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POLYBROMINATED BIPHENYL REDUCTION
DURING PRESSURE COOKING OF CHICKEN

presented by

Susan Katherine Smith

has been accepted towards fulfillment
of the requirements for

M.S. degree in Foods

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POLYBROMINATED BIPHENYL REDUCTION DURING PRESSURE COOKING OF CHICKEN

by

Susan Katherine Smith

A THESIS

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ABSTRACT

POLYBROMINATED BIPHENYL REDUCTION
DURING PRESSURE COOKING OF CHICKEN

by

Susan Katherine Smith

Effects of cooking on polybrominated biphenyls (PBB) were studied. Thigh meat, thigh skin, drumstick and breast (with skin) from half of each chicken fed 0, 30, 45, 60 or 90 ppm of PBB were analyzed raw. Pieces from paired halves were analyzed following pressure cooking separately in deionized water. Cooking yields were obtained and the cooked meat and broth were analyzed separately for PBB.

PBB residues were found in all tissues analyzed with considerable variation in quantity found. Residue levels in tissue increased with increased levels of PBB in feed, and were related to fat level in tissue (wet basis). Levels of PBBs expressed as ppm (solids basis) were lower in cooked samples than in corresponding raw pieces. Part of the PBB lost being found in the drip. PBB recoveries in cooked tissue and broth ranged from 68.1% (thigh skin) to 84.6% (drumstick) with approximately two-thirds found in the cooked meat.

ACKNOWLEDGEMENTS

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INTRODUCTION

The tremendous influx of synthetic compounds and their accompanying waste materials has led to increasing concern over environmental pollutants by people world wide. Of these compounds much attention has been directed toward halogenated hydrocarbons. The nearly ubiquitous presence of chlorinated hydrocarbon residues in many levels of the biosphere is well documented (Risebrough and de Lappe, 1972; Zitko et al., 1972; Martel et al., 1975; Risebrough et al., 1968) and their presence is expected to remain in the ecosystem for an extended period of time. Organochlorine pesticides and polychlorinated biphenyls (PCBs) with their lipophylic properties have been found in the fat of virtually all animals (Holmes et al., 1967; Holden and Marsden, 1967; Reynolds, 1969; Jensen et al., 1969; Koeman et al., 1969; Anderson et al., 1969; Longcore and Mulhern, 1973), and have been detected in human tissues as well (Biros et al., 1970; Yobs, 1972; Hammer et al., 1972; Price and Welch, 1972; Musial et al., 1974; Dyment et al., 1971; Savage et al., 1973; Solly and Shank, 1974; Doguchi and Fukano, 1975; Akiyama et al., 1975). Because of the high degree of biochemical stability (Fries et al., 1973) and lipid solubility these compounds have accumulated through food chains and in the human diet (Fishbein, 1974; Kolbye,

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1972; Fujiwara, 1975; Berglund, 1972; Fries, 1972; FAO/WHO, 1974; Manske and Corneliussen, 1974). Concomitantly, several reports provide evidence that would indicate a substantial proportion of the population of the United States has been exposed to PCBs (Jenlinek and Corneliussen, 1975; Kurtz and Yang, 1975; Dennis, 1975; Humphrey et al., 1976; Hesse, 1975a; Kurtz and Strassman, 1975).

Occurance of contamination of human tissue are generally associated with ingestion of food containing low levels of these compounds. Although precise information concerning toxicity of halogenated compounds at very low levels is not known, the concern is for possible chronic effects of their continual assimulation and accumulation in body fat.

Polybrominated biphenyls (PBBs) are members of the halogenated hydrocarbon class of compounds with structure, reactivity, use and toxicity similar to those attributed to PCBs of higher chlorination. They are not present in the environment in as large amounts as PCBs, and would not be of great concern had not an incredible error occured in Michigan during the manufacture of feed in May of 1973.

Michigan Chemical Corporation manufactured the PBB compounds, Firemaster® Ff-1.[1] These compounds were mistaken for magnesium oxide (trade name Nutrimaster also produced by Michigan Chemical Corp.) and mixed into high protein dairy

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[1] Firemaster® BP-6 mixed with the anticaking agent "Flo-Gard" which is manufactured by PPG Industries and which contains 83% silicone dioxide and a maximum of 7% calcium oxide.

pellets (Jackson and Halbert, 1974; Dunckel, 1975). Through this contamination and consequent cross contamination of other feeds, dairy animals, poultry, swine, and concomitantly animal products were affected. This mistake has cost Michigan farmers untold dollars in loss of animals, income from those animals and the cost of clean up procedures to rid their farms of this contaminant. In the poultry industry alone, approximately two million birds and nearly five million eggs were destroyed. Since this large scale exposure, the circumstances surrounding the accident and the herculian effort to rectify the problem has been published in several reports (Dunckel, 1975; Ball, 1975; Hoeting, 1975; Welborn, 1975; Isleib and Whitehead, 1975).

Toxicity studies have shown PBBs to have similar mode of action to other halogenated compounds, namely PCBs, in that they produced porphyria, liver injury, tremor and loss of weight (Strik, 1973a, b; Jackson and Halbert, 1974; Prewitt et al., 1975; Lee et al., 1975; Aftosmis et al., 1972; Kimbrough et al., 1975), dysfunction of the thyroid (Bastomsky, 1974) effect on various enzymes (Pardini, 1971; Babish et al., 1975), changes in the liver to body weight ratios (Gartnoff et al., 1975), and to alter the level of utilization of corticosteriods (Wasserman et al., 1973), and vitamin A, D and E (Cecil et al., 1973; Wong et al., 1974; Combs et al., 1975).

Feeding studies have shown that bromine levels in biological systems elevate rapidly and then plateau but when the

animals are fed "clean feed" again the levels decrease. This decrease occurs more rapidly in milk, eggs, liver and muscle than in fatty tissue which loses its PBB store much more slowly.

These data raise concern about the possible impact on fish and wildlife in the environment and the health of human beings. Studies concerning the possibility of removing halogenated compounds from food stuffs upon processing thus of great interest. Several studies have been conducted concerning the removal of lipophilic compounds from such products as poultry (Zabik, 1974; Morgan et al., 1972; Ritchey et al., 1967, 1969, 1972; Liska et al., 1967; McCaskey et al., 1968), eggs (Zabik and Dugan, 1971), sausage patties (Funk et al., 1971), bacon (Yadrick et al., 1971), pork loins (Maul et al., 1971), pork muscles (Yadrick et al., 1972), beef loaves with texturized soy (Shafer and Zabik, 1975), dairy products (Murata, 1976), and salmon (Smith et al., 1973). Most of the losses seen were attributed to fat rendering or leaching out during cooking - the more severe the rendering the greater the loss. Different levels of success in elimination of contaminants were seen depending upon the compound worked with, the levels of contamination and the tissue from which the extraction was accomplished.

The purpose of this study was to investigate the effect of cooking on the PBB levels in chicken tissue and chicken broth. The ease of residue removal from skin in contrast to tissue was evaluated by cooking thigh meat and skin separately.

REVIEW OF LITERATURE

Polybrominated biphenyls (PBBs) are fairly new compounds, and are mainly incorporated into plastics as fire retardants for the manufacture of safe end-use products. They are the bromine analogs of the halogenated biphenyl compounds, and share many chemical properties with polychlorinated biphenyls (PCBs) and organochlorine pesticides.

PCBs have been reported to affect a large number of biological systems. To the extent that it has been reported, PBBs affect most of the same systems in a similar fashion.

