Grimmer, G. *Dtsche. Apoth.-Ztg.* **1968**, *108*, 529.
Grimmer, G.; Böhnke, H. *J. Assoc. Off. Anal. Chem.* **1975**, *58*, 725.
Grimmer, G.; Hildebrandt, A. *Chem. Ind. (London)* **1967**, *1967*, 2000.
Haenni, E. D.; Howard, J. W.; Joe, F. L., Jr. *J. Assoc. Off. Agric. Chem.* **1962**, *45*, 67.
Howard, J. W.; Fazio, T. *J. Assoc. Off. Anal. Chem.* **1980**, *63*, 1077.
Insco, M. N. *Anal. Chem.* **1964**, *36*, 2505.
Joe, F. L., Jr.; Salemme, J.; Fazio, T. *J. Assoc. Off. Anal. Chem.* **1982**, *65*, 1395.
Kolarovic, L.; Traitler, H. *J. Chromatogr.* **1982**, *237*, 263.
Lee, R. F.; Sauerheber, R.; Dobbs, G. H. *Mar. Biol. (Berlin)* **1972**, *17*, 201.
Lijinsky, W.; Ross, A. E. *Food Cosmet. Toxicol.* **1967**, *5*, 343.
Lijinsky, W.; Shubik, P. *Science (Washington, D.C.)* **1964**, *145*, 53.
Lintas, C.; DeMattaeis, M. C.; Mesli, F. *Food Cosmet. Toxicol.* **1979**, *17*, 325.
Obana, H.; Hori, S.; Kasimoto, T. *Bull. Environ. Contam. Toxicol.* **1981**, *26*, 613.
Panalaks, T. *J. Environ. Sci. Health, Part B* **1976**, *B11*, 989.
Payne, J. F. *Mar. Pollut. Bull.* **1977**, *8*, 112.
Romanowski, T.; Funcke, W.; Konig, J.; Balfantz, E. *Anal. Chem.* **1982**, *54*, 1285.
Ryan, J. J. In "Chlorinated Dioxins and Dibenzofurans in the Total Environment"; Choudhary, G.; Keith, L. H.; Rappe C., Eds.; Butterworth: Woburn, England, 1983; p 87.
Santodonato, J.; Howard, P.; Basu, D. *J. Environ. Pathol. Toxicol.* **1981**, *5*, 20, 292.
Sawicki, E.; Meeker, J. L.; Morgan, M. J. *Int. J. Air Water Pollut.* **1965**, *9*, 291.
Sirota, G. R.; Uthe, J. F., Department of Fisheries and Oceans, Halifax, Nova Scotia, Canada, personal communication, 1982.
Suess, M. J. *Sci. Total Environ.* **1976**, *6*, 239.
Tilgner, D. J. *Food Manuf.* **1970**, *87*, 47.

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Determination of Polycyclic Aromatic Hydrocarbons in Canadian Samples of Processed Vegetable and Dairy Products by Liquid Chromatography with Fluorescence Detection

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An HPLC–fluorescence method was developed and used for the determination of 15 polycyclic aromatic hydrocarbons (PAH) in samples of milled wheat, finished cereals, milk powders, malt, spinach, and cooking oils. Results showed that the bran portion of milled wheat as well as finished bran cereal had a considerably higher PAH content than other fractions or finished products. The use of direct heating for drying milk powders and malt was found in some cases to lead to elevated levels of PAH, and these were found to correlate with levels of nitrosamines present. Three samples of spinach had very low levels of PAH while two of three types of cooking oils had levels of carcinogenic PAH in the low micrograms per kilogram range.

It has been amply demonstrated in the literature that plant food can become contaminated with polycyclic aromatic hydrocarbons (PAH) through environmental pollution particularly via the air and through food processing such as direct drying (Howard and Fazio, 1980). Because of the potential for PAH contamination of vegetables, we initiated a limited survey of selected products, available in Canada. The results, reported herein, will help to estimate the total exposure to PAH for Canadians.

