Bull. Environ. Contam. Toxicol. (1987) 39:498–505

Bulletin of Environmental Contamination and Toxicology

# Rapid Estimation of 4,4'-Dichlorobenzilic Acid in Human Urine After Dicofol Exposure

S. N. Deshmukh,[1] H. N. Nigg,[2,*] J. H. Stamper,[2] C. R. Bryan,[2] J. P. Toth,[3] A. P. Nielsen,[4] and M. Royer[5]

[1]Hindustan Insecticides Ltd., Hans Bhawan, G Floor, Wing 1, Bahadur Shah Zafar Marg, New Delhi 110002, India; [2]University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, Florida 33850; [3]IFAS Organic Mass Spectrometry Facility, Department of Food Science and Human Nutrition, University of Florida, Gainesville, Florida 32611; [4]Exposure Assessment Branch, HED(TS-T69c), OPP/EPA, 401 M St., SW, Washington, D.C. 20460; and [5]U.S. EPA, Releases Control Branch, HWERL, Woodbridge Avenue, Edison, New Jersey 08837

Dicofol (1,1-bis(4-chlorophenyl)-2,2,2-trichloroethanol) is used in Florida citrus for control of the citrus rust mite and spider mites. In 1983, the U.S. Environmental Protection Agency began a special review of dicofol because of the possible environmental effects arising from DDT-type compounds in the formulation (U.S. EPA 1984). One consideration in the EPA review was possible human exposure to these compounds. One facet of an exposure experiment is monitoring urinary metabolites of pesticides. Human studies were complicated by the lack of data on the urinary products of dicofol in both humans and small animals.

Spectrophotometric methods for the microdetermination of dicofol residues in agricultural products have been described (Gordon et al. 1963, Hughes 1961, and Gunther and Blinn 1957). Other studies have described column chromatography and thin-layer chromatographic methods (Gillett et al. 1964, Kovacs 1966), but these methods are either not specific, were designed for the parent compound only, or are not sensitive enough for urine analyses.

Black et al. (1971) developed a gas chromatographic analysis (GLC) for dicofol. Unfortunately, because dicofol is quantitatively converted to p,p'-dichlorobenzophenone during the gas chromatographic analysis, the method is unsuitable if dicofol and dichlorobenzophenone are both present in an extraction of a urine sample.

Brown et al. (1969) used a thin-layer chromatographic separation of dicofol and dichlorobenzophenone in the extracts prepared from tissues and fluids of orally and intraperitoneally dosed rats. The analytes were recovered from the silica gel G plates and quantified with gas chromotography. They found 4,4'-dichlorobenzophenone, DDE, 4,4'-dichlorobenzhydrol, and dicofol. A high-performance liquid chromatographic (HPLC) separation of dicofol and

*Send reprint requests to H. N. Nigg at the above address.

dichlorobenzophenone is feasible (Kvalvag et al. 1979) as well as a florisil column separation method (Morgan 1968).

1,1-bis(4-chlorophenyl)1,2,2,2-tetrachloroethane (tetrachloro-DDT) is used as the starting material in the manufacture of dicofol and may be a contaminant in the formulation. This compound, DDE, DDT, and other DDT-type formulation contaminants may be partially excreted as 4,4'-dichlorodiphenylacetic acid (DDA) in humans (Roan et al. 1971). These compounds are primarily stored in adipose tissues and slowly excreted (Morgan and Roan 1971). DDA could also be an excretory product of dicofol. Brady et al. (1980, 1982) suggested another compound, 4,4'-dichlorobenzilic acid (DBA), as a human urinary metabolite of dicofol and chlorobenzilate. The methods described above would not detect DDA or DBA in urine as these compounds are not readily analyzed by gas-liquid chromatography. The purpose of these experiments was to develop a routine analytical method for 4,4'-diclorobenzilic acid in human urine.

