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The Secretary of the Expert Committee on Malaria has the honour to communicate herewith a paper by Professor Dr. Maria E. ALESSANDRINI,* of the Chemical Laboratory, Istituto Superiore di Sanità (Director: Professor D. Marotta), Rome.

(This paper has been published in Italian in the Annali di Chimica Applicata, 1948, and in the Rendiconti dell'Istituto Superiore di Sanità 1948, XI: 521-530. It deals with a simple field method which has already been largely applied in Sardinia in the campaign for anopheline eradication, and which will probably be found useful by other workers. The method was presented by Dr. J.R. Busvine at the laboratory meeting of 1 July 1948 of the Royal Society of Tropical Medicine and Hygiene, in London.)

A RAPID METHOD FOR THE DETECTION AND DETERMINATION OF
SMALL QUANTITIES OF DDT ON SPRAYED SURFACES

To obtain good results in an anti-malaria campaign using DDT spray technique, which has in general successfully replaced all other methods hitherto used, it is important to know the residual quantities of DDT on surfaces sprayed with petroleum solutions, with water emulsions, with wettable powders or treated with fogs, at any time after treatment. Such knowledge enables one to detect neglect or

*The author has kindly supplied a certain number of chromatic scales one of which is attached herewith.

oversight in the treatment, to reconcile various differences of opinion relative to treatment, to compare the efficiency of different methods of application, and to avoid waste in the use of costly materials.

In order to accomplish this, it is necessary to have a rapid, sensitive method at hand which may be easily used, even in the field where elaborate means are unavailable, to detect and eventually accurately to determine small quantities of DDT on various sprayed wall surfaces in houses, barns, pig-sties, caves, cellars, etc.

Several colorimetric methods are known which are very sensitive and may be applied more or less easily and rapidly in a laboratory, such as those by the following: S.W. CHAICHIN (1), G.L. BAILES & M.G. PAYNE (2), M.S. SCHLECTER, S.E. SOLOWAY, R.H. HAYES & H.L. HALLER (3-4).

These methods are used for samples containing DDT powder only, or powder mixed with inert substances, and are easily applicable to sprayed surfaces also, although some of them are not so specified. The method used by S.W. CHAICHIN, for instance, gives a yellow coloring which may be caused by the presence of other substances, and cannot therefore be used for surfaces sprayed with solutions of DDT in heavy mineral oils (as may be used in the treatment of bridges, caves, etc.) because the coloring obtained in such a case is, rather than yellow, quite brown.

As far as the author knows, through personal trials, the only method which may be applied in the field using a minimum of equipment is that of H.A. STIFF JR. & J.C. CASTILLO (5 and 6), but it is limited to the mere qualitative detection. This method is based on a red coloration which is produced, when DDT is present, in a solution of Xanthidrol in anhydrous pyridine.

The method is simple, sensitive and rapid, but apart from the fact that it is not specific, as F. IRREVERE and N.C. SHAPLESS have ascertained (7) and the same authors admitted, it presents the great drawback of having a sensitive reaction to moisture.

The authors advise adding 1-2 tablets (about 125mg. each) of potassium hydroxide to the solution in order to absorb moisture, but this amount is insufficient at times, especially in damp places where as many as from 7-8 and even 10 tablets are required. This undoubtedly is inconvenient, and even more so if the person who is to use the method is not a chemist. Besides, pyridine does not remain anhydrous very long, and must be dehydrated and re-distilled frequently. It is also difficult to find in Italy; when available, it is very expensive. Moreover, although the method is sensitive in theory, in practice the same authors advise making a test on a not too small surface of about 77 cm² (10.3 cm. x 7.5 cm).

For the above-mentioned reasons, and because of an urgent request from officials of E.R.L.A.A.S. for a practical method (ERLAAS - Ente Regionale per la Lotta Anti Anofelica in Sardegna - an organization working in conjunction with the Rockefeller Foundation, and undertaking a complete eradication of anophelines from the island of Sardinia), the author has tried to find another method which might better suit the purpose.

