AROCLOR 1242 INHALATION AND INGESTION BY SPRAGUE-DAWLEY RATS

Ann C. Casey

School of Public Health, University at Albany, Rensselaer, New York, USA and St. Regis Mohawk Tribe Environmental Division

David F. Berger, John P. Lombardo

Department of Psychology, SUNY at Cortland, Cortland, New York, USA

Anne Hunt

Department of Counseling Psychology, University at Albany, Albany, New York, USA

Fred Quimby

Department of Veterinary Pathology, Cornell University, Ithaca, New York, USA

PCBs have been considered to be almost nonvolatile and insoluble in water. However, recent studies have shown the importance of their slight solubility in water and capability to enter the atmosphere and disperse throughout the global environment. This preliminary study was designed to measure uptake and observe any physiological changes in Sprague-Dawley rats. The PCB product Aroclor 1242 is the major pollutant of the Hudson River, NY, and New Bedford Harbor, MA. The rats were exposed for 30 d to 0.9 μg/m³ via inhalation and 0.436 μg/g (ppm) in the food. The inhalation of PCBs gave a greater PCB uptake than ingestion. Both routes of administration caused significant serum thyroid hormone elevations. Histopathologic changes were observed in the urinary bladder, thymus, and the thyroid after both exposure regiments. Rearing and ambulation were significantly decreased in both exposure regiments in an open field behavior test.

Polychlorinated biphenyls (PCBs) were first synthesized in 1889, and in the 1920s were developed for large-scale use by the Swan Corporation, which was later acquired by Monsanto Chemical Company in 1935 (Risebrough & Brodine, 1971). Initially referred to as chlorinated diphenyls, PCBs were mass produced for industrial usage beginning in 1929. In the

Received 9 June 1998; sent for revision 8 July 1998; accepted 21 October 1998.

This project was funded in part by the Cortland Alumni Foundation and in part by a National Institute of Environmental Health Sciences Superfund Basic Research Grant, ES4913 and ES05950. We express our thanks to Dana Wagemaker and Randy Williams for the PCB analysis, Terry Troha and Dr. Robert Rej for the hormone assays, and Noel Kinder for her dedicated animal care. We express our gratitude to Dr. Brian Bush for his knowledge and guidance in preparing this article and making this project a reality.

Address correspondence to Ann C. Casey, St. Regis Mohawk Tribe Environment Division, Rt. 37 Box 8A, Hogansburg, NY 13655, USA.

Journal of Toxicology and Environmental Health, Part A, 56:311–342, 1999

0098-4108/99 $12.00 + .00

United States, large-scale manufacturing, distribution, and usage continued until these substances were banned in 1977 due to widespread concern that PCBs were associated with health risks (U.S. Department of Health and Human Services, 1993). However, PCBs were implicated in various health effects long before 1977. Only 7 years after PCBs were first mass-produced, companies such as Halowax Corporation and General Electric Corporation reported incidents of chloracne and jaundice in workers and customers (Drinker et al., 1937).

Three fatal cases of jaundice in workers exposed to vapors from a mixture of tetra- and pentachloronaphthalenes with approximately 10% refined chlorinated diphenyls (known today as PCBs) prompted Halowax Company to commission a study of the effects of PCBs (Drinker et al., 1937). Drinker and his colleagues found that rats that inhaled or ingested these chlorinated hydrocarbons were adversely affected. The rats exposed to just the chlorinated diphenyl had moderate swelling of the liver cells, with increased granularity and hyalinization. When carbon tetrachloride and ethyl alcohol were given to test liver function, the PCB-exposed rats exhibited enhanced hepatotoxicity. Drinker et al. concluded that "the chlorinated diphenyl is certainly capable of doing harm in very low concentrations and is probably the most dangerous of the chlorinated hydrocarbons. These experiments leave no doubt as to the possibility of systemic effects from the chlorinated naphthalene and chlorinated diphenyls" (Drinker et al., 1937, pp. 296–298).

The evidence is mounting that PCBs are volatilizing from bodies of water such as the Great Lakes, and sediments and soil in urban areas like Chicago (Pearson et al., 1996). Recent laboratory studies have shown that PCBs are volatilizing from sediments into the atmosphere (Chiarenzelli et al., 1996). Once in the atmosphere, the PCBs are then transported by prevailing winds to areas far beyond their points of origin (Pearson et al., 1996). This is of great concern due to their long half-lives and tendency to bio-accumulate (Safe, 1987). A recent study done in the United Kingdom suggests that PCB exposure from indoor air could be a significant contribution to total PCB exposure (Currado & Harrad, 1997). These concerns were discussed in a recent publication, in which inhalation of PCB-contaminated air (average daily intake 100 ng) and meat/fish/poultry (average daily intake 210 ng; 85% of the daily intake was from the fish load) consumption were thought to represent the greatest sources of exposure for the general population (ATSDR, 1996). In contrast, for dioxin-like compounds (PCDD/PCDF), beef has been shown to contribute exceedingly to the total daily intake by food consumption, with fish as a minor contributor (Schecter et al., 1994).

While ingestion of PCBs is recognized to be a primary route of exposure in humans and wildlife, very little is known about the effects of inhalation (ATSDR, 1996). What is known about the effects of inhalation is from aerosol exposures derived from a very few studies conducted following accidents in the workplace (Wolff, 1985). In these studies the expo-

sure was acute and at very high doses. In addition, one study lacked a control group and the exact concentrations of PCBs were unknown.

Few PCB inhalation studies have been carried out since Drinker's pioneering work in 1937. Two inhalation studies that are noteworthy are Treon et al. (1956) and Benthe et al. (1972). Treon et al. (1956) volatilized Aroclor 1242 from a heated well and passed air over it at 500 L/min into a 600-L exposure chamber. The animals were exposed to 8.60 μg/L, 6.83 μg/L, and 1.9 μg/L of Aroclor 1242. In the first experiment, no abnormalities were found by gross necropsy. In the second experiment, deaths in the test and control animals occurred and were attributed to pneumonia, an increase in the rat liver weights of the exposed group, and degenerative changes in the viscera of both groups. In the third experiment, deaths were attributed to infectious pulmonary disease in the exposed group; the rest of the survivors except two had normal viscera. Benthe et al. (1972) aerosolized Pydraul A200 (trade name for hydraulic fluids produced by Monsanto containing Aroclor 1242 and phosphate esters) at a concentration of 30.4 g/m^3. Pydraul at this concentration was readily absorbed by the rat through the lungs and reached maximum concentration in the liver and brain 2 and 24 h, respectively, after a single 30-min dose. However, none of these studies examined endpoints such as behavioral changes, or thyroid hormone levels, and comprehensive histopathology was not preformed.

It has been shown (ATSDR, 1996) that in occupational exposure, 80% of the uptake was due to inhalation. At present, safe air levels for PCBs content are defined in the United States by the National Institute for Occupational Safety and Health (NIOSH) as 1000 ng/m^3 for an 8-h workday, and the U.S. Food and Drug Administration (FDA) currently permits the consumption of fish that contain PCB levels of 2 mg/kg (ppm) (U.S. Department of Health and Human Services, 1993). The majority of studies examining the effects of ingested PCBs in rats have involved doses of PCBs larger than those allowed by the FDA and than those that commonly occur in the environment. In contrast, the study described here was designed to investigate environmentally relevant, subchronic, low-level exposure of a commercial PCB product inhalation exposure.

In addition to the hepatic effects of PCB shown by Drinker et al. (1937) and by numerous other investigators since, harmful effects of PCB on other mammalian systems have been discovered (Waid, 1986). Aroclors have now been definitively shown to be carcinogenic to rats (Mayes et al., 1997). Several endocrine-linked effects have also been demonstrated: (1) reduction in brain dopamine in rats and nonhuman primates (Seegal & Schantz, 1994), (2) changes in thyroid hormones (Porterfield, 1994), and (3) a possible adverse effect on human sperm motility (Bush et al., 1986). Behavioral changes were observed in rats exposed to Aroclor 1254 by Overman et al. (1987) and by Daly et al. (1989) eating Lake Ontario fish containing Aroclors 1254/1260. These effects have recently been attributed to a possible interference with the thyroid hormone system (Seegal &

Schantz, 1994). Studies with female rats clearly show induction of uterine development in developing rat pups by PCB derived from a landfill (Hansen et al., 1995). Related effects on the immune system have also been reported (Silkworth & Grabstein, 1982).

The endpoints of the present study included (1) measuring the efficacy of uptake and distribution of PCB congeners in the body, (2) determining any indications of behavioral change, (3) determination of thyroid function, and (4) a preliminary microscopic assessment of physiological changes occurring from exposure to PCB vapor via-inhalation or by PCB ingestion. The effects were assessed by four distinct means: detailed congener-specific PCB analysis, histopathology, measurement of serum levels of total thyroxine (T4) and triiodothyronine (T3), and a simple behavioral measure (open field) to detect any gross impairment of exploratory behavior. Sprague-Dawley rats were employed, as preliminary work showed them to be the least contaminated with PCB and DDE. This strain of rat is also well documented in behavioral assessment using open field testing. Young rats were chosen to more clearly detect any body weight gain changes occurring as a result of exposure.

MATERIALS AND METHODS

In the present study, inhalation and dietary exposure to Aroclor 1242 at levels below the NIOSH and FDA standards were studied in adolescent male Sprague-Dawley rats. Exposure to PCB vapor and PCB-contaminated food was undertaken, while allowing the animals to live in as normal conditions as possible, so that substantial stress would be limited, to minimize erroneous behavioral, endocrine, and immune reactions. Whole-body exposure was employed rather than nose-only inhalation in order to prevent the severe stress that would accompany restraint for 23 h/d (Leavens et al., 1996). This exposure regimen is widely employed for vapors, where deposition of test compounds on fur or skin is of lesser concern than with aerosol or particulate exposure.

Two exposure routes were employed to compare and contrast the widely investigated food exposure results with the inhalation of PCB vapor. The food exposure was a positive control. The nonexposed rats served as controls for both groups and for the diet, which contained added corn oil.

Animals

Twenty-four adolescent (31-d-old) male Sprague-Dawley rats were used (Taconic Farms, Germantown, NY). The animal care and treatment protocol was approved in accordance with established guidelines for animal subjects at Cortland College, State University of New York. Upon arrival, the rats had a 2-wk acclimation period. They were housed 2 per cage in metabolism cages (Whahmann, Baltimore, MD) and maintained

on a 12-h light/dark cycle with free access to food and water. Prior to the commencement of the experiment, the air and water were tested for PCB content. There were no detectable PCB levels found in the air (<1 ng/m^3) or the water (<1 ng/L). The rats, weighing 167 ± 12 g at 45 d of age, were randomly assigned to one of the 3 exposure groups (n = 8): inhalation, ingestion (Aroclor 1242), or the control group. The day prior to exposure a 23-h food deprivation schedule was commenced; during the 1-h rest period each pair of rats was presented with 30 g powdered food in glass sponge cups. There was adequate food to satisfy them without any sign of fighting or dominance behavior between the pairs. The next day the controls and the inhalation group were fed 30 g of food containing corn oil (1 g/30 g of powdered food) for the next 30 d. The food was Agway Proline rat ration. The ingestion group was fed 30 g of Aroclor 1242-spiked food (0.436 µg/g; Table 1).

Characteristics of Aroclor 1242

Aroclor 1242 is a complex mixture of PCB congeners ranging from mono- to octachlorobiphenyl species (Erickson, 1997). The lot (1 qt) of Aroclor 1242 was obtained from Monsanto in 1971; since then it has been fully characterized by our laboratory. Coplanar congeners present were IUPAC PCB numbers 81, 77, 123, 118, 114, 105, 126, 167, 156, and 157 (Hong et al., 1993). Impurities of PCDF and PCDD (total congeners) have been detected in Aroclor 1242 at a concentration of 0.6 µg/g and of <0.002 µg/g, respectively (Erickson, 1997; Hong et al., 1993). The presence of PCDFs is noteworthy; however, the toxic equivalent (TEQ) is 0.0008 µg/g, which makes its contribution minimal when compared to the coplanar PCB TEQ contribution of 25 µg/g (Hong et al., 1993).

The PCB congener patterns of the vapor phase and the liquid phase of Aroclor 1242 are strikingly different with regard to the penta or higher chlorinated PCB congeners. The liquid phase contained a larger proportion of these higher chlorinated PCB congeners than the vapor phase (Table 1). The higher chlorinated PCB congeners are less volatile, as shown by their longer retention time on the gas chromatograph. It was expected that their contribution to the inhalation dose would be lower than the ingestion dose.

Generation of Test Atmosphere

The generation of a vapor-phase test atmosphere was based entirely on the evaporation of the liquid PCB mixture (Figure 1). The term *vapor* refers to the gaseous state of volatile substances that can coexist as a gas and a liquid at atmospheric pressure and room temperature. Aroclor 1242 has an average molecular weight of 261, and Graham's law of gaseous diffusion can be used to predict a diffusion rate. Experimentally, the evaporation rate at 25°C of Aroclor 1242 was determined to be 0.029 g/m^2/h (Metcafe et al., 1986).