## MATERIALS AND METHODS

All solvents were of high purity and suitable for trace analysis. Some analyses were performed on a Hewlett-Packard 5730A GC equipped with $^{63}$Ni electron-capture detector. The 1.8-m silanized-glass column was packed with 4% SE30/6% SP 2401 on 100/120 mesh Supelcoport (Supelco, Inc.). The carrier gas was nitrogen (47 mL/min), oven temperature 200°C detector and inlet temperatures were 250°C. Oxidized samples were analyzed on a Tracor 540 gas chromatograph equipped with a Hall detector in the halogen mode. The packing material and column were identical. The Tracor 540 carrier gas was UPC helium (25 mL/min) and the detector gas was UPC hydrogen at 30 mL/min. The detector and inlet temperatures were 250°C, injector 250°C, and the column oven temperature was 220°C. Injections were by autoinjection. All injections were made in 5 uL of isooctane. Quantification was by a daily five point standard curve constructed concurrently with sample injections. Standard compounds were obtained from the U.S. EPA repository in Research Triangle Park, NC except for the p,p'-dichlorobenzilic acid which was a gift from Ciba-Geigy, Greensboro, NC.

Twenty mL of urine were fortified with standard compounds in 10 uL of acetone. The urine sample was placed in a separatory funnel and 100 mL of acetonitrile and 50 mL of distilled water were added. This mixture was extracted three and sometimes four times with 100 mL of hexane each time. The combined hexane extractions were reduced to dryness at 40°C on a rotary evaporator and transferred to a storage vial by using 10 mL of hexane. The extracts were analyzed without further purification.

For 4,4'-dichlorobenzilic acid and 4,4'-dichlorodiphenylacetic acid, a separate 20 mL urine sample was fortified, and DBA and

DDA were subjected to oxidization to 4,4'-dichlorobenzophenone for gas chromatography. DBA and DDA do not readily gas chromatograph as parent compounds. All glassware was rinsed in hexane prior to use. Twenty mL of urine and 25 mL of glacial acetic acid were added to a 500 mL separatory funnel. One gram of chromium trioxide was added incrementally, and the funnel was swirled intermittently for 20 min. One hundred mL of deionized water were added and the funnel was swirled to mix. This mixture was extracted twice with 100 mL of hexane by inverting the funnel 12 times. The hexane fractions were combined and taken to dryness at 40°C on a rotary evaporator. The final residue was transferred in three steps into 10 mL of isooctane. The isooctane fraction was analyzed with a Hall detector without further cleanup. Each oxidation set included a blank urine. Various oxidation times were tried and the possible metabolites and contaminants of dicofol were put through this procedure as well.

Identification by gas-liquid chromatography was performed on a Perkin-Elmer 8320 chromatograph with electron capture detection. The capillary columns were OV-1 (10 m x 0.25 mm), 0.5 um film thickness and BP-5 (25 m x 0.25 mm, 0.25 um film thickness). The carrier gas was hydrogen with an inlet pressure of 7 psi (OV-1) and 13 psi (BP-5). Makeup gas was nitrogen at a flow rate of 60 mL/min. The temperatures for the OV-1 column were: detector 250°C, column oven 175°C, inlet 250°C. For the BP-5 column, temperatures were: detector 260°C, column oven 210°C, inlet 230°C. All injections were in 2 uL of isooctane.

Mass spectral data were obtained with a Finnigan 4500 GC/MS system. Compounds were separated by using a 30 m x 0.25 mm fused silica bonded-phase capillary column (DB-5, J & W Scientific). The injection port temperature was 250°C, and injections were either split (ca. 100:1) or splitless (0.5 min injection purge), depending on sample concentration. The GC oven was programmed from 50 to 300°C at 10°C/min, with an initial hold of 0.5 min. The carrier gas was helium. Solid p,p'-dichlorobenzilic acid was introduced via a direct insertion probe programmed from 40 to 300°C at 20°C/min. The ion source temperature was 150°C, and electron energies of 70 and 100 eV were used for electron impact (EI) and chemical ionization (CI) mass spectra, respectively. CI spectra were obtained with methane as the reagent gas at a pressure of 0.2 torr. Methylations of DBA and DDA urine fortifications and exposed worker urine were according to Skow and Bicking (1986).

