In contrast to the behavior of **4c**, O-methylation of sweet amide **5b** led to a tasteless product, **5g**. The CD parameters of **5g** differed significantly from those of the parent compound and reflected an obvious conformational difference in solution. The absence of IHB in **5g** is probably the reason for enhanced conformational mobility.

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Polychlorinated Biphenyls: Congener-Specific Analysis of a Commercial Mixture and a Human Milk Extract

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On the basis of the relative retention times and response factors of all 209 synthetic polychlorinated biphenyls (PCBs), this paper reports the first congener-specific analysis of a commercial PCB preparation, Aroclor 1260, and the PCB composition of a human milk extract. The analysis indicates that Aroclor 1260 contains nearly 80 different PCB congeners with the major components identified as 2,2',3,4',5',6-, 2,2',4,4',5,5'-, 2,2',3,4,5,5'-, and 2,2',3,4,4',5'-hexachlorobiphenyl and 2,2',3,3',4,4',5-, 2,2',3,3',4,5,6'-, 2,2',3,4,4',5,5'-, and 2,2',3,4',5,5',6-heptachlorobiphenyl. In contrast, the major PCB components of the human milk fraction were the 2,4,4'-tri-, 2,4,4',5-tetra-, 2,2',4,4',5-penta-, 2,3',4,4',5-penta-, 2,2',3,4,4',5'-hexa-, 2,2',4,4',5,5'-hexa-, 2,2',3,3',4,4',5-hepta-, and 2,2',3,4,4',5,5'-heptachlorobiphenyls. The significance of congener-specific PCB analysis is discussed in terms of the structure–activity effects on PCB persistence, bioaccumulation, and toxicity.

Polychlorinated biphenyls (PCBs) are highly stable industrial chemical products that are synthesized by the direct chlorination of biphenyl. Commercial PCBs are distinguished by their stability and resistance to breakdown by acids, bases, oxidation, and reduction, their miscibility with numerous organic solvents, their nonflammability, and their excellent electrical insulation properties. Because of these highly desirable physical properties PCBs have enjoyed widespread use as industrial fluids, flame retardents, diluents, hydraulic fluids, and dielectric fluids for capacitors and transformers. Due to their widespread use, careless disposal practices, and environmental stability, PCBs have been widely identified in diverse environmental matrices including fish, wildlife, and domestic animals, rivers, lakes, and oceans and their underlying sediments, aquatic and marine flora, air, rain, and snow (Risebrough et al., 1968; Fishbein, 1972; Buckley, 1982; Ballschmiter et al., 1981; Wasserman et al., 1979). It was also apparent from several analytical studies that PCBs preferentially bioaccumulate in the food chain and residues are routinely detected in human adipose tissue, blood and human milk (Wasserman et al., 1979; Cordle et al., 1978; Holdrinet et al., 1977; Safe, 1982). Thus, the chemical stability of PCBs is paralleled by their environmental stability and potential for environmental transport, and it is evident from analytical surveys that PCBs are the

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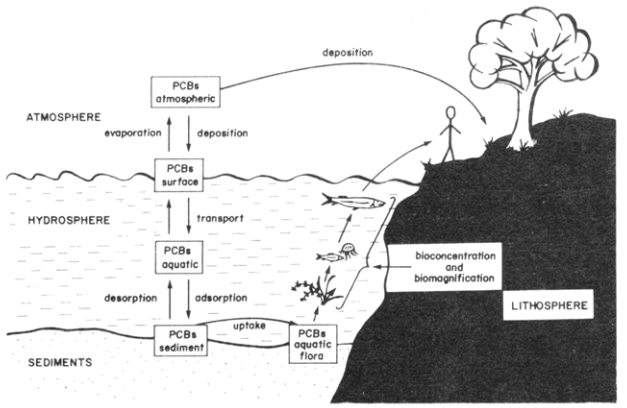

Figure 1. Environmental fate of PCBs.

most ubiquitous industrial chemical pollutant in the global ecosystem.

