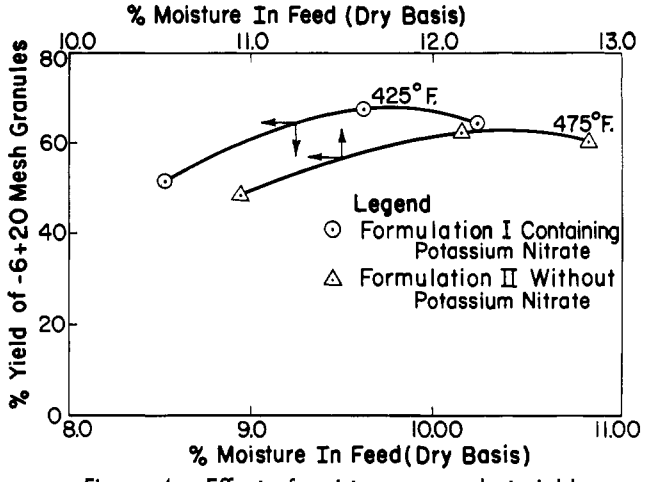

Figure 4. Effect of moisture on product yield

Table IV. Averaged Maximum Variation in Product Nutrient Content

Formulation	N	P_2O_5	K_2O
I	0.962	1.112	1.360
II	1.144	1.396	1.642

a maximum yield of approximately 62% at 12.2% moisture. The more pronounced dependence of yield on moisture in Formulation I is probably due to a higher solubility of the salt phase. Table III shows the nutrient distribution of both formulations in the various sized fractions of the granules. The averaged maximum variation of nutrient content in the product was lower for Formulation I than for Formulation II for all nutrients (Table IV).

The extent of agglomeration could be controlled by the mixing time after the addition of the water. Formulation II formed large agglomerates in the batch reactor, while Formulation I formed more uniformly sized granules. This was primarily due to the lower moisture requirement of the salt solution phase of Formulation I. This lower moisture resulted in a drying time of only 30 minutes at 425° F. for an average product moisture content of less than 1%; Formulation II required 45 minutes drying at 475° F.

Conclusions

Because of the high solubility of potassium nitrate at the elevated temperatures of granulation, Formulation I, containing potassium nitrate, required only 9.6% moisture for a maximum yield of 67.6% in the −6 +20-mesh size fraction. Formulation II, without potassium nitrate, required 12.2% moisture for a maximum yield of 62.3%. This yield was considered good, since no recycle was employed.

For a product with an average moisture content of less than 1%, Formulation I required a drying time of 30 minutes at an inlet gas temperature of 425° F; Formulation II required 45 minutes at 475° F.

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INSECTICIDE MODE OF ACTION

Absorption and Binding of DDT by the Central Nervous System of the American Cockroach

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It is hypothesized that DDT interferes with nervous function by forming a charge-transfer complex with a component of nerve. Studies on the kinetics and equilibria of DDT penetration into and out of nerve cords indicated the formation of complexes of DDT with two components of the cord and having dissociation constants of approximately 6×10^{-6}M and 1.5×10^{-7}M. At 10^{-5}M DDT, about 83% of the DDT in the cord is complexed. Two complexes have been partially purified on Sephadex and DEAE-cellulose columns. One contains protein and is extractable by butanol.

The chlorinated hydrocarbons are presumed to owe their toxicity against insects to effects upon the nervous system because tremors and later paralysis are the most prominent symptoms of poisoning, and because profound disturbances can be shown electrophysiologically in nerve preparations from poisoned insects. The basis of these effects has never been experimentally demonstrated. However, Mullins (7) has suggested that DDT and related compounds owe their properties to precise fit in a hypothetical intermolecular lattice, and Gunther *et al.* (3) have proposed that Van der Waals binding to a protein in nerve is involved. From the effects of DDT upon negative afterpotentials in single axons and the modification of these effects by applied

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ions, Narahashi and Yamasaki (*8*) have suggested that the immediate cause of the disturbed function is interference with potassium transport. The authors have speculated (*10*) that DDT forms a charge-transfer complex with a component of the axon, thus disrupting transmittance. The present study was designed to determine whether DDT does in fact bind to a component of nerve.

