Defence Science Journal, Vol.1, No.2, January1950, pp. 175-186

TOXICOLOGY OF INSECTICIDES—SOME RECENT WORK AT THE T.D.E.L. (S), KANPUR.

By Mr. S. K. Ranganathan, Control Officer, Technical Development Establishment, Laboratory (Stores), Kanpur.

Introduction.

1. The chemical control of insect pests whether in the field or in the warehouse or in the home is now an established practice in civilised countries of the world, and its contribution particularly to the well-being of the human race is an acknowledged fact. Although the history of insecticides would seem to date back to about 1000 B.C. it appears that until the last few decades, such information as was available was rather empirical and lacked scientific precision. Often in the past, the list of quack remedies against insect pests assumed such alarming proportion, that the entomologists were often inclined to be sceptical about the concept of chemical insecticides. Happily, recent developments in the methods of assessment of insecticides have in their turn given fresh impetus to the search for more potent insecticides in a rational manner and while the search continues, confidence in such of them as are now available is based on sound scientific knowledge.

2. Insecticides are generally divided into three classes. These are : (1) Stomach poisons, (2) Contact poisons and (3) Fumigants. The effect of stomach poisons is manifest only when the (masticating) insects swallow them along with their food. Arsenicals, fluorine compounds, etc., fall in this category. The second class of insecticides kill by contact and would appear to be specially suited for insects whose mouth parts have been transformed into a sucking apparatus. Certain sulphur and copper preparations and natural substances such as fish oils, mineral oils, nicotine (tobacco), pyrethrins (pyrethrum), rotenone (from derris root), etc., are used as contact insecticides. The synthetic insecticides like DDT, (Dichlorodiphenyl-trichloroethane) and "Gammexane" (Benzene hexachloride) also belong to this class of compounds. The third group, fumigants, act in the gaseous state through the respiratory system and examples are hydrocyanic acid, carbon disulphide, chlorinated hydrocarbons like ethylene dichloride, methylbromide, etc.

3. In recent years the study of each of the above three groups of compounds has constituted a specialised branch in itself, with its own specific laboratory methods of assay and estimation of toxicity. At the Technical Development Laboratory (Stores), Kanpur, work has now been in progress for the last few years on the methods of application and study of toxicity of various contact insecticides to different types of insects. The object of the present paper is to discuss briefly the principle behind the toxicological experiments, describe the methods of assay and state such outstanding results as have been obtained recently.

Principles of Insecticide Toxicology.

4. Insecticide Toxicology is concerned with the study of the potency of insecticides assessed by means of the reactions it produces in living insects (Biological assay). This takes the form of a *quantitative* study of different insecticides applied against a single insect species, or single chemical against different species, or a single insecticide against a single species with controlled variations of the environment. Such a study has its impact on the mechanism of insecticidal action, and opens up problems of fundamental physiological importance but these latter are less obviously associated with the needs of economic entomology. It is however well to stress here that certain aspects of toxicological studies are not amenable to mathematical precision. Nevertheless the results obtained in these are of considerable practical importance.

5. One type of assay in toxicological work, which has found wide usage is that dependent upon the *quantal* or all-or-nothing response. A quantitative measurement of certain responses, however desirable it may be is often not practicable, since they do not permit of any gradation. They could only be expressed as 'occurring' or 'not-occurring'. The most common example of this kind of response is death, and in practical toxicology of insecticides, this is the only response that often matters. The statistical treatment of quantal assay data is carried out by the method of Bliss[1] and is known as *Probit Analysis*.

6. *The Sigmoid Curve.*—In any population of insects, given adequate numbers and a graded dosage scale, a large proportion of the individuals exhibit susceptibilities to a toxic substance near to the mean response. Nearer either limit of maximum and minimum response, the susceptibilities are fewer and fewer. The distribution of responses (Sigmoid) is usually skewed with a much wider range of variation amongst the resistant individuals than among the susceptible ones. This appearance is due to the arithmetic scale of the abscissa with the limitation of zero dosage on the susceptible side and the theoretical possibility of infinite variation on the resistant side. When values along the abscissa are plotted on a logarithmic scale there is no such limitation on the side of susceptibility and much of the skew disappears (Fig. 1 & 2), and the resulting sigmoid curve is normal. The central part of the curve is nearly straight but the lower and upper ends tend to become asymptotic with respect to the limits of toxicity namely zero and hundred percent mortality. While a curve of this type makes it possible to estimate the M.L.D. (LD 50) with a fair measure of accuracy, it is generally entirely inadequate at more extreme values (or mortality). In economic entomology, it is often of great importance to know the dosage for mortalities in the region of 95 per cent.

7. *Probit transformation.*—In the probit transformation of Bliss [1], it is possible to determine the high percentages of mortality. In this transformation any point on the mortality curve less than 100 per cent (95, 99, 99.9) can be compared as accurately as the 50 per cent point if the abscissae are plotted logarithmically and the ordinates plotted with empirical "probits" derived from certain