Numerous gas chromatographic studies using packed or capillary columns have confirmed the complexity of all commercial PCB formulations (Sawyer, 1978; Webb and McCall, 1976; Burse et al., 1983; Newton and Laski, 1983; Kerkhoff et al., 1982; Jensen and Sundstrom, 1974; Sissons and Welti, 1971; Ballschmiter and Zell, 1980; Mullin et al., 1981; Albro et al., 1981; Albro and Parker, 1979). Some of the difficulties inherent in PCB analytical protocols are similar to those encountered in the analysis of related organochlorine pesticides and pollutants such as lindane, DDT, DDE, hexachlorobenzene, dieldrin, and related hexachlorocyclopentadiene-derived insecticides. Diverse extraction and cleanup procedures have been devised to preferentially remove coextractives that are present in different matrices and interfere with routine quantitative gas chromatographic (GC) and GC–mass spectrometric (MS) analysis. In contrast to the organochlorine pesticides, the qualitative analysis for PCBs presents several unique problems. Most analytical schemes for PCBs use the various commercial PCB preparations as quantitative reference standards. The PCB concentrations are estimated by comparing the relative intensities of several diagnostic peaks observed in the commercial reference standards and in the analyte. The accuracy in determining PCB levels is highly variable and matrix dependent; for example, the PCBs that are present in many waste industrial fluids or in (retro-filled) transformers usually resemble a specific commercial mixture and comparative GC analysis can yield accurate quantitative results. In contrast, gas chromatographic analysis of PCBs in extracts from diverse environmental matrices clearly indicates that these mixtures can be strikingly different from the commercial PCB analytical reference standards. These differences in composition reflect the major differences in the physical properties (e.g., water solubility and volatility) and biodegradability of the individual PCBs present in the commercial mixtures. Figure 1 illustrates some of the effects that would alter the composition of a commercial PCB preparation introduced as a pollutant into an aquatic or marine environment; these include physical partitioning between the water–sediment and water–air interfaces, sediment desorption processes, and biomagnification and bioconcentration with aquatic forms of life and the food chain. Thus, it is not surprising that the composition of PCB extracts from these environmental matrices can vary widely and often do not resemble any commercial mixture (Kerkhoff et al., 1982; Jenson and Sundstrom, 1974; Mullin et al., 1981; Hansen, 1979; Harvey and Steinhauer, 1974; Wolff et al., 1982). Quantitative analysis on these mixtures is usually determined by pattern or peak matching methods using artificially reconstituted mixtures of different commercial PCB formations. At best, these results are only semiquantitative estimates of the total PCB levels in these environmental samples.

High-resolution glass capillary GC analysis can provide a solution to some of these analytical problems. The high resolving power of coated silica or quartz capillary columns offers a method that can separate the PCBs present in most samples; the identities of the individual peaks must then be determined by using synthetic standards and by retention index addition methods (Ballschmiter and Zell, 1980). This latter technique predicts the relative retention times (RRTs) of specific PCBs and has been used to assign the structures of the individual PCB congeners present in diverse analytes (Kerkhoff et al., 1982; Jensen and Sundstrom, 1974; Sissons and Welti, 1971; Ballschmiter and Zell, 1980; Mullin et al., 1981, 1984; Albro et al., 1981). This method relies on the RRT values that have been determined for the limited number of available synthetic PCB standards. However, accurate quantitation of the individual PCB components in a mixture can only be accomplished by comparing the observed RRT and peak height (or area) data for a PCB–containing extract and the RRT and molar (or weight) response factors for synthetic PCB standards.

It was apparent to us that high-resolution PCB analysis must not only incorporate a high-resolution separation method but must also provide results which will confirm identities and concentrations of each individual PCB present in any mixture (Mullin et al., 1981). Moreover, since several reports clearly indicate that the toxicity of PCBs are structure dependent (Poland and Knutson, 1982; Parkinson and Safe, 1981; Safe et al., 1982; Poland et al., 1979; Yoshimura et al., 1979; Goldstein et al., 1977), the capability for isomer-specific PCB analysis will be an important method for assessing the potential environmental and human health impact of PCBs. This analytical approach requires the synthesis and characterization of all 209 PCB congeners and determination of their GC RRTs and molar response factors. We have recently completed the synthesis of the 209 PCB congeners (Mullin et al., 1984), and these standards can now be used for congener-specific PCB analysis. This paper compares the composition of a human milk PCB extract with commercial Aroclor 1260, which is used as a low-resolution quantitative standard for the GLC quantitation of many PCB mixtures derived from environmental matrices.

