is lost after general rearrangement of cleaved disulfide bonds through interchain or intrachain mechanisms in this system remains to be determined. The conclusion is that freezing in the presence of R-SH discriminately disrupted the determinant groups of peanut proteins; the conarachin system was more sensitive to reduction than α-arachin, and some of the antigenic determinants were protected in whole extracts.

The thiol study on the major peanut proteins reported by Cherry and Ory (1973) showed drastic electrophoretic modifications on disk gels after adding R-SH that were not as readily discernible in the present study. It should be pointed out, however, that dissociation of variable sized subunits (possibly having equal net charges) can be detected through the "sieve" effect on acrylamide gels; this is not necessarily true in agar electrophoresis. Hence, in view of the disk study, perhaps some determinant groups are maintained by interchain disulfide bonds.

ACKNOWLEDGMENTS

The author thanks Jack J. Bergquist for the photography and John P. Cherry for supplying some of the treated samples.

LITERATURE CITED

Cherry, J. P., Ory, R. L., *J. Agr. Food Chem.* **21**, 656 (1973).
Daussant, J., Neucere, N. J., Yatsu, L. Y., *Plant Physiol.* 44, 471 (1969).
Dechary, J. M., Talluto, K. F., Evans, W. J., Carney, W. B., Altschul, A. M., *Nature (London)* **190**, 1125 (1961).
Freedman, M. H., Sela, M., *J. Biol. Chem.* **241**, 2382 (1966).
Grabar, P., Williams, C. A., *Biochim. Biophys. Acta* **10**, 193 (1953).
Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.* **193**, 265 (1951).
Neucere, N. J., *Anal. Biochem.* **27**, 15 (1969).
Sela, M., Schecter, F., Schecter, I., Borex, F., *Cold Spring Harbor Symp. Quant. Biol.* **32**, 537 (1967).
Wall, J. S., *J. Agr. Food Chem.* **19**, 619 (1971).

Navin J. Neucere

Southern Regional Research Center
New Orleans, Louisiana 70179

Received for review January 22, 1974. Accepted April 29, 1974. Southern Regional Research Center is one of the facilities of the Southern Region, Agricultural Research Service, U. S. Department of Agriculture. Use of a company and/or product named by the Department does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

Evidence of Chlorodibenzo-*p*-dioxin and Chlorodibenzofuran in Hexachlorobenzene

Three commercial hexachlorobenzene preparations were analyzed for toxic impurities. The impurities were separated from the hexachlorobenzene by fractional crystallization followed by alumina chromatography. The contaminants were identified by electron capture gas chromatography and by gas chromatography–mass spectrometry. The levels of the impurities were determined when the standards were available. Pentachlorobenzene was the major contaminant in all preparations. A number of other compounds were found including hepta- and octachlorodibenzofuran and octachlorodibenzo-*p*-dioxin. It is important that any studies which are conducted to evaluate the toxicity of hexachlorobenzene take these findings into consideration.

Hexachlorobenzene (HCB) is used as a fungicide to control bunt of wheat. HCB is also encountered as a waste by-product from manufacturing plants which produce chlorinated hydrocarbons (*Chem. Week*, 1973).

Cattle in two Louisiana parishes were recently found to have high levels of HCB in the adipose tissue (*Chem. Week*, 1973). Residues of HCB have also been found in human adipose tissue (Curley, *et al.*, 1973; Brady and Siyali, 1972; Acker and Schulte, 1970) and blood (Acker and Schulte, 1970; Siyali, 1973). In Turkey a human poisoning outbreak occurred in 1955 when grain treated with HCB was inadvertently consumed. Symptoms of the poisoning in Turkey included porphyria cutanea tarda with hypersensitivity of the skin to sunlight, hyperpigmentation and hypertrichosis, hepatomegaly, weight loss, osteoporosis, and enlargement of the thyroid and lymph nodes (Schmid, 1960; DeMatteis *et al.*, 1961). Rats fed a diet of 0.2% HCB showed marked enlargements of the hepatocytes (Medline *et al.*, 1973).

