Nearly all the reports upon the use of these substances in the field have rightly stressed their extremely toxic nature. In so doing they have tended to throw the issues out of perspective. Garlick (1950) has rightly drawn attention to the fact that many thousands of square miles have been sprayed with parathion without any accidental poisoning. The results of the present investigation offer some information on this issue. Fifty sprayers have been examined during the height of the spraying season. Between them they had sprayed hundreds of acres of fruit trees and thousands of trees. Only three of them exhibited even the slightest indication of absorption. Thirty factory workers or laboratory workers were examined. Only the use of the most stringent criterion of departures of cholinesterase levels from normality permitted the recognition of any abnormality in nine of them. Ten workers who had been engaged continuously in the manufacture of parathion for two years had normal levels of blood cholinesterase.

These facts demonstrate quite clearly that the organo-phosphorus insecticides may be manufactured and applied without risk provided manufacturer, consumer, and their employees co-operate by the rigid adherence to every form of safety device and procedure which has been developed for that end.

The observations here reported provide no grounds for complacency, and those who use or are responsible for encouraging the use of these materials should remember that freedom from accidents in this country during the past year has been the result of the heed given to early warnings rather than to the innocuous nature of the materials handled.

Summary

The cholinesterase levels of 80 men and women exposed to organo-phosphorus insecticides in the field or factory have been determined.

Three criteria of departure from normal variation of enzyme levels have been described. On the basis of these standards abnormal variations which are probably attributable to insecticide absorption have been recognized in 12 individuals.

The departures from normality were very slight. No serious case of poisoning was observed.

These observations emphasize the value of the test, and their significance in relation to the incidence of insecticide poisoning in field and factory workers is discussed.

We wish to acknowledge the generous help and co-operation which we received from those firms and workers, both agricultural and factory, who made this research possible. We are also very appreciative of the careful technical assistance we received from Miss A. M. Eyres. Acknowledgment is also made to the Chief Scientist, Ministry of Supply, for permission to publish this paper. Crown copyright reserved. Reproduced with permission of the Controller of H.M. Stationery Office.

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FAILURE OF ORAL D.D.T. TO INDUCE TOXIC CHANGES IN RATS

BY

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Dichloro-diphenyl-trichloroethane (D.D.T.) remains one of the most widely used of the synthetic insecticides. Appreciable quantities may be found on food, and one report shows that fat taken from ordinary citizens not considered to have been unduly exposed to D.D.T. may contain up to 30 p.p.m. (parts per million) of the insecticide (Laug, Kunze, and Prickett, 1951). At the present time nothing is known about the action of D.D.T. beyond the fact that it interferes in some way with the normal function of the nervous system. A detailed search failed to reveal any evidence of interference with a number of enzyme systems (Judah, 1949). For this reason special importance must still be attached to histological evidence of tissue damage or change occurring in animals exposed to D.D.T.

The pathological changes occurring in animals dying of acute poisoning by D.D.T. have been described, and the most striking lesion is a severe liver necrosis (Cameron and Burgess, 1945). Despite this obvious damage to the liver tissue animals in chronic poisoning die from causes associated with injury to the central nervous system and do not show the characteristic signs of acute liver injury. Fitzhugh and Nelson (1947) claimed that they could see characteristic liver lesions in rats that had received a diet containing 100–800 p.p.m. of D.D.T. for a period of two years. The changes they found were hypertrophy of the centrolobular hepatic cells with increased oxyphilia, increased basophilia, and margination of cytoplasmic granules and a tendency to hyalinization of the remainder of the cytoplasm. Only those animals receiving 400 p.p.m. or more of D.D.T. showed any clinical evidence of poisoning.

Laug, Nelson, Fitzhugh, and Kunze (1950) fed rats for 15–27 weeks on diets containing 1 to 50 p.p.m. D.D.T. and claimed that similar lesions developed in the liver cells of rats receiving as little as 5 p.p.m. D.D.T. These changes were much less prominent in the livers of other rodents and were never found in dogs, cats, monkeys, or the larger experimental animals. The conclusion they drew that as little as 5 p.p.m. of D.D.T. in the diet can produce recognizable changes in the structure of the hepatic cells of the rat has obvious and serious implications bearing on the question of what should be the maximum permitted limit of D.D.T. in foodstuff for human or animal consumption. The implication that a concentration of D.D.T. might be harmful has been accepted in America (Dunbar, 1949).

In 1947 a series of long-term feeding experiments on rats, fowls, sheep, pigs, and cattle were started by the Agricultural Research Council.* A large number of rats

*The Toxicity Subcommittee of the Research and Development Co-ordinating Committee on Insecticides initiated and supervised this work.

were used and they received different doses of D.D.T. Apart from those animals dying from intercurrent disease, with or without signs of D.D.T. poisoning, some of each group were killed at different times. This report outlines our observations on histological material prepared from these animals. Although the method of administration differed from that used by Laug *et al.* (1950), the dose levels were comparable and the experimental animals and the period of exposure to D.D.T. were the same. As our findings are quite different from those of the American workers we feel that they should be published because of the interest that has been aroused by the American claims.