TABLE 1. PCB Congener Concentrations and Doses Via Food and Air and Resulting Doses Received by Sprague-Dawley Rats

Congener	IUPAC number	Food (ng/g)	Air (ng/m^3)	Control (ng/g)	Food dose (ng/d)	Air dose (ng/d)	Food (μg/kg/d)	Air (μg/kg/d)
2	1	13.5	42.5	0[a]	202	4.3	1.0	0.02
4	3	0.0	24.1	0	0	2.5	0.0	0.01
2/2 + 26	4 + 10	14.5	113.2	0	217	11.6	1.1	0.06
24 + 25	7 + 9	3.2	17.5	0	48	1.8	0.2	0.01
2/3	6	6.0	26.0	0	91	2.7	0.5	0.01
2/4 + 23	8 + 5	29.7	132.8	0	445	13.6	2.2	0.07
26/2	19	4.7	24.7	0	70	2.5	0.4	0.01
3/4	13	5.0	9.9	0	76	1.0	0.4	0.01
25/2	18	31.2	95.9	0	468	9.8	2.3	0.05
4/4 + 24/2	15 + 17	39.8	110.1	0	597	11.2	3.0	0.06
236 + 26/3	24 + 27	3.3	8.6	0	49	0.9	0.2	0.00
26/4 + 23/2	32 + 16	38.8	100.3	0	581	10.2	2.9	0.05
245	29	0.4	0.5	0	7	0.1	0.0	0.00
25/3	26	3.2	4.4	0	48	0.4	0.2	0.00
24/3	25	1.6	2.1	0	23	0.2	0.1	0.00
25/4	31	0.0	33.3	0	0	3.4	0.0	0.02
24/4	28	26.0	33.2	0	390	3.4	1.9	0.02
34/2 + 25/26	33 + 53	24.3	27.6	0	365	2.8	1.8	0.01
24/26 + 23/4	51+22	18.9	20.6	0	283	2.1	1.4	0.01
236/2	45	2.8	3.2	0	42	0.3	0.2	0.00
23/26	46	2.2	2.2	0	32	0.2	0.2	0.00
25/25	52	11.6	8.8	0	174	0.9	0.9	0.00
24/25	49	9.9	6.9	0	149	0.7	0.7	0.00
24/24 + 246/4 + 245/2	47 + 75 + 48	10.3	6.3	0	154	0.6	0.8	0.00
23/25	44	14.1	8.2	0	211	0.8	1.1	0.00
236/3 + 34/4 + 23/24	59 + 37 + 42	24.7	12.5	0	371	1.3	1.9	0.01
26/34 + 234/2 + 236/2	71 + 41 + 64	12.0	5.9	0	179	0.6	0.9	0.00
23/23	40	3.8	1.5	0	57	0.2	0.3	0.00
245/3	67	1.0	0.0	0	15	0.0	0.1	0.00
235/4	63	1.1	0.0	0	16	0.0	0.1	0.00
235/26 + 245/4	94 + 74	14.2	3.2	0	213	0.3	1.1	0.00
25/34	70	10.8	2.5	0	162	0.3	0.8	0.00
24/34 + 236/25	66 + 95	14.3	2.1	0	214	0.2	1.1	0.00
236/24	91	1.5	0.0	0	22	0.0	0.1	0.00
23/34 + 234/4	56 + 60	10.7	1.7	0	161	0.2	0.8	0.00
235/25	92	0.5	0.0	0	8	0.0	0.0	0.00
236/23	84	1.6	0.0	0	24	0.0	0.1	0.00
235/24 + 245/25	90 + 101	3.3	0.2	0.438	50	0.0	0.2	0.00
245/24	99	2.1	0.0	0.259	32	0.0	0.2	0.00
2346/3 + 235/23	109 + 83	0.8	0.0	0.528	12	0.0	0.1	0.00
245/23	97	1.9	0.0	0.139	28	0.0	0.1	0.00
234/25	87	2.0	0.0	0	30	0.0	0.1	0.00
236/236	136	0.0	0.0	0	0	0.0	0.0	0.00
236/34 + 34/34	110 + 77	4.7	0.1	0.316	70	0.0	0.4	0.00

TABLE 1. PCB Congener Concentrations and Doses Via Food and Air and Resulting Doses Received by Sprague-Dawley Rats (*Continued*)

Congener	IUPAC number	Food (ng/g)	Air (ng/m³)	Control (ng/g)	Food dose (ng/d)	Air dose (ng/d)	Food (μg/kg/d)	Air (μg/kg/d)
234/23	82	0.8	0.0	0	12	0.0	0.1	0.00
2356/25	151	0.0	0.0	0	0	0.0	0.0	0.00
235/236 + 2346/25	135 + 144	2.6	0.0	0	39	0.0	0.2	0.00
2356/24 + 235/34	147 + 107	0.0	0.0	0	0	0.0	0.0	0.00
345/24	123	0.0	0.1	0.626	0	0.0	0.0	0.00
245/34	118	3.2	0.1	0	48	0.0	0.2	0.00
2356/23	134	0.0	0.0	0.153	0	0.0	0.0	0.00
2345/4	114	0.0	0.0	0	0	0.0	0.0	0.00
235/245	146	0.0	0.0	0.136	0	0.0	0.0	0.00
245/245 + 234/236	153 + 132	0.0	0.0	0.392	0	0.0	0.0	0.00
234/34	105	1.1	0.0	0	17	0.0	0.1	0.00
2345/25 + 2356/236	141 + 179	0.0	0.0	0	0	0.0	0.0	0.00
2345/24	137	0.0	0.0	0	0	0.0	0.0	0.00
2346/236	176	0.0	0.2	0	0	0.0	0.0	0.00
234/235	130	0.0	0.0	0	0	0.0	0.0	0.00
234/245 + 236/345 + 2356/34	138 + 164 + 163	0.8	0.1	0.686	11	0.0	0.1	0.00
2346/34	158	0.0	0.0	0.257	0	0.0	0.0	0.00
2345/23	129	0.0	0.0	0	0	0.0	0.0	0.00
2356/235	178	0.0	0.0	0	0	0.0	0.0	0.00
2345/246 + 2356/245	181 + 187	0.0	5.6	0.477	0	0.6	0.0	0.00
2346/245	183	0.0	0.0	0	0	0.0	0.0	0.00
234/234	128	0.0	0.0	0	0	0.0	0.0	0.00
245/345	167	0.0	0.0	0	0	0.0	0.0	0.00
23456/25	185	0.0	0.0	0	0	0.0	0.0	0.00
2345/236	174	0.0	0.1	0	0	0.0	0.0	0.00
2356/234	177	0.0	0.0	0	0	0.0	0.0	0.00
2346/234 + 2345/34	171 + 156	0.0	0.0	0	0	0.0	0.0	0.00
2346/2356	201	0.0	0.0	0	0	0.0	0.0	0.00
2345/235	172	0.0	0.0	0	0	0.0	0.0	0.00
2345/245	180	0.0	0.0	0.408	0	0.0	0.0	0.00
2356/345	193	0.0	0.0	0	0	0.0	0.0	0.00
23456/236	199	0.0	0.0	0	0	0.0	0.0	0.00
2345/234 + 23456/34	170 + 190	1.6	0.0	0	24	0.0	0.1	0.00
2345/2356	199	0.0	0.0	0	0	0.0	0.0	0.00
23456/245 + 2345/2346	203 + 196	0.0	0.0	0	0	0.0	0.0	0.00
23456/234	195	0.0	0.8	0	0	0.1	0.0	0.00
2345/2345	194	0.0	0.0	0.09	0	0.0	0.0	0.00
23456/2345	206	0.0	0.0	0	0	0.0	0.0	0.00
Total		436.0	900.0	4.766	6550	91.9	32.8	0.46

Note. Congener structures show the position of the chlorine, with rings separated by /.
[a]A value of zero indicates less than the detectable level (see text).

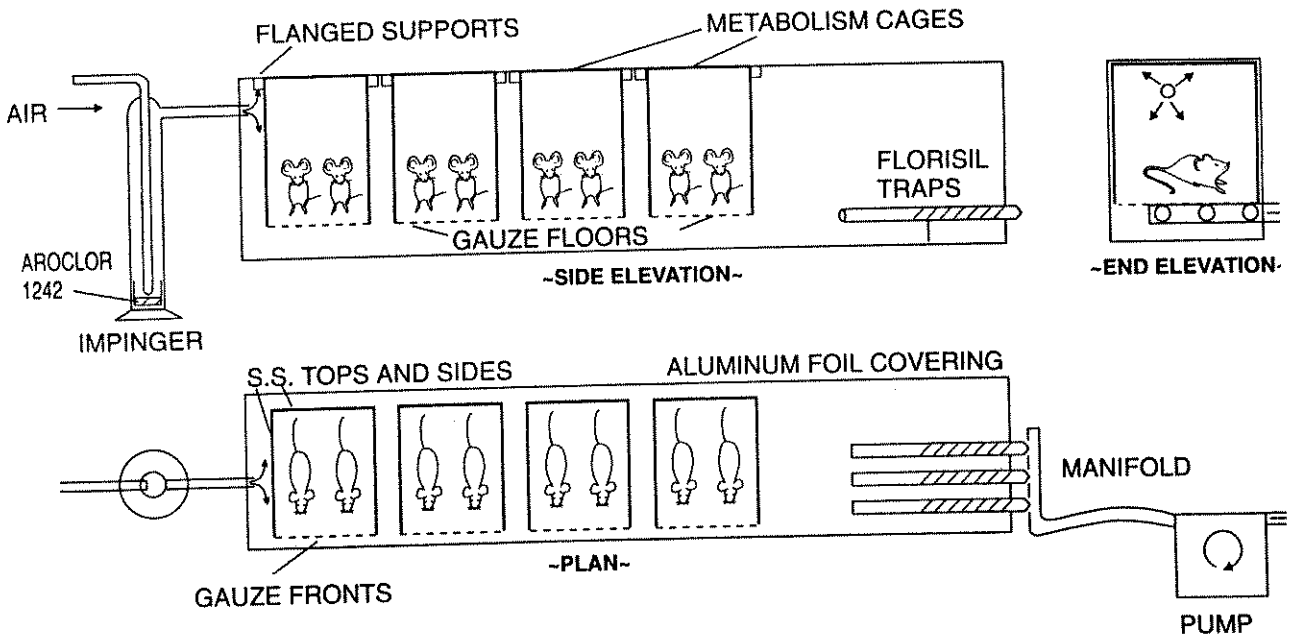

FIGURE 1. Dosing system used for administering Aroclor 1242 vapor to rats.

The vapor phase was produced by drawing air over the liquid Aroclor 1242 product contained in a 500-ml glass impinger at room temperature. The air was drawn through the impinger by producing a very slight negative pressure (140 dyn/cm^2 or 0.14 g/cm^2 caused by 9 L of air/min (184 μpoises at 25°C) passing through a limiting orifice of 1 mm radius 2 mm long). Inside the sealed inhalation chamber, air was drawn through three Florisil-packed tubes (10-ml graduated glass disposable pipettes) situated at the far end of the chamber using a Gast vacuum pump (model 1531-107K-G557X, Benton Harbor, MI). This system was designed to overcome several potential problems. First, because negative pressure determined by the limiting orifice in the stem of the impinger was employed, and vapor was produced by air demand only, possible contamination of the facility was avoided. Second, in the event of air leakage into the chamber via poor seals, which would cause dilution of the atmosphere, the exit concentration of PCB in the atmosphere was measured, not the concentration entering the chamber, using Florisil traps. Since the air from the impinger first impinged upon a stainless steel plate (comprising the side of the first cage; Figure 1), the flow would be turbulent, causing good mixing in the chamber. If leaks of the chamber did occur, the leaked air would be mixed into the chamber atmosphere before reaching the outlet tubes. The animals near to the entry port should not have experienced a higher concentration than animals further away, although rotating the cages daily also guarded against this possibility. Furthermore, leakage was unlikely as the pressure drop of <1 g/cm^2 or 0.001 atm was so low. The 9 L/min flow produced approximately 3 changes of air in the chamber per hour.

This system did not create aerosol droplets from the surface of the pool of Aroclor 1242 surface (35 cm^2, 1 cm below the impinger orifice), as no surface ripple was visible. Since the system drew air over the pool of Aroclor instead of blowing air into the impinger, a nebulizing effect of mist/droplets was not created. It was important not to create an aerosol since low-level vapor exposure was required for the experimental protocol.

Because behavioral experiments were to be carried out on the animals postexposure, a normal 12-h light cycle was required; this was achieved by having the front face of the cage made of polyethylene. Ideally, glass should have been used for this purpose since, unlike metals, glass, and Teflon, polyethylene absorbs PCB vapor strongly; however, the actual concentrations that the animals were exposed to were known (Table 1) because vapor concentrations were measured downstream of the chamber. Lower vapor concentrations were discernable only on the first 2 d of the experiment, possibly caused by absorption by the polyethylene. Each group of animals was housed similarly, except that the control and ingestion groups were in unsealed polyethylene-fronted chambers open to the atmosphere of the room, which had 15 changes of air per hour.