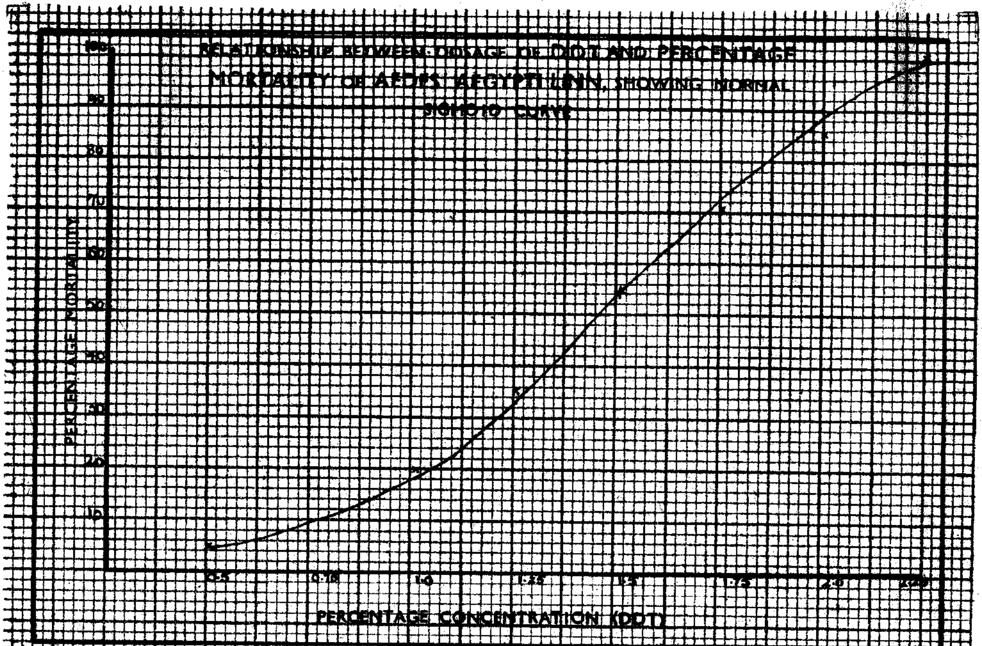

RELATIONSHIP BETWEEN DOSAGE OF DDT AND PERCENTAGE
MORTALITY OF AEDES AEGYPTI LINN. SHOWING NORMAL
SIGMOID CURVE
PERCENTAGE MORTALITY
100
90
80
70
60
50
40
30
20
10
0.5
0.75
1.0
1.25
1.5
1.75
2.0
PERCENTAGE CONCENTRATION (DDT)

Fig. 1

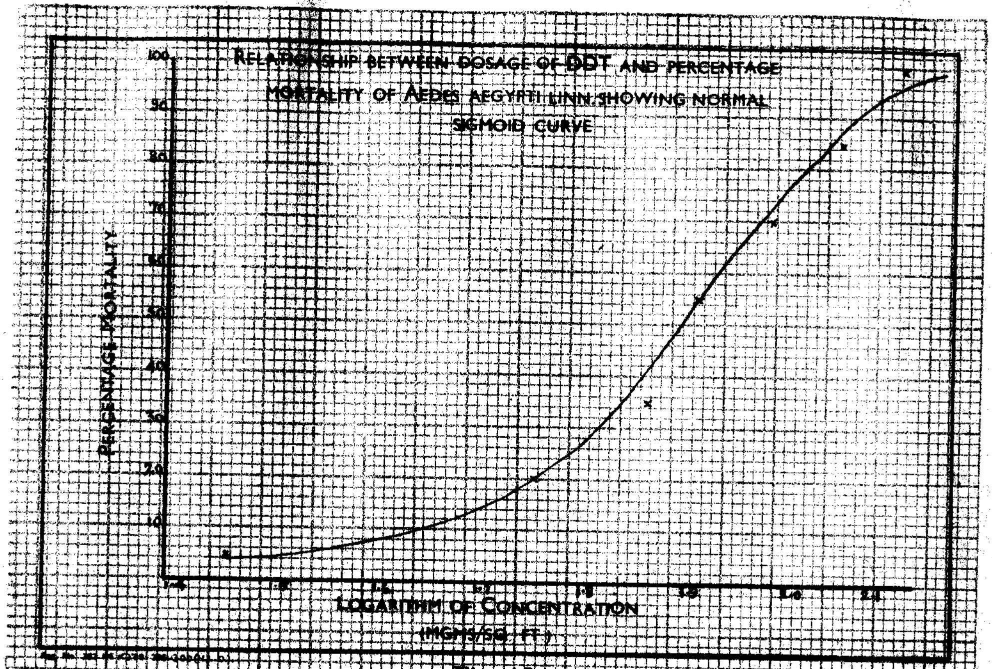

RELATIONSHIP BETWEEN DOSAGE OF DDT AND PERCENTAGE MORTALITY OF AEDES AEGYPTI LINN SHOWING NORMAL SIGMOID CURVE
PERCENTAGE MORTALITY
100
90
80
70
60
50
40
30
20
10
1.6
1.7
1.8
2.1
LOGARITHM OF CONCENTRATION
(MGMS/SQ. FT.)

Fig. 2

CONTACT TOXICITY OF DDT TO AEDES AEGYPTI LINN, SHOWING TRANSFORMATION OF THE SIGMOID CURVE TO A STRAIGHT LINE

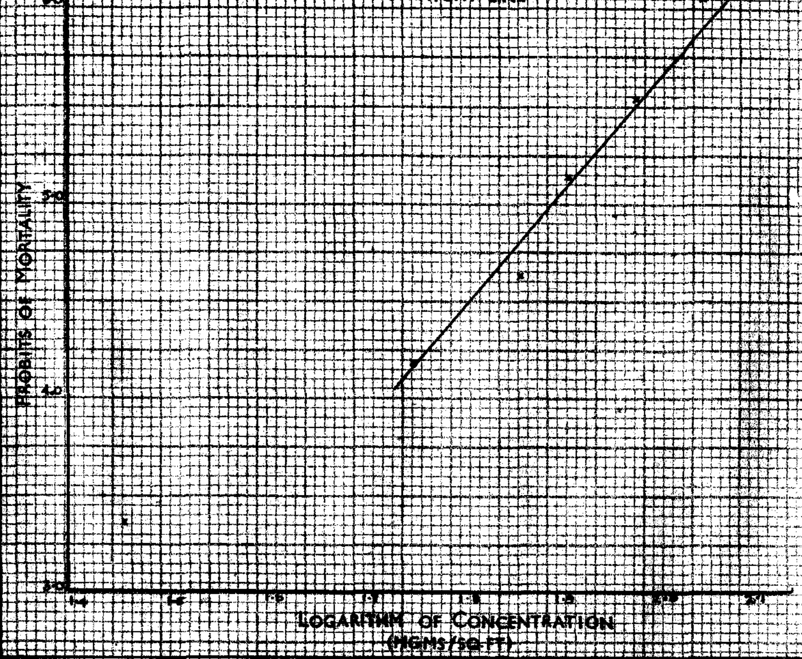


Fig. 3

tables of "normal equivalent deviations". The observed median mortality is given the value 5.00 in an arbitrary scale, the value in either direction being calculated in a symmetrical manner. The value of 5.00 is equated to 0.00 of the usual statistical table of deviates to avoid the use of negative values for kills below the median. When the probit of the observed mortality is plotted on the ordinate and logarithm of the dosage on the abscissa, a straight line results which represents the original sigmoid mortality curve (See Fig. 3).