Materials and Methods

The abdominal nerve cord of adult male cockroach (*Periplaneta americana*) was used throughout this investigation. The cockroach was dissected in Ringer solution (components: 9.13 grams of NaCl, 0.0233 gram of KCl, 0.0378 gram of $CaCl_2$, 0.024 gram of NaH_2PO_4, and 0.256 gram of Na_2HPO_4 in 1 liter of distilled water), and a portion of an abdominal nerve cord, excluding the first thoracic and the last abdominal ganglia, was transferred into 1 ml. (or 0.5 ml. in some cases) of a suspension in 1% ethanol of $10^{-5}M$ C^{14}-DDT (ethane-labeled) with a specific activity of 4.93 mc. per mmole.

The samples were allowed to stand at 25° C. for various time intervals. The cords were then taken out and washed briefly with fresh saline, and the excess moisture was removed with a filter paper. The nerve cords were then dissolved in 1 ml. of hot 50% Hyamine. To a 1-ml. portion of the sample, 9 ml. of dioxane counting solution (*4*) were added, and radioactivity was counted in a Packard liquid-scintillation spectrometer.

Sephadex columns were prepared by suspending Sephadex G-50 coarse grade gel in 0.9% NaCl and packing it as 30 × 1 cm. columns. Active diethylaminoethylcellulose (Standard DEAE-cellulose, Carl Schleicher and Schuell Co.) was suspended in $1N$ NaOH and neutralized with $1N$ HCl, then washed with distilled water and $0.0175M$ sodium phosphate buffer (pH 7) until the wash became pH 7.0. It was then packed as a 25 × 1.5 cm. column. The DEAE-column elution program was as follows: 100 ml. each of (I) $0.0175M$ sodium phosphate buffer (pH 7.4), (II) $0.1M$ buffer, (III) $0.1M$ buffer + $0.4M$ NaCl (1:1), (IV) $2M$ NaCl, and (V) $4M$ NaCl

Results

Penetration of DDT into Isolated Nerve Cord. An isolated nerve cord was weighed and allowed to stand in 0.5 ml. of $1 \times 10^{-5}M$ C^{14}-DDT for various time intervals, and the amount of DDT penetrating the cord was measured. The average cord weight was 2 mg., and the data given as "per cord" have been adjusted proportionately on a weight basis. The 100% penetration value was obtained by immersing five nerve cords for 24 hours in a solution containing 17.6 μg. of C^{14}-DDT per gram of cord. The results (Figure 1) indicate the presence of two clearly visible phases of penetration: first, a quick phase which takes place within the initial 1 minute, and, second, a slow phase. The second phase follows first-order kinetics. The fast uptake is responsible for approximately 30% of the total pickup.

To test the reproducibility of this assay method, six nerve cords were independently assessed for their total DDT-penetration rate. The result was satisfactory, the standard error being 3.5% of the average (actual figures: 186.8 ± 6.5 c.p.m.).

Outward Diffusion of DDT from the Nerve Cord. To test the possibility that DDT is tightly bound to certain substances in the nerve cord, five nerve cords were first allowed to stand in $10^{-5}M$ DDT suspension for 10 minutes. Thereafter, the external liquid was first removed by a filter paper, and cords were quickly transferred into 1 ml. of saline where they were allowed to stand for various time intervals. The radioactivity in the ambient water was then assessed. The results (Figure 2) show that the outward diffusion of DDT also takes place in two steps. The initial fast diffusion takes place within 30 seconds. The rate of diffusion thereafter became almost negligible, indicating that some portion of DDT is tightly bound to the nerve cord and, therefore, cannot be extracted by simple diffusion.

Effect of DDT Concentration on Uptake. The above experiments indicate that the rate of DDT passage into and out of the nerve cord does not follow simple first-order kinetics, though the slow phase of each reaction seems to fit such kinetics. Another convenient way to detect any deviation from the simple diffusion theory is to test the rate of diffusion at various concentrations, since the rate of diffusion, if it follows simple diffusion theory, is expected to be directly proportional to the external concentration of the diffusing compound (*2*).

As is shown in Figure 3, the amount of DDT taken up by the nerve cord in 10 minutes is not propo tional to the concentration of DDT, although the amount increased proportionally at concentrations higher than $5 \times 10^{-5}M$, with a linear relation between uptake and concentration. The plateau found in this experiment suggests saturation of a binding site responsible for a part only of the total binding of DDT. To establish the possible role of the extracellular space in the nerve cord (*11*), DDT was added to a nerve-cord homogenate. Unabsorbed DDT was eliminated from the solution either by adding 40 mg. of dry Sephadex G-50 gel, or by passing the final solution through a Sephadex column which retains all free DDT (see below). The result indicates an identical reaction rate of absorption by the homogenate with that by the intact nerve cord (Figure 4).