EXPERIMENTAL SECTION

The individual PCB analytical standards were synthesized as described and the retention times and response factors were determined relative to the standard, octachloronaphthalene (Mullin et al., 1984, 1981). The high-resolution capillary gas chromatography was performed by using a Varian Model 3700 gas chromatograph equipped with a ^{63}Ni electron capture detector. A 50-m fused silica capillary column (0.2-mm i.d.) coated with SE-54 (Hewlett-Packard) was used to separate the PCB isomers and congeners. The oven temperature was programmed at a rate of 1.0 °C min^{-1} from 100 to 240 °C. The injector and detector temperatures were 270 and 330 °C, respectively. Sample volume, 6.0 μL, was injected by using an automatic sampler with splitting in the injector (10:1 split ratio, vented from 0.75 to 1.75 min). The hydrogen carrier gas was held at a constant pressure of 2.25 kg cm^{-2} to give the optimized linear velocity ($\bar{\mu}$) at 100 °C of 45 cm s^{-1}. The retention times (RT) of the PCBs were expressed relative to that of the octachloronaphthalene (OCN, RT = 124.9 min) standard; the relative response factors (by weight)

Table I. Quantitative and Qualitative Analysis of PCBs in Aroclor 1260 and a Human Breast Milk Extract

congener name[a]	% in Aroclor 1260	% in Human Milk[b]	congener name[a]	% in Aroclor 1260	% in Human Milk[b]
PCB-018	0.12		PCB-118	0.49	6.5
PCB-017	0.05		PCB-134	0.35	
PCB-024	0.01		PCB-114		0.33
PCB-016	0.04		PCB-131	0.07	
PCB-029	0.02		PCB-122	0.12	0.53
PCB-026	0.02		PCB-146	1.3	1.9
PCB-028	0.04	8.8	PCB-153	9.6	12.
PCB-021	0.01		PCB-141	2.5	0.29
PCB-033	0.09	2.2	PCB-176	0.33	
PCB-053	0.04		PCB-137	0.22	0.87
PCB-022	0.01	0.65	PCB-130		0.59
PCB-045	0.07		PCB-138	6.5	10.
PCB-046	0.02	0.25	PCB-158	0.70	0.55
PCB-052	0.25	1.9	PCB-129	0.20	
PCB-043	0.02		PCB-178	1.2	
PCB-049	0.06	0.66	PCB-175	0.49	
PCB-048	0.29	0.37	PCB-187	4.5	1.5
PCB-044	0.11	0.78	PCB-183	2.3	1.4
PCB-037	0.04	2.9	PCB-128	0.47	0.33
PCB-042	0.04		PCB-167	0.16	0.85
PCB-041	0.25	1.3	PCB-185	4.1	0.11
PCB-040	0.03		PCB-174	5.5	0.39
PCB-100	0.02		PCB-177	1.9	0.61
PCB-074	0.03	11.	PCB-171+202	1.2	0.37
PCB-070+-076	0.15	0.61	PCB-156	0.45	4.87
PCB-095	2.7		PCB-173	0.06	
PCB-091	0.07		PCB-200	0.78	
PCB-056+-060	0.14	0.71	PCB-157		0.47
PCB-084	0.65		PCB-172	0.78	0.31
PCB-101	2.5	0.97	PCB-180	9.1	5.3
PCB-099	0.13	4.8	PCB-193	0.47	0.19
PCB-119		0.08	PCB-191	0.10	0.90
PCB-083	0.04		PCB-199	0.33	
PCB-097	0.45		PCB-170	6.8	5.3
PCB-087	0.45	0.82	PCB-201	2.9	0.85
PCB-085	0.13		PCB-203	3.1	0.79
PCB-136	1.4		PCB-196	2.5	0.18
PCB-110	1.7	1.0	PCB-189	0.15	2.4
PCB-154	0.02		PCB-195	3.1	0.31
PCB-082	0.11		PCB-207	0.08	
PCB-151	2.5	0.59	PCB-194	1.7	0.48
PCB-144+-135	1.5	0.51	PCB-205	0.11	0.06
PCB-107	0.03	0.31	PCB-206	0.85	0.24
PCB-149	7.4		PCB-209	0.06	0.09

