nitrogen aeration, may be involved in retention at the higher temperature but further work is needed to explain this difference.

In Table IV the desorption of acrylonitrile is given for the treatment and aeration at 0 °C. The desorption time was 42 days for wheat, 35 days for corn, and 21 days for peanuts. Since acrylonitrile desorbed very slowly from wheat treated at 25 °C so that complete desorption required 21 days aeration (Table II) a study was made to find ways of hastening desorption. Wheat was oven dried at 110 °C for 2–6 h before and after treatment with the fumigant to test the effect of removing moisture from the grain and also nitrogen was flushed over wheat prior to treatment to remove any volatile materials that might absorb fumigant (Table V). When wheat was oven dried for 4 h after treatment with the fumigant the desorption time was reduced from 21 to 5 days. If the wheat was dried before fumigation the fumigant desorbed in less than 7 days, and if the dried wheat was stored in a humid atmosphere to reestablish its initial moisture content before fumigation the acrylonitrile was still desorbed in 6 days. This suggested that moisture itself had little influence on retention of acrylonitrile. In this drying procedure in 6 h 12% moisture was removed from the wheat. When wheat was flushed with nitrogen at a flow rate of 50 cm^3/min from 1 to 6 days before fumigation, the desorption time for acrylonitrile was also reduced. Flushing with nitrogen for 1 day, prior to fumigation, reduced the residual acrylonitrile so that only 1 ppm remained after 14-days aeration and when flushing was extended to 6 days no acrylonitrile was present after 7-days aeration.

At the end of 7-days aeration grain was flushed with nitrogen for 1 to 6 days and contained 1.6 and 0 ppm of acrylonitrile, respectively. These amounts are well below the residue in grain, fumigated the same way, but without nitrogen flushing, after aeration at 25 °C.

When passing the nitrogen stream through a cold trap several volatile substances from the grain were found to be condensed in the trap. These were separated into individual components by gas chromatography but they are, as yet, unidentified. The reduced levels of acrylonitrile residue found in wheat subjected to aeration or heat prior to fumigation may have been related to removal of these volatile materials that served to bind the fumigant.

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Determination of Hexabromobenzene in Rat Tissues by Gas–Liquid Chromatography

A method for the determination of hexabromobenzene (HBB) in rat tissues has been evaluated. The recovery from spiked samples is close to 100%. No cleanup procedure is necessary before gas–liquid chromatography on Chromosorb W coated with 4% SE-30 and 6% QF-1 or OV-210. The minimum limit of detection is 0.01 ng of standard HBB and 0.1 ppm of HBB in rat tissues.

Hexabromobenzene (HBB) is a fire retardant used in plastics, textiles, and woods (Negishi et al., 1972; Raley, 1972; Mischutin, 1974; Pashin et al., 1974). Its close relative hexachlorobenzene (HCB) is a fungicide and appears to be ubiquitous (see Vos et al., 1972). Related compounds, polybrominated biphenyls, which are also used as a fire retardant, caused considerable damage in the U.S. dairy and cattle industry (Jackson and Halbert, 1974; Carter, 1976). Another related compound, hexabromobiphenyl, was observed to induce liver porphyria in several avian species (Strik, 1972). We have been studying the effects of HBB in conjunction with HCB on rats. Therefore, before quantitating HBB residues in rat tissues, the HBB analytical procedure was evaluated. The analytical method for pentachloronitrobenzene (PCNB) and its metabolites (Kuchar et al., 1969) was evaluated for HBB and was modified so that a portion of the aqueous homogenate used for enzymatic analysis can be used also for HBB residue determination. The method was also scaled down for economy and ease of handling.

MATERIALS AND METHODS

The tissues were ground with redistilled water in a glass homogenizer to obtain 20% (w/v) homogenate. The aqueous homogenate or distilled water was spiked with an HBB standard in hexane. The solvent was evaporated gently under nitrogen; then the homogenate was mixed thoroughly before extraction.