## RESULTS AND DISCUSSION

Brown et al. (1969) found dichlorobenzophenone, dicofol and dichlorobenzhydrol in rat urine. It was consequently important to extract one urine aliquot prior to oxidation because gas chromatography does not differentiate between dicofol and dichlorobenzophenone due to on-column decomposition of dicofol

to the latter compound. This assured that any 4,4'-dichlorobenzophenone found after oxidation came from DBA or perhaps DDA and not from field contamination of urine with dicofol.

Table 1 presents the data for extraction of unoxidized urine. Dichlorobenzhydrol was recovered at only 23% with the hexane extraction and had the added disadvantage of having one-tenth to one-twentieth the sensitivity to electron-capture detection compared to dicofol and the other metabolites.

Table 2 presents oxidized urine data. Dichlorobenzilic acid was recovered as dichlorobenzophenone with a 78% yield. The apparent dichlorobenzophenone (DBA equivalent) must be multiplied by 1.18 to allow for the decrease in molecular weight during the conversion of dichlorobenzilic acid to dichlorobenzophenone. For DDA, the conversion factor is 1.12. Dichlorobenzhydrol converted to dichlorobenzophenone at 19% efficiency. Parent dichlorobenzhydrol was recovered at 16% after oxidation. DDD, DDE, DDT, and tetrachloro-DDT did not oxidize to 4,4'-dichlorobenzophenone, and the parents were recovered at generally low levels. The levels added to urine for these reactions were high (250-500 ppb) in order to obtain <u>any</u> possible conversion. Recovery of 4,4'-dichlorobenzophenone was about 65% when it was added prior to oxidation. Most importantly, DDA converted to 4,4'-dichlorobenzophenone at only 5%.

The confirmation of structures was as follows: standard p,p'-dichlorobenzophenone (4,4'-DCBP) displayed an EI mass spectrum consistent with its presumed structure. Presumed 4,4'-DCBP obtained from oxidation of 4,4'-dichlorobenzilic acid displayed an identical mass spectrum. Presumed 4,4'-DCBP obtained by combining oxidized exposed worker urine, a simple Sep-pak® procedure and concentration, had the same mass spectrum as authentic 4,4'-DCBP. The presumed 4,4'-DCBP from workers' oxidized urine had the same retention time and co-chromatographed with authentic 4,4'-DCBP on the 10 m OV-1 (retention time 2.1 min) and the 25 m BP-5 (retention time 4.9 min) capillary columns. It also had the same retention time and co-chromatographed with authentic 4,4'-DCBP on the BP-5, temperature-programmed from 50 to 220°C at 12°C/min (retention time 15.4 min). Worker urine did yield a smaller GC peak which had the same retention time as 2,4'-dichlorobenzophenone. Methylation of a water sample fortified with DBA yielded one product whose EI and CI mass spectral profiles were consistent with the methyl ester of 4,4'dichlorobenzillic acid. Methylation of worker urine yielded the same compound by GC and EI and CI mass spectrometry. The mass spectrum of methylated DDA did not resemble this product. Methylated DDA had a retention time of 7.5 min on the BP-5 capillary column; methylated DBA and methylated worker urine both showed one major peak at 8.5 min. We conclude that worker urine contained DBA and not DDA.

Table 1. Dicofol and DDT-type metabolite recoveries from 20 mL of human urine with hexane extraction.