[a] Congener names adapted from Ballschmiter and Zell (1980). [b] Human milk sample collected and extracted by the Michigan Department of Public Health under Cooperative Agreement CR807192 with the Large Lakes Research Station, U.S. Environmental Protection Agency.

for the PCBs were expressed relative to that of OCN (RRF = 1.0 for 1 ng of OCN) by using integrated peak areas. (note: RRF = (height of congener peak/concentration of congener peak)/(height of internal standard peak/concentration of internal standard peak). Table I summarizes the percent composition of a human milk PCB extract and Aroclor 1260. The analysis of Aroclor 1260 and the human milk PCB extract has previously been reported (Mullin et al., 1981) prior to the availability of all the PCB congeners as analytical standards. To assure the accuracy of the data, the total PCB concentration in the Aroclor 1260 was calculated and compared to the expected value (32 ng/mL found and 30 ng/mL expected), yielding 94% accuracy.

RESULTS AND DISCUSSION

Analytical Data. In a previous study 195 of the 209 PCB congeners have been separated by using a 50-m fused silica capillary column coated with SE-54 (Mullin et al., 1984). Five pairs of isomers, namely, 31/28, 56/60, 70/76, 203/196, and 135/144, and two pairs of nonisomeric congeners, 202/171 and 81/145, were not completely resolved by using this column, and several other separation methods are currently being investigated [note: the numbering scheme of Ballschmiter and Zell (1980) is used for PCB congener identification]. Preliminary results in our laboratory suggest that only isomers 31/28, 70/76, and 203/196 are components of the commercial PCBs and only the latter pair of octachlorobiphenyls are potentially persistent in the environment. In addition, isomers 31/28 and 203/196 can be separated on the capillary column although quantitation of the isomer pairs is not possible if both compounds are present.

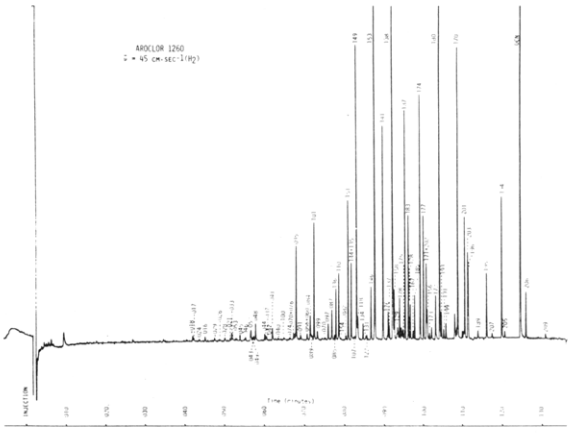

Figure 2. Congener-specific GC analysis of the commercial PCB, Aroclor 1260.

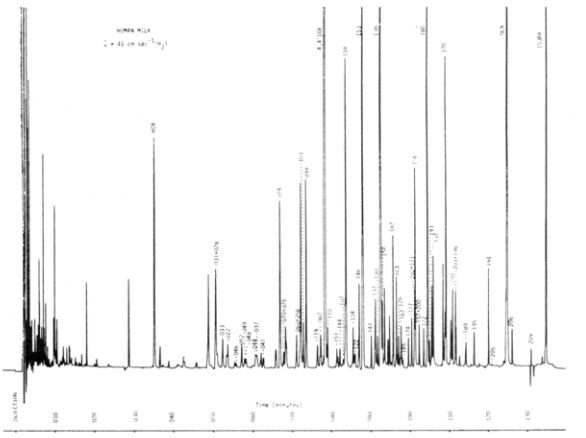

Figure 3. Congener-specific GC analysis of a human milk extract from the State of Michigan (note the occurrence of 2,2',4,4',5,5'-hexabromobiphenyl, which is present due to the PBB contamination in Michigan).