Three extraction procedures were evaluated. In each extraction, 0.2 ml of homogenate was used. The homogenate was mixed with 5 ml of acetonitrile before adding 5 ml of water. The solution was then shaken with 5 ml of hexane (procedure 1). The homogenate was mixed with 5 ml of water and shaken with 5 ml of hexane (procedure 2) or benzene (procedure 3). The solution was shaken in a test tube with a screw cap lined with Teflon for 0.5 or 1 h on a mechanical shaker (Buchler Instrument). After shaking, the solution was centrifuged and the solvent layer analyzed by gas–liquid chromatography (GLC).

Procedure 1 was used to extract the HBB residue from various tissues of rat pups, which nursed on dams that were fed rat diet containing 80 ppm of HBB.

Analysis of HBB was performed by using a gas–liquid chromatograph equipped with a tritium detector (Aerograph HyFi Model 600). Glass columns 0.4 cm i.d. × 90, 105, and 120 cm long were packed with Chromosorb W (AW) coated with 4% SE-30 + 6% QF-1 (column A) or OV-210 (column B). Unless indicated, the oven was maintained at approximately 195 °C while the nitrogen gas flow was 35 or 50 ml/min depending on the column

Table I. HBB Residue Level ± Standard Error of the Mean (ppm) for Rat Dams and 17-Day Old Pups That Were Nursed on Dams Fed a Diet Containing 80 ppm of HBB

	17-day old pups				
Dam liver	Liver	Kidney	Heart	Spleen	Brain
4.8 ± 0.7	14.3 ± 0.5	2.6 ± 0.2	3.1 ± 0.1	2.7 ± 0.1	1.5 ± 0.1

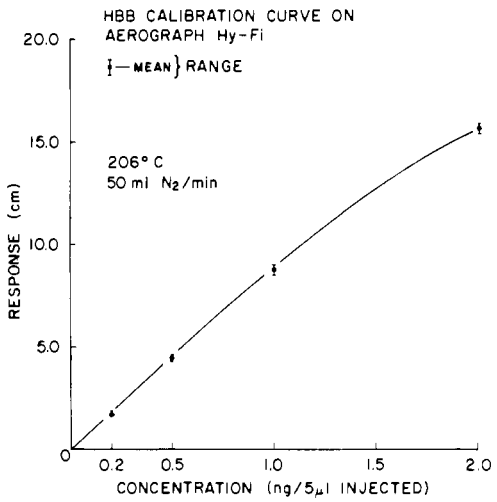

Figure 1. A typical calibration curve for HBB obtained by using a glass column, 0.4 cm i.d. × 105 cm long, packed with Chromosorb W (AW) coated with 4% SE-30 and 6% QF-1.

used and the elution time desired. The injection volume was 5 μl.

RESULTS AND DISCUSSION

Figure 1 shows a typical HBB calibration curve obtained by using column A. The detection was linear up to 2 ng of HBB at 205 °C oven temperature. Similar linear curves were obtained with a 120-cm long column at 215 °C; there was a slight reduction of the response of the detector as the amount of HBB injected increased up to 10 ng. By using a 90-cm long column at 195 or 205 °C, linear calibration curves were also obtained. The minimum limit of detection was 0.01 ng of standard HBB and 0.1 ppm of HBB in rat tissues.

Figure 2 shows the HBB peak (no. 12) cochromatographed with some organochlorine pesticides on column B. Similar chromatograms were obtained with column A. Column B was preferred since it was stable at a temperature up to 275 °C. The amount detected (80 ng) was comparable to the amount detected for *p,p*-DDT (50 ng) or *o,p*-DDT (38 ng).

The unknown peak from the HBB standard was eluted earlier (Figure 2, peak no. 5). This peak increases when the standard solution was left for several weeks on a bench top under the normal lighting conditions in the laboratory. This unknown component appeared to be more polar than HBB. Further studies will be carried out to quantitatively recover and identify this compound.

On the average, procedures 2 and 3 gave recoveries of 56 ± 10 and 84 ± 10%, respectively, whereas procedure 1 gave an approximately 99 ± 1% recovery. The HBB contaminant was partially recovered from the aqueous medium.

