

could be a useful adjunct to potato breeding in these circumstances.

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## ENTOMOLOGY

### Influence of Relative Humidity on the Uptake of Insecticides from Residual Films

It is now well known that the effectiveness of insecticides absorbed by certain soils is much greater at high than at low humidity. Analytical results<sup>1-3</sup> have shown that this is not due to an increase in concentration of the toxicant in the superficial layers of the soil.

It has been suggested<sup>1,2</sup> that water is adsorbed preferentially on the soil particles, with consequent displacement of the insecticide molecules and increase in their mobility. Under these conditions the toxicant was thought to be more readily available to the insect.

The increased availability of dieldrin in humid conditions has now been confirmed by the direct determination of toxicant pick-up by insects from residual films on glass.

Insects were exposed in glass tubes, the inner surface of which (200 cm<sup>2</sup>) had been treated with dieldrin labelled with carbon-14. A gentle flow of air was led through the tubes during exposure to prevent accumulation of insecticide vapour and condensation of insecticide directly on to the insects<sup>4</sup>. Pick-up of toxicant by the insects was thus achieved mainly by contact with the treated surface. The relative humidity was varied by feeding either dried or humidified air into the exposure tubes (relative humidity < 10 or > 90 per cent respectively). The exposure tests were carried out using dieldrin-resistant strains of *Musca domestica* and *Anopheles gambiae*, and a normal susceptible strain of *Aedes aegypti*.

After exposure, extracts of both the insects and the remaining insecticide residue on the glass were examined by a liquid scintillation counting technique. The total recovery of radioactive material, in terms of dosage applied, amounted to 95 per cent.

Table 1 shows that uptake of insecticide was always higher under humid than under dry conditions, and provides a quantitative confirmation of previous findings, indicating a greater availability of toxicant at higher relative humidity.

Table 1. UPTAKE OF <sup>14</sup>C-DIELDRIN UNDER DRY AND HUMID CONDITIONS

Species	No. of insects	Residue (μg)	Exposure time (h)	Insecticide recovered from insects (μg)		Ratio of uptake humid/dry
				Dry	Humid	
<i>Musca domestica</i>	10	1	24	0.480	0.769	1.6
<i>Aedes aegypti</i>	20	0.1	17	0.022	0.069	3.1
<i>Anopheles gambiae</i>	30	1	15	0.067	0.606	9.0

It is interesting to note that the influence of humidity on the uptake of dieldrin is least with *Musca domestica*, which walks the most actively during exposure, and most pronounced with *Anopheles gambiae*, which seldom walks at all. *Aedes aegypti* is intermediate in both respects.

The most likely explanation for this inverse relationship is that insects walking over the treated surface pick up the insecticide mechanically from a large area compared with that contacted by the stationary insects. The rate of pick-up by the stationary insects would depend largely on the mobility of the insecticide molecules in the residual film, and under these conditions the greater influence of relative humidity would be expected.

The results obtained with residual films on glass were confirmed in further exposure tests on dieldrin-impreg-

nated filter paper, which showed that the uptake of toxicant by *Anopheles gambiae* was 2.2 times higher under humid than under dry conditions. It seems likely, therefore, that the effect of humidity is not restricted to specific substrates. Furthermore, similar observations have been made with γ-BHC and with DDT on dried soils, and it would appear that we are dealing with a fairly general phenomenon. The influence of relative humidity should, therefore, receive careful consideration in the interpretation of bioassay tests of the kind described here.

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## MICROBIOLOGY

### Endotoxoid Preparations

THE methods which are used for the preparation of non-toxic but highly antigenic toxoids from Gram-positive bacterial toxins could not be applied to the detoxification of toxic *O*-antigens of Gram-negative micro-organisms.

During investigation of the relationship between chemical structure and various biological properties of Gram-negative bacterial *O*-antigens, it was found possible to abolish selectively one or more biological activities by a variety of chemical treatments<sup>1</sup>. Endotoxic *O*-antigens soluble in trichloroacetic acid were isolated from *Serratia marcescens* cultivated on beef infusion broth. The lyophilized preparations were purified by extraction with methanol, a procedure which removes inert impurities but does not influence the biological properties of the endotoxins<sup>2</sup>. The purified materials were then subjected to chemical procedures. Those treatments which diminished the mouse lethal toxicity while retaining the reactivity with homologous rabbit *O*-antiserum were further investigated. Three methods were found to be especially effective.

(1) 100 mg of the foregoing endotoxin refluxed for 60 min with 10 ml. of 2 per cent boron trifluoride in anhydrous methanol resulted in the almost complete dissolution of the material in this solvent. When the reaction mixture was diluted with 4 volumes of water and dialysed against distilled water for 48 h, the material showed less than one-tenth of the original lethal toxicity.

(2) 100 mg toxic *O*-antigen preparation was refluxed for 60 min with 20 ml. of 0.02 M potassium methylate in anhydrous methanol. The insoluble residue was filtered on a glass filter, washed with methanol and dried in a vacuum desiccator. This preparation was practically free from any toxic properties.

(3) Our earlier observation showed that the use of a powerful dissociating agent formed by concentrated pyridine and formic acid in a 2 : 1 ratio dissolves bacterial glycolipids at boiling water bath temperature without altering their mouse lethal toxicity. A 10 per cent aqueous solution of the same reagent under identical conditions in 60 min not only dissolved the *O*-antigen preparation but detoxified it as well. 100 mg endotoxin and 10 ml. of concentrated pyridine and formic acid in a 2 : 1 ratio were first stirred in a boiling water bath. After 15 min 90 ml.

Table 1. TOXICITY AND ANTIGENICITY OF THE PREPARATIONS

Preparation	LD <sub>50</sub> (μg)	Precipitated (μg)	
		Antibody N/μg preparation*	Rabbit serum titre after immunization†
Parent <i>O</i> -antigen	160	5.4	1 : 1,280
Endotoxoid 1	2,300	1.2	1 : 320
Endotoxoid 2	4,000	9.4	1 : 1,280
Endotoxoid 3	2,700	11.0	1 : 1,280

\* Measured by the ultramicro-bromsulphthalein test.

† Determined by passive haemagglutination.