8. A "provisional regression line" is the first estimate of the transformed dosage-mortality curve, a straight line drawn through the points without calculation. This line may be used as per method indicated by Bliss[1] to initiate the arithmetical process of estimating a better fitting line (Fig. 3). The empirical probits plotted for a carefully conducted experiment often lie so close to a straight line that there is generally no necessity to improve on the provisional line. Only experience of the subject and of the experimental technique used can be a sound guide in this matter. The log LD 50 or 90 or 95 can be directly read from the line.

9. *Determination of Residual effect—(Persistence).*—Before proceeding to state examples of utilisation of the above statistical technique in the interpretation of certain results obtained in the laboratory recently, it would be well to refer to a second aspect of toxicology which may be regarded as complimentary to the evaluation of LD 50 or LD 90 of an insecticide. This is concerned with the assessment of the life of the insecticide (film), on surfaces or what is generally known as the residual effect of the insecticide. The method of assessment consists in assaying at regular intervals the toxicity of the insecticidal film. The standard of effectiveness has to be judged in terms of not only the mortality produced but also the period for which the insects were in contact with the insecticide. Arbitrary standards are possible, but these would naturally vary from one insect species to another. This type of work would only involve the tracing of relationship between mortality and the period of storage of insecticidal film, and vigorous analysis of the results is neither necessary nor practicable.

10. *Biological assay.*—While chemical and physical reactions are measurable with a high degree of precision biological reactions, especially when they involve whole organisms which in themselves are widely variable, admit of large "errors". In any one experiment many variable factors may be present. In laboratory work the variability of each must be reduced as much as possible. Temperature generally plays a large part in influencing the effectiveness of insecticides. Humidity is also of importance, but barometric pressure differences are usually disregarded in toxicological work. Variability in the test insects as a result of stage of development age, etc., should be eliminated. As far as possible, a pure culture of the test insects should be used. The size of sample requisite for reliable results depends upon the experimental plan and technique but "observed mortalities will fall more and more closely on a smooth mortality curve as the size of the groups increases". Special precautions have been taken at the T.D.E.L.S. in

the breeding and preparation of the test insects for bio-assay of insecticides. Details are given below for two representative insect species.

11. *Breeding of* Culex fatigans *Wied.*—The mosquitoes are bred in a room conditioned at 85 ± 1°F and 72 ±3 per cent R.H. To obviate any variation in resistance to insecticides due to genetic factors, the colony was initially raised from one egg raft obtained from a single gravid female brought from the field. Egg rafts are collected from the breeding cages daily and transferred to cylindrical glass dishes, each containing 300 c.c. of tap water. The larvae hatch out after 30 hrs. and the population of the larvae is adjusted to about 300-400 in each dish, containing in addition to water 0.15 gm. of powdered brewer's yeast. After the second larval stage, the concentration of the yeast in each dish is increased to 0.2 gm. Additions of yeast to the larval dishes are made daily. The pupation commences on the eighth day of hatching of the larvae. The pupae are collected every two hours daily and each two hourly collections are placed in suitable cages. The adults emerge 36 hours later and are matured for 36 ± 2 hours longer when the normal, healthy *females* are picked out and used as test insects.

12. *Breeding of* Tribolium castaneum *Herbst*.—The beetles are bred in a conditioned room at a temperature of 77 ± 1°F. and 70 ± 1 per cent R.H. Sterilised whole wheat flour (Atta) passed through 24 mesh sieve is used as the breeding medium. (i) *Main cultures.* The cultures are derived from the progeny of a single female. Adults are kept in breeding jars, one hundred in each, along with wheat flour and allowed to breed for three weeks, when they are removed and destroyed. The newly emerged adults are removed at fortnightly intervals. A set of main culture is started every week from the emerged beetles. (ii) *Sub-cultures.* Young beetles 0-2 weeks old are kept in batches of 500 each in jars containing wheat flour for 3 weeks and then removed and used for test. Thus at the time of test, the insects are 3-5 weeks old.

13. *Details of technique using* Culex fatigans *Wied.*—(i) Filter *Paper technique.*—The principle of this technique is to make adult mosquitoes walk on filter paper impregnated with a solution of the insecticide in a high boiling solvent e.g. liquid paraffin[3] 'Whatman' filter paper No. 1 is used and the method of impregnation has been standardised to give always a uniform increase in weight so that actual weighing is eliminated. 20 insects are eventually confined by means of a glass funnel over the impregnated filter paper laid on a glass plate. The insects do not rest on the (inner) sides of the funnel and the time spent by the insects in flight as against resting on the filter paper is insignificant. The insects are confined for a predetermined period in accordance with the plan of the experiment the knock-down noted and then removed. The mortality of the exposed insects is recorded after 24 hours.

(ii) *Box technique*[4].—The principle of this technique is to expose the test insects in a chamber composed of six glass plates 3¼″ × 4¼″ × 1/16″, the inner surfaces of which had been treated uniformly with the insecticide. The treatment is carried out by spraying the requisite quantity of the insecticide, in solution or emulsion state through a Potter's tower head[4] into a closed rectangular chamber and allowing the mist to settle on glass plates disposed horizontally on the bottom of the chamber. Insects two-thirds of whose wings had been earlier clipped under anaesthesia are released into the box made of six glass plates and after a definite period of exposure are transferred to a Petri dish, covered over with a lamp chimney, and the mortality after 24 hours recorded. Controls are run concurrently with experimental assays. This consisted of exposure of insects (with wings clipped) to unsprayed plates for the same contact period as in the assay.

This technique was evolved at the T.D.E.L. (S), Kanpur and has been of great use in estimating the residual effect of insecticides (*vide* para. 9 above).

14. *Details of technique using* Tribolium castaneum Herbst.—Batches of 20-50 insects (with suitable number of replicates) are confined for 24 hours on plates (glass or cement) on which the insecticidal film has been deposited uniformly. At the end of this period, the insects are carefully removed and kept in small Petri-dishes with a little wheat flour. Daily count of mortality upto a period of seven days is generally made. At the time of every exposure, control assay is carried out on plates which had received no treatment.

15. *Some recent results.*—A short summary of some of the more important investigations carried out at the T.D.E.L. (S), Kanpur on mosquitoes and the stored product pest, *Tribolium castaneum* Herbst is provided in the Appendix to this paper. It is, however, intended to draw attention in the body of the paper to two very recent investigations that have shown results of great practical importance. These are (i) Reactivation of surfaces treated with DDT by storage in a teakwood box, leading to the discovery of tectoquinone as a synergist for DDT and (ii) the effect of pretreatment on persistence of DDT on cement surfaces. In the first of these two investigations the test insect was *Culex fatigans* Wied and, in the second *Tribolium castaneum* Herbst.