Bearing in mind that the DDT tetranitroderivative diluted in benzene, as already noted (8), gives an intense colouring, not only with a solution of anhydrous sodium methylate, as ascertained by M.S. SCHLECTER, S.B. SOLOWAY, R.H. HAYES and N.L. HALLER (3 and 4), but also with an alcoholic solution of potassium hydroxide which is not completely anhydrous, it seemed logical to utilize this peculiarity in designing a suitable method. The results were satisfactory.

Therefore by making a thorough nitration of material scraped from a small sprayed surface, extracting the products of the nitration with benzene, giving it practically normal alcoholic potassium hydroxide treatment, a colouring is obtained which varies from light blue to bright blue, and finally to a purple with a strong bluish fluorescence, according to the presence of lesser or greater quantities respectively, of DDT. With pure pp' DDT, the colouring obtained is of a brilliant and more intense purple compared with that obtained with technical DDT which is less intense and tends more towards a greenish blue for lesser quantities, and a less brilliant and darker purple for greater ones. All these colours change slowly at room temperature, and more rapidly when heated, to green, to yellowish green and finally to yellow for lesser quantities, and to brownish yellow for greater quantities. At the end of 24 hours or even less, colourings of more or less intense yellow are obtained which remain unaltered for several days. At times, quantities of DDT, greater than the 0.001 grams originally present, cause formations of small red crystals to separate, after 24 hours. This method is extremely sensitive, and gives a weak but decided colouring even with only 2 gammas (γ) of DDT.

As to the nature of the products formed - this will be discussed in a following paper. In a preceding article (9), only the qualitative method was discussed, with the intention of furnishing quantitative references soon afterwards. These references are accordingly discussed in the experimental section of this paper, after hundreds of field and laboratory checks by the author and other interested parties.

It is possible to say that the blue product is a potassium salt. In fact, due to acidification, the colour disappears and reappears owing to the addition of the alkalis. When the blue compound is taken into consideration, the red crystalline product which is soluble in yellow in the alkaline benzene-alcoholic solution, should be a dehydrochlorinated product. The dehydroalogenation should occur very slowly at room temperature and in a benzene solution.

FIELD TEST FOR SURFACE DDT

To detect and to determine DDT in the field, in a few minutes and with a minimum of equipment, the following reagents and materials are necessary:

Sulphonitric Mixture: This should be freshly prepared for each series of tests, cautiously uniting 10 ml. of concentrated sulphuric acid (about d.l.,84) with 14-15 ml. of fuming nitric acid (D. about 1,52).

Pure Benzene.

Alcoholic Potassium Hydroxide: About normal (about 56 grams of potassium hydroxide per litre).

A small cylindrical separator of about 25 ml.; a few dry test tubes; a few graduated cylindrical tubes of 5 ml.; calibrated glass test tubes having a diameter of exactly 7,2 mm., and about 10 cm. in length; a small alcohol lamp; a test tube holder; a thermometer from 0° to 150°; a chromatic paper scale, prepared as will be indicated later. All this material may be carried in a small portable case constructed for the purpose.

Procedure of the test: This is to be carried out in the following manner:

Using a small knife, scrape about 25 cm² (a small square of about 5 cm.) of sprayed surface (wall, cement, brick, stone or plaster) and gather the powder on a small piece of cardboard. In gathering the samples, attention must be taken not to make a too superficial scraping, in which case part of the DDT might not be removed. Owing to the great sensitiveness of the method, a much

smaller surface might be scraped. One square centimeter should be more than sufficient. However, since the spray may not have been evenly distributed, it is better to scrape a larger surface in order to secure a medium sample.

According to the author's personal experience, the surface of 25 cm.² mentioned above is the most advisable. However, if spraying has been carried out recently and well, (in which case about 2 grams of DDT per square metre and consequently about 0.005 grams in the 25 cm.² of scraped surface should be present), it is advisable to take only an aliquot part of the powder for the analysis (even 1/4 or 1/5) in order to avoid a colouring too intense for exact comparison with the chromatic scale. If, on the contrary, the surface has been sprayed some time ago, 1/2 or even all of the sample should be taken.