| Chemical | Fortification level (ppb) | Replications | % Recovery Mean ± SEM |
|---|---|---|---|
| 4,4'-dichlorobenzophenone | 5 | 3 | 78 ± 4 |
| 4,4'-dichlorobenzophenone | 5 | 4 | 99 ± 2 |
| 4,4'-dichlorobenzophenone | 10 | 3 | 101 ± 12 |
| 4,4'-dichlorobenzophenone | 10 | 4 | 142 ± 5 |
| 4,4'-DDD | 5 | 3 | 78 ± 4 |
| 4,4'-DDD | 5 | 4 | 70 ± 4 |
| 4,4'-DDD | 10 | 3 | 58 ± 2 |
| 4,4'-DDD | 10 | 4 | 57 ± 5 |
| 4,4'-dicofol | 5 | 3 | 86 ± 6 |
| 4,4'-dicofol | 5 | 4 | 100 ± 9 |
| 4,4'-dicofol | 10 | 3 | 73 ± 4 |
| 4,4'-dicofol | 10 | 4 | 80 ± 4 |
| 4,4'-DDE | 5 | 3 | 56 ± 1 |
| 4,4'-DDE | 5 | 4 | 82 ± 3 |
| 4,4'-DDE | 10 | 3 | 79 ± 2 |
| 4,4'-DDE | 10 | 4 | 67 ± 1 |
| 2,4'-dicofol | 5 | 3 | 85 ± 10 |
| 2,4'-dicofol | 5 | 4 | 83 ± 13 |
| 2,4'-dicofol | 10 | 3 | 73 ± 11 |
| 2,4'-dicofol | 10 | 4 | 72 ± 12 |
| 4,4'-Tetrachloro-DDT | 5 | 3 | 104 ± 10 |
| 4,4'-Tetrachloro-DDT | 5 | 4 | 70 ± 7 |
| 4,4'-Tetrachloro-DDT | 10 | 3 | 78 ± 7 |
| 4,4'-Tetrachloro-DDT | 10 | 4 | 76 ± 10 |
| 4,4'-Dichlorobenzhydrol | 250 | 3 | 23 ± 10 |

Brady et al. (1980, 1982) oxidized DBA to 4,4'-dichlorobenzophenone with 5% potassium permanganate in 20% sulfuric acid. This method was not satisfactory in our hands. The method described here was suggested by Dr. Y. Iwata (University of California, Riverside) and is a modification of the method of Blinn et al. (1954) for chlorobenzilate in citrus fruit. The method is rapid, avoids some of the interferences generated by potassium permanganate (Blinn et al. 1954, also noted interferences with permanganate), and obviates laborious methylation procedures. There are standards readily available

Table 2. Dicofol and DDT-type metabolite recoveries from 20 mL human urine after oxidation.

| Compound | Fortification level (N) (ppb) | Recovery of parent cmpd. (%) | Recovery of DCBP* (%) |
|---|---|---|---|
| 4,4'-Dichlorobenzilic acid | 40 (6) | 0 | 78 ± 6.0* |
| 4,4'-dichlorobenzhydrol | 500 (6) | 16.2 on 1 sample 0 on other 5 | 19.9 ± 1.7 |
| 4,4'-DDA | 50 (5) | 0 | 4.8 ± 2.2 |
| 4,4'-DDD | 500 (7) | 33 ± 2.3 | 0 |
| 4,4'-DDE | 250 (7) | 14 ± 2.6 | 0 |
| 4,4'-DDT | 500 (7) | 35 ± 4.2 | 0 |
| 4,4'-Tetrachloro-DDT | 500 (6) | 41 ± 3.7 | 0 |
| 4,4'-dichlorobenzophenone | 500 (10) | 89 ± 3.8 | N/A** |
| 4,4'-dicofol | 500 (8) | 55 ± 5.3 | Only product |
| Chlorobenzilate | 500 (10) | 81 ± 7.9 | 0 |

*Values are mean ± SEM

**Not applicable

for 4,4'-dichlorobenzilic acid and dichlorobenzophenone. There is no standard available for methylated-dichlorobenzilic acid.

This method has one disadvantage. Technical staff should be well-trained in the hexane extraction step. Emulsions form easily, and these are very difficult to break, resulting in poor recovery of 4,4'-dichlorobenzophenone.

