Bull. Environm. Contam. Toxicol. 20,782-785 (1978)

Identification of a Reduced Form of Chlordecone (Kepone®) in Human Stool

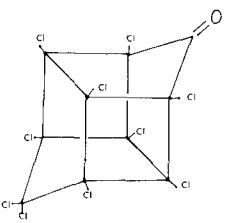
Robert V. Blanke[1], Marc W. Fariss[1], Philip S. Guzelian[2], A. R. Paterson[3], and Danne E. Smith[3]

[1] Department of Pathology, [2] Department of Medicine, Medical College of Virginia, Virginia, Commonwealth University, Richmond, Va. 23298, and [3] Allied Chemical Corporation, Morristown, N.J. 07960

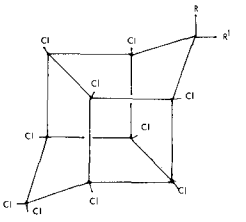
The pesticide ingredient chlordecone (decachlorooctahydro-1, 3, 4-metheno-2H-cyclobuta [c, d] pentalen-2-one; Kepone®; hereafter referred to as CD) was first developed by Allied Chemical Corporation in 1952. Later the compound was made by a small independent company in Hopewell, Virginia. In mid-1975, toxicity developed in workmen exposed to massive quantities of CD during the manufacturing process (MARTINEZ et al., 1976).

In conjunction with this incident, experimental and clinical studies were initiated to examine the pharmacokinetics of CD. It was found that negligible quantities of CD were excreted in urine, saliva or perspiration. The major excretory pathway of CD was in bile into the gastrointestinal tract. In addition, CD showed a fat/blood concentration ratio of 7:1 and a liver/blood ratio of 15:1 (COHN, et al., 1976). The fat/blood ratio of CD is considerably lower than reported for other organochlorine pesticides such as DDT (280·1) (MORGAN and ROAN, 1974). Within the blood, CD was confined to the plasma fraction but CD was not found in ultrafiltrates of plasma within a pH range of 6.4 to 9.5.

These findings suggest that, unlike mirex (I) (dodecachlorooctahydro-1, 3, 4-metheno-1H-cyclobuta [c, d] pentalene) and other organochlorine pesticides, CD shows polar characteristics. These are due to an apparent inductive effect on the carbonyl group which becomes hydrated easily (II) (GILBERT et al., 1966a; GRIFFIN and PRICE, 1964).



CHLORDECONE (CD)



I. $R = R^1 = Cl$
II. $R = R^1 = OH$
III. $R = H$ $R^1 = OH$

Based on these observations and the known chemical reactivity of the carbonyl group in CD (MCBEE et al., 1956; GILBERT, et al., 1966a, 1966b) one would predict that metabolites of CD, if they exist, would probably involve attack at the carbonyl group. Even though the chemical structure of CD suggests that biotransformation of this pesticide is a feasible event, we have no knowledge of metabolites of CD having been identified in mammals, other than a report of uncharacterized radioactive "metabolites" in bile and feces from rats treated with ^{14}C-CD (MATTHEWS and MORALES 1977).

0007-4861/78/0020-0782 $01.00

Methods

The organochlorine substances in human stool from patients with a diagnosis of CD toxicity were assayed by a procedure involving solvent extraction, clean-up and subsequent gas-liquid chromatography utilizing an electron capture detector (BLANKE et al., 1977). A broad peak eluting after CD was observed in some stool extracts (Figure 1). This late eluting peak was identified as decachlorooctahydro-1, 3, 4-metheno-2H-cyclobuta [c, d] pentalen-2-ol (III) by gas chromatography/chemical ionization mass spectrophotometry[1]. Authentic III, prepared by the reduction of CD with $LiAlH_4$ (GILBERT, et al., 1966a) chromatographed identically with the late eluting peak in human stool extracts on 3% OV-1 (Figure 2).

