioassays, though other beetles from stored grain (*Tribolium* spp.) have been. *Tyrophagus* has not been tested as a bioassay organism, but acaricides have been evaluated against it (*7*). *Artemia* has been used as a test organism in the bioassay of nine toxicants (*8*).

To insure uniformity, methods of obtaining and exposing the test organisms were standardized as far as possible. *Drosophila* adults were obtained within 24 hours of emergence (*9*). *Cryptolestes* were removed from cultures at intervals such that adults 4 days old or less were obtained. These organisms were exposed, at 78°F in the dark, to a dry film of toxicant on the bottom of 1.25-oz. salve jars. Evaluations were made at 24 hours, with a further reading at 48 hours for *Cryptolestes*. The method of exposure for *Artemia* and *Tyrophagus* is given in detail elsewhere (*10*), time of exposure for these organisms being 24 hours.

Dosage-mortality relationships were developed by the log-probit method outlined by Finney (*11*). Thirty-one insecticides and specific acaricides in highly purified form were tested. These included the majority of pesticides in common use and also a few less well known ones because of their close chemical relationship to more common toxicants.

Log dosage-probit mortality regression lines can be used in a number of ways as a possible means of distinguishing toxicants. The angle of the slope of the line is typical for a given toxicant for any particular organism under standard conditions. It should also be possible to develop a typical toxicity ratio at the LD_{50} level, the amount required for this degree of effectiveness in various organisms being compared with the LD_{50} dosage for a base organism. Such methods were rejected because they require developing a regression line anew for each organism every time an unknown is tested, though these methods could be used in extremely difficult cases.

The method tested was that of establishing a base dose of an unknown toxicant in solution for one organism at the LD_{50} level. This dose was then applied to the other three organisms so as to yield specific mortalities within calculable limits. Thus, by starting with a standard dose, a compound could be "fingerprinted" by its effect on other organisms. With this method any of the organisms that is suitably sensitive will serve to establish a base dose. If the compound is sufficiently specific in its toxicity for several organisms, it can be separated on the first application of the dose standardized at the 50-percent mortality level for one of the organisms. *Drosophila* appears to be the most useful of the organisms tested for establishing a base dose for most toxicants. Exceptions are the specific acaricides, which do not affect *Drosophila*.

Figure 1 illustrates the separation of

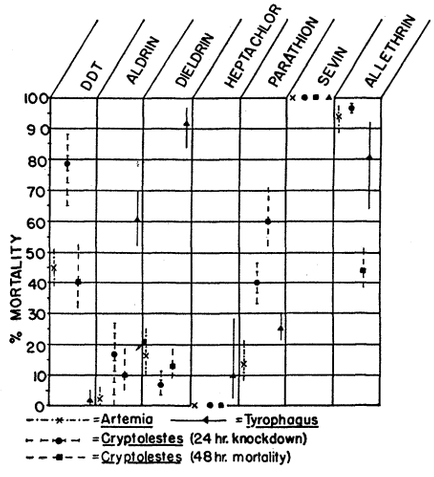

Fig. 1. Toxicity, for three organisms, of seven insecticides at the LD_{50} level for *Drosophila*.

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

some representative pesticides (*12*) when the LD_{50} for *Drosophila* was used as the base dose. The vertical extension of each percentage of mortality represents the 95-percent confidence interval under our test conditions. It is obvious from this example that even closely related pesticides can be distinguished. For instance, with the *Drosophila* base dose, aldrin can be readily distinguished from dieldrin by its effect on the mite *Tyrophagus*. Allethrin can be confused with these two toxicants when tested on the basis of this single criterion, but clarification occurs in the effect of these toxicants on *Artemia* and *Cryptolestes*. Needless to say, there are instances where careful cross-checking of the effectiveness in all organisms is necessary to separate the 31 compounds tested; this can be done by selecting a different organism to establish a base dose.

Certain toxicants tend to separate out as groups; this indicates a chemical relationship or suggests similarity in mode of action. Chlordane and related cyclodiene derivatives form similar mortality patterns, as do several organophosphorus compounds. In like manner, all the specific acaricides affect only *Artemia* and *Tyrophagus*.

It will usually be necessary to remove waxes and other interfering substances from plant extracts before using this method. It is hoped that this procedure can be used for identification of an unknown toxicant, subject to confirmation by chemical procedures.