PCBs are a class of large volume and multi-use industrial chemicals which are known to be widely distributed in the global ecosystem. Many of the toxic effects of these chemicals have been extensively studied in both animals and humans. PBBs, on the other hand, have received little attention because their industrial use is limited and they are not recognized as significant environmental contaminants. As a consequence, the toxicological research conducted with these chemicals has been limited in scope.

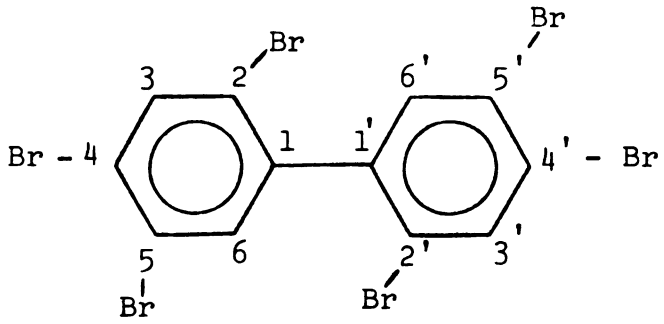
This review presents the uses, characteristics and toxicities of PBBs, and attempts to summarize the similarities of properties of PBBs and PCBs. Due to the durth of studies concerning the effect of processing on PBB levels in foods, evaluation of the effect of processing on PCB and chlorinated hydrocarbon pesticide levels in foods are also reviewed.

Polybrominated Biphenyls

Chemical and Physical Properties - Uses

Due to the chemical and physical properties of PBBs (particularly that of thermal stability) they provide effective and economically feasible plastisizers and fire retardants for incorporation into flame resistant polymers.

Michigan Chemical Corporation manufactured a product called Firemaster® BP-6. BP-6 is a mixture of brominated biphenyl with an average of approximately 63% hexabromobiphenyl, 14% heptabromobiphenyl, 10.5% pentabromobiphenyl, 2% tetrabromobiphenyl and others unidentified (Kerst, 1974). Recent analysis with nuclear magnetic resonance (NMR) and chemical studies have identified the principle component of Firemaster® BP-6 as 2,2',4,4',5,5' - hexabromobiphenyl as depicted below (Sudstrom et al., 1976). General properties



of BP-6 are: solid at room temperature, with a softening point of 72°C; decomposing at 300-400°C; very low solubility in water (11 ppb at 25°C); soluble in most organic solvents; and a vapor pressure of 5.2×10^{-8} mmHg at 25°C (Kerst, 1974).

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The production, distribution and usage of PBBs has not been as widespread as PCBs; and unlike PCBs, may not be physically located in a position of chemical reactivity. BP-6 has been used extensively in thermoplastics. Some examples of major uses are in business machines, electrical products and fabricated products (Table 1). PBBs are not used in food or feed, nor are they used in products that come in contact with human skin as in flame retarding fabrics (Kerst, 1974).

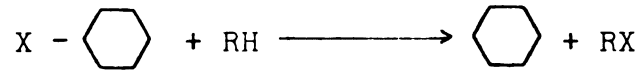
Of the possible degradation routes of halogenated aromatic compounds in the environment, photolytic degradation is especially important since these materials do not appear to be readily metabolized by microorganisms (Norris et al., 1975). Studies indicate that BP-6 undergoes reductive de-halogenation when exposed to UV light under certain conditions (Ruzo and Zabik, 1975). This reaction has also been shown to occur in mono- and di-brominated biphenyls (Bunce et al., 1975) and octabromo-biphenyls (Norris et al., 1974). This photoreactivity seems to take place about 7 times more rapidly in brominated compounds than the corresponding chlorinated biphenyls (Ruzo and Zabik, 1975). This may be due to steric interference of the bromines and the lower C - Br bond energy (Ruzo and Zabik, 1975; Bunce et al., 1975), which is 71.0 k cal/mole compared to the C - Cl bond energy of 85.6 k cal/mole (Kerst, 1974). Presumably ortho halogens preferentially cleave from PBBs upon photoexcitation. Photodegradation of halogenated aromatics in water with UV light seems to occur through

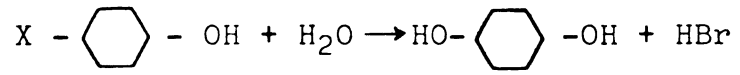
Table 1. Specific industrial uses and approximate allocations of Fire Master® BP-6 produced.[1]

Industrial Use	Approx. allocation of total Fire Master® BP-6 produced	Examples
	%	
Business machines and industrial equipment	48	Typewriter, calculator and microfilm reader housings; business machine housings
Electrical	35	Radio and TV parts, thermostats, shaver and hand test housings
Fabricated products	12	Projector housings, movie equipment cases
Transportation	1	Miscellaneous small automotive parts, i.e. electrical wire connectors, switch connectors, speaker grills
Miscellaneous	4	Small parts for electrical applications, motor housings, components for industrial equipment

[1] Kerst, 1974.

hydroxylation leading to the formation of phenolic compounds as seen below (Joschek and Miller, 1966). Once photodegrada-

Photoreduction 

Photohydroxylation 

tion of a polyhalogenated aromatic molecule in water is initiated, it should accelerate as electron with drawing halogens are replaced by electron releasing hydroxyls. Also as hydroxyl replacement of halogen proceeds, the resulting species would be expected to absorb more strongly in the longer wave lengths and this ultimately could result in the rupture of the aromatic ring (Norris et al., 1974). Michigan Chemical Corporation contends that BP-6, after initial degradation has proceeded, would degrade relatively rapidly then to carbon dioxide, water and bromine ion (Kerst, 1974).

Residues in the Environment

The chemical characteristics of PBBs are similar to those of PCBs, of higher chlorination, and the chlorinated hydrocarbon pesticides such as DDT. Thus, one can make strong inferences about the behavior of PBB in the environment based on the past experience with PCB and DDT (Fries, 1975a).

Important characteristics are high solubility in fat, low solubility in water, high resistance to breakdown by

living organisms or chemical processes in the environment. Therefore, after PBB is introduced into the environment it will persist. Initially most of it will remain in the general area in which it was introduced. However, as time passes, there are a number of processes by which PBBs can be redistributed to other areas. After the initial disaster in Michigan of the introduction of BP-6 into animal feed, this feed was distributed to a number of farms through normal marketing channels. PBB was redistributed further by the movement of animals, animal food products and byproducts. Also, PBB attached to soil particles can move both by water and wind erosion to distant areas. Some small fraction of PBB can volatilze and can subsequently wash out in rain or other precipitate (Fries, 1975a).

To illucidate information concerning the movement of PBB, Hesse (1975b) studied the extent of contamination produced by what was the major Michigan Chemical Corporation PBB production site located on the Pine River at St. Louis, Michigan. Water sampling revealed concentrations which ranged from 3.2 µg/l in samples taken 75 yards downstream from the plant to 0.01 µg/l in samples from 8 miles below the site. Concentrations at sample sites 12 and 20 miles downstream were below the sensitivity limit of 0.01 µg/l. PBB concentrations in near shore stream sediments in the area of plant out falls were as high as 77,000 µg/kg. Downstream from the St. Louis Reservoir the sediment concentrations showed a gradual decline from 6,200 µg/kg 1/4 mile downstream, to 100

µg/kg 24 miles downstream. Elevated PBB levels were found in Pine River fish ranging up to a maximum of 1.33 mg/kg/ in carp captured in the reservoir in the vicinity of Michigan Chemical Corporation. Sampling 3 miles downstream showed continued high concentrations of 1.25 mg/kg and measurable concentrations (0.09 mg/kg) in carp captured 8 miles downstream. These levels in local fish populations were sufficiently high to issue health warnings against their consumption.