Inhalation Exposure

The exposures were carried out in a standard five-level rack of metabolism cages, the middle level of which was modified into a sealed chamber with heavy-gauge aluminum foil and duct tape (Figure 1). A second layer of polyethylene sheet, again sealed with duct tape, backed up the aluminum foil. The front of the chamber was covered with a polyethylene sheet allowing light into the chamber. The chamber dimensions were 165 × 35 × 30 cm (volume 173 L). In the chamber there were four cages (24 × 18 × 18 cm) with stainless steel mesh fronts and bottoms and stainless steel sides and top; these slid into the top of the rack on stainless steel flanges. Each housed two rats with a space for a fifth cage. This space was used for the Florisil traps, which protruded into the bottom of the chamber (ID 0.9 cm, length 26 cm). The manifold system drew 9 L air/min through the chamber. This system generated an atmosphere containing 900 ± 110 ng/m^3 of PCBs, as determined by gas chromatography–electron capture detection (GC-ECD) based congener-specific analysis of the Florisil traps on alternate days (n = 17) for the entire study (Figure 2). This resulted in the rats inhaling an average of 0.64 μg/kg/d, given an average respiration rate of 74 mL/min (107 L/d) (Altman & Ditter, 1971) and an average rat weight of 200 g (starting rat weight 167 ± 12 g, final rat weight 232 ± 35 g).

The 9-L/min flow through the inhalation chamber represents 540 L/h; the 8 rats would inhale 35 L/h, so that the air supply was judged to be

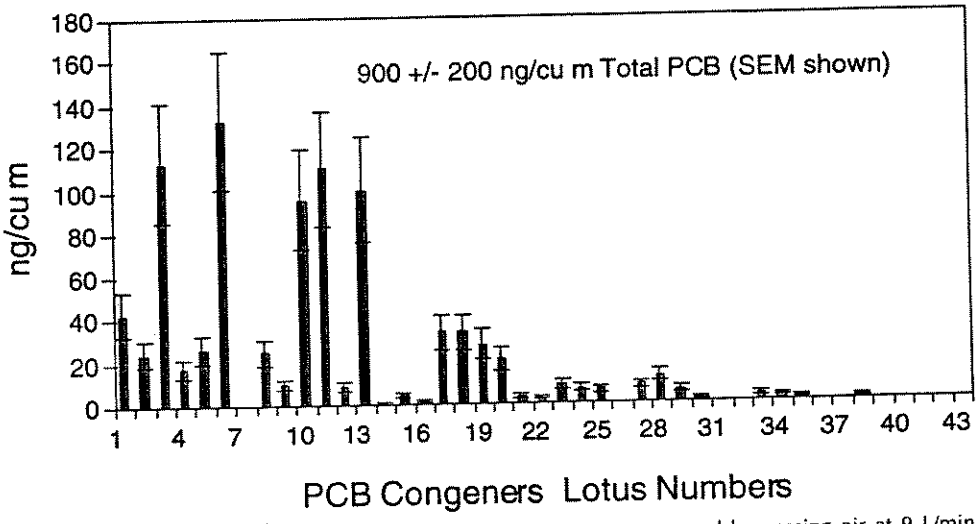

FIGURE 2. Mean PCB congener pattern measured daily in air generated by passing air at 9 L/min over a pool of Aroclor 1242 liquid with surface area of 32 cm^2 for 30 d. Lotus numbers are the spreadsheet serial numbers of the congeners as they emerge from the chromatograph, ordered as shown in Tables 1 and 2.

adequate with regard to carbon dioxide and oxygen content, although some condensation of water was visible in the inhalation group chamber, indicating some increase in humidity and temperature. The animal loading (MacFarland, 1976) increased from 0.8% to 1.07% during the experiment.

Ingestion Exposure

Rat ration was weighed into 30-g portions and then corn oil (1 ml) containing Aroclor 1242 (1.3 μg/ml) was mixed into it thoroughly with a spatula to give a final concentration of 0.436 μg/g (ppm). The rats in each cage consumed the 30 g of ration completely during the 1-h rest period; it was assumed they consumed 15 g each. This method of exposure was more convenient than preparing a bulk diet before the experiment, and it assured an accurate measure of the daily PCB intake. The inhalation and control groups were fed the same powdered Agway ration containing the same quantity of corn oil. During the 1-h rest period (hence the exposure was for 23 h daily) the Durosorb pads on the cage bottoms were changed and the water bottles were filled. The food dishes were removed at the end of the hour to prevent ingestion of food that might become contaminated by PCB absorbed from the vapor. This procedure resulted in the ingestion group being exposed at a rate of 45 μg/kg/d. No differences were present in the amount of food consumed by the groups, since all food was consumed during the rest period.

Collection for Analysis

At the end of the 30-d period, all animals were killed via stunning and decapitation. Blood was collected directly after decapitation, allowed to clot for 40 min, and centrifuged for 15 min to yield serum, stored at –20°C until analysis. Dissection was carried out to avoid cross-contamination of tissue with highly PCB-contaminated materials such as fat, skin, or fur. The tissues collected for PCB analyses were brain, abdominal fat, lung, and liver. The tissues were stored at –20°C until analysis. The histological examination was performed on the adrenal gland, urinary bladder, stomach, thymus, lacrimal glands, spleen, lymph nodes, colon, skin, testes, secondary sex glands, liver, and pancreas. The tissues were fixed in 10% buffered formalin and were later paraffin embedded, sectioned at 7 μm, and stained with hematoxylin and eosin for histological analysis.

Open Field Behavior

On the day following the last day of exposure, the rats were tested individually in an open field device that consisted of a cylindrical white wall (100 cm diameter, 65 cm high) enclosing a gray plywood floor divided into 18 segments with a circle in the center. A fluorescent fight was placed above the device, which was bathed in loud white noise (85 dB). The animals' behavior in this mild stress situation was evaluated for 3 min

for the following endpoints: (1) ambulation—the number of segments entered with all feet was counted; (2) rearing; (3) urination; and (4) number of fecal deposits.

Determination of PCB

The tissues, sera, and Florisil traps were analyzed by congener-specific analysis. This was performed by capillary GC with electron capture detector (ECD) (Bush et al., 1983, 1989). Briefly, the tissue samples were mixed with anhydrous sodium sulfate to remove water. The tissue and sodium sulfate were then ground together with 20 ml hexane:acetone (1:1) with an SDT Tissumizer (Tekmar, Cincinnati, OH) for 1 min. The hexane supernatant was then pipetted into a volumetric flask. The extraction was repeated 2 more times and the combined extracts were diluted to 50 ml; a 2-ml aliquot was removed for a gravimetric measurement of percent lipid. The remaining extract was transferred to a Kuderna-Danish (KD) evaporator (to achieve concentration without PCB loss) and concentrated to approximately 2 ml on a steam bath. The extract was transferred quantitatively to a 1 cm × 15 cm chromatography column containing 10 g calibrated 4% deactivated Florisil (US Silica, Silver Springs, WV) with a 2-g layer of sodium sulfate on top of the Florisil. The sample was then eluted with hexane and 50 ml was collected, transferred to a KD evaporator, evaporated to 1 ml, and pipetted into a 2-ml GC vial for analysis. For the extraction of PCB from the Florisil traps, the three traps were eluted with 18 ml hexane and the first 15 ml was collected. The three extracts were combined and 1 ml was taken for analysis.

The samples were analyzed on a Hewlett-Packard 5890 GC equipped with a nickel-63 ECD using a fused silica column with cross-linked 5% phenylmethylsilicone coating (0.33 μm film thickness, 0.25 mm internal diameter, Hewlett Packard Ultra II). The oven temperature was held at 100°C for 2 min, then raised up to 160°C at 10°C/min, then 1°C/min to 190°C, and then 2°C/min to a final temperature of 270°C. The temperature was held at 270°C for 10 min until all congeners were eluted. Ninety-six PCB congeners were identified and measured. The microprocessor of the GC was calibrated with the a mixture of Aroclors 1221, 1016, 1254, and 1260 (200 ng/ml of each) fortified with hexachlorobenzene (HCB), *p,p'*-DDE, and mirex at 5, 10, and 10 ng/ml, respectively, and 3,3',4,4'-tetrachlorobiphenyl at 20 ng/ml. This mixture has been previously characterized (Bush et al., 1989; Frame, 1996). The data analysis was carried out with a personal computer using FILESERVER to transfer the data into a LOTUS 123 spreadsheet (Lotus Development Corp., Cambridge, MA). Quality control for the analysis was ensured as described previously (Bush et al., 1983, 1989) and by spiking each sample with DDE to standardize retention times. A replicate of a sample was run every 20 samples to ensure the consistency of the sample extraction. The average minimum detectable level (MDL) ($p < .05$ that the reported level is zero) (type I

error) for a sample weighing 1 g was 0.02 ng/g (U.S. EPA, 1984). It should be noted that at one quarter of this value, the chance the congener is present is >87% (type II error).

Thyroid Hormone Analyses

Total thyroxine (TT4) and total triiodothyronine (TT3) were determined with veterinary DPC Coat-A-Count Total radioimmunoassay kits (Diagnostic Products Corporation, Los Angeles, CA). The blood was collected at the time of dissection in a glass vacutainer tube, was centrifuged for 15 min, and the serum was separated from the red cells and pipetted into a clean Eppendorf tube. The procedure required 200 µl serum. The samples were stored at –60°C until analysis. The samples were removed from the freezer and allowed to warm to room temperature. The samples, controls, and calibrators (100 µl each) were pipetted into the DPC tubes. One milliliter iodinated triiodothyronine (for T3 analysis) or 1 ml iodinated thyroxine (for T4 analysis) was added to each tube. The samples were vortexed for 30 s and then incubated for 2 h in a 37°C water bath. After the incubation the samples were decanted and read for 1 min on a Wallac Wizard 1470 gamma counter. The standard curves were plotted and sample concentrations were calculated using Wallac MultiCalc software.

RESULTS

Daily Health Observations

All animals survived the 30-d exposure period. During the 30 d of exposure, the rats showed no overt signs of illness. Starting rat weight at age 45 d was 167 ± 12 g, and the final rat weight at age 75 d was 232 ± 35 g. Weight gain of control rats (85 ± 18 g) was similar to that reported by others (94 ± 12 g) for somewhat larger male Wistar rats (100–120 g) (Harris et al., 1993). There was no significant percentage weight gain difference between the control and ingestion groups (39 ± 4% and 40 ± 7%, respectively). However, there was a diminished percentage weight gain between the control and inhalation groups (39 ± 4% and 33 ± 4%).

PCB Analysis

Figure 2 illustrates the composition and reproducibility of the vapor exiting from the inhalation chamber (numeric values are given in Table 1). The compositions of the inhaled air and of the diet are illustrated in Figure 3, showing differences in the relative congener levels, particularly for the higher chlorinated congeners. The relationship between vapor concentration and concentration in the liquid PCB phase (concentration differential) is shown in Figure 4. This illustrates the difference in lipophilic characteristics between air and a lipid phase of lower and higher chlorinated congeners.

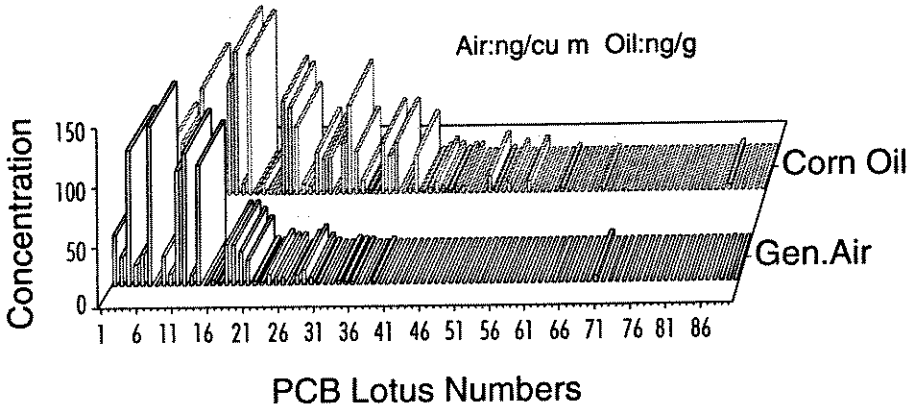

FIGURE 3. Three-dimensional comparison of the PCB congener composition of generated air and food. Congeners are represented in the order they elute from the gas chromatograph (Lotus numbers), which is approximately in order of increasing lipophilic character.

PCB congener concentrations found in the four tissue types examined are shown in Table 2. The cerebral cortex concentrations are illustrated in Figure 4; this is presented to demonstrate the consistency of the uptake for the individual rats in this experiment. The control rat brains showed two congeners at low levels, 2,4,4'-trichloribiphenyl (IUPAC number 28) and 3,4,4'-trichlorobiphenyl (IUPAC number 37) that were present in the rats when obtained from the supplier.