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References and Notes

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Temperature Fluctuations Accompanying Water Movement through Porous Media

Abstract. Temperature measurements were made at localized sites in bentonite, kaolinite, and small glass beads during infiltration. The temperature of the medium was observed to rise gradually and then to drop sharply as the infiltrating water approached and reached the measurement site. Temperature fluctuations were observed to be about 9°C for bentonite, 4°C for kaolinite, and 0.1°C for the glass beads.

The detection of water movement at localized sites in porous media, though frequently difficult, is usually possible. Determining whether the water arrives as a vapor or as a liquid is much more difficult, and often may be impossible. Velocity measurements which distinguish between liquid flow and vapor flow are even more complicated, but these measurements are precisely what is needed to investigate the kinetics of water movement in unsaturated porous media.

This is an old problem and one which received the early attention of soil physicists, although a satisfactory solution is still lacking. Apparently, Bouyoucos (*1*) was the first to place an air gap in the medium to distinguish liquid and vapor movement of water in soils. He arranged the apparatus containing the soil so that water vapor could diffuse unimpeded across a small air gap which completely interrupted liquid flow. This method was also used, with some modification, by Lebedeff (*2*) and by Taylor and Cavassa (*3*). By measuring the change in the distribution of a small amount of salt added to the medium, Gurr *et al.* (*4*) distinguished vapor flow from liquid water flow. More recently, Rollins *et al.* (*5*) used an external capillary on a closed soil-water system to measure vapor movement induced by temperature gradients. Measurements were taken at the steady state when the liquid flow through the capillary was presumed to equal the vapor flow through the unsaturated soils. In an excellent review of the whole problem of water and heat flow through unsaturated media, Philip and deVries (*6*) have discussed the experimental procedures and results of these investigations. They concluded that experimental methods have not satisfactorily distinguished between liquid and vapor transfer because of various complications inherent in each of the methods.

The measurement of temperature fluctuations due to the heat effects accompanying phase changes offers a means of differentiation and measurement of vapor and liquid flow. Let us visualize a liquid front advancing through a porous medium. Water molecules continually evaporate from the liquid and diffuse into the voids ahead, colliding with and being sorbed by the medium. Thus, one may predict that the advancing liquid front will be cooled, and that the medium ahead of the liquid front will be warmed. Hence, a temperature sensor located ahead of an advancing liquid front should show a gradual temperature rise due to sorption and condensation of water vapor followed by an abrupt temperature drop due to the arrival of the cooled liquid front.

To check the validity of this prediction, a series of experiments was performed. Ultrasmall thermistors and a potentiometer of high sensitivity made the experiments possible. Bentonite, kaolinite, and glass beads (*7*) were used as the porous media. Several small, cylindrical, Plexiglas sample holders were fabricated with provisions for embedding the thermistors in the medium during the preparation of an experiment and for controlling the introduction of water. Thermistor circuits which generated self-heating effects of less than 0.01°C were used in all the experiments. During the course of an experiment, voltage drops across the thermistors were continuously monitored by a two-pen Bristol recording

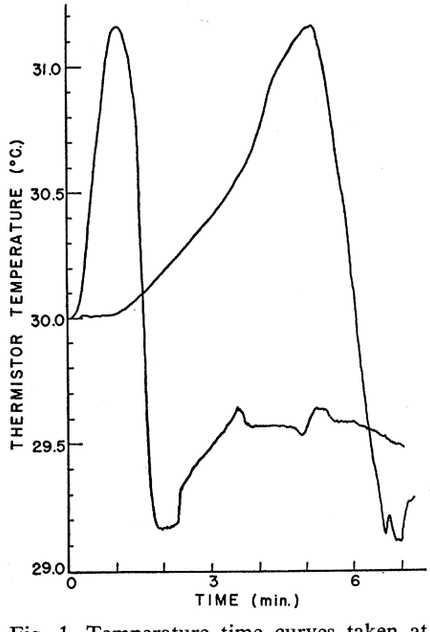

Fig. 1. Temperature–time curves taken at two sites in a kaolinite sample during infiltration by water. The curves were obtained by means of two thermistors embedded in the kaolinite sample. The temperature sensed by the thermistors is the ordinate and the time required for each thermistor to respond after the introduction of the water is the abcissa. The thermistor nearest the source of the infiltrating water gave the first response.