EXPERIMENTAL SECTION

Reagents. All solvents were distilled in glass or HPLC grade. The polycyclic aromatic hydrocarbons used in this work are listed in the preceding paper (Lawrence and Weber, 1984). Dimethyl sulfoxide (Me_2SO was either Baker analyzed grade (J. T. Baker) or Aldrich Gold Label Me_2SO (Aldrich Chem. Co.).

Samples. All commercial samples were purchased from local outlets. The wheat milling fractions were obtained from an experimental milling study and the malt samples obtained through commercial producers for the brewing industry.

Apparatus. High-pressure liquid chromatography (HPLC) was carried out by using an Altex Model 110A pump and a 5-μm Spheri-5 RP-18 reversed-phase column (25 cm × 4.6 mm i.d.) (Brownlee Laboratories) at ambient temperature with a mobile phase (isocratic) of water/acetonitrile (30/70 v/v) at a flow rate of 2.0 or 3.0 mL/min. The compounds were detected with a Schoeffel Model FS 970 fluorescence detector connected in series with a Waters Model 440 UV detector set at 254 nm. The fluorescence wavelengths were 250 nm (excitation) and >370 nm (emission).

Sample Analysis. The extraction and cleanup procedure was carried out exactly as described earlier (Lawrence and Weber, 1984). The method was based on the procedure developed by Grimmer and Böhnke (1975) with some modifications such as Florisil cleanup (Basu and Saxena, 1978) and Me_2SO partition (Haenni et al., 1962). The method involves initial saponification with alcoholic KOH, an aqueous/cyclohexane partition, and then Florisil cleanup. A Me_2SO/hexane partition was employed before final HPLC analysis.

RESULTS AND DISCUSSION

The methodology used proved to be adequate for the quantification of the PAH down to 0.1 μg/kg or less in the products examined. Recoveries of PAH carried through the complete method generally were greater than 75% at the 1 μg/kg level, the exceptions being BaP (38%), DahA (61%), Per (28%), and An (24%). These compounds were found to be partially lost during the Florisil cleanup step. Since the recoveries for the other 11 PAH were acceptable, no attempts were made to alter the cleanup procedure to

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Table I. PAH in Milled Wheat Fractions (μg/kg)[a]

compd	flour (1)	flour (2)	rough wheat	clean wheat	bran
vomitoxin[b]	ND[c]	~1000	~700	~700	~1000
FL	3.4	1.5	2.4	2.8	28
PY	5.7	2.6	5.2	5.7	20
DMP	1.9	0.5	0.4	0.5	3.6
BaA	0.8	0.3	0.5	0.5	2.5
Per	0.2	0.1	0.1	0.1	0.6
BaP	0.1	0.1	0.1	0.1	0.5
total PAH	12.1	5.1	8.7	9.7	55.2

[a] Averages of duplicates. [b] Values in μg/kg. [c] ND = not detected.

Table II. PAH in Breakfast Cereals (μg/kg)[a]

compd	puffed wheat	whole wheat	wheat bran	corn bran	flaked milled corn	whole grain oats
FL	5.4	7.4	18	1.9	1.4	2.9
PY	5.4	8.5	21	2.2	3.4	2.8
BbFL	0.2	0.1	0.9	0.2	0.1	-
DMP	0.9	1.1	4.8	0.7	0.4	0.5
BaA	0.8	0.8	3.7	0.6	0.3	0.4
Per	0.2	0.3	1.5	0.1	0.1	0.1
BaP	*b*	0.1	0.8	0.2	-	-
DacA	-[c]	0.3	3.8	0.3	-	-
DahA	3.0	-	3.6	-	-	-
Pi	1.4	-	0.5	0.3	-	0.2
IP	3.0	-	1.4	0.2	-	-
total PAH	20.3	18.6	59.5	6.7	5.7	6.9

[a] Averages of duplicates. [b] Interfering peak. [c] Dashes indicate <0.1 μg/kg.

improve the recovery for these compounds.