The isomer specific GC analysis of commercial Aroclor 1260 is illustrated in Figure 2 (note: the resolution of isomers 196 and 203) and represents the first such report for any PCB mixture. Figure 3 illustrates the high-resolution isomer-specific analysis of the PCBs extracted from a human milk sample obtained in the State of Michigan. This gas chromatogram does not resemble the pattern of any commercial PCB, and pattern matching methods would not yield meaningful quantitative results. However, the high-resolution isomer-specific GC approach permits quantitation of all the individual PCB components present in this mixture. Several PCB congeners, including 2,2',4,4',5,5'-hexa- (no. 153), 2,2',3,4,4',5'-hexa– (no. 138), 2,2',3,3',4,4',5-hepta– (no. 170), and 2,2',3,4,4',5,5'-hepta- (no. 180), are major components of both Aroclor 1260 and

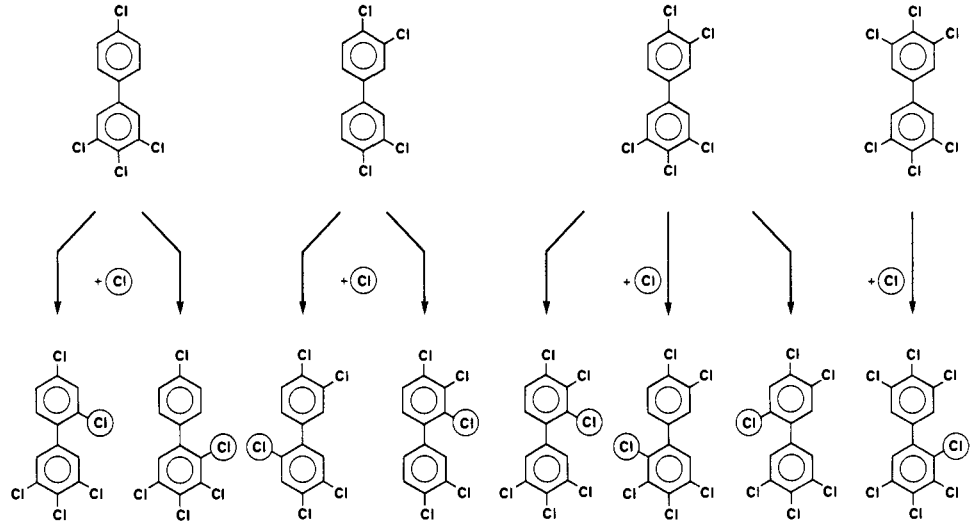

Figure 4. Structures of the most active coplanar PCBs and their mono-*o*-chloro-substituted analogues.

the human milk extract. These four PCB congeners possess several common structural features including (a) six or more chlorine atoms per biphenyl moiety and (b) the presence of only three different substitution patterns (i.e., 2,4,5-, 2,3,4,5-, and 2,3,4-) on both phenyl rings. PCBs no. 153 and 180 do not contain adjacent unsubstituted carbon atoms and are therefore resistant to metabolic breakdown (Matthews and Dedrick, 1984), and their persistence in human tissues is not unexpected. The results also suggest that the higher chlorinated PCBs (no. 138 and 170) that contain a 2,3,4-trichlorophenyl group are also resistant to metabolism and environmental breakdown and readily bioaccumulate in human tissues.