Mean PCB congener concentrations in each tissue were compared using Student's *t*-test:

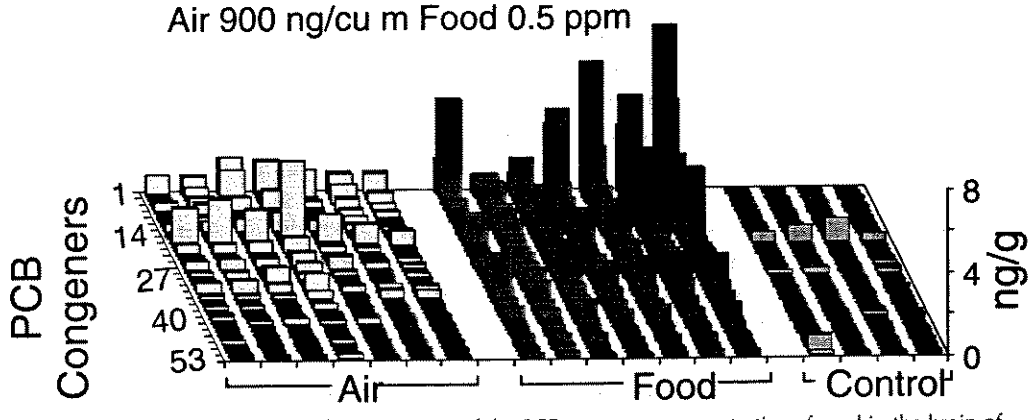

FIGURE 4. Three-dimensional presentation of the PCB congener concentrations found in the brain of individual rats after exposure. (1) Inhalation from a total PCB concentration of 900 ± 110 ng/m^3 in air for 30 d. (2) Ingestion from a food concentration of 436 ng/g total PCB in food. (3) Brains from control rats. IUPAC PCB numbers are given.

TABLE 2. Mean PCB Congener Concentrations (ng/g wet weight) in Rat Tissues After Inhalation and Food Exposure

Congener structure	IUPAC number	Fat		Cerebral cortex		Liver		Lung	
		Air	Food	Air	Food	Air	Food	Air	Food
2	1	6.50	14.30[a]	1.14	3.16[a]	0.00	0.00	1.07	3.20[a]
4	3	0.00	0.00	0.73	2.89[a]	0.00	0.00	0.00	0.72
2/2 + 26	4 + 10	0.82	0.00	0.20	0.72[a]	0.00	0.00	0.10	0.07[a]
24 + 25	7 + 9	1.56	0.52[a]	0.35	0.34	0.15	0.07[a]	0.17	0.18[a]
2/3	6	2.12	0.90[a]	0.09	0.16[a]	0.04	0.02	0.11	0.05[a]
2/4 + 23	8 + 5	10.50	5.54[a]	0.48	1.09[a]	0.56	0.32[a]	0.28	0.17[a]
26/2	19	0.00	0.00	0.10	0.16[a]	0.03	0.00	0.06	0.06
3/4	13	0.00	0.00	0.35	0.29	0.12	0.12	0.08	0.00
25/2	18	3.23	1.62	0.24	0.48[a]	0.09	0.04	0.08	0.05[a]
4/4 + 24/2	15 + 17	4.50	1.12	0.36	0.65[a]	0.46	0.07[a]	0.21	0.11[a]
236 + 26/3	24 + 27	0.00	0.00	0.04	0.08[a]	0.02	0.03	0.02	0.03
26/4 + 23/2	32 + 16	4.07	2.49	0.45	0.83[a]	0.15	0.07[a]	0.11	0.12
245	29	0.00	0.00	0.02	0.03	0.00	0.00	0.01	0.01
25/3	26	0.77	0.53	0.06	0.07	0.03	0.01[a]	0.03	0.03
24/3	25	0.11	0.05	0.03	0.03	0.02	0.03	0.03	0.05[a]
25/4	31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24/4	28	103.88	99.47	1.72	2.23	1.83	1.77	2.57	3.27
34/2 + 25/26	33 + 53	2.62	2.51	0.31	0.51[a]	0.08	0.06	0.08	0.06[a]
24/26 + 23/4	51 + 22	1.96	0.59	0.37	0.40	0.04	0.00[a]	0.06	0.06[a]
236/2	45	0.08	0.18	0.06	0.10[a]	0.00	0.00	0.02	0.02
23/26	46	0.00	0.00	0.07	0.09	0.04	0.03	0.00	0.02
25/25	52	3.98	7.34	0.27	0.47[a]	0.16	0.23[a]	0.34	0.52[a]
24/25	49	2.71	6.25	0.19	0.24	2.78	2.49	0.14	0.21[a]
24/24 + 246/4 + 245/2	47 + 75 + 48	21.70	41.06	0.41	0.64[a]	1.12	2.06[a]	0.60	1.33
23/25	44	1.90	2.69[a]	0.22	0.32	0.10	0.09	0.08	0.11[a]
236/3 + 34/4 + 23/24	59 + 37 + 42	1.55	2.67[a]	0.32	0.34[a]	0.20	0.15	0.08	0.14[a]
26/34 + 234/2 + 236/4	71 + 41 + 64	1.24	2.54[a]	0.21	0.24	0.07	0.08	0.04	0.07[a]
23/23	40	0.00	0.00	0.03	0.07	0.00	0.00	0.02	0.01
245/3	67	0.00	0.00	0.12	0.01[a]	0.00	0.00	0.00	0.03
235/4	63	1.09	1.66[a]	0.01	0.02	0.08	0.11[a]	0.08	0.20[a]
235/26 + 245/4	94 + 74	39.60	120.76[a]	0.50	1.50	2.04	3.89[a]	0.92	3.74[a]
25/34	70	1.93	4.37[a]	0.07	0.13[a]	0.02	0.02	0.04	0.06[a]
24/34 + 236/25	66 + 95	16.89	45.72[a]	0.28	0.55[a]	1.60	2.72[a]	0.65	2.46[a]
236/24	91	0.00	0.00	0.00	0.03[a]	0.01	0.00	0.00	0.00
23/34 + 234/4	56 + 60	7.41	26.92	0.12	0.37	0.48	1.01[a]	0.52	3.12[a]
235/25	92	0.00	2.31	0.00	0.06[a]	0.01	0.02	0.01	0.05
236/23	84	0.00	0.00	0.03	0.06[a]	0.00	0.01	0.00	0.00
235/24 + 245/25	90 + 101	3.64	12.11	0.14	0.29	0.33	0.43	0.15	0.42[a]
245/24	99	12.55	39.11	0.17	0.53[a]	1.50	3.01[a]	0.46	1.63[a]
2346/3 + 235/23	109 + 83	0.00	0.00	0.14	0.12[a]	0.54	1.87[a]	1.34	1.81[a]
245/23	97	0.00	1.22	0.05	0.08[a]	0.00	0.12[a]	0.00	0.08
234/25	87	0.25	3.88	0.04	0.11	0.10	0.22[a]	0.05	0.19[a]
236/236	136	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
236/34 + 34/34	110 + 77	0.65	4.03[a]	0.10	0.23[a]	0.02	0.02	0.04	0.13[a]
234/23	82	0.00	0.00	0.04	0.02	0.00	0.00	0.01	0.02
2356/25	151	0.00	0.00	0.03	0.06[a]	0.00	0.01	0.02	0.02
235/236 + 2346/25	135 + 144	0.80	0.63	0.05	0.05	0.09	0.09	0.05	0.05

(Table continues on next page)

TABLE 2. Mean PCB Congener Concentrations (ng/g wet weight) in Rat Tissues After Inhalation and Food Exposure (*Continued*)

Congener structure	IUPAC number	Fat		Cerebral cortex		Liver		Lung	
		Air	Food	Air	Food	Air	Food	Air	Food
2356/24 + 235/34	147 + 107	0.00	1.44	0.00	0.00	0.00	0.00	0.00	0.11
345/24 + 236/245	123	1.07	2.98[a]	0.10	0.18[a]	0.00	0.09	0.00	0.00
245/34	118	14.86	61.57[a]	0.24	0.86[a]	0.66	1.65[a]	0.41	1.85[a]
2356/23	134	0.37	0.00	0.01	0.03[a]	0.00	0.00	0.00	0.00
2345/4	114	1.86	3.56	0.02	0.09[a]	0.45	0.68[a]	0.01	0.19[a]
235/245	146	3.49	4.99[a]	0.04	0.07[a]	0.09	0.12[a]	0.10	0.17
245/245 + 234/236	153 + 132	25.85	44.69[a]	0.45	0.76[a]	1.27	1.46	1.61	2.22[a]
234/34	105	3.76	18.69[a]	0.09	0.36[a]	0.18	0.48[a]	0.12	0.95[a]
2345/25 + 2356/236	141 + 179	0.31	0.09	0.02	0.04[a]	0.00	0.00	0.12	0.26
2345/24	137	0.59	1.63[a]	0.00	0.03[a]	0.10	0.40[a]	0.06	0.10
2346/236 + 234/235	176 + 130	0.00	0.00[a]	0.02	0.00[a]	0.00	0.00	0.00	0.00
234/245 + 236/345	138/164	22.36	37.35[a]	0.46	0.65[a]	0.92	1.31[a]	0.83	1.56
2346/34	158	1.14	2.91[a]	0.08	0.10	0.07	0.24[a]	0.24	0.68
2345/23	129	0.00	0.00[a]	0.01	0.01	0.01	0.22[a]	0.00	0.00
2356/235	178	0.56	0.82[a]	0.00	0.02[a]	0.01	0.00[a]	0.02	0.02
2345/246 + 2356/245	181 + 187	7.20	9.12[a]	0.13	0.17[a]	0.26	0.32[a]	0.34	0.44[a]
2346/245	183	2.85	3.37[a]	0.05	0.09[a]	0.15	0.18[a]	0.14	0.18
234/234	128	2.30	4.58[a]	0.03	0.18[a]	0.12	0.19[a]	0.05	0.16
245/345	167	0.00	0.00[a]	0.00	0.01[a]	0.00	0.11	0.01	0.03
23456/25	185	0.00	0.00[a]	0.00	0.01[a]	0.00	0.00	0.00	0.02
2345/236	174	0.00	0.00[a]	0.02	0.06	0.00	0.02	0.01	0.02
2356/234	177	4.34	4.57[a]	0.08	0.14[a]	0.14	0.16[a]	0.10	0.13[a]
2346/234 + 2345/34	171 + 156	2.09	2.28[a]	0.08	0.16[a]	0.14	0.23[a]	0.08	0.05[a]
2346/2356	201	0.75	0.75	0.01	0.07[a]	0.05	0.12[a]	0.04	0.09[a]
2345/235	172	1.67	1.20	0.03	0.09[a]	0.02	0.05[a]	0.05	0.07[a]
2345/245	180	9.13	9.94	0.18	0.23[a]	0.33	0.36	0.24	0.29[a]
2356/345	193	0.00	0.00	0.00	0.01	0.00	0.00	0.02	0.02
23456/236	199	0.11	0.00	0.00	0.02	0.00	0.21	0.03	0.05
2345/234 + 23456/34	170 + 190	6.21	8.05[a]	0.70	0.43	1.42	2.10	0.80	1.29[a]
2345/2356	199	4.02	4.60	0.15	0.14	0.23	0.24	0.15	0.18
23456/245 + 2345/2346	203 + 196	2.87	3.20	0.11	0.11	0.24	0.24	0.12	0.15
23456/234	195	1.77	2.67[a]	0.09	0.07	0.12	0.14	0.07	0.10
2345/2345	194	2.08	2.43[a]	0.06	0.06	0.18	0.19	0.09	0.10
23456/2345	206	0.98	1.27[a]	0.03	0.05[a]	0.17	0.18	0.08	0.12
Total		386.25	697.26[a]	14.03	26.16	22.25	33.00	16.68	36.29[a]
Fat (%)				8.48	6.88	3.24	3.05	5.01	4.43[a]

Note. A concentration of zero indicates the congener was not detected or <.01 μg/g. See text for minimum detectable levels. Data were rounded to two decimal places for clarity.

[a]Concentrations by the two exposure routes are discernibly different ($p < .05$, $n = 8$ for each route).

where X_1 is the mean via inhalation, X_2 is the mean via food, S is the standard deviation of the mean via inhalation, and n is the number of subjects ($n = 8$). All concentrations were significantly different from control concentrations ($p < .01$). Significantly different concentrations between inhalation and ingestion ($p < .05$, $t > 1.9$) values are marked in Table 2.