Methods

Male and female albino rats were used when they were six weeks old, and each animal received a single dose of D.D.T. dissolved in oil six days a week. The basic dose was calculated in the following way:

Actual LD50 for rat (single dose)	300 mg./kg.
Calculated LD50 for man (70 kg.)	21 g.
Recommended dosage for treated grain	0.005%
Daily consumption of D.D.T. by a man eating 500 g. of bread a day	0.025 g.
Fraction of LD50 consumed	1/840
Basic rate of dosage for rat	1/840 × 300 (LD50) = 0.36 mg./kg.

Groups of rats were given the basic dose, and 10 times and 100 times the basic dose. If a rat of average weight (250 g.) is assumed to consume 20 g. of food a day, the following rough equivalent for these doses in terms of parts per million D.D.T. in the diet can be calculated:

	Basic dose ..	..	= 0.36 mg./kg. ≡ 3.5 p.p.m.
10×	" "	..	= 3.6 mg./kg. ≡ 35.0 "
100×	" "	..	= 36.0 mg./kg. ≡ 350.0 "

The dose of D.D.T. dissolved in arachis oil was placed on the back of the tongue of the rat and was immediately swallowed. The control animals received arachis oil only. Rats from each group were deliberately killed 33, 43, and 60 weeks after the start of the experiment. At one stage of the experiment an epidemic of pasteurellosis swept through the animal house and there were a number of incidental deaths not attributable to D.D.T. poisoning. Other animals were killed at different times during the experiment either because they had severe symptoms of poisoning by D.D.T. or had otitis media or abscesses round the jaws. The group of rats killed 43 weeks after starting D.D.T. were from a second experiment in which there were no incidental deaths.

Histological material was obtained from 123 experimental and 30 control rats. Small pieces of tissue were taken from the liver, spleen, kidney, heart, adrenals, bone marrow, and, in selected cases, from the lung and brain. They were fixed at once in 10% formol saline and 10% formol alcohol, but some were fixed in Helly's fluid. Paraffin sections were prepared in the usual way and stained with Ehrlich's acid haematoxylin and eosin and with van Gieson. In most cases fat staining with Sudan III was also done.

The animals from which the material was obtained were from the groups fed at different levels of D.D.T. for different periods. The numbers from each are given in the Table.

Source of Histological Material from Rats Fed D.D.T. for Long Periods

1. *Number of healthy rats deliberately killed*

Daily Dose of D.D.T.	Period of Treatment (Weeks)			Total No. of Animals
	33	43	63	
0.36 mg./kg. ..	6	6	6	18
3.6 " ..	6	8	6	20
36.0 " ..	6	5	6	17
Nil	6	14	6	26

2. Rats dying from intercurrent disease

Total number that had received D.D.T.	..	68
Number killed or dying with signs of poisoning	..	11
Controls	..	4
Total	..	72

Results

The organs of the rats that had received D.D.T. showed no consistent changes. Special attention was paid to the liver of those rats that were deliberately killed at different times after starting the administration of D.D.T. There was slight focal necrosis in 3, portal infiltration in 8, a tapeworm cyst in 1, and some regenerative change in 1. Thus changes were seen in 13 out of 55 rats. Among the 26 control rats focal necrosis was observed in 7 cases and portal infiltration in 11.

A special search was made for signs of regeneration in the liver—namely, areas showing loss of normal lobular pattern, irregular size of liver cells, and presence of double nuclei. If these had been found we might have concluded with reason that D.D.T. had produced damage at some stage, with subsequent recovery. This, however, was not the case. Among the 68 rats dying from intercurrent infections or killed because they were ill, the livers of two female rats showed changes that could be associated with D.D.T. poisoning. Both rats had received 10 times the basic dose (3.6 mg./kg. daily, or approximately 35 p.p.m.) for 45 and 60 weeks. Both presented centrolobular necrosis, and this was attributed to D.D.T. because careful search failed to disclose the additional changes due to infection. The appearance did not suggest prolonged damage but rather an acute disturbance starting shortly before death. With these exceptions no characteristic lesions could be found even in the rats dying with the signs of severe D.D.T. poisoning. Lipoid pneumonia and pulmonary oedema caused the death of 12 rats in this group; these conditions were no doubt the result of aspiration of the oily solution of D.D.T.

A special search was made for the hepatic lesions which had been described by Fitzhugh and Nelson (1947) and Laug *et al.* (1950) as characteristic of chronic D.D.T. poisoning. Basophilic cytoplasmic granules are a common feature in rat liver cells, and their margination is sometimes observed in both experimental and control groups. No significant difference in the extent of these changes could be made out between the two groups. No convincing evidence of hypertrophy of liver cells was found in rats that had received D.D.T. Increased cytoplasmic oxyphilia and hyalinization of the cytoplasm were not observed in any animal.