16. *Reactivation of surfaces treated with DDT by storage in a Burma teak box* [5].—The genesis of this investigation was accidental. During early work on the evaluation of insecticides (*vide* Appendix) it was noticed that a Burma teak box 12″ × 10″ × 4″ in which glass plates with feeble activity were stored between exposure to mosquitoes had their activity raised very considerably. Initial experiments were directed towards the confirmation of the effect. Table I records the results of a typical experiment carried out in this connection. The technique of assessment was as described in para. 13(ii) above.

TABLE I.

Exposure Tests on Plates treated with DDT effect of Storage in Burma Teak Box.

DDT Concentration 35 *mg./sq. ft.*

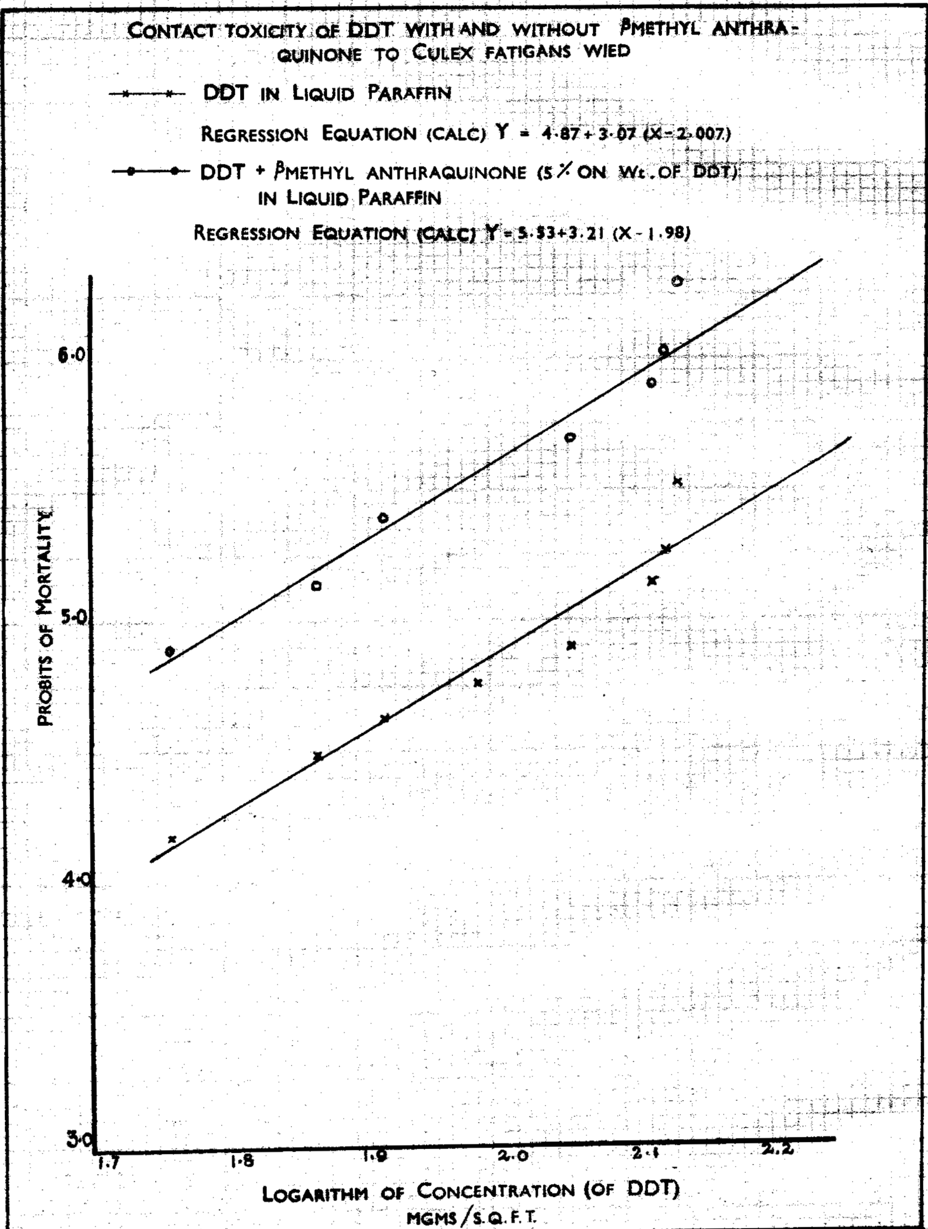
Age of deposit in days	Contact period in minutes	Percentage mortality*	Storage conditions in between exposures
1	20	100	Initial exposure.
15	60	50	Assembled on the bench.
19	60	35	Do.
24	60	100	In the box.
30	60	30	Assembeld on the bench.
40	60	75	In the box.
47	60	35	Assembled on the bench.
62	60	0	Do.
70	60	55	In the box.

*Mortality in control in all cases was zero.

The striking enhancement in the mortality rate of insects when exposure is carried out on plates after their storage in the (Burma teak) box will be readily noted.

17. The assessment of likely causative factors was then undertaken. Careful experiments showed that light, darkness or humidity did not play any role. Boxes of identical dimensions but made of timbers like (i) Mango (*Mangifera indica, L.*), (ii) Pine (*Pinus longifolia* Roxb.), (iii) Shisham (*Dalbergia sisso,* Roxb) and (iv) C.P. Teak were tested side by side with the Burma teak box in order to examine the value of specificity of the reactivating effect in relation to these timbers and it was found that the effect was manifest *only* when the plates were stored in the Burma teak box. A second, freshly made Burma teak box also showed the effect thereby proving that the effect was generally for Burma teak and that it was not confined to the first box with which experiments had been conducted. Another striking result in relation to the effect was that the latter was completely suppressed if, during storage of the plates, a continuous stream of air was passed through the box. Finally it was shown that plain untreated plates did not acquire any toxicity as a result of storage in the box.

18. *Tectoquinone (B-methyl anthraquinone) A synergist for DDT.*—With the above results in hand, it was of great interest to trace the underlying mechanism of activation. A systematic examination of the chemical composition of teak was called for. A study of literature showed that Kafuku and Sebe had isolated from the



Reg. No. 382 HE (C)'50-205-300'50 (H.L.O.)