Kuchar et al. (1969) recommended the use of sodium sulfate for PCNB and its metabolites. However, in this experiment, sodium sulfate did not have any effect on the efficiency of extraction of HBB nor the quality of the chromatographic background. Shaking the solution for 0.5 h was not sufficient to extract HBB quantitatively; the recovery was about 82%. The recoveries with 1-h shaking were 99 ± 1 and 97 ± 1% from the water and liver, respectively; the difference between these values was not statistically significant (Student's *t* test).

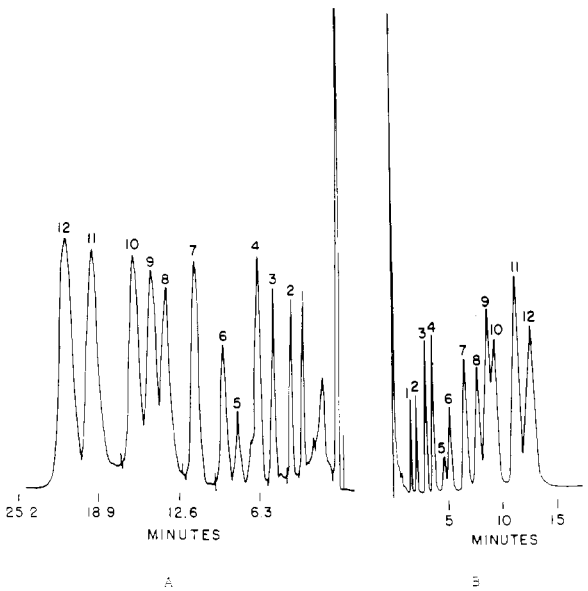

Figure 2. Typical chromatograms of HBB cochromatographed with some organochlorine pesticides: (A) on a column, 0.4 cm i.d. × 183 cm long, packed with Chromosorb W (AW), 60–80 mesh, coated with 4% SE-30 + 6% OV-210; (B) on a column 0.4 cm i.d. × 105 cm long, packed with Chromosorb W (AW), 80–100 mesh, coated with 4% SE-30 + 6% QF-1. Peak numbers, the amount detected in parentheses, are: (1) hexachlorobenzene (2.08 ng); (2) lindane (3.13 ng); (3) heptachlor (6.45 ng); (4) aldrin (6.72 ng); (5) unknown; (6) heptachlor epoxide (6.34); (7) *p,p*-DDE (12.60 ng); (8) dieldrin (12.75 ng); (9) *o,p*-DDT (38.05 ng); (10) *p,p*-TDE (25.20 ng); (11) *p,p*-DDT (49.76 ng); (12) HBB (80.00 ng).

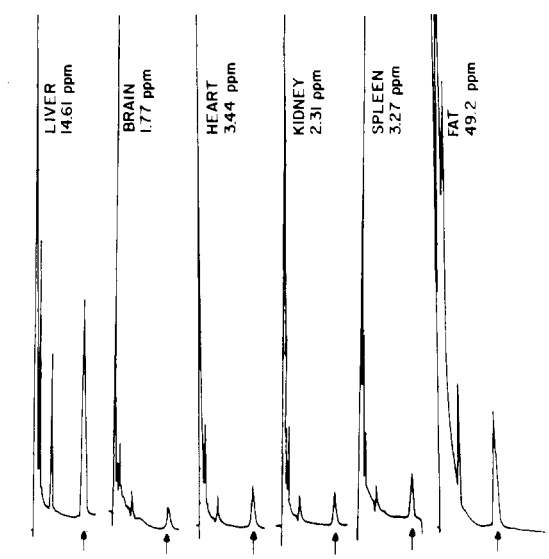

Figure 3. Typical chromatograms of HBB in various tissues of the 17-day old rat nursed by dams that fed on a diet containing 80 ppm of HBB, and a typical chromatogram of HBB from a fat sample obtained from a mature rat that fed on a diet containing 160 ppm of HBB. Column B was used at ~195 °C.