A number of food samples of plant origin were analyzed for PAH. These included samples that might conceivably contain PAH either by environmental contamination or during processing, via direct flue drying of grain products, powdered foods, and tea leaves. Table I compares results for a variety of milled wheat fractions obtained from different samples of wheat grown in southern Ontario, a relatively industralized area of Canada where PAH contamination from the atmosphere might be expected. These same samples had been analyzed previously for the mycotoxin vomitoxin by a modified Scott method (Scott et al., 1981). No correlation between absolute vomitoxin level and PAH content was observed nor was it expected. The only similarity found was that PAH levels were higher (about 5–10 times) in the bran fraction than in other milled fractions. This is also generally true for vomitoxin (Scott, 1983), although it is not shown in Table I since each sample is from a different wheat source. These elevated levels in the bran might be expected since PAH or mycotoxin contamination would likely be the worst on the outer portions of the grain. Figure 1 compares chromatographic results for a bran and flour sample. It can be seen that the overall patterns are very similar, differing only in quantity of individual components present.

Several types of breakfast cereals were analyzed for PAH, and the results are shown in Table II. The wheat products contained higher levels of PAH than either the corn or oats cereals. The wheat bran cereal contained significantly more PAH than any other of the cereals examined, having a total of 59.5 μg/kg, including 13.3 μg/kg of carcinogenic PAH (BaA, BaP, DacA, DahA, IP). The high levels in the wheat bran cereal reflects the relatively high levels found in the bran milling fraction from Table I. Also, the whole wheat cereals contained higher levels of PAH than the milled wheat fractions of Table I possibly due to the presence of approximately 20% bran in the former. Figure 2 compares chromatographic patterns obtained for a bran flakes sample and a bran milling fraction. The chromatograms are qualitatively very similar.

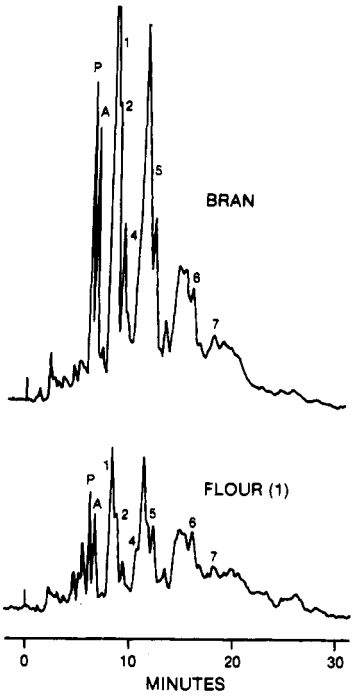

Figure 1. Comparison of HPLC–fluorescence chromatograms for bran and flour. Mobile phase flow rate, 2 mL/min. Other conditions as described in the text. P = phenanthrene; A = anthracene. Numbers refer to compounds listed Table I of the preceding paper.

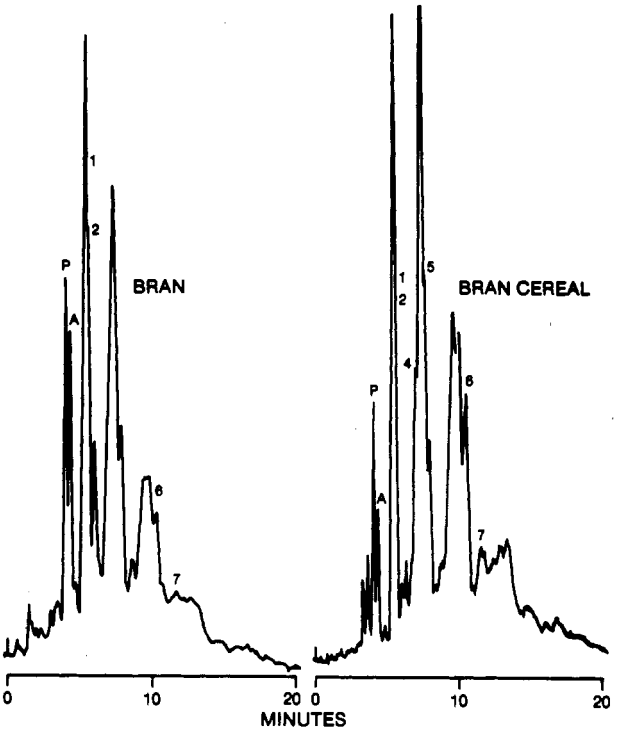

Figure 2. Comparison of HPLC–fluorescence chromatograms for a bran milling fraction and a bran flakes cereal. Conditions as described in the text. P = phenanthrene; A = anthracene. Numbers refer to compounds listed in Table I of the preceding paper.