Discussion

Rats receiving 0.36, 3.6, and 36 mg. of D.D.T. per kg. for six days a week have been carefully examined after 33, 43, and 60 weeks of treatment, but no histological evidence of any changes in the liver cells has been

obtained. Such changes as were seen—focal necrosis or portal infiltration—were found to an equal extent in the control animals of the same age which had received arachis oil only. Among the 68 rats dying during the course of the experiments liver necrosis characteristic of acute poisoning with D.D.T. was seen in two. This suggested that there had been an acute lowering of the animal's tolerance to D.D.T. owing to intercurrent infection. No rat showed any histological evidence that could be called characteristic of chronic D.D.T. poisoning. The findings of Fitzhugh and Nelson (1947) and Laug *et al.* (1950) have not been confirmed. Sections of normal rat liver were compared after fixation in 10% formalin, 10% formol saline, and Helly's fluid for various periods and staining with haematoxylin and eosin. Basophilic cytoplasmic granules are often observed in the normal rat liver cells. Margination of basophilic cytoplasmic granules are common fixation artifacts. They are seen especially in formalin-fixed tissues and are more marked if the tissue is deliberately badly fixed. Formalin is not a suitable fixative for cytological study. The photomicrographs that illustrate the paper of Laug *et al.* (1950) suggest badly fixed tissues.

Summary

Rats given 0.36, 3.6, and 36 mg. D.D.T. per kg. daily for up to 60 weeks showed no lesions that were characteristic of chronic poisoning with D.D.T. The incidence of naturally occurring pathological lesions was equally distributed among the rats receiving D.D.T. and among the control rats receiving arachis oil alone. No significant structural disturbance has been found in the kidneys and heart.

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Replies to a questionnaire on student health sent out to universities in 53 countries had been received by the International Association of University Professors and Lecturers from 23 countries at the time of the Basle conference in 1949. The Association had decided at a previous conference that the universities should undertake a fuller responsibility for the health of their students, and recommended that arrangements should be made to examine students both before and regularly after entry. The replies made it quite clear that importance is generally attached to chest examinations; on the other hand, most universities are against any exacting psychological examination of the ordinary student's mental health, and an indirect approach is favoured. Prague uses intelligence tests as part of the entrance examination. Out of 91 universities having student health schemes, only about 40 provide physical education for those who do not take part in athletics. Closer supervision of lodgings and hostels is advocated, and the importance of adequate nourishment is stressed. Instruction in personal and social hygiene is also advised, and the establishment of suitable amenities for looking after students who are ill; some universities have sick wards in their residence, others utilize the State health service. Full details of the conditions existing in various universities are published in *Communication 16, Report on Student Health Services* (I.A.U.P.L., London, W.C.2, price 1s.).

PRELIMINARY FIELD TRIALS ON A NEW SCHIZONTICIDE

BY

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"B.W. 50-63" is one of a series of 2,4-diamino-pyrimidines, being 2,4-diamino 5-*p*-chlorophenyl 6-ethyl pyrimidine. Certain similarities which this compound and proguanil showed in their antagonistic action on pteroyl-glutamic acid suggested that it might have antimalarial activity (Falco *et al.*, 1949), and this was shown to be the case (Falco *et al.*, 1951).

At the time these field trials started, laboratory experiments had shown that this drug was a successful schizonticide against *Plasmodium gallinaceum* in chicks, *P. cynomolgi* in monkeys, and *P. berghei* in mice. Toxicity tests on chicks had shown that, weight for weight, the drug had a toxicity similar to that of proguanil, but as its effective dose promised to be small its active dose would be safer in practice. B.W. 50-63 was 60 times as active as proguanil against *P. gallinaceum* and 200 times as active against *P. berghei*. The drug was also active against the blood-forms of *P. cynomolgi* in monkeys, but had no pronounced action upon the exoerythrocytic stages (Falco *et al.*, 1951). It was decided to test this drug in accordance with the method introduced by Bruce-Chwatt (1951) and recommended at the World Health Organization's Conference on Malaria in Africa (1951). A number of African children naturally infected with malaria, of age group 5-10 years inclusive, were used, drawn from schools in the vicinity of Lagos.

Observations of splenic enlargement were made on standing children by a seated observer, and the results were classified in accordance with Hackett's classification (Hackett, 1944). Parasite rates and densities were calculated from observations made on thick films, "negatives" being determined only after the examination of 200 thick-film fields. Parasite density counts were made by determining the parasite/leucocyte ratio from the thick film and calculating from that the number of parasites per c.mm. The rates recorded are those generally accepted. The indices recorded are:

1. *The average enlarged spleen*: an index of the size of splenic enlargements in a group. It is obtained by multiplying the number of individuals in each spleen size group (Hackett's classification) by the group number, and dividing the sum of these products by the total number of those examined who are found to have enlarged spleens.
2. *The parasite density index*: an index of the density of parasitaemia in a group. It is obtained by multiplying the number of individuals in each parasite density group (Bruce-Chwatt, 1951) by the arbitrary group number, and dividing the sum of those products by the total number of individuals examined.

A preliminary test was carried out on a selected group of 12 indigenous schoolchildren, from a suburb of Lagos, whose bloods showed the presence of numerous *P. falciparum* trophozoites on D-1 and D day. On D day a single tablet of 25 mg. of the drug was taken by each child by mouth in the presence of the examiner. This preliminary test was carried out against control groups of untreated children and of children treated with 300 mg. proguanil in a single dose, orally,