The results given in Figures 3 and 4 cover two widely separated ranges in which saturation phenomena were observed. In the higher range (Figure 3), saturation of a component (α) was achieved at about $2 \times 10^{-5}M$, the calculated dissociation constant—i.e., half-saturation value—being $6.3 \times 10^{-6}M$. The quantity of this component

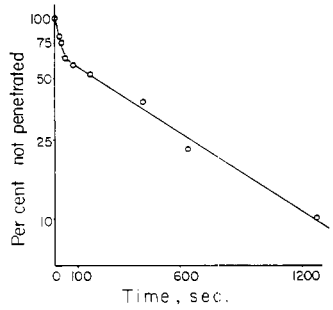

Figure 1. Per cent uptake of DDT (external concn., $1 \times 10^{-5}M$) by abdominal nerve cord of the American cockroach against time

Data represent per cent of the value for "infinite time," as measured in 24 hours' equilibrium experiment (17.6 μg./gram). Dotted line is extrapolation of slow phase to estimate its contribution in early seconds

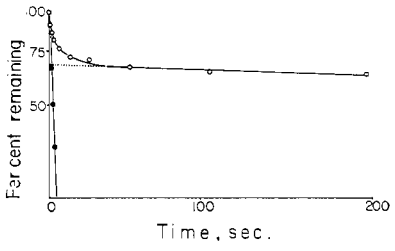

Figure 2. Outward diffusion (open circles) of DDT from the nerve cord

Cords had previously been immersed in $1 \times 10^{-5}M$ DDT and then were transferred into plain saline solution to study amount of DDT diffusing out. Contribution of the fast phase (closed circles) calculated by subtracting extrapoloted contribution (dotted line) of slow phase from observed points

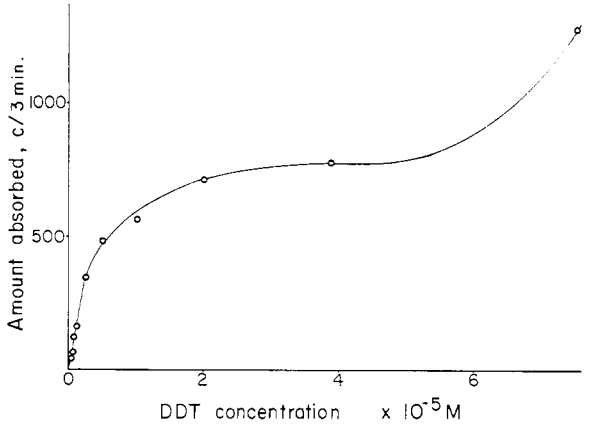

Figure 3. DDT total uptake by the nerve cord as a function of DDT concentration

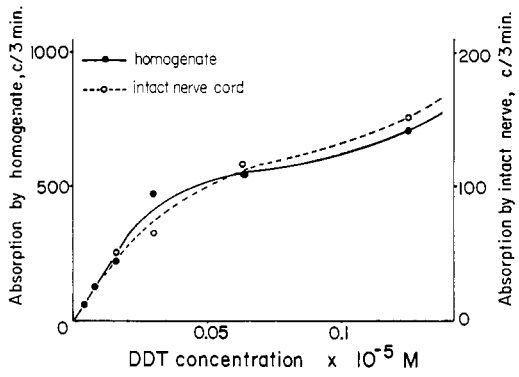

Figure 4. DDT total uptake by the nerve and nerve homogenate (5 cords) as a function of DDT concentration

Free DDT removed by Sephadex gel filtration

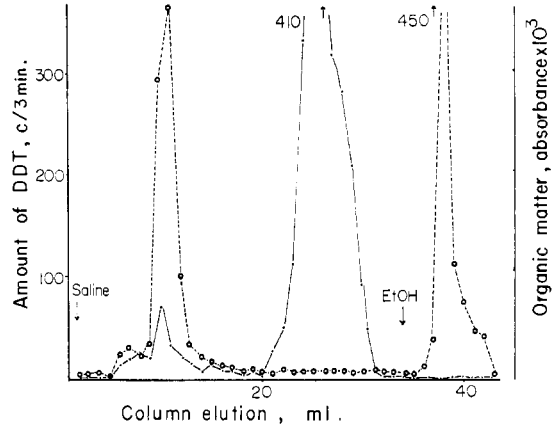

Figure 5. Sephadex column elution of DDT complex (peak 1) and unbound DDT (peak 2, broken lines, open circles)

Organic matter is expressed by unbroken lines and closed circles. Upward arrows indicate peak is off page, values as shown