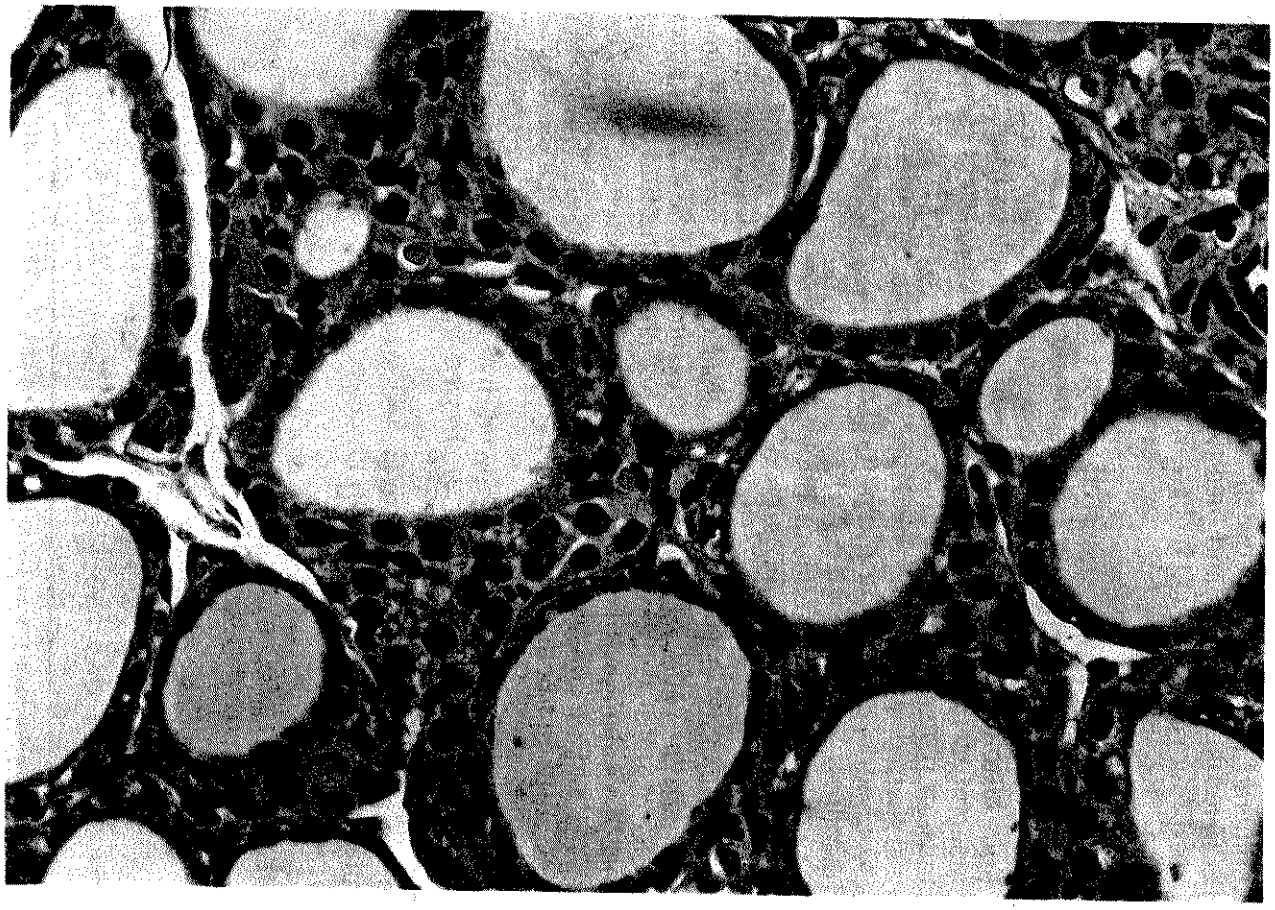

FIGURE 5. Histopathology of thyroid tissue from control rats.

Thyroid

The mean thyroid hormone response is shown in Table 3 (standard errors of the mean are shown). Analysis of variance (ANOVA) analysis of the results for both compounds showed significant difference between groups at $p < .01$. When pairwise t-tests were carried out, there were marked differences ($p < .01$) between controls and both exposure groups and also between inhalation and ingestion exposed groups, with the inhalation exposure groups being highest in both cases.

Histology

Histologic changes were observed in both the inhalation and ingestion exposure groups. The control rats tissues appeared to be normal. In spite of the observation of tearing in the vapor-exposed rats, there were no obvious histologic lesions in the lacrimal glands. No obvious lesions were apparent in the liver, lymph nodes, colon, skin, testes, secondary sex glands, or pancreas. Histopathological change were observed in the following: thyroid gland of the inhalation group ($n = 8$), urinary bladder changes in both exposures (air $n = 8$, food $n = 6$), and thymus changes in both exposures (air $n = 7$, food $n = 7$).

Most notable among the histopathological changes observed was the increased intracellular vacuolization of follicular epithelial cells of the thyroid gland in air-exposed rats (Figures 6 and 7). Tall follicular epithelial cells surrounding a reduced amount of colloid characterized the thyroid glands. Epithelial cells contained numerous intracytoplasmic vacuoles. The nuclei of these cells contained compact chromatin and prominent nucleoli. These changes are characteristic of actively secreting thyroid follicular cells and are consistent with the observed elevated serum levels of thyroxine (T4) and triiodothyronine (T3). This change was not apparent in the PCB-fed rats.

Both the air and food groups showed thymic atrophy. The thymus at 40× magnification (Figures 8 and 9) showed a reduced ratio of darkly staining cortex compared to the medulla.

The urinary bladders of both exposed groups exhibited epithelial hyperplasia. The fed rats showed 8–11 layers of cells, compared to 3–5

TABLE 3. Total Triiodothyronine (TT3) and Thyroxine (TT4) Concentrations in Controls and Rats Exposed to 0.46 μg/kg/d of Aroclor 1242 via Inhalation and 32.8 μg/kg/d via Ingestion

Exposure groups	TT3 (ng/dl)	TT4 (μg/dl)
Control	68 ± 4.75	5 ± 0.27
Ingestion	90 ± 2.84[a]	6 ± 0.26[a]
Inhalation	113 ± 2.64[a]	9 ± 0.36[a]

Note. Values are mean ± SEM.
[a]Discernibly different from control ($p < .05$).

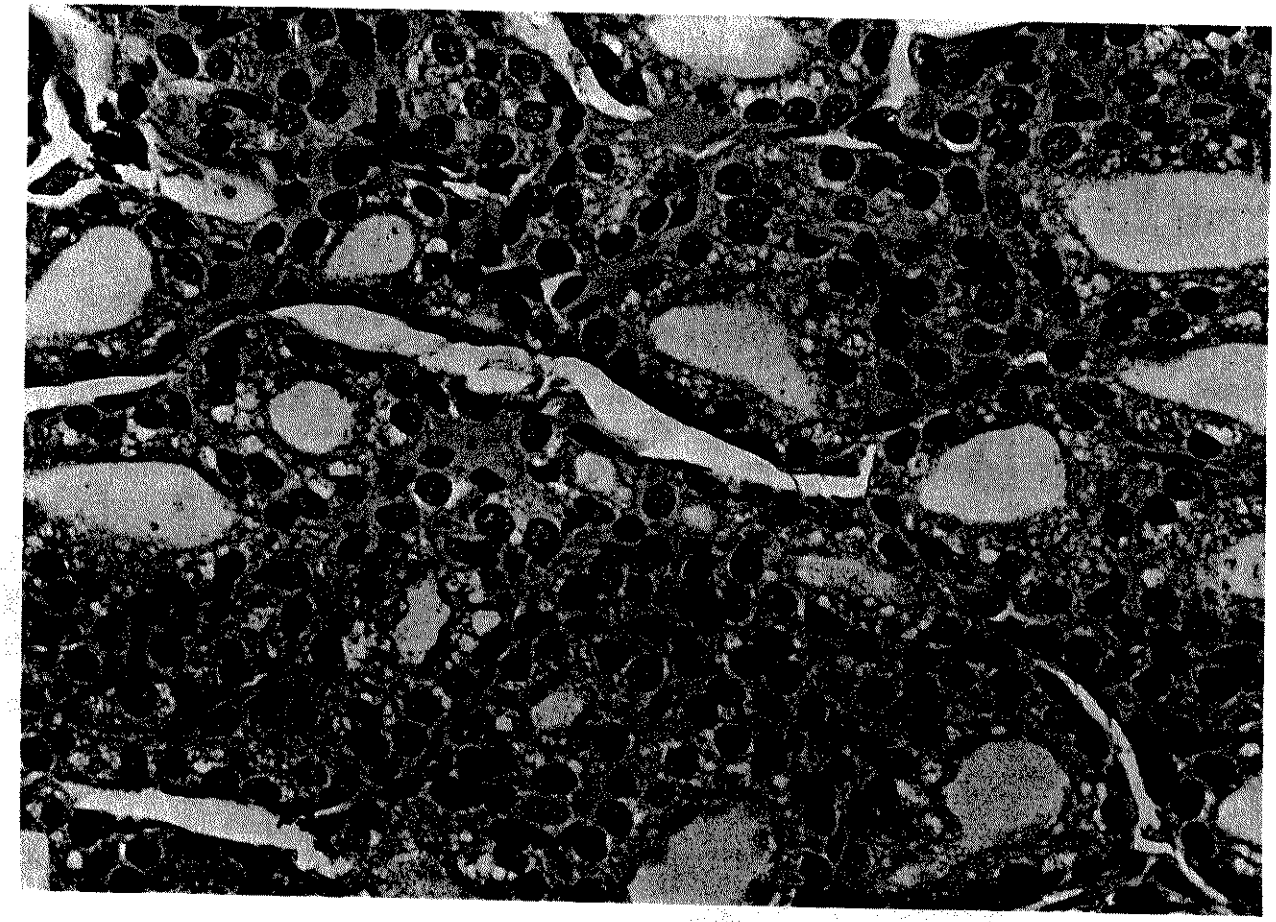

FIGURE 6. Histopathology of thyroid tissue from air exposed rats at the same magnification as Figure 5.

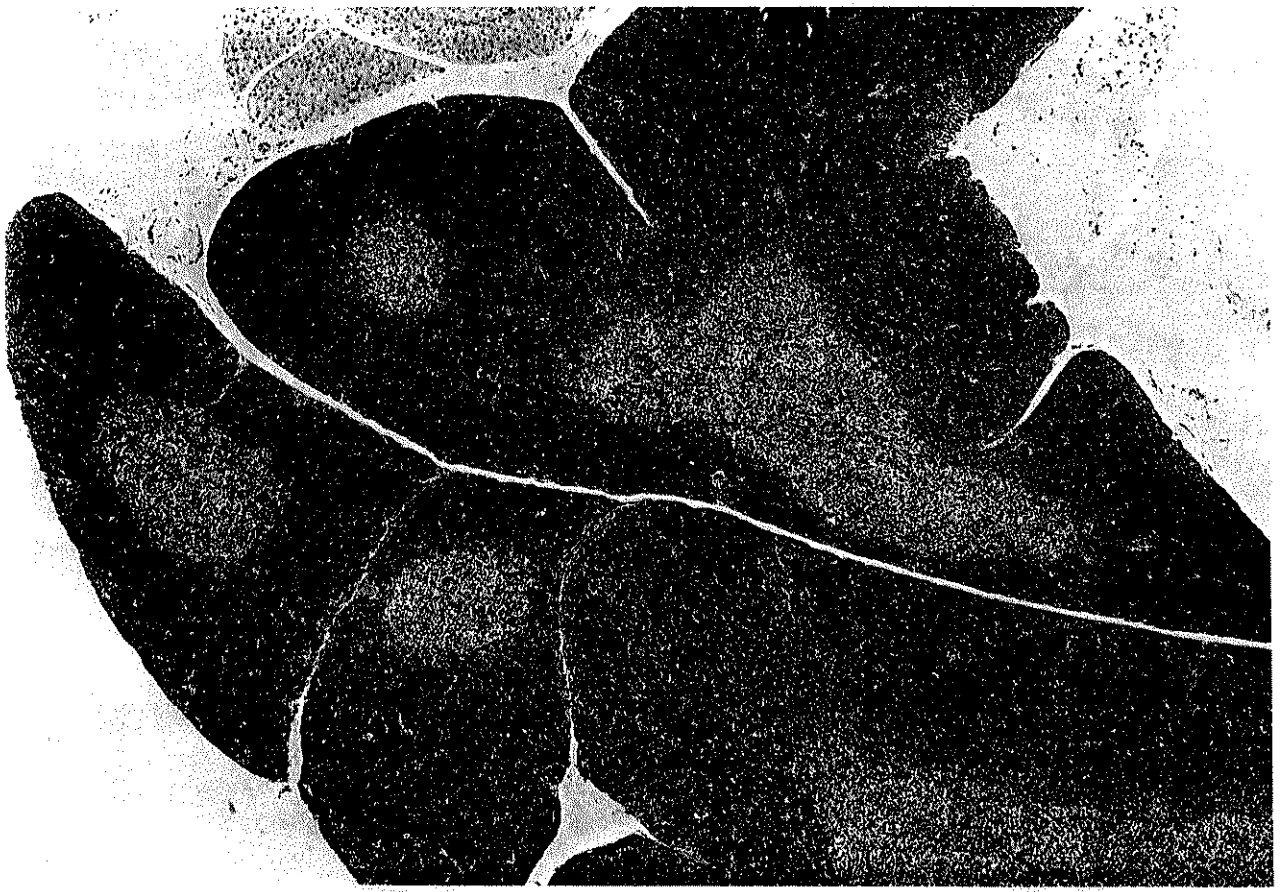

FIGURE 7. Histopathology of control rat thymus tissue at 40×.