In studies on milk powders, infant formula, and malt samples, a correlation between PAH and nitrosamine content was found. It is known that nitrosamines can be

Table III. PAH in Dried Milk and Malt Products (μg/kg)[a]

	product						
	milk powder						
compd	skim milk A	skim milk B	skim milk C	infant formula	barley malt A[c]	barley malt B[c]	barley malt C[c]
NDMA	0	0	0.3	1.0	0	13	150
FL	–[b]	–	1.5	8.0	0.8	2.6	26
PY	–	–	2.1	4.8	1.1	3.2	48
BbFL	–	–	0.2	0.7	0.1	0.1	0.5
DmP	–	–	0.9	0.7	0.1	0.2	6.2
BaA	–	–	0.3	1.7	0.1	0.2	4.2
Per	–	–	0.1	0.6	0.1	0.1	0.4
BaP	–	–	0.1	1.2	–	–	0.3
DacA	–	–	1.0	0.7	–	–	0.4
DahA	–	–	1.1	3.0	–	–	1.2
Pi	–	–	0.2	0.7	–	–	0.3
IP	–	–	0.2	1.2	–	–	0.4
DaeP	–	–	–	–	–	–	–
An	–	–	–	0.3	–	–	–
total carcinogenic PAH[d]	–	–	2.7	8.1	0.1	0.2	6.5

[a] Averages of duplicates. [b] <0.1 μg/kg. [c] Samples from different sources. [d] BaA + BaP + DacA + DahA + IP + DaeP + An.

formed during drying processes where direct heating with flue gas is employed (Spiegelhalder et al., 1980). This same process has been implicated in producing PAH (Fornal et al., 1970; Rohrlich and Suckow, 1970; Howard and Fazio, 1980). Table III shows results obtained for milk powder, infant formula, and malt samples and also includes results of nitrosamine analysis (nitrosodimethylamine, NDMA) by the method of Sen (Sen and Seaman, 1981). In all cases PAH content varied with NDMA level. All samples were dried by the direct method, with the exception of malt sample A, which was dried indirectly. The wide variation in PAH and NDMA content is a reflection on the actual drying conditions employed. Rohrlich and Suckow (1970) found that the BaP content of directly dried wheat increased between 6- and 130-fold depending upon the degree of exposure. The results in Table III (skim milk A and B, malt A) suggest that indirect and, under the proper conditions, direct drying can be effectively used with little or no PAH or NDMA formation. However, in the case of the infant formula and malt C where the level of carcinogenic PAH is relatively high, concern is raised that PAH and NDMA levels may be elevated significantly by certain types of direct drying. Others have also found that the type of fuel used in direct drying has a large effect on PAH levels (Bolling, 1964; Hutt et al., 1978). Joe et al. (1982) analyzed 30 directly dried malt samples and found 18 to contain low levels of PAH (≤1.6 μg/kg carcinogenic). Indirect or electric drying would most certainly reduce PAH levels. It has already been shown that indirect heating of wheat and rye reduces PAH formation (Fornal et al., 1970). This is also shown in Table III for malt A compared to the others. Figure 3 compares chromatograms of a reagent blank (the observed peaks are mainly from the Me_2SO used), a negative skim milk powder, and an infant formula. The difference in PAH content can be readily observed in the two samples.

It has been reported that leafy vegetables grown in industrial areas can contain 10 times or more BaP than the same varieties grown away from industrial areas (Grimmer, 1982). We analyzed three commercially available spinach samples; one fresh, imported and two canned products, one of which was imported. All three had very low residues of PAH, the only carcinogenic one observed being BaA in the range of 0.1–0.5 μg/kg. In contrast, three types of tea leaves commonly used in Canada contained significantly