Another major PCB present in the human milk extract (4.87%), no. 156, is a minor component of Aroclor 1260 and other commercial PCBs (Jensen and Sundstrom, 1974; Ballschmitter and Zell, 1980) and has previously been identified as a major PCB contaminant of Japanese human milk extracts (Safe, 1982). The four remaining major PCB congeners identified in the human milk extract, no. 28, 74, 99, and 118, are minor components of Aroclor 1260 (<0.49% for all four isomers). It is likely that these pentatrichlorinated PCB congeners are derived from the lower chlorinated PCB formulations; however, it is noteworthy that with the exception of congener no. 28, all of these compounds also contain 2,4,5-trichloro substitution on one of the phenyl rings and a *p*-chloro group on the second phenyl ring. This high-resolution analytical study has also identified 2,4,4'-trichlorobiphenyl as a major PCB component and confirms a previous report that identified this compound in a Japanese human milk extract (Yakushiji et al., 1979). The reasons for the persistence of this congener are not apparent. It was also of interest to note that several other compounds including no. 95 (2.7%), no. 149 (7.4%), no. 185 (4.1%), no. 174 (5.5%), and no. 195 (3.1%) comprise 22.8% of the PCBs present in Aroclor 1260 but are minor components (0.81%) of the human milk PCB extract. With the exception of no. 195, all of these compounds possess a 2,3,6-trichloro- or 2,5-dichloro-substitution pattern on at least one of their phenyl rings, and because of the two adjacent unsubstituted carbon atoms rapid metabolic degradation of these congeners would be expected (Matthews and Dedrick, 1984). The lack of persistence of no. 195 is not clear; however, it has been reported for a series of hexachlorobiphenyl isomers that in some animals there is a decrease in tissue persistence of PCBs with increasing *o*-chloro substituents (Sparling and Safe, 1980). The effects of structure on the fate of PCBs in the environment, in biological samples, and in human tissues are currently under investigation in our laboratory, and this high-resolution analytical approach will play a critical role in delineating the environmental fate of individual PCB congeners.

High-Resolution PCB Analysis: Toxicologic Implications. The proposed mechanism of action of the toxic halogenated aryl hydrocarbons such as the PCBs and polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), naphthalenes (PCNs), and azobenzenes (PABs) has been derived from studies on the activities of the most toxic member of this group, namely, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related PCDDs (Poland and Knutson, 1982; Poland et al., 1979; Poland and Glover, 1980). Results obtained from several studies are consistent with a mechanism of action for the toxic halogenated aryl hydrocarbons in which the first step is their reversible binding to the cytosolic receptor protein. It was also apparent that three PCB congeners that are the most toxic, namely, 3,3',4,4'-tetra-, 3,3',4,4',5-penta-, and 3,3',4,4',5,5'-hexachlorobiphenyl (Kohli et al., 1980; Ozawa et al., 1979; Biocca et al., 1981; Marks et al., 1981; McKinney et al., 1976; Yoshihara et al., 1979; Silkworth and Grabstein, 1982) are minor to trace components of the commercial PCBs and are unlikely to be the sole contributors to the toxicity of these mixtures (Jensen and Sundstrom, 1974; Sissons and Welti, 1971; Kamops et al., 1979).

Several previous studies have reported the correlation between the rank order of the AHH induction potencies and receptor binding avidities for the toxic halogenated aryl hydrocarbons and their toxicities (Parkinson and Safe, 1981; Poland et al., 1979; Safe et al., 1982), and this suggests that the two biologic assays can be used as indicators of toxicity. Our research initially focused on the activity of PCBs as inducers of AHH in immature male Wistar rats, and rat hepatoma H-4-II-E cells in culture and their binding affinities to rat hepatic cytosolic receptor protein (Safe et al., 1982; Bandiera et al., 1982; Sawyer and Safe, 1982).

