Measurement of Endrin Following Epidemics of Poisoning

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Between 3 June and 15 July 1967, four explosive outbreaks of acute poisoning with the insecticide endrin occurred in Doha, Qatar and Hofuf, Saudi Arabia. They resulted in the hospitalization of 874 persons and the death of 26 persons. The outbreaks probably caused a lesser degree of poisoning in many others who recovered without seeking medical aid. A thorough epidemiological study reported by Weeks (1) revealed that flour in a lower deck of each of two ships had been contaminated by an emulsifiable concentrate of endrin carried on an upper deck of the same hold. The episodes were unrelated, but leakage in each instance probably occurred when the insecticide was unloaded by net. The flour, which was taken off later at different ports, led to poisoning when separate lots of it were made into bread and eaten.

This paper reports only our chemical studies concerned with the outbreaks. It describes the method used, and summarizes the chemical evidence that endrin from contaminated flour was, in fact, the cause of illness. The paper also presents available measurements pertaining to the distribution of endrin and its rate of loss in man.

Methods

The samples were collected in each country by consultants sent by the World Health Organization. An organophosphate insecticide was thought by local authorities to be the cause of the illness. A WHO team found an insecticide formulation (BHC) on a shelf in a bakery in Doha, Qatar. This insecticide formulation was also suspected as the probable contaminant. Selected benzene[1] extracts of bread and flour were analyzed by flame photometry and electron-capture gas-liquid chromatography for organophosphates and for chlorinated hydrocarbons. The findings were negative for organophosphates and γ-BHC in the flour and bread. The insecticide formulation was found to contain 11.75% γ-BHC (1,2,3,4,5,6-hexachlorocyclohexane) as the active ingredient. However, a compound in the bread and flour was isolated and identified as endrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-endo-5,8-dimethanonaphthalene). This identification was confirmed

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[1] Pesticide quality benzene used.

by electroconductometric gas-liquid chromatography, thin-layer and paper chromatography, infrared and NMR spectroscopy.

The experimental details are given in three parts: the extraction procedures, the gas chromatographic procedures, and the confirmational analysis.

Extraction. The bread samples were thoroughly ground in a mortar. These ground samples (150-875 mg) were quantitatively transferred to 15-ml glass-stoppered centrifuge tubes using 2 ml pesticide quality isopropanol and 4 ml pesticide quality benzene. They were extracted by shaking in a Scientific Products Delux Mixer.[2] After centrifugation, the isopropanol-benzene mixture was aspirated and transferred to another 15-ml centrifuge tube. Hexane-extracted distilled water was added. The benzene phase was separated from the aqueous isopropanol phase.

Flour samples were thoroughly mixed and extracted by the same procedure. The benzene extracts of the broad and flour samples were diluted or concentrated to appropriate volume and held for analysis by gas-liquid chromatography.

The plasma samples were extracted by the method of Dale *et al.* (2) and the urine samples by the method of Cueto and Biros (3).

A few samples of stomach wall, liver, and kidney taken at autopsy during the first outbreak in Doha, Qatar were the only tissues received. These samples were weighed (374-654 mg) and transferred to tissue grinders. Each sample was homogenized 3 times with 2 ml pesticide quality n-hexane. The hexane extracts were combined, centrifuged, concentrated to appropriate volumes, and held for analysis by gas-liquid chromatography.

Gas Chromatography. The gas chromatographic analyses were completed on a Micro Tek Model MT 220 instrument and a Varian Model 1525 equipped with tritium electron-capture detectors. The Micro Tek MT 220 instrument was operated with a 4' x 1/4" aluminum column packed with 3% Dow 220 on 70/80 mesh, acid-washed, DMCS-treated, Chromosorb G. The temperatures of the column, the inlet injection port, and the detector were 175°C, 200°C, and 200°C, respectively. The carrier gas was nitrogen at 60 psi with a flow rate of 60 cc/min. The detector was operated in the d.c. mode (15 v). The Varian instrument was operated with a 5' x 1/8" Al column packed with 3% OV-17 on 70/80 mesh, acid-washed, DMCS-treated, Chromosorb G. The temperatures of the column, the inlet injection port, and the detector were 175°C, 198°C, and 200°C, respectively. The carrier gas was nitrogen at 60 psi with a flow rate of 120 cc/min. The detector was operated at a fixed potential (d.c. 90 v). Other

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[2] Use of trade names is for identification purposes only, and does not constitute endorsement by the Public Health Service, or the U.S. Department of Health, Education, and Welfare.

columns used were 3% QF-1 and 3% DEGS. Best results were achieved on the 3% OV-17 and 3% Dow 200 columns. The endrin standard gave three peaks on the 3% Dow 200 column with retention times of 9.0, 17.4, and 20.2 minutes, respectively. This same pattern was observed in the sample extracts. Possible chemical degradation of the compound on the column might account for the presence of three peaks. On 3% OV-17 one peak was observed at 14.5 minutes.

Confirmational Analysis. The identification of the contaminant (endrin) was confirmed by subjecting flour extracts to electroconductometric gas-liquid, thin-layer, and paper chromatography. A Micro Tek Model 2000 instrument equipped with a Coulson Electrolytic Conductivity Detector operated in the oxidative mode for chloride determination was used. The column and other operating parameters were the same as those with the Micro Tek MT-220 instrument. The observed retention times were identical to those mentioned earlier.

Five grams of flour were placed in a 15-ml centrifuge tube, 8 ml isopropanol or 6 ml acetone was added, and the mixtures were shaken vigorously. The tubes were then centrifuged at 2000 rpm for 5 minutes. Paper chromatography of spots from 100 ul aliquots of the supernatant liquid detected 50 ppm of endrin. Concentration of a 2.0-ml aliquot to 0.2 ml before spotting 100 ul increased the sensitivity to 5 ppm. The paper chromatograms were developed by two-phase ascending systems including:

System A, Immobile: 5% v/v paraffin oil in ether. Mobile: Acetone - water (3:1). Development time, 1 hour.