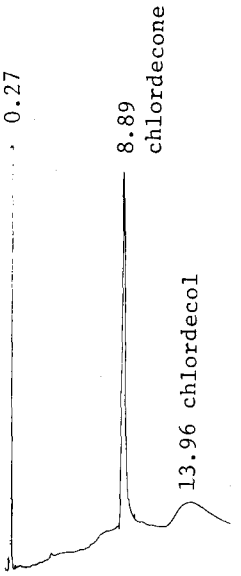


Figure 1. GLC-ECD trace of chlordecone and chlordecol extracted from human stool. Hewlett-Packard Model 5833A; ^{63}Ni ECD; 4% SE-30/6% OV-210; 1.8m x 2mm glass column, 5% methane in argon 20ml/min; Injection 200°C; Detector 300°C; column program 170°/min, 10°/min to 230°C.

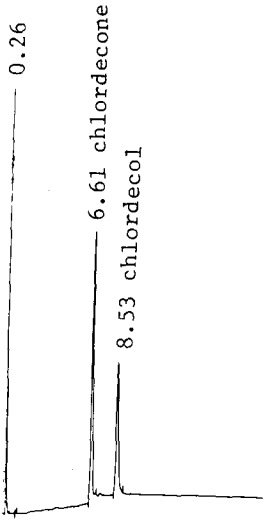


Figure 2. GLC-ECD trace of authentic chlordecone and chlordecol. GLC conditions are identical to those shown in Figure 1 except the column is 3% OV-1.

[1] Performed by EPA, HERL, Research Triangle, N.C.

To confirm this observation extracts from additional human stool specimens were examined by gas chromatography/methane chemical ionization mass spectrophotometry using the multiple ion detection mode. Ions at m/e 472.7 ($^{35}Cl_9{}^{37}Cl$), 474.7 ($^{35}Cl_8$, $^{37}Cl_2$), 476.7 ($^{35}Cl_7$, $^{37}Cl_3$), and 478.7 ($^{35}Cl_6$, $^{37}Cl_4$) were monitored. This cluster represents the ions resulting from loss of water from the protonated molecular ion containing ten chlorine atoms. This is the most intense cluster of ions in the methane chemical ionization mass spectrum of III and shows relative intensities of 1.00, 1.25, 1.09 and 1.00 respectively.

A procedure for measuring III quantitatively in human stool is presently being developed. Nevertheless, integration of GLC peak areas of CD and III in human stool extracts designed to recover CD, shows the two compounds to be present in approximately equal amounts. This indicates that III comprises a major fraction of the CD derived material present in human stool.

Discussion

As was predicted from the chemistry and biological behavior of CD, an altered form of this compound has been characterized in human stool. The reactive, hydrated carbonyl group is the point of attack resulting in reduction to the corresponding alcohol. We propose the common name "chlordecol" for this substance. It is unknown whether this compound is formed in the liver and excreted into the bile or whether reduction of CD is mediated by bacteria in the intestine. It should be noted, however, that cytoplasmic, aldo-keto reductases have been described in a variety of mammalian tissues including human liver and intestine (BACHUR, 1976).

Dechlorinated photodecomposition products of CD and mirex have been described (ALLEY et al., 1974) and a metabolite of mirex in the rhesus monkey has recently been reported which appears to be similar to such a dechlorinated derivative probably arising from bacterial action in the lower gut or in the feces (STEIN, et al., 1976). Similar dechlorinated derivatives of CD have, thus far, not been identified in extracts of human stool.

It is possible that conjugates of chlordecone or chlordecol may be present and that these compounds may represent the "metabolites" in the report of MATTHEWS and MORALES (1977). Evidence for the presence of conjugates of chlordecol in rat bile and feces or in human bile is, at present, equivocal. Further studies are in progress to identify the site(s) of biotransformation of CD in the body and mechanism(s) involved in the reduction of chlordecone to chlordecol *in vivo*.

Acknowledgements

We wish to acknowledge the generous cooperation of Drs. E. Oswald and R, Moseman, of the Health Effects Research Laboratory, Environmental Protection Agency, Research Triangle, NC, and Mr. F. D. Griffith, Jr., Pesticide Residue Laboratory, Virginia Consolidated Laboratories, Richmond, VA for assistance in aspects of this study.

This work is supported in part by a contract (ES-6-2117) and grant (ES-01519) from NEIHS, NIH and by a grant from Allied Chemical Corporation. Dr. Guzelian is the recipient of a Clinical Investigator Award in Gastroenterology from NIAAMD, NIH (5K08-AM-00128).

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