A comprehensive study of all 209 PCBs was not feasible; however, several reports indicated that the most potent AHH inducers are substituted at both para and two or more meta positions. It was concluded that the introduction of *o*-chloro substituents would reduce biphenyl ring coplanarity and the AHH-inducing activity of the PCB congeners. These structural considerations suggest that the 4,4'-dichloro-substituted biphenyls, namely,

4,4'-di-, 3,4,4'-tri-, 3,3',4,4'-tetra-, 3,4,4',5-tetra-, 3,3',4,4',5-penta-, and 3,3',4,4',5,5'-hexachlorobiphenyl are the most likely PCB congeners that induce AHH. Initial studies confirmed that only four of these compounds (3,3',4,4'-tetra-, 3,4,4',5-tetra-, 3,3',4,4',5-penta-, and 3,3',4,4',5,5'-hexachlorobiphenyl) induce AHH and bind to the cytosolic receptor protein (Sawyer and Safe, 1982; Bandiera et al., 1982). The predictive potential of these two assays is confirmed by frequent reports that confirm the toxic potencies of the coplanar PCBs (Safe et al., 1982; Poland et al., 1979; Yoshimura et al., 1979). It is known that ortho-substituted biphenyls exhibit less coplanar conformational character due to steric interactions; thus it was assumed (Poland et al., 1979; Goldstein et al., 1977) that *o*-chloro substituted PCBs would not bind to the receptor protein and not elicit toxic and biologic effects mediated through this protein. We synthesized and tested all the mono-ortho derivatives of the four most active coplanar PCBs (see Figure 4), and the results confirm their AHH induction and receptor binding activities and clarifies the identities of those PCBs that are present in commercial Aroclors and exhibit the mixed-type enzyme induction properties (i.e., induction of PB plus MC type activity) comparable to those observed for Aroclors 1254 and 1260. The mono-ortho coplanar PCBs that have previously been identified in commercial PCBs include 2,3,3',4,4'-penta-, 2,3',4,4',5-penta-, 2',3,4,4',5-penta-, 2,3,3',4,4',5-hexa-, and 2,3,3',4,4',5,5'-heptachlorobiphenyl (Ballschmiter and Zell, 1980; Jensen and Sundstrom, 1974; Sissons and Welti, 1971; Mullin et al., 1981; Albro et al., 1981) and two of these compounds, 2,3',4,4',5-penta- and 2,3,3',4,4',5-hexachlorobiphenyl, have been identified as major components of the human milk PCB extract. Although the toxicities of the mono-ortho coplanar PCBs have not been thoroughly investigated, it is apparent that the effects of many of these compounds resemble those reported for 2,3,7,8-TCDD. For example, 2,3',4,4',5-penta-, 2,3,3',4,4'-penta-, 2,3,3',4,4',5-hexa-, and 2,3,3',4,4',5'-hexachlorobiphenyls cause thymic atrophy in rats (Parkinson et al., 1983). 2,3,3',4,4'-Pentachlorobilphenyl administered to mice and rats results in a wasting syndrome (weight loss), edema, liver lipid accumulation, extensive hepatic damage, and splenic atrophy (Yamamoto et al., 1976); 2,3',4,4',5-pentachlorobiphenyl and Aroclor 1254 cause 100% embryo mortality in eggs from pullets receiving the PCB in their diet at a level of 20 ppm (Ax and Hansen, 1975), whereas several PCB congeners that do not induce AHH were inactive at the same dose level; administration of 2,3',4,4',5-penta- and 2,3,3',4,4',5-hexachlorobiphenyl to rats caused increased liver weights, increased liver lipids, and thymic atrophy (Yoshihara et al., 1979). Most of the mono-ortho coplanar PCBs induce AHH and cause thymic atrophy in responsive C57BL/6J mice but do not elicit these effects in the nonresponsive DBA/2J mice (Parkinson et al., 1982; Robertson et al., 1984).

These data indicate that most of the mono-ortho analogues of the coplanar PCBs elicit toxic effects that resemble (qualitatively) those caused by 2,3,7,8-TCDD, and some of these congeners (2,3',4,4',5-penta- and 2,3,3',4,4',5-hexachlorobiphenyl) have been identified in Aroclor 1260 and the human milk PCB extract examined in this study. Future research should establish the quantitative contributions of this group of compounds to the toxic and biologic effects of commercial PCBs and the PCB residues that persist in human tissues.