A study undertaken at Michigan State University by Jacobs et al. (1976) evluated the persistance in the soil and plant uptake from the soil of PBB. It was shown that PBBs were extremely persistent with only one pentabromobiphenyl isomer showing any significant disappearance after 24 weeks of incubation in selected soils. Consistent with this finding, PBBs were detected in soils from a field which had received manure from a PBB contaminated dairy herd 10 months earlier (Jacobs et al., 1976). Orchard grass and carrots grown in soil contaminated with PBBs showed none or only very minor uptake of PBBs, respectively. Jacobs et al. (1976) concluded that the potential hazards from PBB contaminated soils are low since not only were the PBBs not taken up by the plants, they were also not leached below the depth of their incorporation. Filonow et al. (1976) and Islieb et al. (1974) concurred that PBBs do not leach down into the soil.

Michigan Chemical Corporation (1970) has predicted that under normal use of PBBs, its migration in the soil would be minimal; based on the fact that BP-6 is known to be tightly

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bound into the plastic into which it is incorporated. BP-6 is expected to react in a manner which is similar to the more highly chlorinated PCBs. These exhibit an extremely small migration tendency.

Residues in Biological Systems

Accidental substitution of Firemaster® BP-6 in place of magnesium oxide in animal feed resulted in widespread contamination of cattle, poultry and swine during the fall of 1973 and the winter of 1974 (Jackson and Halbert, 1974; Gutenmann et al., 1975; Detering et al., 1975b; Prewitt et al., 1975; Islieb and Whitehead, 1975; Fries et al., 1975).

The first herd reported to be affected has been described by Jackson and Halbert (1974). In September, 1973, the 400 head Halbert herd, located near Battle Creek, Michigan, experienced a problem with milk cows refusing to eat and a dramatic deterioration of animal health. Extensive testing was carried out by the Michigan Department of Agriculture (MDA). Finally, in late April, 1974, George Fries in the USDA laboratory at Beltsville, Maryland identified the feed contaminating compound as PBB, and the problem was traced back to its origin.

Upon identification of PBB as the unkown contaminant, an intensive effort was made (by the Food and Drug Administration (FDA), Detroit District; MDA; Michigan Department of Public Health; persons at Michigan State University and others) to exclude contaminated food and feed from further

use. The second objective was to learn as much as possible of the human, animal health and environmental consequences of PBB contamination.

Tolerances were established on May 10, 1974 by the US Food and Drug Administration. They were: meat and milk, 1.0 ppm; eggs, 0.1 ppm; and feed 0.3 ppm. These tolerances were revised downward on November 4, 1974 to: milk and meat, 0.3 ppm on fat basis; and eggs and finished feed, 0.05 ppm. Recently (August, 1977) a newly revised tolerance of 0.02 ppm in meat has been established. These action guidelines allowed the MDA to determine how many herds and flocks in the state were contaminated above the guideline levels. This was necessary so these units could be quarantined and provision could be made for disposal of animals, birds and products rendered useless by contamination. Tables 2 and 3 show portions of the May 10, 1976 PBB contamination Status Report prepared by the MDA.

The efforts of monitoring for PBB by the USDA, MDA, FDA, and public health authorities of the food supply have been continuous since PBB contamination was discovered in 1974. No foods are being offered for sale which are above the guidelines set by the FDA. For example, no trace of PBB has been found in processed milk in Michigan since early June 1974 (Cooperative Extension Service Bulletin, April, 1976; Islieb, 1976).

Concern has been directed toward levels of residues in, and excretion of, PBBs from affected animals - from the

Table 2. Livestock known to be contaminated.[1]

Livestock Involved Since May 3, 1974		Known Premises at Low Level Contamination		
Animal	Number	Animal	Number of Premises	Number of Animals
Cattle	32,000	Cattle	468	19,000
Swine	6,192	Swine	64	400
Sheep	1,468	Sheep	6	250
Poultry	1,920,000	Poultry	183	55,000
Goats	2	TOTAL	721	
Rabbits	35			
Horses	2			
Ducks and Geese	2			

[1] From PBB contamination status report. Michigan Department of Agriculture, May 10, 1976.

Table 3. Livestock and products destroyed because of PBB contamination.[1]

Livestock Buried at Kalkaska Site		Products Destroyed	
Animal	Number	Item	Amount
Cattle	23,214	Feed	865 ton
Swine	4,053	Cheese	17,944 lbs
Sheep	1,371	Dry milk products	34,000 lbs
Goats	2	Butter	2,634 lbs
Rabbits	32	Eggs	4,847,227 eggs
Horses	2		

[1] From PBB contamination status report. Michigan Department of Agriculture, May 10, 1976.

standpoint of safety of human consumption of such animals and their products. Several studies concerning PBB residues have shown that PBB is excreted into the milk of cows and eggs of chickens (Gutenmann et al., 1975; Fries et al., 1975; Willet and Irving, 1975). Willet and Irving (1975) found PBB appeared in the plasma, milk and feces of cows after experimental intraruminal doses of the product were given. Gutenmann and Liska (1975) reported the distribution of PBB residues in the tissues of cows with the highest concentration found in fatty tissues.

A two year rat study conducted by Norris et al. (1974) providing 0.1 mg decabromodiphenyl oxide (DBDPO)/kg/day in the diet, revealed the bromine concentration reached a plateau in the liver within thirty days while the concentration in adipose tissue slowly increased. A comparable octabromobiphenyl (OBBP) study (Norris et al., 1974) revealed liver and adipose tissue bromine levels increased rapidly with no plateau reached in the 180 day study. Neither compound produced an accumulation in other tissues. Fries et al. (1975) concurred with this data and found a higher percentage PBB in milk fat, due to the increased percentage of fat content.

Several other studies also observed this same concentration of PBB in fat. Fries et al. (1973b) observed that PBB was accumulated in body fat by hens and cows at levels above those of the diet. PBB levels in eggs declined rapidly when hens were switched to clean feed, although body fat levels remained high. PBB in milk fat also exceeded the concentration in the diet, and diminished upon removal of PBB from the

cow's feed. Detering et al. (1975c) found the content of PBBs in blood, milk and body fat of cows contaminated 7-9 months previously showed that body fat and milk fat contained 600 and 300 times, respectively, of that which the blood contained. Fries et al. (1973a) demonstrated that residues of PBBs in animal systems behave similarly to the residues of PCBs with a comparable degree of halogenation.

In examining the excretion rate of Firemaster® BP-6, PCB and DDE into the milk of cows, Fries and Marrow (1975) found that the half life of PBB in milk was 58 days. The decay rate and steady state excretion levels were similar to those for PCBs and chlorinated hydrocarbons.

Studies done by Ringer and Polin (1975) showed that the accumulation of PBB in the egg of the hen is in direct relation to the level in the diet, and reaches a constant concentration in the egg as soon as the yolk is fully formed in the ovary (taking 7-10 days). The concentration is 1.5 times that in the diet. The biological half-life of PBB in the egg, once the drug has been removed from the diet, is about 17 days. Nearly 60% of the daily intake of PBB in feed goes directly into an egg (yolk portion). That which remains goes into fat depots, liver, muscle, or is excreted in the droppings (about 10-11% of daily intake is excreted). The results of these feeding studies are similar to those found with chlorinated hydrocarbon insecticides (Cummings et al., 1965).