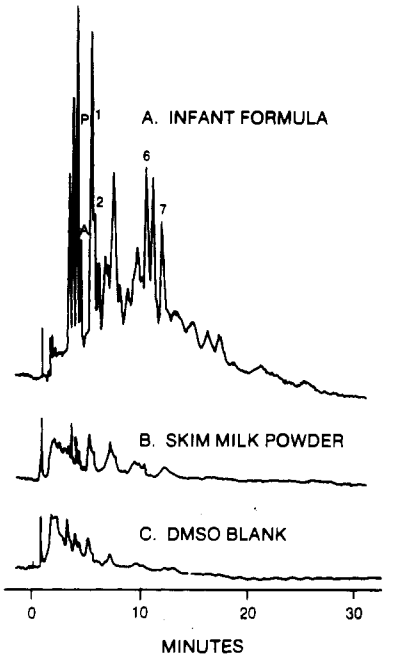

Figure 3. Comparison of chromatograms obtained from (A) infant formula, (B) skim milk powder, and (C) Me_2SO (DMSO in the figure) reagent blank. Conditions as described in the text. P = phenanthrene; A = anthracene. Numbers refer to compounds listed in Table I of the preceding paper.

Table IV. PAH in Cooking Oils (μg/kg)[a]

	cooking oil		
compd	A (canola and/or soya)	B (soya)	C (corn)
FL	0.2	0.4	7.5
PY	–[b]	0.2	1.4
BbFL	t[d]	0.1	–
DMP	0.2	0.1	–
BaA/Ch	0.1	0.3	0.3
Per	t	0.2	0.1
BaP	t	0.2	0.3
DacA	t	0.3	2.0
DahA	t	0.7	1.1
Pi	t	–	–
IP	t	0.5	0.5
An	t	0.1	0.1
DaeP	t	0.3	0.3
DPA	0.2	0.2	–
total carcinogenic PAH[c]	0.1	2.4	4.6

[a] Average of duplicates. [b] Dashes indicate <0.1 μg/kg. [c] BaA + BaP + DacA + DahA + IP + An + DaeP. [d] t = trace observed, not quantifiable.

high residues of BaA (7.7–11.3 μg/kg) and BaP (3.3–4.2 μg/kg). High levels (9.5 μg/kg) of BaP in tea leaves have been reported elsewhere (Lintas et al., 1979) although in the tea infusion the concentration of BaP was extremely low (0.2 μg/kg).

Table IV lists results obtained for three samples of cooking oils. Sample A (containing canola and/or soybean oil) had minimal content of PAH while samples B and C were significantly higher (2.4 and 4.6 μg/kg carcinogenic PAH, respectively). Kolarovic and Traitler (1982) used a caffeine complexation method for extraction of PAH from vegetable oils for capillary gas chromatographic analysis. They reported carcinogenic PAH levels much higher in the samples of soybean and rapeseed (canola) oils they analyzed (approximately 20 and 95 μg/kg, respectively). Grimmer and Hildebrandt (1967) earlier found carcinogenic PAH levels of 18.9 μg/kg for rapeseed oil and

12.1 μg/kg for soybean oil in the samples they analyzed. The source of PAH in the oils is presumably from initial environmental contamination of the vegetables with the derived oils essentially retaining the PAH.

CONCLUSION

From a survey of selected food items from plant or dairy origin, it appears that Canadians are subjected to similar levels of PAH as reported in other countries. Wheat bran products seem to be significantly higher in PAH content than other types of cereal grain foods. Some vegetable oils may also contain low levels. Processing of certain food items by direct heating methods can lead to a significant increase in PAH content of the finished product, depending upon the type of fuel used, the combustion conditions, and the type and degree of exposure of the food to the flue gases. Some correlation between PAH and nitrosamine levels in direct dried milk products and malt was found.

Registry No. NDMA, 62-75-9; FL, 206-44-0; PY, 129-00-0; BbFL, 30777-19-6; DMP, 1576-67-6; BaA, 56-55-3; Per, 198-55-0; BaP, 50-32-8; DacA, 215-58-7; DahA, 53-70-3; Pi, 213-46-7; IP, 193-39-5; DaeP, 192-65-4; An, 191-26-4; DPA, 1499-10-1.