Fig. 4

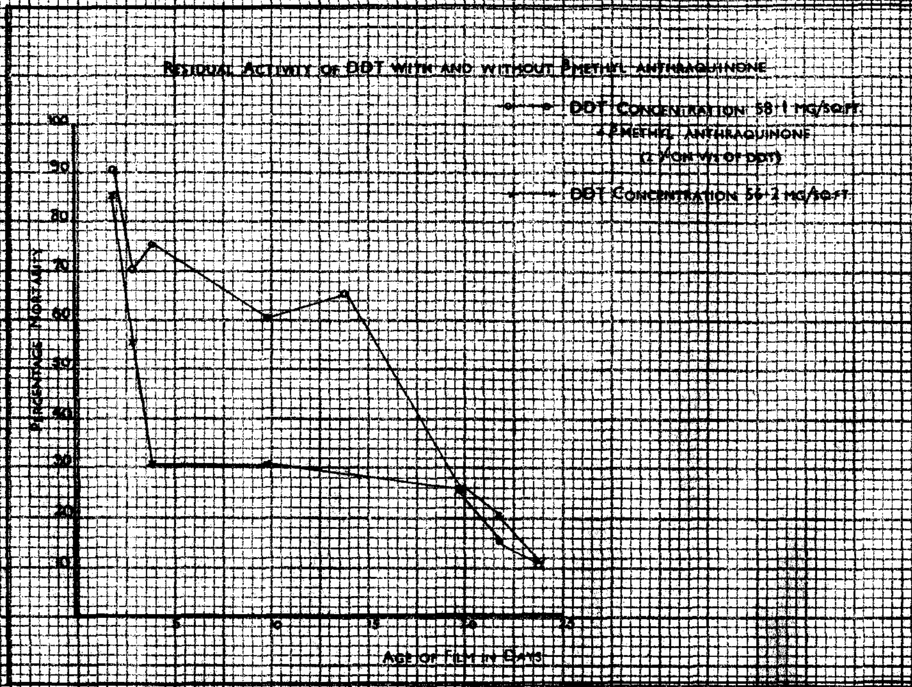

Residual Activity of DDT with and without β methyl anthraquinone
DDT Concentration 58·1 mg/sq.ft.
+ β methyl anthraquinone
(2 % on wt of DDT)
DDT Concentration 56·2 mg/sq.ft.
Percentage Mortality
100
90
80
70
60
50
40
30
20
10
5
10
15
20
25
Age of Film in Days

Fig. 5.

resin of teak (*Tectona grandis* L.) *inter alia* tectoquinone m.p. 174-175° and established the identity of this substance with B-methyl anthraquinone. It was considered of interest to study the capacity if any possessed by this substance for activating DDT.

19. B-methyl anthraquinone was synthesised in the laboratory. In the first experiment, parallel toxicity tests with (i) pure pp' DDT (ii) DDT containing B-methyl anthraquinone (5 per cent on the weight of DDT) and (iii) B-methyl anthraquinone in requisite concentrations serving as control for (ii), were carried out against *Culex fatigans* adult females as per technique described in para. 13(i) above. The contact period was 60 minutes. In all the concentrations investigated, B-methyl anthraquinone did not produce any kill. The dosage-mortality relationship for DDT and DDT B-methyl anthraquinone is shown in Fig (4). The results show very clearly that this quinone has a remarkable synergistic effect on DDT. The LD 50 for (i) DDT and (ii) DDT + B-methyl anthraquinone are 112.2 and 64.6 mg./sq. ft. respectively.

20. In the second experiment the residual effect of DDT as influenced by B-methyl anthraquinone was sought to be investigated (*vide* technique described in para. 13(ii) above). DDT (58.1 mg./sq. ft), DDT (in equivalent concentration) + 20 per cent B-methyl anthraquinone on the weight of DDT and tectoquinone by itself were examined. The duration of the test was about three weeks and the contact period was 10 minutes throughout. The quinone by itself did not prove toxic at any stage. The results are recorded graphically for the other two treatments in Fig. (5). The DDT film containing B-methyl anthraquinone loses toxicity much less rapidly than DDT film alone. A 50 per cent. mortality is maintained by the former for about two weeks whereas the latter fails in this respect in about three days.

21. The practical importance of the above results are far reaching both in relation to Economic Entomology and the study of mechanism of insecticidal action of DDT. It is intended to study (1) other quinones, as activators. (2) synergistic action, if any, of such quinones on insecticides other than DDT, (3) physicochemical aspects of activation due possibly to increased cuticular permeability, reduction of interfacial tension between the cuticle and the insecticide, etc.

22. *The effect of pretreatment on persistence of DDT on cement surfaces.*—In the Appendix to this paper, attention has been drawn to the results obtained with DDT and Gammexane (Benzene hexachloride) on cement surfaces against *Tribolium castaneum* Herbst. One result which has not been stated there arose out of an investigation which like the one associated with teak wood box described earlier had its genesis in an unusual way. In assessing various concentrations of DDT on cement surfaces, unexpected behaviour was shown by certain replicates, and this was ultimately traced to the fact that the cement blocks used in the evaluation had been earlier employed for testing camdistemper and camoil paints. It was considered of interest to investigate the effect of pretreatment separately with (i) linseed oil and (ii) glue/potassium dichromate mixture on the toxicity and persistence of DDT films.

Concurrent work in U.K. [8] on the effect of pretreatment of brick surfaces with various materials like sodium silicate, gelatin, linseed oil, etc., on the toxicity (and persistence) of pyrethrins in shell oil base has supported the findings at T.D.E.L. (S) with DDT, in so far as a comparison is possible.

23. Cement blocks were used in the investigation. The rate of application of linseed oil and glue/dichromate mixture (6 per cent solution) were 3.15 gms. and 1.57 gms./sq. ft. respectively. The treatment with the insecticide was carried out on the pretreated surfaces by the mist deposition technique described in para. 13(ii) above. Two preparation of DDT, one a solution in Kerosene Grade II, and the other an oil-water emulsion were used for treatment. The eventual deposit of the insecticide was about the same in both cases of application viz. 150 mg./sq. ft. The entomological assay was carried out as described in para. 14 above. Exposures on plates were carried out monthly for a total period of 20 months.