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Extraction of a High-Protein Isolate from Jerusalem Artichoke (*Helianthus tuberosus*) Tops and Evaluation of Its Nutrition Potential

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The herbage of Jerusalem artichoke (*Helianthus tuberosus, L*), a plant native to North America, has been demonstrated as a good source for the preparation of a protein isolate that is high in lysine and appears to be a high-quality protein. On the basis of chemical composition, it appears that the resulting residues may provide good ruminant feed.

In a changing world with an increasing population it is essential that the most efficient use of available land be made for the production of food and feed. There is considerable interest in the utilization of renewable carbohydrate resources for the production of alcohol for fuel. The Jerusalem artichoke *Helianthus tuberosus*, with its vigorous growth habit, is receiving attention both as a source of sugar for alcohol production and for possible utilization as a fructose sweetener. This plant, when grown for its tubers, can yield as much carbohydrate as sugar beets or corn grain (Fleming and Groot Wassink, 1979). The forage yield is also very high although a maximum yield of both forage and tubers cannot be obtained simultaneously. The herbage yields, which are approximately equal to the tuber yields, present a disposal or utilization problem (Stauffer et al., 1975). A good deal of promotional claims are available for this native American crop, but only meager information is found in the scientific literature concerning its use as an animal feed. There has been sporadic interest in its cultivation. Early studies were not encouraging and reported yields of only 5–6 tons/acre each for the tubers and the tops (Anderson and Kiesselbach, 1929). In addition, Boswell et al. (1936) reported that harvesting the herbage during the growth period before harvest of the tubers would reduce the tuber yield. Perhaps the greatest deterrent to acceptance as a new crop was that the tubers did not store well and that the principal sugar obtained from the tubers was fructose, which was not in demand as a sweetener at that time. More recently, however, Dorrell and Chubey (1977) have reported yields of tubers as great as 26 tons/ha and one new experimental variety has produced between 38 and 60 tons/ha (Chubey and Dorrell, 1982). In neither case was the herbage yield reported. Preliminary field tests in Nebraska showed that the yield of tubers decreased when forage was harvested at the early bloom stage but chemical analyses of the early harvested forage compared favorably with that of alfalfa (O'Keefe, 1982). Farmers in Minnesota as well as in Nebraska reported no feeding problems with silage made from the herbage. The present study was undertaken when samples of Jerusalem artichoke forage were brought to our service laboratory for forage evaluation. The high protein content of the green forage appeared to be a good source for protein isolate that might have possible uses for diet enrichment.

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MATERIALS AND METHODS

The French white variety of Jerusalem artichoke was grown on marginal soil type without any fertilizer treatment or irrigation at Waseca, MN, during 1983 and the first cutting of the foliage was obtained after six weeks of growth. The second and third cuttings followed at 4-week intervals. The plants were cut about 30 cm from the ground. The entire aerial part (including stems, leaves, and shoots) was composited and chopped before analyses and protein isolation.

Compositional Analyses. Total nitrogen was estimated by the Kjeldahl procedure 46-12 approved by the American Association of Cereal Chemists in 1976 (AACC, 1969). Nitrate nitrogen was determined by the ion-selective electrode method described by Hill and Rawate (1982). Protein was estimated as (total nitrogen – nitrate nitrogen) × 6.25.

Methods described by the Association of Official Analytical Chemists (AOAC, 1980) were used for the determination of ash, crude fiber, ether extract, calcium, magnesium, iron, and zinc and also for solubilization of phosphorus. Phosphorus was determined by the molybdenum blue method (Fiske and Subbarow, 1925). Amino acid analyses were carried out on a 6 N HCl hydrolysate with a Beckman 120C amino acid analyzer as previously described by Hill and Rawate (1982).

Preparation of a Protein Isolate. As in the case of amaranth (Hill and Rawate, 1982), when the total herbage was used as the starting material, pressing alone would not expel sufficient juice for protein isolation. Therefore, it was necessary to prepare an aqueous extract by macerating the chopped herbage with an equal volume of water in a 1 gal size waring blender. A procedure that permits good protein yield in outlined in Figure 1. The protein was

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