SPECTROPHOTOMETRIC DETECTION OF TRICRESYL PHOSPHATE (TCP) IN FOODSTUFFS

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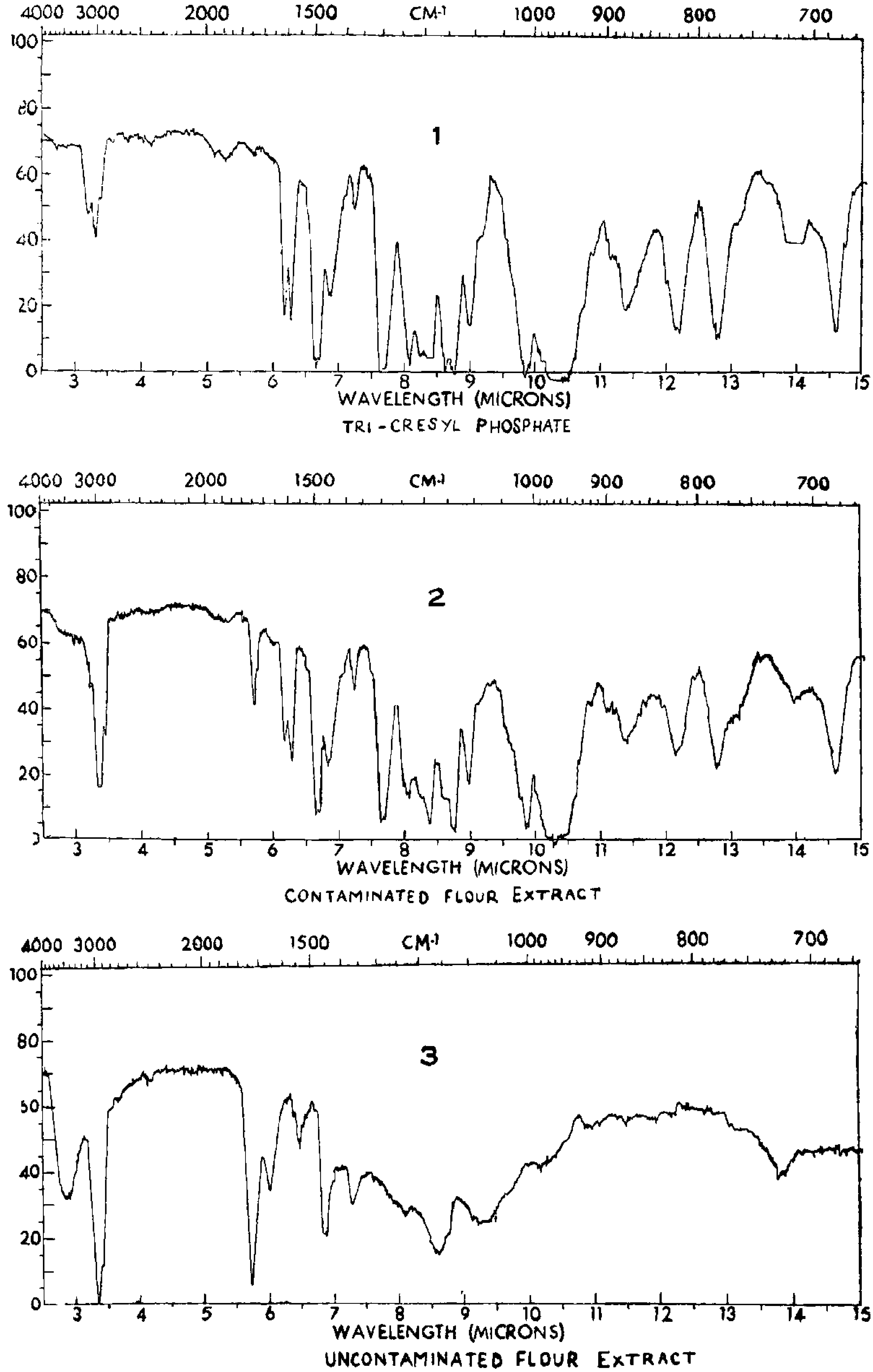
INCREASING use of insecticides in pest control and inadequate methods of their storage and transport have frequently resulted in serious contamination of foodstuffs. This is responsible for an alarming increase in cases of food poisoning and is causing considerable anxiety. Recently, severe cases of illness and even death due to food poisoning have been reported from different parts of India. Various kinds of food materials were stated to have been contaminated, *viz.*, wheat flour, milk-powder, edible oils, etc. Though the actual contaminant has not been definitely identified in most cases, it was suspected that certain organic phosphorus compounds like 'folidol' and 'tricresyl phosphate' were involved in specific cases. For these reasons, the development of efficient and rapid methods for the detection and estimation of pesticide residues in foods has assumed considerable importance.[1-3] In general, the procedure consists of, (*i*) extraction of the contaminant from the foodstuff with an organic solvent, *e.g.*, benzene, chloroform and petroleum ether, (*ii*) concentration of the extract and removal of interfering substances by partition between solvents and/or chromatography on adsorbents like alumina, celite or florisil, and (*iii*) detection and determination of the insecticide by physical, chemical, bioassay or microbiological[4] methods. Vapour phase chromatography has also been used[5] for detection purposes.