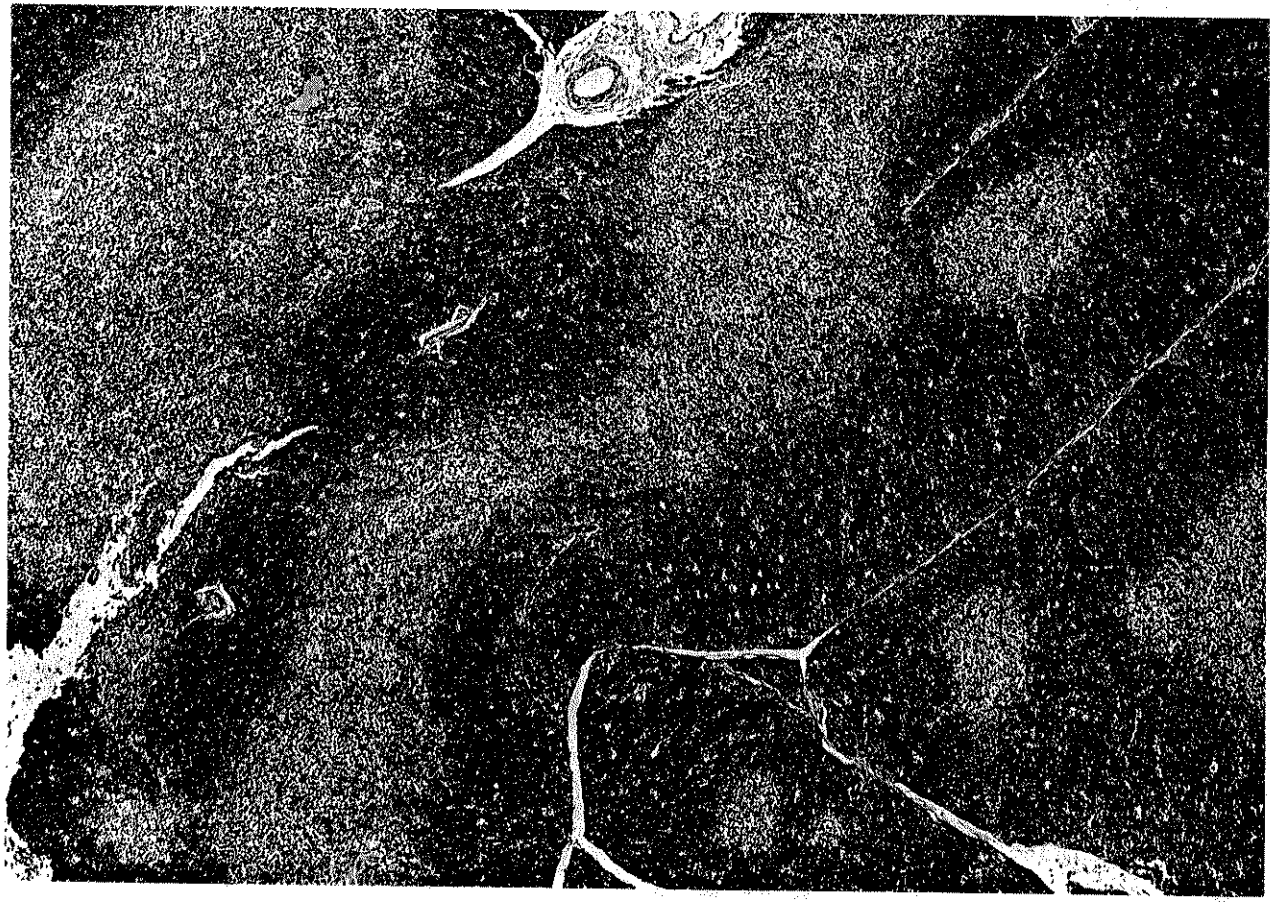

FIGURE 8. Histopathology of inhalation rat thymus tissue at 40×.

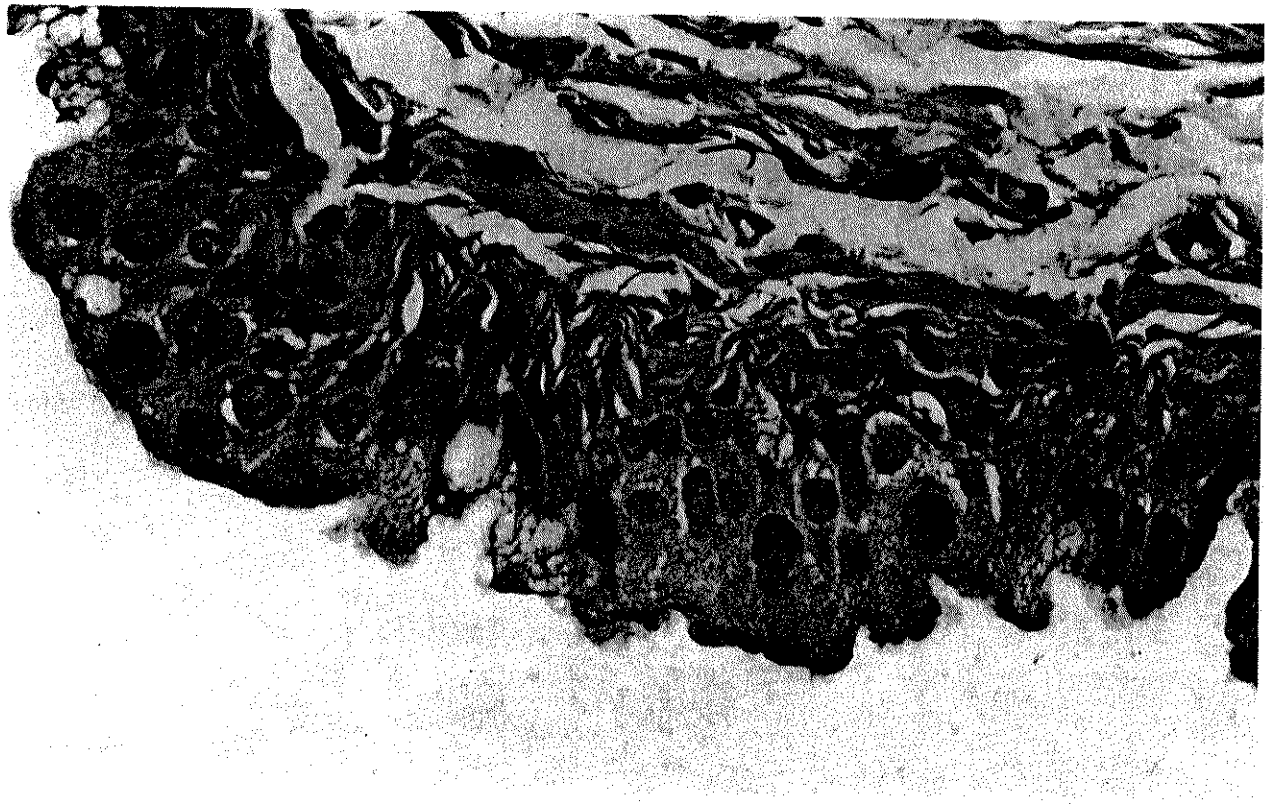

FIGURE 9. Urinary bladder at 450× of control rat.

for the control rats, at 450× (Figures 10 and 11). It is apparent that there was an increase in plasma in intermediate and superficial cells. The surface plasma membrane was more darkly staining. The urinary bladders on dissection of the inhalation and ingestion groups appeared opaque, while control bladders had the normal degree of transparency.

Open Field Behavior

Each rat was submitted to an open field test on the morning after termination of exposure before sacrifice in the afternoon. There was a significant difference between control and both groups of exposed rats in rearing, ambulation, and production of boli. The exposed groups were less active on all counts in the order control, inhalation, and fed groups (Table 4).

DISCUSSION

Daily Health Observations

Overall there were no signs of ill health. All the groups ate the 30-g ration during the 1-h rest period with no overt signs of starvation or aggression. In comparing the weight gain of rat pairs, the mean percentage gained by the larger rats (in all pairs) showed a discernable difference from that gained by the smaller rats (39 ± 6 and 34 ± 4; $t = 3.3$, $p < .01$), so dominance may be a factor in the overall experimental variance. However, there was a discernable difference between the percentage weight gain of the inhalation group versus both the control and the ingestion groups (29 ± 4 and 33 ± 4; $t = 3.6$, $p < .01$).

Although the number of air changes per hour (i.e., 3.1) was lower than that recommended in the guidelines of the National Research Council for care and use of Laboratory Animals (Institute of Laboratory Resources, 1996; i.e., 10–15), our animal loading is only 0.8 to 1.07% compared with 5% in the recommendations, so it is probable that the air changes were sufficient to maintain an adequate supply of oxygen and to prevent the buildup of carbon dioxide and ammonia. Barrow and Dodd (1979) measured ammonia levels in chambers with animal loads of 1–5% with 8, 16, and 24 air changes per hour. The urine was collected on absorbent pads as in the present experiment. At 1% loading with 8 changes per hour, ammonia levels reached equilibrium at 0.48 ± 0.7 ppm after 4 h. At the highest animal loading, the ammonia level reached 2.4 ± 0.38 ppm after 4 h but continued to increase. In 1983, Schaerdel et al. determined that at 100 ppm, urine-derived ammonia produced extremely small blood concentrations of ammonia and had no other measurable effects in rats. In the present experiment the Durosorb pads were changed daily during the 1-h rest period, but it is not known whether the microbial generation of ammonia from urine would be similar to that observed in Barrow and Dodd (1979) and Schaerdel et al. (1983).

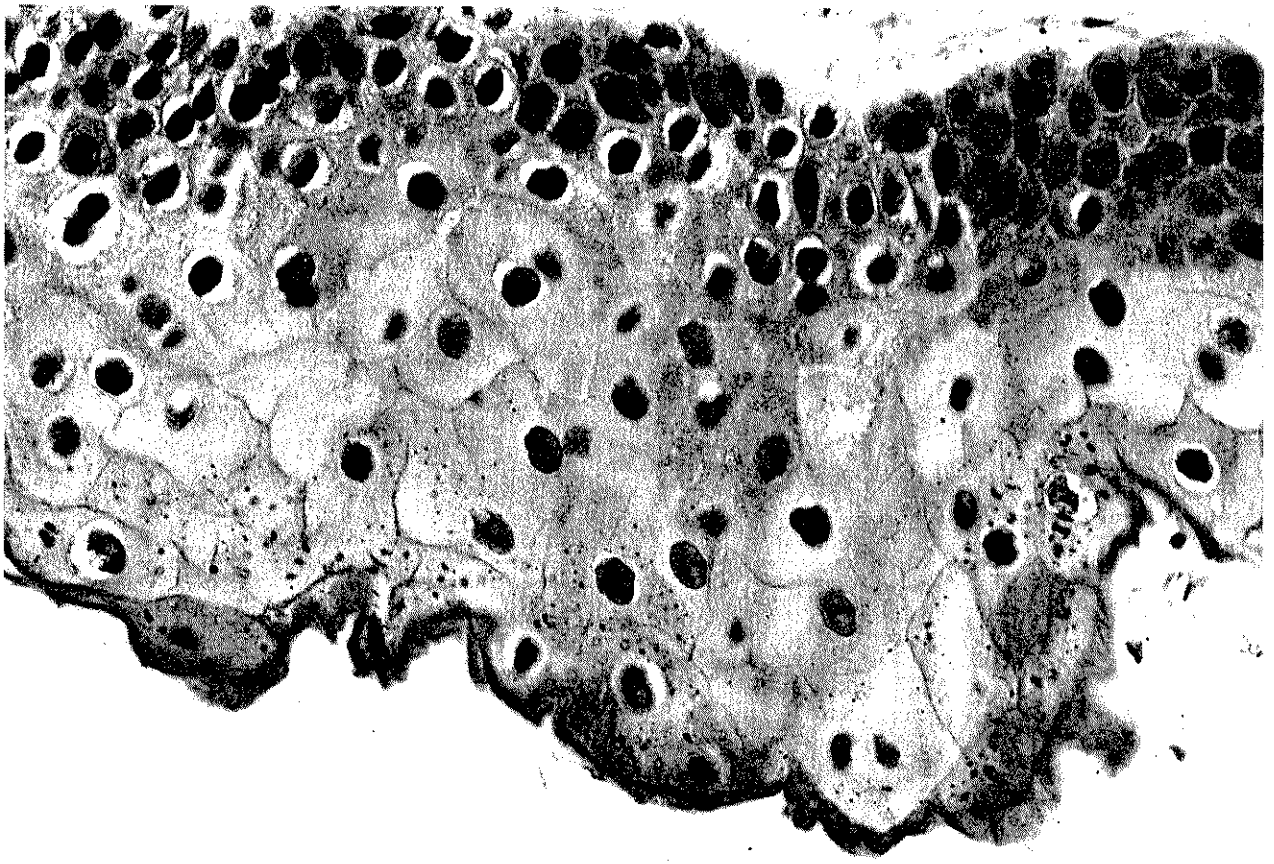

FIGURE 10. Urinary bladder at 450× of rat fed Aroclor 1242.