Stadelman et al. (1965) studied the persistance of

selected hydrocarbons after a measured exposure and found up to twenty-six weeks were required to completely remove residues from hen abdominal fat and egg yolk. Liska et al. (1964) looked at DDT residues in eggs and tissues of chickens on low DDT level rations. The residue levels in both eggs and fat increased sharply, thus the fatty tissue concentrated the contaminant.

Feeding studies show bromine levels in biological systems build up, seem to level off and remain stable, and then when the animals are again on "clean feed" the levels decrease. More rapid decreases occur in milk, eggs, liver and muscle than in adipose tissue which loses its PBB store much more slowly.

Toxicity of Polybrominated Biphenyls

The primary concern with the toxicity of PBBs revolved around its structural similarity to PCBs. Since both human and animal toxicological data are available for PCBs, comparisons are made between the two groups of compounds.

For brominated biphenyls limited toxicity data are only available on mixtures containing predominantly hexa- and octa-bromobiphenyl. Both mixtures differ sufficiently in isomeric composition so that their toxic effects may quantitatively be quite different, and also different from PCBs. The problem of toxic contaminants such as dibenzofurans and naphthalenes, has not yet been resolved for both PBBs and PCBs.

Aftosmis et al. (1972a, b) described toxicity of the more highly brominated PBB mixture. This mixture was principally octabromobiphenyl (OBB). Liver weight increase was dose-related, and bromine accumulated in liver, fat and muscle tissue. Bromine levels remained elevated after 18 weeks on "clean feed" with no decrease in level in fat tissue; however, levels in liver and muscle decreased. Histopathological changes in the liver of rats receiving 100 ppm and 1,000 ppm OBB were observed. Abnormal fetuses were observed in litters from mothers on 1,000 ppm and 10,000 ppm OBB diets, but no conclusion as to compound relationships of these abnormalities was reached by the authors. Bromine was diluted in fetuses of mothers receiving OBB in their diets. Hexabromobiphenyl (HBB) was shown to be more toxic than OBB by acute skin absorption when applied at 35 percent in corn oil paste. Also, HBB, OBB and decabromobiphenyl (DBB) were compared by direct interperitoneal injection (in corn oil) in rats. After one week, HBB (especially a preparation containing lower bromologs) accumulated in body fat far more than OBB or DBB. These observations caused PBBs to be rejected as candidates for flame retardants in polyester fibers (Aftosmis et al., 1972a, b).

The work of Norris et al. (1974) demonstrated hematological changes, liver enlargement, and liver and kidney lesions at all levels of OBB (1.0, 01, 0.01%) in the diet. Decabromobiphenyl oxide was less toxic. Lee et al. (1975) also induced enlargement of the liver due to hypertrophy of

liver cells in rats fed 100 and 1,000 ppm OBB for 2 and 4 weeks, respectively. Microscopic lesions and pathological changes also occured in the liver. Analysis of the tissues revealed a dose related build up of bromine predominantly in the fat as well as the liver.

The available data suggests PCBs and PBBs containing six or fewer halogen atoms are readily absorbed from the gut of higher animals. The available data also implies that PCBs are not excreted to an appreciable extent prior to metabolism to more polar compounds, and that long term PCB storage is in the skin and adipose tissue (Matthews and Anderson, 1975a).

Laboratory studies have demonstrated that the rate of PCB metabolism and thus excretion is approximately inversely proportional to the degree of PCB chlorination so long as there are two adjacent unsubstituted carbon atoms on the biphenyl ring. When two adjacent unsubstituted carbon atoms are not present the biological half-life of the given PCB may be a matter of years, and accumulation of high tissue concentrations with continued exposure is inevitable (Matthews and Anderson, 1975b). The major constituent of Firemaster® BP-6 does not have two adjacent unsubstituted carbon atoms, and the corresponding PCB has been shown to have an extremely long half-life in the laboratory rat and probably the human population as well.

Poor metabolism and excretion of PBBs may lead to long retentions, predominantly in adipose tissue, with accumulation to very high levels on continued exposure. Whether this would

lead to sufficient recirculation of the chemicals to cause toxic effects on target organs is presently not known.

Toxicological evaluation by way of the oral lethal dose (LD_{50}) comparison of PBB with other halogenated compounds shows that it has low acute toxicity. The data with regard to acute toxicity in rats given in Table 4 suggests PBBs are "practically non-toxic." The varying degree of toxicity of PBBs in animals is most often correlated to the concentration of these chemicals at which the animals are exposed. The LD_{50} dose for Japanese quail exceeds 1.0 g/kg (Strik, 1973), for rats the value found is in the range of 21.5 g/kg, and the acute derimal LD_{50} for rabbits is between 2.15-10.0 g/kg (Michigan Chemical Corporation, 1970). Patch application produced slight erythema and edema. No irritative effects were produced in the eyes of albino rabbits by application of the test material, and no mortalities resulted from inhalation of a PBB dust concentration of 71.1 mg/liter (Michigan Chemical Corporation, 1970). Based on these results, PBB was described by Michigan Chemical Corporation as non-toxic by ingestion or dermal application, not a primary skin irritant or a corrosive material, not an eye irritant, and not highly toxic by inhalation exposure.

However, PBBs are fat soluble and, as already stated, accumulate in the body. Field experience with highly contaminated feed (2,000-4,000 ppm) indicates there is toxicity especially in younger animals and those not lactating or producing eggs (Hoeting, 1975).

Table 4. LD_{50} experimental test results for selected substances when fed orally to rats.[1]

Substance	Mg/Kg Body Weight
Dieldrin	40
DDT	285
PCB	10,000
Fire retardant - PBB	21,500
Heptachlor	90
Lindane (benzene hexachloride delta isomer	1,000
Metathione	1,156
Methoxychlor	5,000
Sodium chloride (table salt)	3,000

[1] Cooperative Extension Service, East Lansing, MI. April, 1976.

The clinical symptoms of animals involved in the Michigan PBB contamination incident are complex and confusing because there seems to be little consistency between the levels at which the animals were exposed and the symptoms they exhibit. Most dairy animals exposed to toxic levels of PBB showed symptoms of malnutrition and starvation which couldn't be readily distinguished from poor rations. Chemical analysis is the only positive method of determining PBB exposure. However, as the following studies demonstrate, PBBs have a similar mode of action as halogenated compounds in that they produce porphyria, liver injury, tremor, loss of weight and retarded growth.

Mammalian Toxicity

Preliminary results of studies (Gartoff et al., 1975) with rats to determine biochemical toxicologic characteristics of PBB were reported by Kolbye (1975). These studies were initiated in October, 1974 by scientists from the Bureau of Foods, with rats being fed diets containing various concentrations (5, 50 and 500 ppm) of Aroclor® 1254 (PCB) or BP-6 (PBB). The results obtained indicate that both PCB and PBB cause dramatic alternations in normal biochemical and physiological processes. These effects occurred over many levels of biological organization, from macroscopic retardation of growth to submicroscopic changes in the relationships between cellular molecules. Many of these specific biochemical effects have been reported in previous work on the toxicology of PCB (Mehlmann et al., 1974a, b; Mackerer et al.,

1973). It was found that the most obvious effect of PCB and PBB on these rats was a retardation of growth seen at the high level of exposure (500 ppm fed). Growth depression is a constant finding in studies where high levels of PCB were fed to lab animals (Mehlmann et al., 1974a, b; Allen and Abrahamson, 1973; Buckner et al., 1974; Koller and Zinkl, 1973; Kimbrough et al., 1972), and was a symptom reported in the farm animals ingesting PBB contaminated feed. The depressed growth of rats found by Gartoff et al. (1975) study was due primarily to decreased feed efficiency and secondarily to decreased feed consumption and feed efficiency.