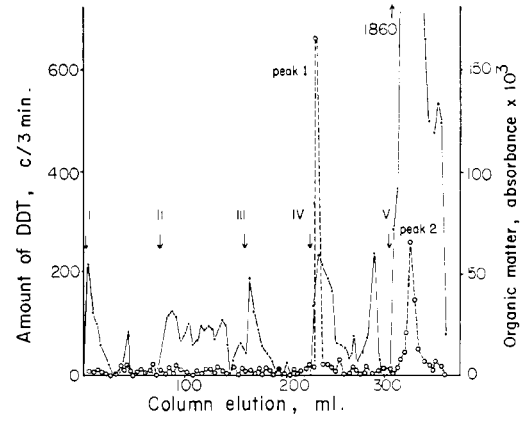

Figure 6. DEAE-cellulose column elution of DDT complexes

Downward arrows indicate position where each stepwise elution occurred: (I) 0.0175M sodium phosphate buffer (pH 7.4); (II) 0.1M buffer; (III) 0.1M buffer + 0.4m NaCl (1:1); (IV) 2M NaCl; (V) 4M NaCl. Symbols as in Figure 5

was equivalent to 18.6 mμmole of DDT per gram of cord weight. In a lower range, a second component (β) saturating at about $5 \times 10^{-7}M$ was observed, with a dissociation constant calculated at $1.5 \times 10^{-7}M$, and a quantity equivalent to 9.3 mμmoles per gram of cord. These dissociation constants are appropriate only if each mole of component binds one mole of DDT. At higher concentrations there was a linear relationship between concentration and uptake, suggesting diffusion into a phase or component (γ) which is not saturated even by $6.4 \times 10^{-4}M$ DDT. It is possible that under these concentration conditions, a nonspecific lipid phase is involved.

The γ contribution at $10^{-5}M$ was calculated by extrapolating to zero the upper part of the curve in Figure 3, and reading off the value at $10^{-5}M$. The α component was saturated even at concentrations below $10^{-5}M$ (Figure 4). Its contribution was obtained by extrapolating to zero the upper part of the curve in Figure 4, reading off the combined β and γ contributions at the saturation value (about $5 \times 10^{-6}M$), and subtracting it from the total absorption at that value. The β contribution at $10^{-5}M$ was obtained by subtracting the α and γ contribution from the total absorption at $10^{-5}M$. These calculations give approximate contributions at $10^{-5}M$ of 9, 74, and 17% for the α, β, and γ components, respectively. Possibly this value for the γ component corresponds roughly with the finding in the experiment shown in Figure 1 (which was performed at $10^{-5}M$), that about 30% of the radioactivity goes in very fast; Figure 2 suggests that about 30% comes out very rapidly. Probably, this rapid penetration and loss is associated with the γ fraction, which does not involve binding to any component. By contrast, the loss of activity from the α and β fractions is extremely slow, and one cannot distinguish between them. If, for both α and β, formation of complexes occurs—e.g.,

$$\mathrm{DDT} + \alpha \underset{k_2}{\overset{k_1}{\rightleftarrows}} \mathrm{DDT} \cdot \alpha$$

then the low values for the k_2's (presumably corresponding to the low rates of loss) are compatible with the very low values for dissociation constant, K, for $K = \frac{k_2}{k_1}$.

Column Separation of DDT-Complex. An attempt was made to separate the binding substances which react with DDT at $1.0 \times 10^{-5}M$. The homogenate was first allowed to stand with DDT for 10 minutes at 25°C., then the mixture was quickly brought to 0° C. A 1-ml. aliquot of the homogenate containing five nerve cords, equivalent was then added to a Sephadex column. A DDT complex was eluted as a peak coinciding with a small peak of organic matter [measured as absorbance at 280 mμ (Figure 5)]. The main organic peak was eluted much later, and unbound DDT itself could only be eluted by 96% ethanol. The DDT complex was further purified on a DEAE-cellulose column. The chromatogram obtained (Figure 6) clearly indicates that the DDT complex is eluted at two places (peak 1 and peak 2, respectively). The latter fraction was associated with a larger quantity of organic matter. It is not possible to state the connection between the two column fractions and the α, β, and γ components described above.