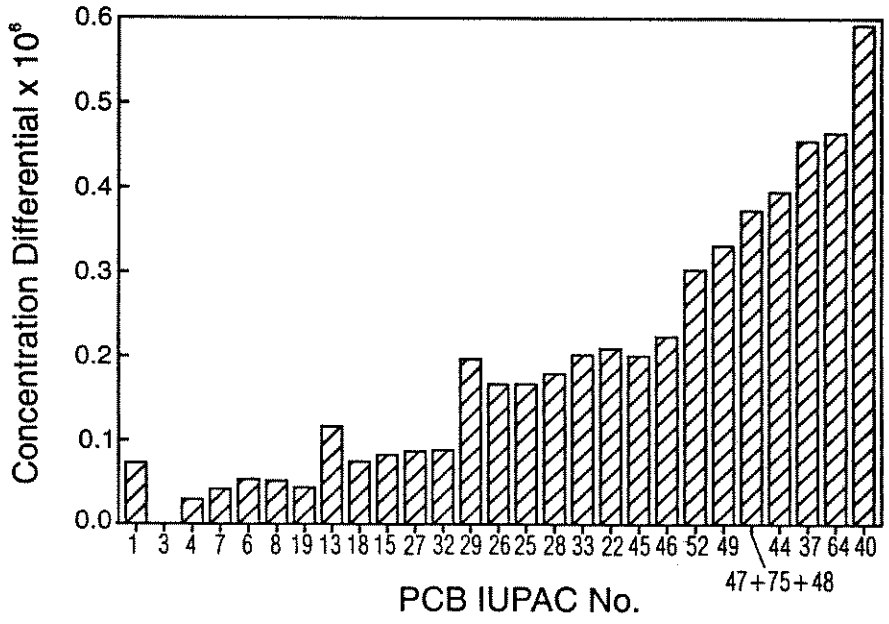

FIGURE 11. PCB congener concentration differentials between air and liquid Aroclor 1242 during passage of air at 9 l/min over a surface area of 32 cm^2.

Thyroid Measurements

Collins and Capen (1980) observed pathological changes in the thyroid gland similar to those observed here. They fed rats 5, 50, and 500 ppm Aroclor 1254 for 4 wk, and also observed a statistically significant increase in serum T3 at 5 ppm, which dramatically decreased at 50 and 500 ppm. The changes observed in the present study were produced at approximately one-tenth of the lowest PCB food concentration used by Collins and Capen (1980), and while the changes observed here may be reversible when exposure is terminated, permanent harm to brain development of young rats could result from variations in thyroid hormones levels of the magnitude observed in our work (Porterfield, 1994).

Histological Changes

The present work includes an extensive histological examination of the rat tissues as well as thyroid hormone measurements. Few animal tox-

TABLE 4. Open Field Behavioral Results

Exposure groups	Rearing	Ambulation	Boli
Control	10.8 ± 7	53.4 ± 10.5	3.1 ± 1.5
Ingestion	5.8 ± 2.4[a]	31 ± 7.5[a]	2.6 ± 2[a]
Inhalation	5.9 ± 3.3[a]	42.6 ± 12[a]	1.8 ± 1[a]

[a]Discernibly different from control ($p < .05$).

icity data on Aroclor 1242 exist, especially at low doses by inhalation. The New York State Department of Health (NY DOH) permitted air concentration at a New York State office building that was contaminated by an electrical transformer fire is approximately equal to the concentration used here (NY DOH, 1996). The histological changes observed in our study may well be reversible, but the doses used are similar to or lower than permitted environmental exposures. For example, the U.S. FDA permitted concentration of fish ingestion 2 ppm is 4 times greater than the level used here. In addition, previous studies on PCBs (Overman et al., 1987; Seegal et al., 1990, 1991) were carried out at oral doses of >3 mg/kg/d using neonatal and adults rats and nonhuman primates. In the majority of studies, endpoints related to liver cytochrome activity (Wolff, 1985; Hansen et al., 1995; Baker et al., 1977) behavioral and neurotransmitter changes in certain brain regions (Daly, 1991; Seegal et al., 1991), thyroid (Collins & Capen, 1980), and immunological measurements (Silkworth & Grabstein, 1982) were made; changes were discernible in all sets of measurements at the doses employed.

The exposure to PCBs derived from Aroclor 1242 by either ingestion or inhalation elicited significant histopathological effects on the thyroid and thymus. The effects of exposure to PCBs and furans on the thyroid and thymus have been well documented and are consistent with other studies (Collins & Capen, 1977, 1980; Chu et al., 1994). However, the dose used in this study was one-tenth of the doses where discernible effects were reported in the previous two studies.

The thyroid lesions have been previously described in PCB and furan orally exposed rats (Collins & Capen, 1977, 1980). The orally exposed rats in our study showed no histopathological changes, as reported by Collins and Capen (1977) with a dose of 5 ppm. The inhalation-exposed group showed the same histopathological characterization as the 50 and 500 ppm orally exposed rats in the Collins and Capen (1980) study. This type of lesion was confirmed with the elevations of serum TT3 and TT4. This elevation could have been a transient effect due to an early overstimulation of the thyroid. A longer study or a higher dose might have reproduced the decreases in circulating serum TT3 and TT4 seen by Collins and Capen (1977, 1980). A study of thyroid functions (T4 and T3) in 123 Yusho patients 16 yr after exposure did show significantly higher T4 and T3 compared with controls (Murai et al., 1987).

The histopathological effect on the thymus was the same in both exposure regimes. The reduced cortical volume and the medullary atrophy have also been reported by others (Collins & Capen, 1977; Chu et al., 1994). It is not known if exposure at this age will have an effect on the immune system; however, in other studies adult rats were exposed and no effects were found in the immune system, whereas prenatal exposure altered immune function.

Open Field Behavior

The open field test is one of the most traditional and widely used methods for the assessment of exploratory behavior and the emotional states of rats. Normal exploratory behavior, measured by the open field tests, was clearly affected by the PCB intake when compared to the control group; no marked difference could be discerned between the two exposed groups.

The exploratory behavior was slowed with the reduced number of ambulations and rearing in the exposed groups. The emotionality of the rats in the exposed groups was also affected by the reduced numbers of defecations. This indicates that in future studies, more sensitive tests may reveal more clearly the behavioral locus that is being affected, as in the work of Daly et al. (1989) and Daly (1991), which showed effects on delayed gratification.

PCB Uptake

The composition of the vapor exiting the inhalation chamber was remarkably constant (Figure 2). No change in composition over 30 d was discernable when the concentrations of individual congeners were plotted and regressed against time. The composition of the vapor resembles the composition of the liquid Aroclor 1242 in the food but with the expected relatively higher concentrations of the more volatile congeners (Figure 3). Aroclor 1242 does contain traces of the majority of the higher chlorinated congeners, but this is not evident from Table 1 because the analyses are rounded to improve readability.

Figure 12 illustrates the partition between the liquid PCB phase and the air flowing over it. The concentration of each congener in the air phase will be related to the fugacity in the liquid (or "lipid") phase in a situation, resembling what we have already described between liquid PCB mixtures and water (Bush & Kadlac, 1995). The resemblance to the K_{ow} values for the congeners (Wood et al., 1987) is striking, showing that the more chlorinated congeners have approximately six times the affinity for the lipid-like PCB phase than the less chlorinated PCB. Since the liquid and air are not at equilibrium, the values do not accurately reflect true thermodynamic values. They are, however, helpful in indicating what might occur when PCB congeners are taken up from the vapor by lipids in the lung membranes. Although protein receptor-type binding of one congener (2,4,2'4'-tetrachlorobiphenyl) in mouse lung tissue has been demonstrated (Brandt & Bergman, 1975), simple partitioning to the lipid constituents, and diffusion, will probably be the dominant means of uptake to the blood stream based on our studies of lower organisms (Bush & Kadlac, 1995; Wood et al., 1987).

Although the PCB doses were substantially lower than in the majority of previous toxicological studies in rats, effects were observed which

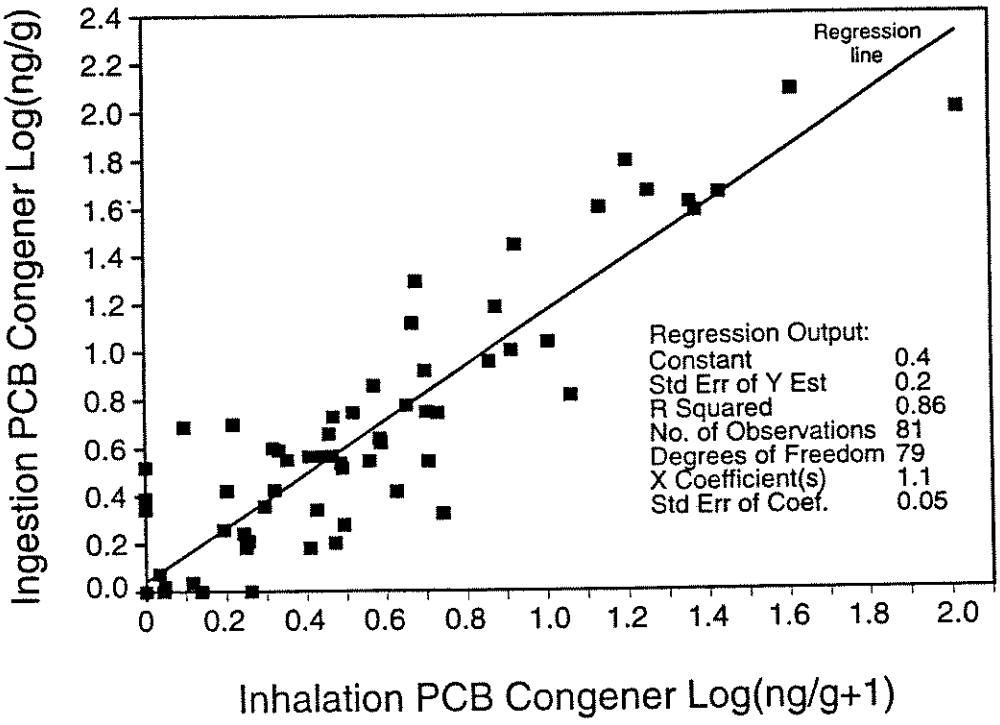

FIGURE 12. Scatter plot of the PCB composition of rat adipose tissue after exposure to Aroclor 1242 by inhalation for 30 d (*x*) and the PCB congener composition of the same tissue-type in rats expose by ingestion of Aroclor 1242 (*y*).

were qualitatively and quantitatively different between groups. The sum of PCB congeners comprising the air dose was 0.46 µg PCB/kg body weight/d, assuming a rat breathing rate of 74 mL/min or 107 L/d and complete absorption from the inspired air, whereas the food dose was approximately 100 times higher (i.e., 32.8 µg PCB/kg/d). Clearly, PCB was transported into the animals, and although the PCB congener patterns differed between tissues, the residue patterns and tissue concentrations from inhalation and from ingestion did not differ greatly. However, the experimental variance was low enough to allow statistically significant differences to be discerned. Although the pattern of airborne PCB differed from the ingested pattern, with the food containing proportionately more of the higher chlorinated congeners, the resultant residues in fat in particular were very similar, except for the higher chlorinated PCBs in the food group. This may be attributed to the more lipophilic character of the more highly chlorinated PCB congeners. Comparison of the uptake via inhalation and via ingestion may be accomplished with a linear model, which compares the two compositions on a congener, by congener basis (Bush & Kadlac, 1995). Figure 12 illustrates the very close match between congener pat-

terns between the two routes of intake in adipose tissue, with $r^2 = .86$. The x coefficient of 1.1 indicates that the adipose tissue was contaminated to a similar extent overall by the two routes of exposure, although on a congener-by-congener basis there were discernable differences as shown in Table 2. Other tissues showed pattern similarities as indicated by the x coefficients obtained by regressing the non-log-transformed data (fat, 1.3; liver, 1.4; brain, 1.8; lung, 1.7); all r^2 values were >.69. Since the food dose is approximately 100 times the air exposure dose, this suggests that inhalation is a considerably more efficient adsorption route for the rat than ingestion. However, toxicokinetic factors may be important, since the inhalation exposure was essentially continuous whereas the food group received one daily bolus dose.

Ten congeners comprised 75.5% and 80.5% of the residue in fat via inhalation and ingestion, respectively; these are congeners that have previously been shown resistant to mammalian liver hydroxylation, having 4,4′-chlorine substituents (Baker et al., 1977; Brandt & Bergman, 1975; Shain et al., 1986). The surprisingly high level of 2,4,4′,5-tetrachlorobiphenyl (IUPAC number 74; 26% and 17%, respectively) caused some concern because the proportion of this congener in both intakes was lower in the original product. Because of this, the identity was confirmed using a second GC column, Apiezon L (Bush et al., 1989). This congener is the second most prevalent in human milk in spite of its low level in the environment (Bush & Kadlac, 1995; Fitzgerald et al., 1996). Its surprisingly high concentration in rat tissues in the present experiment may indicate a novel aspect of mammalian liver metabolism worthy of further investigation. In *Macaca nemistrina* fed Aroclor 1016 (Seegal et al., 1990) 2,4,4′,5-tetrachlorobiphenyl was not an important component of brain tissue (adipose tissue was not analyzed), whereas 2,4,4′-trichlorobiphenyl was the major residual congener. This may indicate a species difference in metabolic capability, with the macaque being vegetarian.