24. The results are shown graphically in Figs. 6-9. The figures represent the average of three replicates. In the beginning all treatments give uniformly high kills but a striking difference is manifest in later exposures. Even from the first month onwards a regular decline in potency of surfaces pretreated with linseed oil and those not pretreated is observed. In the second month, the mortality figures generally fall below 50 per cent and in later months a very fast decline is noted. Furthermore, in the case of plates treated with DDT in kerosene solution, the effect of pretreatment with linseed oil would appear to decrease the efficiency of the insecticide particularly in the initial months. As compared to these, the plates pretreated with glue/potassium dichromate mixture generally maintain a high kill. This effect is observed in the case of deposits from both DDT emulsion and solution upto 11 months, after which the deposits from DDT solution show a sharp fall in potency (to 20 per cent). Those from DDT emulsion continue to give high kills (at least upto 20 months). Quite apart from the effect of pretreatments, in all cases an effective mortality is reached in 3 days after exposure. There is only a slight increase in the intervening period upto 7 days.

25. The importance of the results is far reaching and in view of this, a confirmatory practical trial is being started under depot conditions.

26. *Summary.*—(i) The principles of insecticide toxicology are outlined together with the concept of *quantal* response in relation to insecticides is defined. (ii) The probit transformation of Bliss is discussed. (iii) The exacting requirements of the technique of bio-assay are pointed out. (iv) The breeding of *Culex fatigans* Wied, and *Tribolium castaneum* Herbst in the laboratory for the purpose of bio-assay of contact insecticides as also the methods of assay are described. (v) The peculiar activating effect possessed by Burma teak in activating DDT surfaces (with feeble activity) is described and from this is traced the synergistic action of B-methyl anthraquinone on DDT. (vi) Details of an investigation (using the red flour beetle) on the effect of pretreatment of cement surfaces on the toxicity and residual effect of DDT are described and it is shown

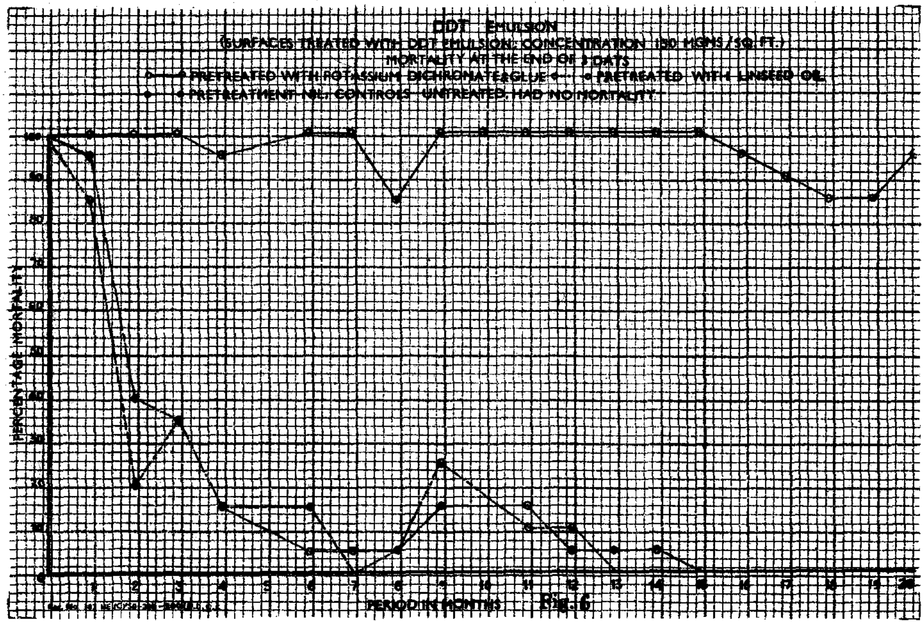
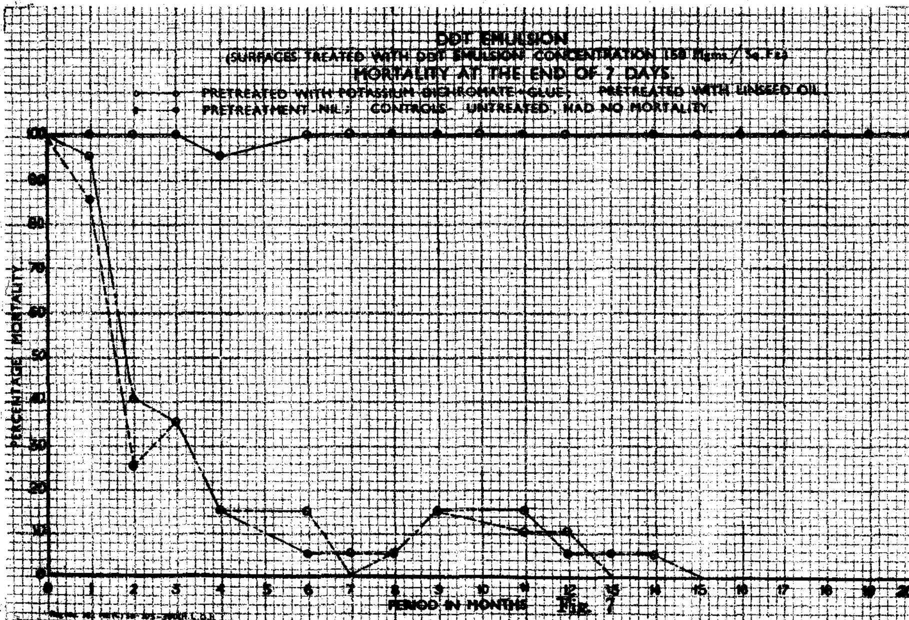

DDT EMULSION
(SURFACES TREATED WITH DDT EMULSION; CONCENTRATION 150 MGMS/SQ. FT.)
MORTALITY AT THE END OF 3 DAYS
PRETREATED WITH POTASSIUM DICHROMATE & GLUE
PRETREATED WITH LINSEED OIL
PRETREATMENT NIL. CONTROLS UNTREATED, HAD NO MORTALITY.
PERCENTAGE MORTALITY
0
10
20
30
40
50
60
70
80
90
100
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
PERIOD IN MONTHS