Characterization of a DEAE Fraction. The peak 1 fraction of the DEAE column purified preparation had a broad shoulder in the UV-absorption spectrum ranging from 245 to 270 mμ. Its radioactivity could be extracted into butanol from aqueous phase, although only approximately 30% of activity could be transferred into ether from an equal volume of aqueous fraction. It is not possible to say whether this transfer

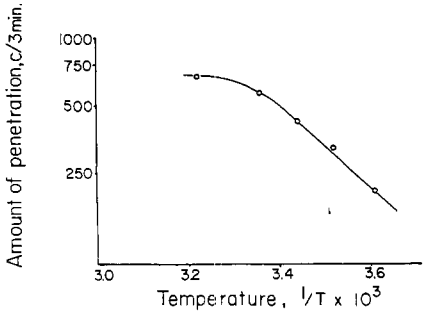

Figure 7. DDT uptake by nerve cord as a function of temperature

represents the whole complex or dissociated DDT produced by the treatment with these organic solvents. The fraction reacted with Folin-Ciocalteu's reagent indicating a presence of 20 to 30 μg. per ml. of protein equivalent, based on calibration with crystalline bovine albumin. Judging from the result of the Sephadex column experiment, the complex has a relatively large molecular size.

Effect of Temperature on Rate of DDT Uptake by the Nerve Cord. DDT has a negative temperature coefficient—i.e., its toxicity is enhanced when the temperature is lowered. To investigate whether this phenomena is related to the gross amount of DDT uptake by the nerve cord, the absorption of $10^{-5}M$ DDT was tested on the intact cords. The relation between temperature and the rate of diffusion can be expressed in a linear fashion when the reciprocal of the absolute temperature is plotted against the logarithms of the amount penetrated (*5*). The result (Figure 7) indicates that the rate of DDT penetration steadily increases from 4° to 25° C. to reach a plateau at 37° C.

Discussion

The question of DDT solubility raises several problems. Saturated solutions in water have been reported as being as high as 1000 p.p.b. ($2.8 \times 10^{-9}M$) (*9*), and probably more correctly as 1.2 p.p.b. ($3.4 \times 10^{-12}M$) or less (*7*). The value can be increased to $1.2 \times 10^{-2}M$ by a lipoprotein (*6*). In the present work, the values given are based on the assumption of true solution, and the DDT behaves as if this were the case.

The rate of DDT outflow from the nerve cord indicated that a portion (approx. 30%) of DDT is rather loosely associated, and the rest is tightly bound to the cord. Apart from this absorption phenomenon, the rate of inward and outward diffusion with respect to time closely follows first-order kinetics. The rate of total DDT penetration also proportionally increases with the external DDT concentrations higher than $5 \times 10^{-5}M$. These facts indicate that the gross behavior of DDT toward the nerve cord is in accord with a simple law of diffusion.

No serious effort was made to identify the binding substance(s), for the amount involved was too small to permit any chemical analysis at present. The most obvious candidates for the binding substances are the lipids of the nerve cord, as DDT is one of the most apolar compounds so far known. The difficulty is that the DDT complex is not easily extracted by ether, though this does not exclude the possibility that a water-soluble lipoprotein is involved in the reaction. However, this is only speculation, and further studies are needed to draw any decisive conclusion.

DDT potency for insects decreases with increasing temperature. Total uptake into nerve cords actually increased with temperature, and total uptake is, therefore not simply correlated with potency. However, the α, β, and γ components were jointly studied in the temperature experiment, and it is entirely possible that the individual components might respond differently.

In conclusion, it has been shown that DDT does indeed complex with components of insect nerve, and these complexes have been isolated. Whether these complexes are of the charge-transfer type (*10*) and whether they play a role in poisoning are matters requiring further study.

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INSECTICIDE REACTION WITH NERVE

Interactions of DDT with Components of American Cockroach Nerve

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THE ACTUAL TARGET of DDT in insects and mammals is considered to be the central nervous system, as judged by symptomological observations (*2*). Electrophysiological evidence (*7*) suggests that DDT blocks the transport of cations (particularly potassium) across the nerve membrane. The mechanism of this blockade is not known, although several hypotheses to explain the interaction of DDT with the nerve membrane have appeared. For instance, Mullins (*6*) has hypothesized that DDT fits into an intermolecular lattice, and Gunther *et al.* (*3*) have postulated binding of DDT to proteins in the nervous system. All of these theories suggest the entry and fixation of the DDT molecule to the nerve components, but do not answer the basic problem of the way in which DDT, thus fixed, can block ion transport.

The authors have suggested (*8*) that DDT acts by forming a charge-transfer complex with a component of the axon, thus destabilizing it. (Complexes of this type may form between two sterically matched molecules, one of which is a good electron donor, the other a good electron acceptor; complex for-

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