LITERATURE CITED

Basu, D. K.; Saxena, J. *Environ. Sci. Technol.* **1978**, *12*, 795.
Bolling, H. *Tec. Molitoria* **1964**, *15*, 137.
Fornal, J.; Fornal, L.; Babuchowski, L. *Przegl. Zbozowa-Mlyn.* **1970**, *14*, 445.
Grimmer, G. *Pharm. Ztg.* **1982**, *127*, 1.
Grimmer, G.; Böhnke, H. *J. Assoc. Off. Anal. Chem.* **1975**, *58*, 725.
Grimmer, G.; Hildebrandt, A. *Chem. Ind. (London)* **1967**, *1967*, 2000.
Haenni, E. O.; Howard, J. W., Joe, F. L., Jr. *J. Assoc. Off. Agric. Chem.* **1962**, *45*, 67.
Howard, J. W.; Fazio, T. *J. Assoc. Off. Anal. Chem.* **1980**, *63*, 1077.
Hutt, W.; Meiering, A.; Delschlagerr, W.; Winkler, E. *Can. Agric. Eng.* **1978**, *20*, 103.
Joe, F. L.; Salemme, J.; Fazio, T. *J. Assoc. Off. Anal. Chem.* **1982**, *65*, 1395.
Kolarovic, L.; Traitler, H. *J. Chromatogr.* **1982**, *237*, 263.
Lawrence, J. F.; Weber, D. F. *J. Agric. Food Chem.* **1984**, preceding paper in this issue.
Lintas, C.; De Mattaeis, M. C.; Merli, F. *Food Cosmet. Toxicol.* **1979**, *17*, 325.
Rohrlich, M.; Suckow, P. *Getreide Mehl* **1970**, *20*, 90.
Scott, P. M., Health Protection Branch, Ottawa, Ontario, personal communication, 1983.
Scott, P. M.; Lau, P. Y.; Kanhere, S. R. *J. Assoc. Off. Anal. Chem.* **1981**, *64*, 1364.
Sen, N. P.; Seaman, S. *J. Assoc. Off. Anal. Chem.* **1981**, *64*, 1238.
Spiegelhalder, B.; Eisenbrand, G.; Preussmann, R. *IARC Sci. Publ.* **1980**, *No. 31*, 467.

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Rapid Isolation and Thin-Layer Chromatographic Screening of Extracts from Boll Weevil (*Anthonomus grandis* Boheman)

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A rapid isolation procedure is described for producing separate lipoidal and polar component extracts from small samples of boll weevils. Tissue grinding and extraction were done simultaneously in a capsular mortar in a high-speed triturating device. The extracting solvent of 2:1 chloroform–methanol and pulverized tissue were transferred from the capsule to a microcentrifuge tube, and water was added to induce phase separation, which was aided by centrifugation. Both extracts were analyzed by thin-layer chromatography on silica gel; the developing solvent was hexane–ethyl ether–acetic acid (90:10:1 v/v) for the chloroform extract and benzene–acetic acid–water–nitromethane (60:40:10:30 v/v) for the aqueous methanol extract. The utility of the method was illustrated by the ability to differentiate individual samples of young and old boll weevils of both sexes taken at different times during the year. Samples differed in the amount of lipoidal and polar components they contained.

Since the pioneering work of Reiser et al. (1953) on the lipid composition of the boll weevil (*Anthonomus grandis* Boheman), increasingly more sophisticated and detailed studies have been made in an effort to gain understanding of the relationship of the insect's lipid content and its physiological state (Brazzel and Newsome, 1959; Guerra et al., 1982). In spite of these efforts, the biochemical regulation of these physiological states, especially diapause, remains, for the most part, unknown.

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In more recent chemical studies (Lambremont et al., 1964; Joiner and Lambremont, 1969; Keeley et al., 1977) workers have utilized modifications of the lipid isolation method of Folch et al. (1957) and conducted analyses of the isolates by either column, gas, or thin-layer chromatography. The essential features of their modified methods include repetitive homogenization and solvent extraction, followed by phase separation over an extended time at low temperature. Although the original and modified isolation methods meet the needs of the analytical scheme for which they were developed, they are too tedious and time consuming for the frequent processing of a large number of small samples (one to five boll weevils) where only semiquantitative results are sought.

The regulatory roles that lipids and other components play in weevil metabolism and physiology are not known