System B, Immobile: N,N'-dimethylformamide, 35% v/v in ether. Mobile: 2,2,4-Trimethylpentane. Development time, 45 minutes.

System C, Immobile: 5% v/v paraffin oil in ether. Mobile: Methyl alcohol-acetic acid-water (80:5:15). Development time, 1 hour.

The spots on the chromatograms were detected by Mitchell's spray for chloride compounds (0.4 g silver nitrate, 2.5 ml 2-phenoxyethanol, and 5 ml acetone, diluted to 50 ml with chloride-free water) (4). The R_f values corresponded to those for standard endrin and were 0.53, 0.89, and 0.45 for systems A, B, and C, respectively.

Thin-layer chromatography was accomplished on aluminum oxide and silica gel plates using pesticide quality n-hexane-ethyl ether (95:5) for development. The R_f values for both samples and standard endrin were 0.35 on aluminum oxide and 0.31 on silica gel.

Verification that the flour sample extracts contained endrin was obtained by spotting the extracts on paper along with separate spots of standard solutions of endrin, dieldrin and a mixture of the two. These papers were then exposed to ultraviolet light for 1 hour. Two-phase chromatography of the papers by two systems of

TABLE 1

Endrin Concentrations Found in Various Samples From Three of the Four Outbreaks

Sample	Endrin concentration (ppm) First Doha outbreak	Third Doha outbreak	Hofuf outbreak
Bread, portions of which had been eaten by patients	48	1,339-1,807	243-537
Flour, used to make the same bread	INA[a]	2,153	3,367
Blood from patients	0.007-0.032	INA	0.008-0.027
Urine from patients	INA	INA	<0.004-0.007
Vomitus from patient	INA	INA	5.24
Tissues (autopsy) from:			
stomach wall	0.16	INA	INA
liver	0.685	INA	INA
kidney	0.116	INA	INA

[a] INA: Information not available.

TABLE 2

Concentration of Endrin in the Urine and Plasma Collected from Acutely Poisoned Patients on the Day of Onset

Patient's initials	Interval from ingestion to symptoms (hr)	Concentration of endrin (ppm) Plasma	Urine
H.N.	3.3	0.008	0.005
F.D.	1.5	0.011	<0.007
A.R.	1.5	0.027	<0.004

different polarity (4) verified that the flour extracts gave the same decomposition products upon exposure to U.V. light as were given by standard endrin.

Our results were confirmed independently at the pesticide laboratory at Perrine, Florida by three separate methods. Portions of flour, bread, and other samples were subjected to thin-layer chromatography, infrared spectrophotometry, and nuclear magnetic resonance spectrometry. The thin-layer chromatograms, IR spectra, and NMR spectra of the samples were compared with the thin-layer chromatograms, IR spectra and NMR spectra of pure endrin and of pure endrin that had been subjected to weathering. Virtually all spots on sample chromatograms were accounted for on the chromatograms of weathered endrin. All bands on the IR spectra of the samples were accounted for on the IR spectra of the weathered endrin also, as were the peaks on the NMR spectra. There was no doubt that the chromatograms and the spectra of the samples were those of endrin or its degradation products.

Results and Discussion

The flour used for making bread was not necessarily the most contaminated. The concentration of endrin in cloth from a stained area of one sack was 194,000 ppm; the concentration in flour from directly beneath the stain was 28,070 ppm; and the concentration in flour from deep within the sack was 4,460 ppm. As shown in Table I, the highest concentration of endrin found in flour known to have been used for making bread eaten by a patient was 3,367 ppm. The lowest concentration of endrin in bread known to have caused poisoning in one of the four outbreaks was 48 ppm. The lowest comparable value of 176 ppm was reported earlier by Coble et al. (5). Table I shows that endrin was measured not only in flour, bread, and vomitus, but also in blood, urine, and tissues from patients. Endrin is not found in comparable samples from the general population or even from workers who manufacture and formlate the insecticide (6).

We received samples of urine and plasma which had been obtained from three acutely poisoned patients on the day of onset of symptoms. Table II shows that endrin was more regularly detected in the plasma and that the concentration was higher than in the urine. The 14 late samples that we received were uniformly negative for endrin. By contrast, dieldrin is easily measured in the blood of people in the general population (2) and was still elevated 14 months after accidental ingestion leading to poisoning (7).

Dieldrin, p,p'-DDT, o,p'-DDT, p,p'-DDE, o,p'-DDE, and BHC were detected in the blood, urine, and tissue (stomach wall). The levels were no greater than those commonly found in the general population, and these compounds were entirely unrelated to the illness.

The chemical evidence presented clearly indicates that endrin was in fact the contaminant that caused the illness and deaths in the four outbreaks of poisoning in Doha, Qatar and Hofuf, Saudi Arabia. Endrin was detected in the blood and urine of patients, and in tissues taken at autopsy. The concentrations in the blood were similar to those found by Coble *et al*. (5). Samples of blood and urine taken 29-31 days after the outbreak were uniformly negative for endrin.

Acknowledgment

Infrared and NMR spectral analysis, electron-capture gas-liquid chromatography and thin-layer chromatography were completed as further confirmational analysis by the Pesticide Research Laboratory, Pesticide Program, Perrine, Florida.

Dr. Wayland J. Hayes, Jr., formerly with the PHS Pesticides Program, gave advice and guidance throughout the study. Dr. Hayes is now with the Department of Biochemistry, Vanderbilt University, Nashville, Tennessee.

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