Fig. 6



DDT EMULSION
(SURFACES TREATED WITH DDT EMULSION CONCENTRATION 150 Mgms / Sq. Ft.)
MORTALITY AT THE END OF 7 DAYS.
PRETREATED WITH POTASSIUM DICHROMATE-GLUE; PRETREATED WITH LINSEED OIL
PRETREATMENT NIL; CONTROLS- UNTREATED, HAD NO MORTALITY.
PERCENTAGE MORTALITY
0
10
20
30
40
50
60
70
80
90
100
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
PERIOD IN MONTHS

Fig. 7

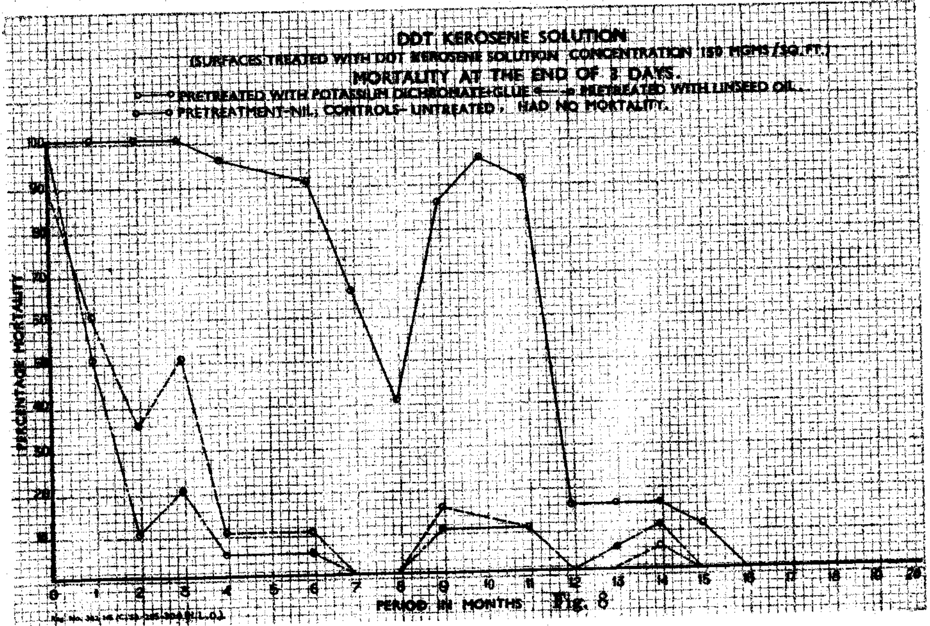
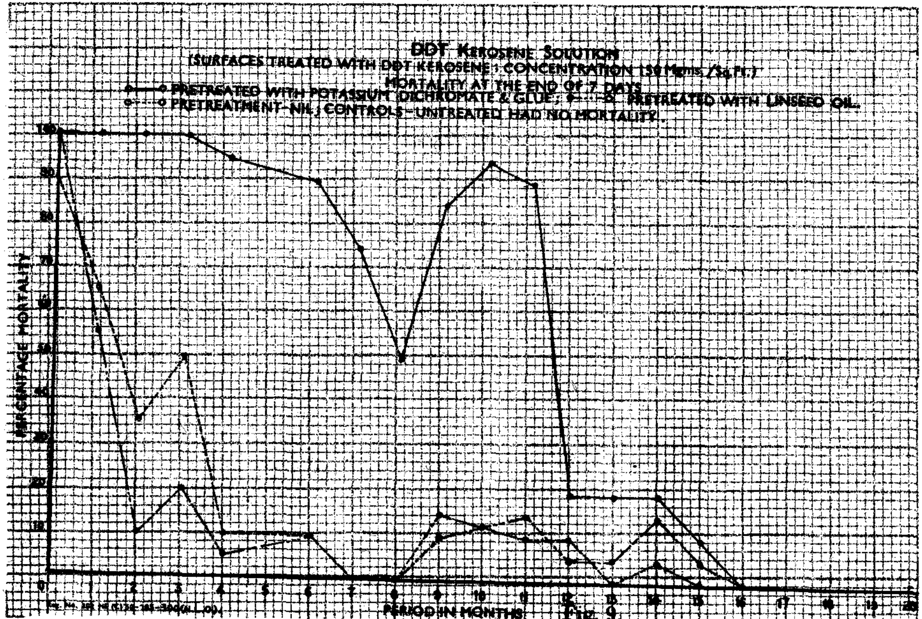

DDT KEROSENE SOLUTION
(SURFACES TREATED WITH DDT KEROSENE SOLUTION CONCENTRATION 150 MGMS/SQ.FT.)
MORTALITY AT THE END OF 3 DAYS.
PRETREATED WITH POTASSIUM DICHROMATE+GLUE
PRETREATED WITH LINSEED OIL.
PRETREATMENT-NIL; CONTROLS- UNTREATED, HAD NO MORTALITY.
PERCENTAGE MORTALITY
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
PERIOD IN MONTHS

Fig. 8



DDT Kerosene Solution
(Surfaces treated with DDT kerosene; concentration 150 Mgms./Sq.Ft.)
Mortality at the end of 7 days
Pretreated with potassium dichromate & glue; Pretreated with linseed oil.
Pretreatment-nil; Controls-untreated had no mortality.
Percentage mortality
Period in months

Fig. 9.

that pretreatment with a mixture of glue and dichromate is of outstanding value in prolonging the residual effect of the insecticide. (vii) In the appendix to the paper are stated briefly the results obtained in certain representative investigations both against *Culex fatigans*, and *Tribolium castaneum*.

References.

1. Bliss, C.I. *Annals. Appl. Biol.*, 1935, *22*, 134, 307.
2. Parkin, E.A. and Green, A.A., *Ibid*, 1943, *30*, 290.
3. Torns, B.A. and Callaway, S. Porton Report No. 2727.
4. Newman, J.F., Aziz, M.A. & Koshi, T. *Proc. Ind. Acad. Sci.* (In press).
5. Aziz, M.A., Koshi, T., and Sankaran, V. *Proc. Ind. Acad. Sci.* (In Press).
6. Kafuke and Sebe, *Bull. Chem. Soc.*, Japan 1932, 7, 1141.
7. *Organic Synthesis* (Coll. Vol. 1), p. 345.
8. Hewlett, P.S. and Parkin, E.A. *Annals Appl. Biol.*, 1947, **34**, 224 ; *Parkin, E.A. & Hewlett, P.S.* Ibid, 1946, *33*, 381.

APPENDIX.

Summary of Representative Investigations on (the Stored Products Pest) Tribolium Castaneum, Herbst.