In April 1962, a serious outbreak of a paralytic disease occurred in the Malda District of West Bengal. The source was traced to wheat flour which was supplied to the local inhabitants. Chaudhuri *et al.*[6] after a detailed study of the clinical symptoms of the disease surmised that the contaminant was probably tricresyl phosphate (TCP). This was confirmed by a chemical examination of the flour samples according to the method of Collins.[7] An oily extract of the flour was hydrolysed with alkali, the hydrolysate acidified and the cresols steam-distilled. The steam distillate was treated with diazotized *p*-nitroaniline and the azo-dye formed estimated colorimetrically. The results were also verified by a determination of the phosphorus content of the extracts. The contamination was reported to be quite heavy, about 15%.

When our attention was drawn to this problem, we felt that a simpler and a more definite method of identifying TCP would be by the use of infra-red spectra.[8,9] Therefore, the flour samples (ca. 100 g.) were extracted with boiling light petroleum (b.p. 60–80° C.) (2 × 250 ml., 2 hrs. each time), the extract was filtered and the solvent removed. The infra-red spectrum of the oily residue was taken (thin film) (0·2 mm. cells) on Perkin-Elmer "Infracord", Model 137 Infra-red Spectrometer. For comparison, similar spectra of authentic TCP (Fig. 1) and of an extract of a sample of uncontaminated flour (Fig. 3) were also taken. Of the fourteen samples examined, two were contaminated with TCP to a small extent. A flour sample, kindly provided by Prof. R. N. Chakravarti, was also examined (Fig. 2); there was no doubt that it was contaminated heavily with TCP.

In order to assess the applicability of the method for the detection of TCP in edible oils, infra-red spectra of sesame oil (pure) as well as those of samples containing known amounts of TCP were taken (thin films). Under the conditions of the experiment, TCP could be detected in about 5% concentration; there is no interference by the fatty portion in the detection of TCP either in wheat flour or in sesame oil. However, when the TCP was present in smaller amounts the detection was not definite; in these cases concentration could be effected by chromatography as explained later.

Attempts were also made to use ultra-violet absorption for a quantitative determination of TCP in flour samples and edible oils. Preliminary studies showed that TCP has a characteristic peak at 265 mμ in hexane solution and this absorption could be used. A standard curve was drawn by plotting optical densities at 265 mμ against concentrations of TCP in purified hexane. The hexane, which is ordinarily available in the market contains considerable amount of impurities absorbing in the ultra-violet. Purification is best done by refluxing with one-third its volume of concentrated sulphuric acid (2 × 4 hrs.), followed by refluxing with a saturated solution of potassium permanganate in 10% sulphuric acid (4 hrs.).


1
WAVELENGTH (MICRONS)
TRI-CRESYL PHOSPHATE
2
WAVELENGTH (MICRONS)
CONTAMINATED FLOUR EXTRACT
3
WAVELENGTH (MICRONS)
UNCONTAMINATED FLOUR EXTRACT

FIGS. 1-3

The hexane layer is then separated and distilled. A source of error in the quantitative determination of TCP from optical density measurements at 265 mμ is the influence of the fatty material. Sesame oil has a low maximum at 286 mμ and its absorption at 265 mμ is relatively small. On the other hand the absorption of the flour extracts has no characteristic maximum and the absorption at 265 mμ is not negligible; for this reason the estimates of the amount of TCP are likely to be higher. Hence it is desirable to make use of chromatography to separate TCP from the fatty portion in the extractives, and this would also, incidentally, concentrate the former. Abdallah and Landheer[5] reported that in insect extracts, the insecticides (DDT, Parathion and Lindane) could be separated from the fat components by chromatography on celite. We studied this method in the present case. In a typical experiment, chromatography on a celite column (58 × 1½ cm.) was carried out, by placing contaminated sesame oil (containing 5% TCP) on top of the column. Elution was done with purified hexane; the eluate was collected in 5 ml. fractions and the optical density of each fraction measured at 265 mμ and 286 mμ, using a Beckmann Model DU Spectrophotometer. The fatty material was eluted first, before the TCP. Similar results were obtained with contaminated wheat flour. The method appears to be a convenient and useful one for the quantitative concentration of TCP when present in small amounts.

In recent years, a considerable number of organophosphorus insecticides have been developed. In addition to having high insecticidal action, they also possess considerable mammalian toxicity.[10] Attempts are being made to develop phosphorus compounds with low toxicity to mammals and some success has been claimed.[11] The problem of contamination of foodstuffs by toxic residues, however, is still very real, and there is need for a close watch on such possibilities to minimise suffering and anxiety. The procedure described above for qualitative detection and quantitative determination of TCP is capable of adaptation to other organo-phosphorus compounds in general. We believe that refinements are certainly possible to extend its applications and sensitivity.

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MICROBIOLOGY AND WORLD FOOD SUPPLIES

AT the eighth International Congress for Microbiology, held in Montreal during Aug., 1962, great concern was expressed at the widening gap existing between the global rates of increase of population and of food supply. The Congress considered how best microbiology might help towards a future need to increase world food production.

One attack on this problem is to improve the fertility of soils, in which connection the following would be helpful: (*a*) A study of thermophilic and mesophilic micro-organisms capable of more rapidly transforming such non-food wastes as sawdust, straw, weeds, leaves, sludge, etc., into suitable organic matter of manurial value. (*b*) Research on the effect of herbicides, fungicides and other chemicals on micro-organisms, in order to attempt to control soil population. (*c*) Further studies on the effectiveness and wider distribution of *Azotobacter*, *Rhizobium* and other micro-organisms capable of fixing atmosphere nitrogen.

Another field of investigation is to find sources of food additional to orthodox agricultural production. It should be realized that micro-organisms should be capable of supplying some of these; for micro-organisms are able to produce edible protein, fat or carbohydrate, and vitamins, from materials entirely inaccessible to human or mammalian digestion, yet do not require agriculturally useful land.