Figure 3 shows typical chromatograms of tissues containing HBB. The samples did not have a background that

interferes with the HBB detection even though the extract was not cleaned up.

Table I shows the levels of HBB in the dam liver and in various tissues of 17-day old pups. The HBB level found in the pup liver is approximately 3 times that of the dam and the standard errors of the mean are very small indicating the reproducibility of the determination.

CONCLUSION

The method for extracting HBB using acetonitrile/hexane partition is quantitative. It is simple and does not require cleanup of the extract. The initial extraction step is compatible with that for analysis of esterase activity since the initial aqueous homogenate can be used in both residue and enzymatic analyses. The method is efficient and time saving.

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A High-Pressure Liquid Chromatographic Method for the Quantitation of Neohesperidin Dihydrochalcone

Neohesperidin dihydrochalcone was resolved from filtered grapefruit juice by high-pressure liquid chromatography (HPLC) using a micro C-18 column and eluting with a water–acetonitrile system. Detection was accomplished at 280 nm.

Neohesperidin dihydrochalcone (NHDHC), a nonnutritive sweetening agent which is 20 times sweeter than saccharin on a molar basis (Horowitz and Gentili, 1963), is prepared from the flavonoid glycosides neohesperidin (Horowitz and Gentili, 1969) or naringin (Krbechek et al., 1968). Since there is a possibility that NHDHC may be employed in the citrus industry as a nonnutritive sweetener of grapefruit juice, a simple rapid analytical assay for this compound became desirable. This paper reports such a procedure.

MATERIALS AND METHODS

Apparatus. A Model ALC 202 high-pressure liquid chromatograph (HPLC) with a Model 6000 A pump and U6K injector (Waters Associates, Milford, Mass.) was used. The recorder was a Texas Instruments Servo/Riter II 2-pen. A Schoeffel UV–visible liquid chromatography analyzer Model SF 770 (Schoeffel Instrument Corp., Westwood, N.J.) was the detector. A Spectra-Physics Integrator (minigrator, Spectra-Physics, Santa Clara, Calif.) was used. A Waters Associates sample clarification kit with 1.2 or 0.45 μm Millipore aqueous filter system was used.

Column. A Waters Associates 30 cm × 4 mm i.d. reverse phase μ Bondapak C-18 column (octadecyltrichlorosilane chemically bonded to <10 μ Porasil packing) was used.

Reagents. The eluting system was water–acetonitrile, 75:25, v/v. Both solvents were degassed.

Sample Preparation. Fresh, hand-squeezed, processed single-strength grapefruit juice or reconstituted concentrate which had been sweetened with NHDHC was filtered.

High-Pressure Liquid Chromatographic (HPLC) Resolution and Quantitation of Neohesperidin Dihydrochalcone. An aliquot (50 μl) of the above filtered juice was injected onto the column with a flow rate of 1.5 ml/min. Detection was accomplished at 280 nm with 0.1 absorbance unit full scale. Integration was conducted at an attenuation of 1.0, peak width setting of 41, and slope sensitivity of 270. The recorder chart speed was 12 in./h.

The quantity of NHDHC in unknown samples was determined from a linear regression equation. This equation was obtained from eight standard samples of NHDHC over the range of 0.1–1.0 μg. These samples were eluted isocratically and detected under the above conditions.

Percent Recovery and Precision. The reliability of the procedure was determined by a series of recovery experiments in which a base sample of grapefruit juice was fortified with known amounts of NHDHC. Five individual samples were fortified with sufficient NHDHC to provide a concentration of 3–15 ppm of NHDHC in 3-ppm increments.

The repeatability of the method was determined by analyzing five aliquots from a grapefruit juice sample containing NHDHC.

RESULTS AND DISCUSSION

This procedure constitutes a simple, rapid, quantitative determination of NHDHC. The time required for a complete analysis was 20 min. The samples are filtered to remove particulate material which may clog the system.