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Detection of *Bis*-(Trichloromethyl) Disulfide in Strawberries

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Captan (N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide) has been used extensively as a valuable fungicide and was approved on a wide variety of fruit and vegetables following its discovery in 1952. This compound is an odorless white solid and is usually produced as a fine powder. It is represented by the formula shown in Figure 1. The toxicity of this trichloro-methylsulfenyl fungicide has been documented (FRY and FISCOR 1978).

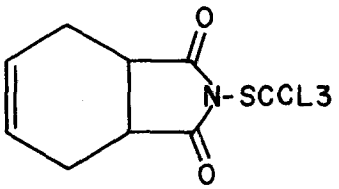

Figure 1. The molecular structure of captan (N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide)

Cytogenetic and lethal studies on captan were reported by TEZUKA et al. (1978). These authors established that there was a significant increase in the frequency of cultured human diploid cells showing stickiness and a severe mitotic inhibition at concentration levels of 3.0 to 4.0 micrograms of captan per mL of medium. Other investigators, (BRIDGES 1975, KADA et al. 1974, SHIRASU et al. 1976), reported that captan induced point mutation in bacteria and cultured Chinese hamster cells. It has been established in the CODE OF FEDERAL REGULATIONS (1976), that tolerances for residues of this fungicide shall not exceed 25 parts per million in or on fresh strawberries. During the assay of captan in fresh strawberries we detected and identified bis-(trichloromethyl) disulfide, a com-

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pound which is structurally similar to the trichloromethylthio moiety of captan. We also established that this compound is present as an impurity in technical captan.

MATERIAL AND METHODS

Samples. Several pounds of fresh whole strawberries were collected from a wholesale market in Florida. This sample was shipped under refrigeration (2-3°C) and was examined for captan within two days of collection.

Captan material. Two types of captan standards were used. The first was a Primary Standard (99.8% purity) obtained from the Environmental Protection Agency, Pesticides Reference Standards Section, Beltsville, Maryland. The second type consisted of a technical material and was purchased commercially from Chevron Chemical Company. The purity of the latter material was labeled to be 50 percent.

Extraction of captan and the impurity. The strawberry sample was subjected to the extraction procedure used by the Food and Drug Administration (AOAC, 1975). In this procedure, an acetonitrile extraction, a petroleum ether partition and a chromatographic separation on a florisil column was used. The impurity detected, in the strawberry extract obtained from the florisil column, was separated in a fraction of ethyl ether in petroleum ether (6+94).

Gas liquid chromatography. The gas chromatograph was equipped with a tritium electron capture detector. Two glass columns each 1.8 meters x 4 mm (i.d.) were used. The first column consisted of 10% DC-200 on 80/100 mesh chromosorb W (HP) and the second contained an equal mixture of 10% DC-200 and 15% QF-1 on 80/100 mesh chromosrob W (HP). The column temperatures were set at 200°C. Nitrogen was used as the carrier gas at a flow rate of 120 mL/min.

Gas-chromatography-mass spectrometry. The GC/MS analysis was performed using a Finnigan 3300 automated mass spectrometer system. Both Electron Impact and Chemical Ionization spectra were obtained. The gas chromatograph connected to the mass spectrometer was equipped with a 1.8 meter x 2 mm (i.d.) glass column containing a 3% OV-101 on 80/100 mesh chromosorb W (HP). For EI, the gas chromatograph was interfaced to the mass spectrometer with a glass, single stage jet separator. Helium carrier gas was adjusted to give a flow rate of 20 mL per minute. For CI, the gas chromatograph was connected directly to the mass

spectrometer and methane was used as both the carrier and ionization gas. The flow rate of the methane gas was adjusted to give a pressure of about 1 mm Hg in the mass spectrometer source. The gas chromatograph columns were used both isothermally at 200°C and temperature programed from 140°C to 220°C at 10°C/min.

RESULTS AND DISCUSSION

Strawberry sample. The unknown impurity contained in the ethyl/petroleum ether fraction gave an electron-capture response with a retention time relative to Aldrin (RRT/A) of 0.19 on 10% DC-200 and a RRT/A of 0.18 on 10% DC-200 + 15% QF-1. GC/MS analysis of this response gave a molecular ion cluster beginning at m/z 298 with an isotopic abundance pattern for a six-chlorine molecule (Figure 2). A mass spectra catalog (EIGHT-PEAK INDEX) contained a single compound which met these criteria. The impurity was identified as bis-(trichloromethyl) disulfide. Its mass spectrum and peak assignments have previously been reported (KAAE and SENNING 1968).