In the present study commercially available HCB was analyzed for toxic impurities which may have been formed during the manufacturing process. The chlorodibenzo-*p*-dioxin (CDD's) and the chlorodibenzofurans (CDF's) were of particular interest because these compounds have previously been found as contaminants in pentachlorophenol (Firestone *et al.*, 1972; Jensen and Renberg, 1972; Villanueva *et al.*, 1973), 2,4,5-T, Silvex, and 2,4-D (Woolson *et al.*, 1972). The toxicities of some of the CDD's have been reported (Rowe *et al.*, 1971; Williams *et al.*, 1972; Higginbotham *et al.*, 1968). In evaluating the toxicity of HCB the impurities in the sample should be determined, since these impurities may contribute to the overall toxicity of the sample.

EXPERIMENTAL SECTION

Three HCB samples were analyzed. Fractional crystallization of each HCB sample from hot benzene was used to separate HCB from the impurities. A total of four or five crystallization fractions were collected from each sample; the last fraction was the supernatant concentrated. Only crystallization fractions 4 and 5 showed any possible CDD or CDF peaks by gas chromatographic analysis.

The impurities in the crystallization fractions were further separated from HCB by alumina chromatography. For each sample crystallization fractions 4 and 5 were adsorbed into 10 g of alumina, then added to the column above the Na_2SO_4 layer. The preparation and elution of the columns are otherwise previously described (Firestone *et al.*, 1972). The alumina used was Fisher No. A-540. Since recovery studies have shown that the CDD's and CDF's elute primarily in fraction 3 and a small amount in fraction 4, these two alumina fractions from each column were concentrated.

The alumina fractions were analyzed by electron cap-

Table I. Contaminants Found in HCB as Determined by Gas Chromatography (ppm)

Sample	Pentachloro-benzene	Octa-CDF	Octa-CDD
A	200	0.35	0.05
B	1,500	2.33	ND[a]
C	81,000	58.3	211.9

[a] ND = not detected. Minimum detectable limit for this sample was <0.2 ppm.

Table II. Per Cent Recovery of Octa-CDD and Octa-CDF from Alumina Columns

	0.1 ppm	10 ppm	100 ppm
Octa-CDF	76	59	80
Octa-CDD	81	94	87

ture gas chromatography for peaks with the retention times of the available CDD and CDF standards. The octa-CDD and octa-CDF standards were purchased from Analabs, Inc., North Haven, Conn. Tetra-CDD was a gift of Dow Chemical Co., Midland, Mich. Hexa-CDD and hepta-CDD were synthesized according to a known method (Pohland and Yang, 1972). The hexa-CDD standard was then purified by preparative gas chromatography. The hepta-CDD was not purified sufficiently to be used as a standard. It was used, however, to determine the presence of hepta-CDD in the sample by comparison of its retention time on the gas chromatograph.

A gas chromatograph with a tritium detector was equipped with a U-shaped glass column 6 ft × 0.25 in. o.d. packed with 3% OV-1 on 80–100 mesh Supelcoport. The temperatures were as follows: column, 220°; detector, 200°; and injector, 235°. The nitrogen carrier flow was 65 ml/min at 52 psi. Under these conditions the octa-CDF standard had a retention time of 24 min.

A gas chromatograph–mass spectrometer was used to confirm the presence of the CDD's and CDF's found by electron capture gas chromatography and to identify the other impurities in alumina fractions 3 and 4. An LKB 9000 was equipped with a mass marker (±0.3 mass unit) and interfaced to a 10 ft × 0.25 in. o.d. coiled glass column packed with 3% SE-30 on 80–100 mesh Chromosorb W, acid washed, DMCS treated. The temperature of the column and flash heater was 230°. The helium flow rate was 70 ml/min at 12 psi. Other conditions were: leak current, 5 μA; filament current, 3.8 A; box current, 50 μA; trap current, 60 μA; ionizing energy, 70 eV.

RESULTS AND DISCUSSION

The major impurity in all the HCB samples was pentachlorobenzene. When the alumina fractions were analyzed by gas chromatography, a peak with the retention time of octa-CDD or octa-CDF was found in each sample of HCB. Analysis by gas chromatography–mass spectrometry was necessary to determine the identity of the peak, since octa-CDD and octa-CDF were unresolved on several gas chromatographic columns. If both compounds were present, an estimate of the relative amount of each was made from the response on the mass spectrometer. The levels of octa-CDD and octa-CDF were then calculated from the gas chromatogram using the peak height method and the appropriate standard.