2,4,4′-Trichlorobiphenyl is the next most dominant congener in the present study. It is a large component of Aroclor 1242 and Aroclor 1016. Both 2,4,4′-trichlorobiphenyl and 2,4,4′,5-tetrachlorobiphenyl are major residual congeners in workers exposed to Aroclors 1016 and 1242 (J. Brown, Jr., personal communication). These data and that of others suggest that some rearrangement or removal of chlorine atoms from the PCB molecule may be occurring in humans and rats (Shain et al., 1986). The relatively high concentration of 2-monochlorobiphenyl and 2,4′-dichlorobiphenyl would support this hypothesis.

The liver PCB composition greatly resembles the fat composition. When the mean congener concentrations in the two tissue types are compared on a scatter plot, an r^2 value of .39 (air) and .61 (food) is obtained with an x coefficient of 15 ± 2 (air) and 20 ± 2 (food). Brain PCB patterns are represented in a three-dimensional form in Figure 5; they resemble the intake compositions more than did the fat or liver residues. The con-

centration data show more significant differences between the fed and inhalation groups for brain than for other tissues examined. This may be attributed to PCB congeners crossing the blood–brain barrier directly via the blood circulation without prior passage through the liver, where extensive hydroxylation by mixed-function oxidases would occur.

The PCB composition of the lung did not show evidence of any affinity of lung tissue for particular PCB congeners as reported when ^{14}C-labeled individual congeners were injected into the mouse (Brandt & Bergman, 1975). Table 2 indicates that the congener pattern was similar to the other tissues but, as could be expected, the congener composition between the ingestion and inhalation groups was also dissimilar for lung tissue ($r^2 = .64$).

CONCLUSIONS

PCB congeners were absorbed via inhalation of Aroclor 1242 vapor. The vapor was produced by the simple passage of air over liquid Aroclor 1242. This illustrates the ease with which Aroclor 1242 evaporates. Living in an atmosphere of 900 ng/m^3 for 23 h/d resulted in tissue concentrations that were approximately half the concentration found when the rats ingested 0.5 ppm in their food. The effect produced by the ingestion route replicated results of other studies, namely, effect on the thyroid and the thymus. Changes produced by inhalation were similar.

REFERENCES

Agency for Toxic Substances and Disease Registry. 1996. The Toxicology of Polychlorinated Biphenyls. Rep. no. 306-88-0608. Atlanta, GA: ATSDR.

Altman, P. L., and Ditter, D. S. 1971. *Respiration and circulation.* Bethesda, MD: Federation of American Societies for Experimental Biology.

Baker, F. D., Bush, B., Tumasonis, C. F., and Lo, F.-C. 1977. Toxicity and persistence of low-level PCB in adult Wistar rats, fetuses and young. *Arch. Environ. Contam. Toxicol.* 5:143–156.

Barrow, C. S., and Dodd, D. E. 1979. Ammonia production in inhalation chambers and its relevance to chlorine inhalation studies. *Toxicol. Appl. Pharmacol.* 49:89–95.

Benthe, H. F., Knop, J., and Schmoldt, A. 1972. Absorption and distribution of polychlorinated biphenyl (PCB) after inhalatory application. *Arch. Toxikol.* 29:85–95 (German).

Brandt, I., and Bergman, A. 1975. Distribution of polychlorinated biphenyls: structural requirements for accumulation in the mouse bronchial mucosa. *Experimentia* 32:497–498.

Bush, B., and Kadlac, M. 1995. Dynamics of PCBs in the aquatic environment. *Great Lakes Res. Rev.* 1:24–31.

Bush, B., Snow J. T., and Connor, S. 1983. High resolution gas chromatographic analysis of nonpolar chlorinated hydrocarbons in human milk. *J. Assoc. Off. Anal. Chem.* 66:248–255.

Bush, B., Bennett, A. H., and Snow, J. T. 1986. Polychlorobiphenyl congeners, *p,p'*-DDE, and sperm function in humans. *Arch. Environ. Contam. Toxicol.* 15:333–341.

Bush, B., Streeter, R. W., and Sloan, R. J. 1989. Polychlorobiphenyl (PCB) congeners in striped bass (*Morone saxatilis*) from marine and estuarine waters of New York State determined by capillary gas chromatography. *Arch. Environ. Contam. Toxicol.* 19:44–61.

Chiarenzelli, J., Scrudato, R. J., and Wunderlich, M. L. 1996. Volatile loss of PCB Aroclors from subaqueous sand. *Environ. Res.* 31:597–602.

Chu, I., Villeneuve, D. C., Yagminas, A., LeCavalier, P., Poon, R., Feeley, M., Kennedy, S. W.,

Seegal, R. F., Hakansson, H., Ahlborg, U. G., and Valli, V. E. 1994. Subchronic toxicity of 3,3',4,4'-pentachlorobiphenyl in the rat. *Fundam. Appl. Toxicol.* 22:457–468.

Collins, W. T., Jr., and Capen, C. C. 1977. Effects of polychlorinated biphenyl (PCB) on the thyroid gland of rats. *Am. J. Pathol.* 89:119–136.

Collins, W. T., Jr., and Capen, C. C. 1980. Ultrastructural and functional alterations in thyroid glands produced by polychlorinated biphenyls compared with iodide excess and deficiency, and thyrotropin and thyroxine administration. *Virchows Arch. B Cell Pathol.* 33:213–231.

Currado, G. M., and Harrad, S. 1997. The significance of indoor air inhalation as a pathway of human exposure to PCBs. *Organohalogen Compounds* 33:377–381.

Daly, H. B. 1991. Reward reductions found more aggressive in rats fed environmentally contaminated salmon. *Neurotoxicol. Teratol.* 103:1356–1365.

Daly, H. B., Hertzler, D. R., and Sargent, D. M. 1989. Ingestion of environmentally contaminated Lake Ontario salmon by laboratory rats increases avoidance of unpredictable aversive nonreward and mild electric shock. *Behav. Neurosci.* 103:1356–1365.

Drinker C. K., Field, M., and Bennett, G. A. 1937. The problem of possible systemic effects from certain chlorinated hydrocarbons. *J. Ind. Hyg. Toxicol.* 19:283–299.

Erickson, M. D. 1997. *Analytical chemistry of PCBs,* 2nd ed. Boca Raton, FL: CRC Press.

Fitzgerald, E. F., Brix, K. A., Deres, D. A., Hwang, S. A., Bush, B., Lambert, G., and Tarbell, A. 1996. Polychlorinated biphenyl (PCB) and dichlorodiphenyl dichloroethylene (DDE) exposure among native American men from contaminated Great Lakes fish and wildlife. *Toxicol. Ind. Health* 12: 361–368.

Frame, G. M. 1996. A collaborative study of 209 PCB congeners and 6 Aroclors on 20 different HRGC columns. 2. Semi-quantitative Aroclor congener distributions. *Fres. J. Anal. Chem.* 365: 1141–1154.

Hansen, L. G., Li, M. H., Saeed, A., and Bush, B. 1995. Environmental polychlorinated biphenyls: Acute toxicity of landfill soil extracts to female prepubertal rats. *Arch. Environ. Contam. Toxicol.* 29:234–243.

Harris, M., Zacharewski, T., and Safe, S. 1993. Comparative potencies of Aroclors 1232, 1242, 1248, 1254, and 1260 in male Wistar rats—Assessment of the toxic equivalency factor (TEF) approach for polychlorinated biphenyls (PCBs). *Fundam. Appl. Toxicol.* 20:45–63.

Hong, C. S., Bush, B., Xiao, J., and Qiao, H. 1993. Toxic potential of non-ortho and mono-ortho coplanar polychlorinated biphenyls in Aroclors, seals and humans. *Arch. Environ. Contam. Toxicol.* 25:118–123.

Institute of Laboratory Animal Resources, National Research Council. 1996. *The Guide for the Care and Use of Laboratory Animals.* Washington, DC: National Academic Press.

Leavens, T. L., Moss, O. R., and Bond, J. A. 1996. Dynamic inhalation systems for individual whole-body exposure of mice to volatile organic chemicals. *Inhal. Toxicol.* 8:655–694.

MacFarland, H. N. 1976. Respiratory toxicology. *Essays Toxicol.* 7:121–154.

Mayes, B. A., Neal, B. H., Peters, A. C., Brown, J. F., Hamilton, S. B., McConnel, E. E., and Moore, J. A. 1997. Tumor responses in Sprague-Dawley rats fed Aroclors 1016, 1242, 1254, and 1260 for two years. Society of Toxicology Annual Meeting abstract no. 804.

Metcafe, D. E., Zukovs, G., Mackey, D., and Patterson, S. 1986. Hazards, decontamination and replacement of PCB. In ed. J.-P. Crine, pp. 6–14. New York: Plenum Press.

Murai, K., Okamura, K., Tsuji, H., Kajiwara, E., Watanabe, H., Akagi, K., and Fujishima, M. 1987. Thyroid function in "yusho" patients exposed to polychlorinated biphenyls (PCB). *Environ. Res.* 44:179–187.

New York State Department of Health. 1996. Binghamton State Office Building Post-Occupancy Environmental Sampling Round 4. NYSDOH rep. no. 6222024. Albany, NY: Center for Environmental Health.

Overman, S., Kostas, J. J., Wilson, L. R., Shain, W., and Bush, B. 1987. Neurobehavioral and somatic effects of perinatal PCB exposure in rats. *Environ. Res.* 44:56–70.

Pearson, R. F., Hornbuckle, K. C., Eisenreich, S. J., and Swackhamer, D. L. 1996. PCBs in Lake Michigan water revisited. *Environ. Sci. Technol.* 30:429–436.

Porterfield, S. P. 1994. Vulnerability of developing brain to thyroid abnormalities: Environmental insults to the thyroid system. *Environ. Health Perspect.* 102(suppl. 2):125–130.

Risebrough, R., and Brodine, V. 1971. More letters in the wind. In *World in a peril: An environmental review,* eds. S. Novick and D. Cottrell, pp. 243–255. Greenwich, CT: Fawcett.

Safe, S., ed. 1987. *Polychlorinated biphenyls (PCBs): Mammalian and environmental toxicology.* New York: Springer-Verlag.

Schaerdel, A. D., White, W. J., Lang, C. M., Dvorchik, B. H., and Bohner, K. 1983. Localized and systemic effects of environmental ammonia in rats. *Lab. Anim. Sci.* 33:40–45.

Schecter, A., Startin, J., Wright, C., Kelly, M., Päpke, O., Lis, A., Ball, M., and Olsen, J. R. 1994. Congener-specific levels of dioxins and dibenzofurans in US food and estimated daily dioxin toxic equivalent intake. *Environ. Health Perspect.* 102:962–966.

Seegal, R. F., and Schantz, S. L. 1994. Neurochemical and behavioral sequella of exposure to dioxins and PCBs. In *Dioxins and health,* ed. A. Schecter, pp. 409–447. New York: Plenum.

Seegal, R. F., Bush, B., and Shain, W. 1990. Lightly chlorinated ortho-substituted PCB congeners decrease dopamine in nonhuman primate brain and in tissue culture. *Toxicol. Appl. Pharmacol.* 106:136–144.

Seegal, R. F., Bush, B., and Brosch, K. O. 1991. Comparison of effects of Aroclor 1016 and Aroclor 1260 on non-human primate catecholamine function. *Toxicology* 66:145–163.

Shain, W., Overmann, S. R., Wilson, L. R., Kostas, J., and Bush, B. 1986. A congener analysis of polychlorinated biphenyls accumulating in rat pups after perinatal exposure. *Arch. Environ. Contam. Toxicol.* 15:687–707.

Silkworth J. B., and Grabstein, E. M. 1982. Polychlorinated biphenyl immunotoxicity: Dependence on isomer planarity and the Ah gene complex. *Toxicol. Appl. Pharmacol.* 65:109–115.

Treon, J. F., Cleveland, F. P., Cappel, J. W., and Atchley, R. W. 1956. The toxicity of the vapors of Aroclor 1242 and Aroclor 1254. *Ind. Hyg. Q.* 6:204–213.

U.S. Department of Health and Human Services. 1993. Toxicological Profile for Polychlorinated Biphenyls. TP-92/16. Agency for Toxic Substance and Disease Registry Division of Toxicology/ Toxicology Information Branch, 1600 Clifton Rd., NE, E29, Atlanta, GA 30333.

U.S. Environmental Protection Agency. 1984. Definition and procedure for determination of the method detection limit. *Fed. Reg.* 49:43430–43431.

Waid, J. S., ed. 1986. *PCBs and the environment.* Boca Raton, FL: CRC Press.

Wolff, M. S. 1985. Occupational exposure to polychlorinated biphenyls. *Environ. Health Perspect.* 60:133–138.

Wood, L. W., Rhee, G.-Y., Bush, B., and Barnard, E. 1987. Sediment desorption of PCB congeners and their bio-uptake by dipteran larvae. *Water Res.* 21:875–886.