In these rats disruptions in the energy metabolism of the cell were produced by even three weeks of exposure to PCB and PBB. Greater disruption of mitochondria energy production was caused by PBB than PCB. Further analysis of the intermediary metabolites in the liver of rats fed PBB and PCB indicated a perturbation of the redox state of the cell. The redox state is intimately related to cellular energy production.

This study also observed (at 50 and 500 ppm level) that increased cell size and resultant liver enlargement were more marked with PBB than PCB. The changes in the liver composition which were associated with liver enlargement were also greater in animals fed PBB. These changes included an increased percentage dry weight and increased percentage liver lipids consisting of increased cholesterol, phospholipid and neutral lipid. The changes seen in the liver

composition with PBB fed are an established part of the now classical response to PCB (Kolbye, 1975). This response includes proliferation and concentric array formation of the smooth endoplasmic reticulum, fatty liver degeneration and induction of microsomal mixed function oxidase (MFO) activity (Allen and Abrahamson, 1973; Bruckner et al., 1974).

Farber and Baker (1974) found that, on a molar basis, hexabromobiphenyl was approximately 5 times more potent than PCB Aroclor® 1254 as an inducer of mixed MFO enzymes at 5 ppm level in rats. A three fold difference was observed by Gartoff et al. (1975) between Firemaster® BP-6 and Arochlor® 1244 in enzyme induction. Among PCBs it appears as if their potency increases with increasing chlorination and chlorine substitution in the para>ortho>meta positions, respectively (Echobichon and Comeau, 1975). Echobichon and Comeau (1975) suggest that since induction of MFO enzymes may result in increased hormone metabolism of carcinogen activation, exposure to the PCBs and PBBs should be limited on that basis alone.

Plasma and serum components were also analyzed in these PCB and PBB fed rats (Gartoff et al., 1975). The only significant effects were a change in blood glucose and cholesterol levels. PCB caused a decreased blood glucose level in animals fed 50 and 500 ppm while PBB caused a slight decrease only at 50 ppm. On the other hand, cholesterol was increased by a diet with 5 ppm PBB, but only by a diet with 500 ppm PCB (Kolbye, 1975).

In a study carried out by Corbett et al. (1975) BP-6 was shown to be weakly teratogenic causing exencephalia in the offspring of the mice that received both 100 and 1,000 ppm dosages. Cleft palate and defective kidneys were noted in offspring at the 1,000 ppm level. BP-6 fed to pregnant rats and mice (100 and 1,000 ppm) resulted in dose dependent decrease in mean fetal weight.

Liver abscesses, enlargement and hepatic pathological changes were the most common symptoms of acute and chronic toxic effects seen in the Michigan cattle (Jackson and Halbert, 1974). These were seen when the level of PBBs in the body fat were approximately 200 ppm. Hematomas and abscesses in the peritoneal and thoracic cavities of these cows were also observed.

From field observations of PBB contaminated cattle, Prewitt et al. (1975) reported that animals which had over 20 ppm in their milk fat at parturition, had unrelaxed pelvic ligaments resulting in difficult labor, and the calves were stillborn or died shortly after birth. Metritis (inflammation of the uterus) and retained placentas, as well as liver and kidney adhesions, were also common. Examinations of dead calves showed PBBs were transferred through the placenta to the fetus and were embryo-toxic (Detering et al., 1975b). PBB concentration in the body fat of these calves was in the range of 50 to 400 ppm.

Avian Toxicity

Strik (1973b) reported hexabromobiphenyl (100 and 250 mg/kg body weight) fed to Japanese quail and chickens induced porphyria, especially in the liver. Hepatic porphyria is characterized by an accumulation of porphyrins in the liver, kidneys, intestine and other organs. Strik (1973b) saw species differences in porphyria and attributed these differences to those within and between species, the physiological state of the animal, dosage, route of administration, age and feeding of the animal. Adequate protein diet seems to provide some protection against porphyria (Strik, 1973b).

Hepatic microsomal oxidase enzymes of Japanese quail were induced by dietary PBB at 10, 20, and 100 ppm (Babish et al., 1975). Egg production was reduced with none of the eggs hatching from quail hens fed 100 ppm PBBs. Forty percent of the embryos died in the first day or two of development. However, when the feed contained 10 and 20 ppm of PBBs no effect was observed. Also, there was no egg shell thinning in any of the treated groups. Cecil et al. (1975) studied the response time to phenobarbitol as indication of microsomal enzyme activity in Japanese quail. Hexabromobiphenyl behaved similarly to the higher molecular weight polychlorinated terephenyls in that it did induce enzyme activity and was less active than the PCBs studied.

Lillie et al. (1974) fed poultry various PCBs and PBBs at 2 and 20 ppm levels. Egg production, feed consumption and

body weight gain of the progeny were reduced by hexabromobiphenyl at the 20 ppm feed level. Toxic symptoms in poultry as enumerated by Ringer and Polin (1977) seem to be liver and thyroid enlargement, decrease in spleen size, a state of anemia (hematocrit and hemoglobin content decreased), edema (manifested as hydropericardium interfering with normal heart function), and depression of egg production, hatchability, progeny survival and progeny growth. Consumption of PBB did not influence egg shell thickness, egg weight or ability of egg to be fertilized by sperm at levels studied. A comparison of PBB and PCB and minimum contaminant levels at which there is an observable toxic effect in chicks and in adult hens is shown in Tables 5 and 6, respectively. Generally, the contamination level at which changes are seen is higher in PBB than in the PCBs tested.

Low Level Contamination

In order to evaluate the effects of low level contamination (levels above the previous FDA guideline of 0.3 ppm PBB in fatty tissue), Deming (1975) visited 72 quarantined low level herds of dairy cattle. Sixty four percent of the herds visited had experience 20-50% milk production drops with a substantial proportion of the drop attributed to high incidence of sterility problems in the herd. Retarded growth of young stock was also significant which Deming believed to be due to some form of hormone interference in these low

Table 5. Comparison of PCB and PBB (ppm in feed) minimum effective level - as seen in chicks.[1]

Affected Parameter	PCB	PBB
Body weight	1254 ↓ 50	↓ 75
Liver weight	1242 ↑ 25	↑ > 25 > 50
Thyroid weight	no effect	↑ 100
Comb weight	1242 ↓ < 100	↓ 50
Testes weight	1242 ↓ 100	↓ 200
Spleen weight	1242 ↓ 100	↓ > 50 > 100
Hematocrit	1254 ↓ 50	↓ 75
Hemoglobin	1242 ↓ 25	↓ 75
Hydropericardium	1242 ↑ 50	↑ 75

[1] Ringer and Polin, 1977.

Legend: Aroclor® 1254 - biphenyl of 54% average chlorination.

Aroclor® 1242 - biphenyl of 42% average chlorination.

↓ - decreased effect due to contaminant.

↑ - increased effect due to contaminant.

Number following arrow indicates ppm of contaminant in feed.