The contaminants found by gas chromatography are given in Table I. No correction has been made for per cent recovery from the alumina columns. Pentachlorobenzene was found in all three samples. Octa-CDF was found in sample B, octa-CDF and octa-CDD in sample C, and octa-CDF, octa-CDD, and hepta-CDF in sample A.

Table III. Other Contaminants in HCB Identified by Mass Spectrometry

Sample	*m/e*	No. Cl	Probable moieties
A	406	7	Heptachlorodibenzofuran
	426	8	Octachlorobiphenyl
	494	10	Decachlorobiphenyl
	342	7	1-Pentachlorophenyl-2,2-dichloroethylene
B	494	10	Decachlorobiphenyl
	424	8	Octachlorobiphenylene
	400	8	Octachloro-1,1'-bicyclopentadienylidene
	270	6	Hexachlorocyclopentadiene
C	460	9	Nonachlorobiphenyl
	494	10	Decachlorobiphenyl
	374	5	Pentachloroiodobenzene
	329	7	Heptachlorotropilium

Table II shows the recovery of octa-CDD and octa-CDF standards from the alumina columns (fractions 3 and 4) at the approximate levels found in the samples.

Other impurities found in the HCB samples are given in Table III. These compounds were identified by mass spectral analysis. The levels could not be quantitated because the standards were not available. The total ion current indicates that the concentrations of these impurities identified by gas chromatography–mass spectrometry are of the same order of magnitude or less than the octa-CDF found in the sample.

LITERATURE CITED

Acker, L., Schulte, E., *Naturwissenschaften* **57**, 497 (1970).
Brady, M. N., Siyali, D. S., *Med. J. Aust.* **1**, 158 (1972).
Chem. Week, **112**(17), 21 (1973).
Curley, A., Burse, V. W., Jennings, R. W., Villanueva, E. C., Tomatis, L., Akazaki, K., *Nature (London)* **242**, 338 (1973).
DeMatteis, F., Prior, B. E., Rimington, C., *Nature (London)* **191**, 363 (1961).
Firestone, D., Ress, J., Brown, N. L., Barron, R. P., Damico, J. N., *J. Ass. Offic. Anal. Chem.* **55**, 85 (1972).
Higginbotham, G. R., Huang, A., Firestone, D., Verrett, J., Ress, J., Campbell, A. D., *Nature (London)* **220**, 702 (1968).
Jensen, S., Renberg, L., *Ambio* **1**, 62 (1972).
Medline, A., Bain, E., Menon, A. I., Haherman, H. F., *Arch. Pathol.* **96**, 61 (1973).
Pohland, A. E., Yang, G. C., *J. Agr. Food Chem.* **20**, 1093 (1972).
Rowe, V. K., Norris, J. M., Sparschu, G. L., Schwetz, B. A., Gehring, P. J., 162nd National Meeting of the American Chemical Society, Washington, D. C., Sept 1971, Abstract PEST-86.
Schmid, R., *New Engl. J. Med.* **263**, 397 (1960).
Siyali, D. S., *Med. J. Aust.* **2**, 1063 (1973).
Villanueva, E. C., Burse, V. W., Jennings, R. W., *J. Agr. Food Chem.* **21**, 739 (1973).
Williams, D. T., Cunningham, H. M., Blanchfield, B. J., *Bull. Environ. Contam. Toxicol.* **7**, 57 (1972).
Woolson, E. A., Thomas, R. F., Ensor, P. D. J., *J. Agr. Food Chem.* **20**, 351 (1972).

Ellen C. Villanueva[1]
Ralph W. Jennings[2]
Virlyn W. Burse[3]
Renate D. Kimbrough*[4]

Environmental Protection Agency
Chamblee Toxicology Laboratory
Chamblee, Georgia 30341
[1] The Coca-Cola Export Corporation
Atlanta, Georgia 30301
[2] Environmental Protection Agency, Region IV
Atlanta, Georgia 30309
[3] Consumer Product Safety Commission
N.W. Atlanta, Georgia 30309
[4] Center for Disease Control, Toxicology Section
Atlanta, Georgia 30333

Received for review February 11, 1974. Accepted May 6, 1974.