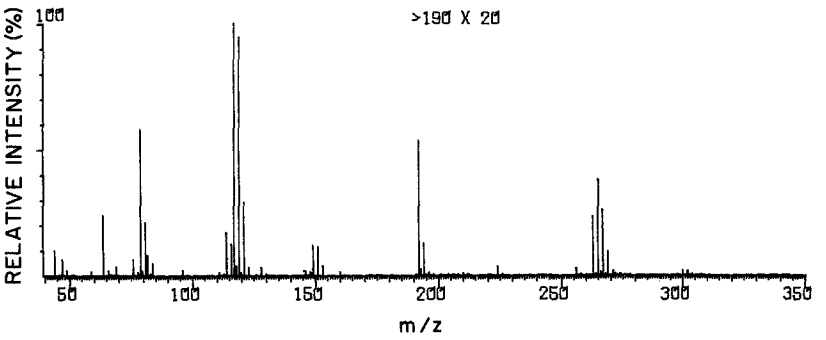

Figure 2. The electron impact mass spectra of the impurity found on strawberries (bis-trichloromethyl) disulfide).

Reference material. The technical captan material and the captan primary reference standards were both analyzed for the presence of the impurity. The analysis of these two captan materials were conducted using the same procedures as described for the strawberry sample. The extract from the technical material contained bis-(trichloromethyl) disulfide as well as two other chlo-

rine/sulfur-containing compounds (Figure 3). The other two compounds were identified by GC/MS as bis-(trichloromethyl) sulfide and bis-(trichloromethyl) trisulfide.

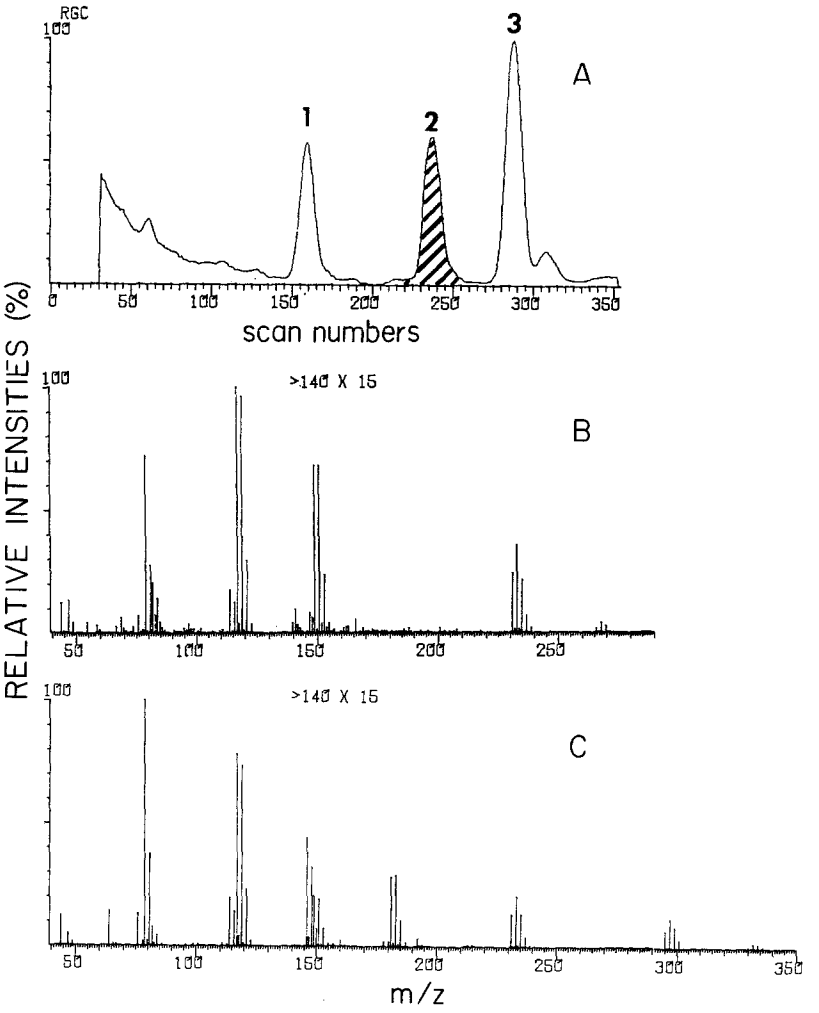

Figure 3. The GC/MS analysis of the ethyl/petroleum ether (6+94) fraction of the technical captan material; (A) Gas chromatography of the extract where peak 1 represents the bis-(trichloromethyl) sulfide, peak 2 (shaded) is the disulfide impurity found on strawberries (see Figure 2 for mass spectrum), and peak 3 represents the bis-(trichloromethyl) trisulfide; (B) EI spectrum of the sulfide compound and (C) EI spectrum of the trisulfide compound.

GLC and GC/MS analysis of the primary standard captan gave no response for the three chlorine/sulfur impurities. This excludes the possibility of thermal degradation on the GLC systems and indicates that the three compounds observed were present as contaminants and/or impurities in technical captan formulations.

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