Table 6. Comparison of PCB and PBB (ppm in feed) minimum effective level - as seen in adult hens.[1]

Affected Parameter	PCB	PBB
Feed consumption	↓ ?	↓ 125
Egg production	1248 ↓ 10	↓ 45
Eggshell thickness	no effect	no effect
Egg weight	no effect	no effect
Fertility	no effect	no effect
Hatchability	1248 ↓ 10	↓ 45
Progeny survival	1248 ↓ 20	↓ 30
Progeny growth	1248 ↓ 20	↓ > 25 < 45
Liver microsomal enzyme induction	↑	↑
Pentobarbital sleeping time	↑	↓

[1] Ringer and Potin, 1977.

Legend: Aroclor® 1254 - biphenyl of 54% average chlorination.

Aroclor® 1242 - biphenyl of 42% average chlorination.

↓ - decreased effect due to contaminant.

↑ - increased effect due to contaminant.

Number following arrow indicates ppm of contaminant in feed.

levels. Body functions suspected to be involved were, metabolism, hormone, and blood cell producing mechanism.

Fries (1975b) reported on a long term observation on the effect of PBB on health and production in dairy cows. Four cows were fed 10 mg PBB/day for 60 days. These cattle were evaluated two years later and it was found that there were no important effects on them as compared to the control group.

A dairy herd health survey was conducted by Meroser et al. (1975), again to assess those herds with low level (those with trace to 1 ppm PBB in body fat or milk fat) contamination; to see if they showed greater health problems than did non-contaminated herds. Based on these data, it was concluded that there were no herd health problems observed that could be attributed to the presence of low levels of PBB.

Human Exposure to PBB Contamination In Michigan

It has been estimated that between the onset of BP-6 contamination in the fall of 1973 and the establishment of the quarantine of affected herds and flocks in the spring of 1974, 8,000-10,000 Michigan residents have been exposed to PBB through the consumption of contaminated eggs, meat, milk and other dairy products (Kolbye, 1975; Schmidt, 1976). A considerable amount of variation in both length and level of exposure has probably occured. As a group, the farm family members have been at greatest risk followed by those individuals who purchased products from contaminated farms on a

regular basis.

In order to determine whether or not persons exposed to PBB contaminated products had suffered any acute adverse health effects, the Michigan Department of Public Health undertook a series of studies in the summer and fall of 1974. Study participants for the exposed group were dairy farm residents from farms which had been quarantined by the MDA. Non-exposed subjects were randomly selected from a list of dairy producers in the same geographical areas where farms had been quarantined.

A total of 298 persons were interviewed for exposure data, illness histories and physical examination and/or blood samples were obtained from 110 persons in the exposed group and 104 persons from the control group. This study revealed no disease, symptoms, or laboratory findings that occured consistently in the exposed group, or that was significantly more frequent among the exposed individuals as compared with the controls. There was no positive relationship detected between PBB blood levels and the occurance of any symptom in the individuals. Physical examination of adults and children showed no unusual abnormalities of the heart, liver, spleen or nervous system. Urinalysis and complete blood count did not reveal a significant excess of unusual abnormalaties related to exposure or PBB levels. Several exposed females delivered normal babies without complication. Tests showed concentrations of PBB in breast milk to be considerably higher than found in paired blood plasma, as high as 175:1,

respectively (Michigan Department of Public Health, 1975b; Humphrey and Hayner, 1975; Fine, 1976).

The concern still remained, however, that there might be long-term effects of the BP-6 contamination. The clinical observations in Japan relating to PCB ingestion suggested skin, liver, nutritional and neurologic changes, seen in individuals ingesting PCBs, might be signs of PBB toxicity. Therefore, a long term epidemiologic evaluation sponsored by the Michigan Department of Public Health, the US Food and Drug Administration and the Center for Disease Control has begun (Michigan Department of Public Health, 1975a). Four thousand persons are taking part in this study and are being followed closely for the development of skin disease, liver disease, metabolic and neurologic changes.

Polychlorinated Biphenyls - Chlorinated Hydrocarbons

Chemistry - Use

Polychlorinated biphenyls are quite stable and are considered "industrial chemicals" with a variety of uses. They had important applications as plasticizers in plastics, and modifiers in many paints and other products, as lubricants, electrical insulators and fire retardants. They were also used in printing inks, textile oils and heat transfer fluids and many other products (US Dept. of HEW, 1969). PCBs have

also been used for mixing with chlorinated insecticides to suppress vaporization and extend their "kill life" (Sullivan et al., 1953; Lichtenstein, et al., 1969). Now the use of PCBs is limited to closed systems for which there is no substitute.

In the US PCBs were manufactured by Monsanto Chemical Company with the trade name Aroclor®; this year their production of PCBs has stopped. Aroclors were mixtures of compounds with the content of individual Aroclors varying from approximately 10-70% chlorine by weight, and the physical properties varying with chlorine content. Those with low percentage chlorine are very fluid liquids; as percentage chlorine increases the products become more viscous or become solids. The series of numbers designating individual Aroclors are as follows: the 1200 series indicates a biphenyl and the last two numbers in the four number series indicates percentage chlorine content. For example Aroclor® 1261 is a biphenyl with 61% chlorine.

PCBs are considered chemically inert, are not hydrolyzed in water, and are resistant to alkalies, acids and corrosive chemicals. They have low volatility and their boiling points range from 278°C for Aroclor® 1221 to 415°C for Aroclor® 1268. All are stable to prolonged heating at 150°C. PCBs are insoluble in water and very soluble in hydrocarbon solvents (Peakall and Lincer, 1970).

Occurance

The nearly ubiquitous presence of PCBs is fully documented. Studies indicate that sources of these chemicals are generally associated with waste disposal materials (Carnes et al., 1972; Schmidt et al., 1971; Fujiwara, 1975; Martell, 1975; Hammer et al., 1972; Nisbet and Sarofim, 1972; Trout, 1972). With sludge disposal taking place by incineration, land fill, and crop or pasture application, the contamination of PCBs in the environment seems obvious.

PCBs and organochlorine insecticides are persistant and accumulate in oil and fat so that the highest levels are found in fatty tissues of birds and in the blubber of certain marine animals. One difference between the two groups of compounds is that, whereas insecticides are found in significant quantities in nearly all species of animal and plant life, significant amounts of PCBs are usually associated with the marine or aquatic environments. Thus, the higest levels of PCBs have been found in fish-eating birds and in marine mammals (Jenson et al., 1969; Anderson et al., 1969; Holden and Marsden, 1967; Peakall et al., 1972; Dennis, 1975).

In the US, minimal human PCB exposure is due to food, air and water; while significant human exposure appears to be limited to sports fishermen consuming fresh water fish from contaminated streams and lakes, and to occupational exposure in industrial workers. A number of studies show that human tissue contains low levels of halogenated compounds. Yobs (1972) reported PCBs, and Biros et al. (1970)

found PCBs and DDE in adipose tissue. Fujiwara (1975) found PCBs, DDT, DDE and several BHC isomers, and Solly and Shanks (1974) reported on PCBs and organochlorine pesticides in human adipose tissue. Also, analysis of human blood showed varying concentration of PCBs, BHC, DDT, and DDE residues (Doguchi et al., 1975; Fujiwara, 1975), depending on the area studied, those with greater environmental contamination had increased levels in the blood analyzed.