This method has been used for the analysis of over 600 human urine samples containing 4,4'-dichlorobenzilic acid from dicofol exposure (results to be reported elsewhere). Extraction of one urine aliquot with hexane assures that the 4'4'-DBCP obtained by oxidation of a second aliquot is not from field contamination with dicofol. The difference in gas chromatographic sensitivity of 4,4'-dichlorobenzophenone and 4,4'-dichlorobenzhydrol combined with extraction and oxidation as separate procedures eliminates 4,4'-dichlorobenzhydrol as a primary urinary metabolite of dicofol. This method can probably be used for the urinary metabolite of chlorobenzilate, as parent chlorobenzilate does not convert to 4,4'-DCBCP (Table 2). Because DDA converts with a low yield to DBCP, this

method may be specifically used for monitoring exposed workers who excrete 4,4'-dichlorobenzilic acid dependent, of course, on the excretion kinetics of the parent compound.

Acknowledgments. Florida Agricultural Experiment Station Journal Series No. 8072. This work was funded in part by a grant from UNIDO/UNDP to SND and from the U.S. Environmental Protection Agency (CR #810743). This paper has not been subjected to the Agency's required peer and policy reviews, and, therefore, the contents and conclusions do not necessarily reflect the views of the Agency and no official endorsement should be inferred.

REFERENCES

Black RF, Kurtz CP, Baum H (1971) Gas-chromatographic analysis of kelthane technical. J Assoc Off Anal Chem 54:1237-1240

Blinn RC, Gunther FA, Kolbezen MJ (1954) Microdetermination of the acaracide ethyl p,p'dichlorobenzilate(chlorobenzilate). J Agric Food Chem 2:1080-1083

Brady SS, Enos HF, Levy KA (1980) Chlorobenzilate residues in urine. Bull Environ Contam Toxicol 24:813-814

Brady SS, Levy KA, Enos HF, Duncan RC, Pfaffenberger CD (1982) Development of methodology for determining human exposure to chlorobenzilate. In Plimmer JR (ed) Pesticide residues and exposure. ACS Symp Ser 182, ACS Washington, DC pp 105-118

Brown JR, Hughes H, Viriyanontha S (1969) Storage, distribution and metabolism of 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethanol. Toxicol Appl Pharmacol 15:30-37

Gillett JW, Van der Gest LPS, Miskus RP (1964) Column partition chromatography of DDT and related compounds. Anal Biochem 8:200-209

Gordon C, Haines L, Martin J (1963) An improved method for kelthane residue analysis with applications for determination of residues in milk. J Agric Food Chem 11:84-86

Gunther FA, Blinn RC (1957) Ultraviolet spectrophotometric microdetermination of the acaricide 4,4'-dichloro-alpha-(trichloromethyl)-benzhydrol-(FW 152). J Agric Food Chem 5:517-519

Hughes J (1961) Colorimetric determination of 'kelthane' residues. Analyst 86:756-757

Kovacs MF Jr (1966) Rapid detection of chlorinated pesticide residues by an improved TLC technique: 3-1/4 x 4" micro slides. J Assoc Off Anal Chem 49:365-370

Kvalvag J, Iwata Y, Gunther FA (1979) High-pressure liquid chromatographic separation of o,p'-and p,p'-dicofol and their dichlorobenzophenone degradation products. Bull Environ Toxicol 21:25-28

Morgan NL (1968) Separation of dicofol (kelthane) and its dichlorobenzophenone degradation product from a standard florisil column. Bull Environ Contam Toxicol 3:254-257

Morgan DP, Roan CC (1971) Absorption, storage, and metabolic conversion of ingested DDT and DDT metabolites in man. Arch Environ Hlth 22:301-308

Roan C, Morgan D, Paschal EH (1971) Urinary excretion of DDA following ingestion of DDT and DDT metabolites in man. Arch Environ Hlth 22:309-315
Skow CAR, Bicking MKL (1986) Direct alkylation of carboxylic in aqueous samples. Chromatographia 21:157-160
U.S. EPA (1984) Notice of Special Review. Fed. Reg. 49:10569
Wolfe HR, Durham WF, Armstrong JF (1970) Urinary excretion of insecticide metabolites. Arch Environ Hlth 21:711-716

Received December 10, 1986; accepted April 23, 1987.