Serial No.	Substance tested	Results	Reference to publication
1	DDT & 666 as dusts ..	(*i*) Kaolin alone has no toxic properties, and works well as an inert carrier.	Report No. Bio/45/9 and Bio/45/28.
	Mixed with Kaolin in various percentages.	(*ii*) DDT in concentrations as low as one part to 399 parts of Kaolin effects hundred percent kill at the end of 50 days. In practice, one pound of DDT would treat nearly 20 thousand pounds grain debris. (*iii*) Calendra adults are more susceptible to DDT. (*iv*) Commercial DDT was slightly more effective than 666 (10%.12% Benzene Hexachloride) against Tribolium adults during the first month but there was little difference later.	J. S. I. R. 1945, 4, 72.
2	DDT & 666 in Wall Washes.	(*i*) DDT in an oil-in-water emulsion with lime wash is rendered completely in active. The deposit becomes in effective within a few days, after application.	Report No. Bio/45/20 and Bio/45/31.
	DDT & 666 mixed with lime & chalk washes in varying quantities and applied on cement concrete surfaces.	(*ii*) The persistence of the insecticidal effects of DDT and 666, when applied as oil-in-water emulsions in chalk wash lasted for over two months. (*iii*) Lime has a serious adverse effect on 666 and to a lesser degree on DDT.	J. S. I. R. 1945, 4, 493.
3	DDT & 666 in glue bound distempers.	(*i*) The persistence of the insecticidal efficiency of a glue bound distemper, incorporating DDT or 666 is correlated with the degree of binding.	Report No. Bio/45/45.
	Distemper containing whiting or chalk, barium sulphate, hide glue and potassium dichromate, was mixed with varying concentrations of DDT and 666.	(*ii*) In equivalent concentrations of crude products, 666 is more effective than DDT. (*iii*) The following concentrations of DDT & 666 were found effective when mixed with distemper (14 mg./sq. ft.) DDT 112 mg./sq. ft. 666 112 mg./sq. ft. 666 66 mg./sq. ft. (*iv*) The insecticidal films were found to produce satisfactory kill in the second exposure (after 15 days)	
4	Gammexane as smoke from pellets.	(*i*) The effective and maximum deposit of smoke is on floor, while deposit on ceiling and walls ineffective. (*ii*) There is rapid loss of insecticide from the surfaces (nearly 50% in 17 days). (17) The nature of surface (wood, glass, etc.) does not make appreciable difference to the activity of deposits.	Report No. Bio/45/50.

Summary of Representative Investigations on (the Stored Products Pest) Tribolium Castaneum, Herbst.

Serial No.	Substance tested	Result	Reference to publication
5	DDT as smoke from 'Moskil' grenades.	(*i*) The deposit consists of liquid droplets, which crystallize very slowly. (*ii*) The deposit upon plates exposed vertically is too light to be of practical value. (*iii*) Useful deposit is produced on horizontal surfaces.	Report No. Bio/45/43.
6	DDT & Gammexane on Jute sacking. Jute sackings treated in hatching oil storage with DDT and 666 in various concentrations, was tested against grain pests.	(*i*) Jute sacking treated with DDT (0·3%) in an emulsion was found to produce 100% kill. (*ii*) The toxicity of the insecticidal film persisted over a period of 14 months. (*iii*) A lower concentration of DDT on finer weave of sacking gave similar kill as with a higher concentration on coarser weave. (*iv*) Gammaxene in concentrations 0·08% and 0·05% was found ineffective.	The report is under issue.
7	Velsicol—solution .. Solution in toluene in varying concentrations tested.	(*i*) In initial stages Velesicol is as effective as DDT against Tribolium adults. (*ii*) In residual effect DDT proves more satisfactory than Velsicol.	
8	Toxaphene as solution Solution in toluene in varying concentrations tested.	(*i*) In initial stages, Toxaphene is as toxic as DDT against Tribolium adults. (*ii*) The residual effect of DDT lasts longer than Toxaphene.	Test report is under issue.

Summary of Representative Investigations on Mosquitoes. (Culex fatigans, Wied).

Substances tested and details	Results	Reference to publication
Efficiency of DDT when dissolved in Kerosene Grade II and III.	Kerosene Grade III itself is highly toxic to mosquitoes, a film on a glass surface remaining active as long as 3 weeks where as kerosene Grade II does not show any toxic properties. Efficiency of DDT in kerosene Grade III is 10 times higher during the first 15 days than a similar concentration of DDT in Kerosene II.	Report No. Bio/46/48.
Efficiency and residual effects DDT applied on glass surfaces against mosquitoes.	Aqueous emulsions of DDT were found superior to DDT solution in Kerosene. Emulsion II containing groundnut oil produces a more active residue than the one without it. Treated glass plates which lose their activity after some period can be re-activated by spraying with a solution of groundnut oil. An initial deposit of 35 mg./sq. ft. on glass surface which normally is inactivated within 3 weeks, is kept active for at least 3½ months by spraying with oil periodically.	Report No. Bio/45/27 & Bio /45/44.
Field trials of indoor spraying with DDT emulsions and Kerosene solutions.	DDT at 114 mg./sq. ft. applied to the internal surfaces of huts produced a marked decrease in the indoor population level of *Anopheles culcifacies* and *A. Fluviatilis*. The decrease in population is maintained over a period of 4 months.	Report No. Bio/45/37 & Bio/46/47.
Insecticidal efficiency of smoke of DDT.	Very little deposit of DDT was obtained on plates hung on walls and those showed poor results while plates kept on the floor and ceiling gave 65 and 55% mortality respectively for 60 minutes contact period.	Report No. Bio/46/50.
Insecticidal efficiency of smoke of 666.	Plates hung on walls did not get sufficient deposit of 666 to exercise effective mortality but plates placed on floor had in heavy deposit and continued to be active for about 2 months.	Report No. Bio/46/50.
Veliscol in Toluene used in varying concentrations on glass plates.	Velsicol proves more toxic than DDT to *Culex fatigans* W. in the initial exposures. The residual effect of DDT however lasts longer but this does not appear to be of practical importance in view of the low mortality.	Report under issue.
Toxaphene in Toluene used in varying concentrations on glass plates.	Toxaphene in equal concentration as of DDT proves less toxic than DDT in the initial exposures but the residual effect of Toxaphone is superior to that of DDT.	Report under issue.