A range of levels of PCBs in human milk has been detected. In Colorado (Savage et al., 1973) levels were not appreciable, while in Japan, the PCB concentrations found were relatively high and it was estimated that babies would ingest between 4.8-5.3 mg/kg body weight of PCBs/day (Fujiwara, 1975). Musial et al. (1974) found that milk from people of provinces of Canada contained PCBs as well as DDT and DDE. Dyment et al. (1971) found PCBs in human milk and serum from Texas and human milk from New Guinea. Other works that have reported the occurance of organochlorine pesticides in human tissue (Price and Welch, 1972; Brown, 1967; Abbot et al., 1968; Dyment et al., 1971) contribute to the evidence of the ultimate disposition of these compounds in man.

The major source of human contamination has generally been associated with ingestion of food containing these compounds (Kolbye, 1972; Fujiwara, 1975; FAO/WHO, 1974). Jelinek and Corneliussen (1975) reviewed the data from the FDA Total Diet Study (1971-1975). They listed three sources of PCB food contamination: 1) environmental contamination -

levels in fish from lakes and streams, 2) industrial accidents - isolated incidents involving leakage and spillage of PCB fluids and other PCB materials on animal feeds, feed ingredients or food, 3) food packaging materials - PCB migration to foods packaged in PCB contaminated paper products (Trout, 1972). Jelinek and Corneliussen (1975) noted that all food classes of the total diet have declined to no occurance and no calculated daily intake of PCBs except in the meat-fish-poultry composites. About 40% of these composites contain detectable PCBs although only traces (below 0.05 ppm) in some have been detected in later years with tighter controls. It is estimated that PCB in the total diet for the general public is 5-10 µg/day (Jelinek and Corneliussen, 1975). Low level findings are primarily due to the fish in these composites.

Animal Toxicity

The range of sensitivity to PCB is enormous. Invertebrates show effects at levels of a few ppb (Duke et al., 1970), while at the other extreme Escherichia coli appear to thrive at thousands of ppm (Keil, 1972). Fish, birds, and mammals fall between these extremes.

The different commercial mixtures of PCBs elicit different toxic responses in animals, and different animal species seem to vary in their susceptibility to the toxic effects of PCBs. Reproduction is severely affected in milk at a

dietary level of 5 ppm Aroclor® 1254 and a slight effect is still noted at a dietary level of 1 ppm (Ringer et al., 1972). In the Rhesus monkey, reproduction was reduced at a dietary level of 2.5 ppm Aroclor® 1248 (Allen, 1975). In rats, a dietary level of 20 ppm Aroclor® 1254 depressed reproduction and in the same laboratory with the same rat strain a level of 500 ppm Aroclor® 1260 was necessary to reduce reproduction (Linder et al., 1974).

Hepatic porphyria has been reproduced in a number of species including the chicken (Vos and Koeman, 1970), rabbit (Vos and Beems, 1971), and the rat (Iverson et al., 1975). Hepatic porphyria occurs along with an increase in aminolevulinic acid synthetase (ALA) in the liver. Mixed function oxidases (MFO) are also induced in the liver, and comparative studies with PCB isomers have suggested that when the 4,4' positions on the biphenyl ring are occupied by chlorine atoms the effect is more pronounced (Ecobichon and Comeau, 1975). MFO enzymes are induced in the rat with as little as 10 ppm in the diet if the animal is exposed for a long enough period of time (Turner and Green, 1974).

Liver enlargement is shown in the rat (Kimbrough, 1975) concomitant with lipid accumulation and increase in cell breakdown with either higher doses or longer exposure. In the primate, in addition to the effect on the liver, the gastric mucosa is affected (Allen and Norback, 1973), the skin is affected and bone marrow is depressed (Allen, 1975). In the rabbit, atrophy of the thymus is seen in addition to

liver pathology (Vos and Beems, 1971). Fluid accumulation occurs in chickens (Vos, 1972; Cecil, 1973) and the lymphatic system is affected in mink (Ringer et al., 1972). Decreased hatchability and teratogenic effects in chick embryos were recorded by Cecil et al. (1974).

Human Toxicity

Adverse human health effects resulting from PCB exposure have come primarily from occupational exposure and from exposure through the ingestion of contaminated rice oil by people in Japan. Schwartz (1936) provided some of the earliest reports of adverse effects due to occupational exposure in the US, in which he described skin lesions and symptoms of systemic poisoning among workers who were reported to have inhaled chlorodiphenyls. There have been numerous reports over the years describing such skin eruptions and of systematic manifestations as well, among marine electricians, machinists, capacitor and transformer manufacturing workers and others occupationally exposed to PCBs. The skin lesions described by Schwartz in 1936 have come to be designated as "chloracne." Part of the chloracne lesion resembles adolescent acne, but is generally more severe and the lesion distribution is inconsistent with adolescent acne. Typical clinical findings in the human exposure to PCBs which occured in Japan in 1968 and which resulted from ingestion of rice oil (contamination as high as 2,500 ppm in the canned oil)

included chloracne and increased pigmentation of the skin, increased eye discharge, transient visual disturbances, feeling of weakness, numbness in limbs and some disturbance in liver function (Kuratsune et al., 1972).

Halogenated Hydrocarbon and Pesticide Residue Removal from Foods

Surveys of food in the market place indicate varying amounts of pesticide residues in many commercial foods (FAO/WHO, 1974; Jelinek and Corneliussen, 1975; Duggan and Weatherwax, 1967; Duggan and Lipscomb, 1969). The total dietary exposure varies from one part of the country to the other and depends upon the dietary habits of the individual or family. Few, if any, foods are completely free from some degree of pesticide residue.

As stated previously PCBs and chlorinated hydrocarbon pesticides have been detected in human tissues and their occurance is generally associated with the ingestion of food containing low levels of these compounds. Although precise information concerning the toxicity of these compounds at very low levels is not known, the interest in removing residue compounds from food stuffs with processing comes from the possible chronic effects of continued assimilation and accumulation of these compounds in body lipids.

Numerous studies have been conducted concerning the amount and procedures most effective in the removal of

pesticides and chlorinated hydrocarbons from our food supply. The effectiveness of molecular distillation in removal of pesticides from milk fat has been evaluated by Bills and Sloan (1967). Heat, deodorization, steam deodorization and freeze drying have been used to study their effect on insecticide residues in milk fat (Kroger, 1967). The effect of processing and storage of dairy products made from milk contaminated with DDT and lindane, were studied by Langlois et al. (1964), and from milk contaminated with organochlorine pesticide residues were evaluated by Li et al. (1970). A study completed by Murata et al. (1976) concluded that spray drying showed promise for the removal of PBBs from milk. Zabik et al. (1971) studied the potential of freeze drying for removal of chlorinated hydrocarbon insecticides from eggs. The success of freeze drying appeared related to both the vapor pressure of the pesticide and the amount of contamination in the whole egg.

Studies of particular interest to this work are of those which showed effectiveness of cooking procedures in removal of chlorinated hydrocarbons from meat. Evidence indicates residues of chlorinated hydrocarbon pesticides are concentrated in fats and fatty tissues of animals; hence, pesticide residue should be reduced when fat is removed during cooking or other preparational procedures.

The effect of cooking on removal of PCB and DDT from Chinook and Coho salmon from Lake Michigan were studied by Smith et al. (1973). Reduction of these compounds from the

salmon steaks was slight; poaching and baking reduced 2-8% of the residues, while baking in nylon bags caused a reduction of 11-16%. No difference was found between Chinook steaks cooked with and without skin. There was no apparent relation between the level of PCB or pesticide residues and concentration of fat in these spawning low fat fish.