Pour the powder into a dry test tube, adding to it 3-4 ml. of the sulphonitric mixture prepared as indicated above. Let it boil very gently for 2 or 2 $\frac{1}{2}$ minutes. The boiling temperature must not exceed 100°C. For the inexperienced worker, it is advisable to keep a thermometer immersed in the mixture during the boiling period. After it has cooled, and while shaking it cautiously, pour the acid mixture into a small separator containing 8-10 ml. of water. Then wash the test tube with 1-2 ml. of water, and pour this also into the separator. Finally, when cool, add to this 3 ml. of accurately measured pure benzene, and shake repeatedly and energetically. Allow the two stratas to separate, remove the aqueous suspension remaining underneath, and gather the benzene solution in another test tube or, better, in a dry, graduated cylinder of 5 ml. Add about 2 ml. ^{of} practically normal alcoholic potassium hydroxide solution, so as to have a total volume of 5 ml. Shake well. (Do not heat to carry out the quantitative

determination as advised for the mere qualitative detection. Even though the heat facilitates and hastens formation of colour, it also causes it to change more rapidly). Finally decant part of the alkaline benzene-alcoholic solution (about 2 ml.) into a small calibrated glass tube having a diameter of exactly 7.2 mm., and a length of about 10 cm. Wait 1 or 2 minutes or even more, until the colouring has reached the maximum intensity, then compare it with the bluish purple colours of the enclosed chromatic scale for technical DDT and read directly from the scale the milligrams of DDT originally present. This refers to the 5 ml. of the alkaline benzene-alcoholic solution, i.e. to the whole sample originally scraped from the surface undergoing examination. In case only an aliquot part of the sample has been analysed, this fact must be taken into consideration.

Furthermore comparing the bluish purple tint obtained from the scale referring to technical DDT (with which sufficiently approximate results are obtained) with the pp' DDT scale, it is possible to get a rough idea of the quantity of pp' isomer present.

After 24 hours, a second comparison between the colouration of the same small tube and the yellow colourations of the scale may be made. In most cases, an almost perfect harmony may be observed between the quantities of DDT indicated by the blue and those by the yellow colourations. As already noted, these remain unaltered for several days, if care has been taken to stopper the small tubes thoroughly. In a few cases, however, the harmony is imperfect, and higher values are obtained with regard to the yellow colouration. This may either be due to an alteration of DDT, or to the presence of foreign substances which have contributed to increase the yellow, not the blue. When, therefore, the two values do not match, the only values to be considered are those obtained from the blue colouration.

For example: concentrated mother solutions, especially the 26% and 32.7% DDT types sent from America for preparation of water emulsions, contain emulsifiers which belong to the "TRITON" group. (Triton X 30, X 100, X 200, X 400, etc.) By "Triton", a mixture of the real and proper emulsifier is meant, varying according to type, with numerous other substances. The exact chemical nature of emulsifiers is not known and is kept secret.

In the above-mentioned mother solutions, the emulsifier generally used is the Triton X 100. The active principle of this Triton is a high molecular weight alcohol (alkylated aryl poly-ether alcohol) in its anhydrous form. This Triton, which the author has had the opportunity of examining, presents itself as an oil, which is soluble in water and in alcohol in all proportions, 50% soluble in ether and in glycerine, and which produces a reddish yellow colour by nitration in the conditions stated above.

In liquid emulsifiers, however, there are small percentages of Triton. Moreover, it is readily absorbed by the walls, while DDT remains on some surfaces in its crystalline form.

If, however, spraying has been recently carried out, traces of Triton may come away together with DDT and give a reaction without any alteration of the blue colour; at most this could be rather more greenish, while the yellow colouring may be slightly increased.

The enclosed chromatic scale,* which is very practical in the field, has been reproduced from the colourations obtained with known quantities of pure American pp' DDT, having a melting point of 108-109°, and American technical DDT having a melting point of 92-93°. These are the most commonly used DDT grades. (The melting point of good technical DDT must never be below 88°. The higher the melting point, the higher the percentage of the pp' isomer.)

