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Chlorobenzilate Residues in Urine

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Chlorobenzilate is a carbinol acaricide introduced by J.R. Geigy in 1952 which has been registered for almost a dozen uses. It is sold under the trade names of Akar^R, Folbex^R, Acaraben^R, Benzilan^R, and Kop-Mite^R.

Chlorobenzilate was the first pesticide to go through the U.S. Environmental Protection Agency's (EPA) full Rebuttable Presumption Against Registration (RPAR) process. In May of 1976, EPA issued an RPAR notice on chlorobenzilate following alleged tumorigenicity in male mice by the National Cancer Institute. In June 1978, the agency issued a "Notice of Determination" which stated that the cancer risk presumption had not been rebutted and called for additional exposure studies to be conducted on applicators and pickers. To carry out one phase of these studies, EPA contracted with the University of Miami School of Medicine to develop an analytical method for monitoring human exposure to chlorobenzilate.

Methods of assessing human exposure to pesticides have included (1) analyses of patches on clothing and skin swabs for dermal exposure, (2) analyses of filters in respirators for inhalation exposure, (3) measuring dislodgable residues on fruit and foliage for picker exposure, and (4) food and ambient air analyses for general population exposure. All of these are measures of exposure but not absorption, and require further estimation of the amount actually absorbed. A more direct approach is to measure the excretion of the parent compound and/or an appropriate urinary metabolite in a timed urine sample. This assumes a knowledge of the rate of excretion which can be readily obtained by using an animal feeding study as a model. We have chosen this approach for our work.

Several authors (BARTSCH et al. 1971, HORN et al. 1955, VETTORAZZI 1975, WHO/FAO 1969) state that the major urinary metabolite in warm blooded animals is 4,4'-dichlorobenzilic acid (DBA). This metabolite can be oxidized to the more stable and more easily gas-chromatographable 4,4'-dichlorobenzophenone (DBP) (it should be noted that DBP is also a metabolite of dicofol). This reaction was used in one of the early chlorobenzilate analytical methods developed by BLINN et al. (1954). We also have used this reaction as the basis of our method.

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METHOD

A gas chromatograph equipped with ^{63}Ni detector was used with a 1.8 m x 4 mm ID glass column packed with 1.5% OV-17 + 1.95% OV-210 on 80/00 mesh Gas Chrom Q. Inlet, column and detector temperatures were 250, 210, and 300°C, respectively; nitrogen carrier gas flow was 60 mL/min.

Oxidizing reagent was 5% $K_2Cr_2O_7$ in 20% H_2SO_4. Keeper solution was 1% paraffin oil in hexane.

Procedure. To 5 mL of urine in a 60-mL separatory funnel is added 5 mL of oxidizing reagent. The funnel mouth is tightly covered with a piece of aluminum foil and the funnel placed in a 90°C oven for 1 h. The funnel is then removed and cooled for 1 h. The oxidized sample is diluted by adding 30 mL distilled water, and partitioned by shaking for 2 min with 10 mL hexane. The aqueous layer is drained into a 125-mL separatory funnel and the partitioning repeated once more with a fresh 10 mL portion of distilled water. The aqueous layer from this partitioning is combined in the 125-mL funnel with that from the first partitioning. To the 125-mL funnel is added 10 mL hexane and partitioning done by shaking the funnel for 1 min. The aqueous phase is discarded and the hexane phase is drained into the 60-mL separatory funnel. The combined hexane phases are then washed by shaking for 1 min with 10 mL fresh distilled water. The aqueous layer is discarded and the hexane layer is passed through a plug of sodium sulfate into a calibrated centrifuge tube. The sodium sulfate is rinsed with a few mL of hexane, combining this with the hexane in the centrifuge tube. After adding 5 drops of keeper solution, the hexane volume is concentrated by a gentle stream of nitrogen while the tube is held in a ca. 40°C water bath. After adjusting the volume in the centrifuge tube to exactly 5.0 mL, injections are made into the gas chromatograph. Dilutions are made to bring any off-scale peaks into the pre-determined linear range of the detector.

RESULTS AND DISCUSSION

The method was applied for the analyses of urines from a rat feeding study and 36 urine samples collected from six citrus grove workers. Pre-dose rat urines and urine from non-exposed humans averaged less than 0.01 ppm as DBP. Recoveries from eight non-exposed human urines fortified with DBA at 0.05 ppm and analyzed along with the citrus worker samples averaged 97% with a range of 84 - 108% and a standard deviation of 9%.

In Figure 1 are shown typical chromatograms of standard DBP, non-exposed human urine and exposed human urine. DBP residues in urines from dosed rats and exposed humans are calculated back to ppm chlorobenzilate by multiplying nanograms DBP found by 1.29 and dividing this figure by the milligram urine equivalents injected. The results of the rat feeding study and the analyses of the citrus worker samples will be published at a later date after completion of all analyses. Publication of the method at this time is for the purpose of making it widely available to other interested investigators.

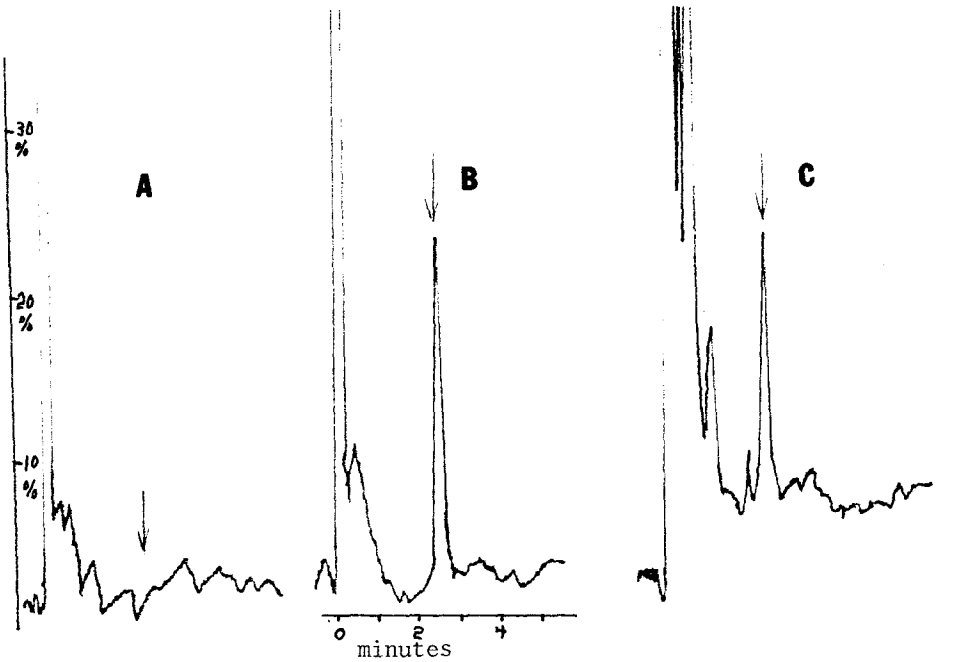

Fig. 1. Typical chromatograms of: A - non-exposed human urine (extract of 5 ml. in 5 ml. hexane), B - DBP standard (0.29 ng.), C - exposed human urine (extract of 5 ml. in 5 ml. hexane). Arrows indicate t_R of DBP.

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