* In the printed chromatic scale, unfortunately, it has been impossible to reproduce the exact colourations, in spite of repeated attempts to improve results. This applies particularly to the colourations obtained with the pp' DDT (for instance the colour corresponding to 0.8 mgm of pp' DDT is darker than that corresponding to higher quantities), while it is not so apparent for the colours concerning technical DDT, for which, therefore, the scale may be fully utilized.

0.1% Ether solutions have been prepared for this purpose, and different quantities of DDT have been taken, always from the same solutions; the ether has been allowed to evaporate at room temperature, reactions being carried out on the remainder of the solution.

Among the different types of technical DDT at 90-95° melting point which have been examined, no differences worthy of mention have been found.

The method has been studied and fully developed bearing in mind its practical use; it is admirably serving its purpose.

The blue colourations which better serve for comparison, are those obtained for quantities of DDT between 0,001-0,0001 grams, but for more practical purposes the author has thought it useful to prepare the scale up to 0,005 grams.

A colorimetric apparatus like Hellige's might also be useful in the field, but at present, a reproduction of the exact colourations by means of coloured glass would not be possible in Italy.

Besides carrying out the determination on sprayed surfaces of walls, cement, brick, stone or plaster, as in the case of the majority of farms, barns, pig-sties, caves, etc., it may be necessary to take samples in other places as, for instance, from wooden, painted or papered walls, or even from glass, (in which case scrapings obviously could not be taken) or from water, where DDT has been sprayed for antilarval work. In the case of walls, where scraping is impossible, a small prepared swab of cotton, previously immersed in ether, is passed several times over the surface treated with DDT. The swab of cotton is then washed with more ether. This is repeated two or three times. The various portions of ether are then poured together and evaporated in a solvent at room temperature. The detection and determination of DDT is then carried out in the usual procedure with the residue.

As for water samples (where it is of great interest to know how long the DDT has remained unaltered since apparently some dehydrochlorination slowly takes place in water) an extraction with ether may also be carried out. Other cases may be similarly treated.

The author has advantageously and repeatedly applied the method also for the determination of small quantities of DDT on leaves and fruit. In such cases, the following procedure is advisable: hold the leaves or the fruit with a pair of forceps or with two fingers. Wash them repeatedly with ether by means of a small sprayer or pipette, gathering the ether in a capsule. The DDT is immediately removed. Care must be taken not to immerse the leaves and fruit in ether because other substances besides the DDT, especially chlorophyll, might dissolve in considerable quantities in ether, and disturb the colourations. Leave the ether to evaporate at room temperature, and follow the usual procedure.

If, however, a laboratory is available and more precise results are desired, determinations may be made using the Pulfrich photometer. To this effect, the author intends to reproduce, at various concentrations, the typical trends of absorption both for pp' DDT and technical DDT as well as for the blue and yellow products, with the relative calibration trends for the filters which may be more suitable.

For the time being, studies with the photometer have been limited to the determination of the mere trend which is typical for absorption of the blue-coloured product obtained by adding alcoholic potassium hydroxide to the pure tetranitroderivative of pp' DDT diluted on benzene. Studies have been carried out with two different concentrations and with filters S 43,45,47,50,53,57,61,75 (Fig.No.1). The values obtained for $\frac{E_{1\%}}{l_{cm}}$ refer to DDT and not to tetranitroderivative.

The different height on the axis of the abscissae of the two trends may be attributed, to a great extent, to a deviation from Beer's law.

SUMMARY

A rapid and very sensitive colorimetric method is described, which can be easily applied in the laboratory or in the field using very little equipment, for the detection and quantitative determination of small quantities of DDT on various surfaces sprayed with petroleum solutions, wettable powders, water emulsions or fogs.

A chromatic scale is reproduced, of great practical utility especially in the field, which enables direct readings of quantities of DDT ranging between 0.0001 and 0.005 grams, based on the colourations obtained by this method.

LITERATURE CITED

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- (9) Ann.Chim.Applicata, 38, 53 (1948)

Fig. 1 -- ABSORPTION CURVES OF THE BLUE-COLOURED PRODUCT (See Text, page 10)
COURBE D'ABSORPTION DU PRODUIT BLEU (Voir texte, page 7)

E. l cm