The effectiveness of curing, heat processing and cooking to reduce dieldrin residue levels in pork bellies was investigated by Yadrick et al. (1971). Dieldrin was reduced 47-80% in the cooked bacon as compared to what was originally present in the uncured samples. Most of the losses were attributed to fat rendering during cooking, although heat destruction, co-distillation, and/or aeration may have occured. No consistent pattern was shown for animal, cooking method, or in curing in reducing dieldrin residue levels.

Funk et al. (1971) determined the effect of three cooking methods (pan frying, baking and microwave energy) on dieldrin residue levels in pork sausage. Sausage patties cooked by the three methods showed no difference in dieldrin residue level, however, differences were noted among animals from which the sausages were made. Analysis of the drip from samples showed residues can be reduced by cooking.

Maul et al. (1971) found that cooked pork loin samples showed lower levels of dieldrin residues than uncooked samples, and residues were present in cooking drip. Ranked in order of increasing percentage of total cooking losses were samples cooked by braising, microwave, roasting and broiling.

In a study completed by Yadrick et al. (1972) dieldrin levels, total lipid composition, and the relative proportion of neutral lipids to phospholipids were determined in selected raw and cooked pork muscles. A consistent reduction in residue levels based on fat occured with roasting. Most of the loss of residues apparently accompanied volatile losses in the meat during cooking, since drip losses were minimal. The dark adductor muscle had a significantly higher ($P<0.001$) level of phospholipid and a lower ($P<0.001$) level of neutral lipid than all other muscles. The dark adductor tended to have the highest dieldrin levels, where the light femoris muscle had the lowest. The data may indicate some preferential deposition of the dieldrin into the phospholipid component of the fat.

Liska et al. (1967) first reported the feasibility of removal of selected hydrocarbon pesticides by cooking. The cooking methods used were more severe than normal procedures might be - simmering at 88-93°C for 2-3 hours and autoclaving at 15 psi for 3 hours. It was found the amount of insecticide residues in raw chicken tissues was related to fat content, and with autoclaving at 15 psi for 3 hours there was a reduction of 10-90% in the amount of residues detected in cooked tissues. This indicated a rendering out of fat and insecticides, however, in the case of DDT the cooking operation caused a change in chemical structure to DDE and DDD.

Ritchey et al. (1967) found DDT and lindane, which had been incorporated into chicken tissues via feed, were reduced

considerably when the birds were cooked by baking and frying. In a later study Ritchey et al. (1969) found DDT incorporated into chicken tissues during the growing period was also reduced during cooking. Total losses of residue were greater when tissues were fried or steamed than when samples were either baked or heated in a closed container. Losses of residues from chicken primarily occured through leaching out of fat during the cooking process. Lindane, endrin, heptachlor, dieldrin and aldrin were fed at 10 ppm to broilers for the 8 week growing period (Ritchey, 1972) and tissue was then cooked by baking, frying or steaming. Losses of these residues occured primarily by leaching with fat and water, although there was some destruction of lindane and heptachlor epoxide by heating.

Morgan et al. (1972) evaluated the levels of lindane, dieldrin, DDT, DDE and DDD in meat and broth from selected parts of hens cooked by simmering and pressure cooking and compared these values to levels in the raw meat. Both cooking methods resulted in similar levels of pesticide reduction. Sixty six percent of the lindane and seventy five percent of the dieldrin was recovered in the broth, therefore, discarding the broth and drippings after cooking could greatly reduce pesticide consumption.

The effect of cooking on PCB residue levels in chicken studied by Zabik (1974) showed cooking by pressure or stewing were similar in effective reduction of PCB levels. Rendering of the PCBs with the fat appears to be the major mode

of removal. Except for adipose tissues, which lost most of its PCB content to the broth, recovered PCBs were about equally divided between cooked samples (drumsticks, breast pieces, thigh meat and thigh skin) and the broth.

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EXPERIMENTAL PROCEDURES

To facilitate the investigation of the effect of cooking on PBB levels in chicken tissue and chicken broth, the Poultry Science Department of Michigan State University donated hens which had been fed PBBs.

Feeding PBBs

Fifteen white Leghorn hens approximately 9 months of age were fed for five weeks a standard cage layer ration (see Appendix) which was contaminated with 0, 30, 45, 60 and 90 ppm of Firemaster® FF-1. Three hens were in each feeding group; each group was housed in colony cages designed to hold 12 birds in a Poultry Science Research and Teaching Center. Water and feed were available ad libitum. Feed was dispensed by a mechanical feeder controlled by a time clock. Feed consumption was not affected by the level of PBB fed and averaged 82, 77, 79, 79, and 81 gm/wk/bird for feed with 0, 30, 45, 60 and 90 ppm PBB, respectively.

At the end of five weeks, the first three groups of hens (those fed feed contaminated with 0, 30, 45 ppm levels of PBBs) were slaughtered. The remaining two groups were placed on clean feed for an additional 8 weeks and then slaughtered.

Slaughter and Preparation

The hens were slaughtered by external severing of the jugular vein, hung in killing funnels, bled, scalded in water at 56°C, hand picked, eviscerated and labeled before being chilled overnight under water. The next morning they were carefully dissected to provide breast pieces, drumsticks, thigh meat, and thigh skin from the right side for raw analyses and those from the left side for cooking and subsequent analyses. The breasts were split so the keelbone remained with the half to be cooked. All samples were wrapped in aluminum foil, labeled as to bird identification, ppm in feed, piece and state in which they were to be analyzed. The samples were then frozen and held at -20°C pending analysis.

Samples, as they were analyzed, were removed from the freezer and thawed at 4-5°C for approximately 24 hours. The raw samples were deboned, the meat and skin of the breast and drumstick were combined, while the thigh and thigh skin were kept separate. Samples were homogenized using a Waring blender; the amount necessary for analysis was removed, and the remainder frozen in glass jars with foil-lined lids.

Cooking

Each chicken piece to be cooked was processed under 15 psi pressure for 15 minutes in 500 ml deionized water, in a 3.8 l aluminum pressure sauce pan. Heating time to reach the

designated pressure was approximately 8 minutes and did not vary significantly from one piece to another. After the pan had cooled 5 minutes the pressure was released. Percentage yields of cooked chicken pieces were calculated as the weight of cooked chicken pieces minus bone divided by the raw piece weight times 100.

Broth percentage yields were calculated as the cooked broth volume divided by 500 ml times 100. Cooked samples were homogenized, placed in glass jars, with foil-lined lids and then frozen and held at -20°C pending analysis. The broth was not frozen to eliminate the possible problem of bottle breakage upon freezing; but rather, held at 4-5°C in glass bottles and analyzed with one month.

Chemical and Residue Analyses

All chemical and residue analyses were carried out in duplicate. The chemicals used were ACS reagent grade and the solvents were redistilled from glass. All glassware was acetone-rinsed following by petroleum ether rinsing before being used. Standard solutions were prepared in petroleum ether using the commercial Firemaster® BP-6, hexabromobiphenyl (Lot No. 5143, Michigan Chemical Corporation, Chicago, IL).

Moisture Analyses

Moisture analyses were carried out in a Hotpack vacuum oven, model 633, at 100°C and a vacuum of 660-711 mm (26-28 in) Hg to a constant weight. Sample size weighed to the nearest 0.1 mg were as follows: 4-6 g for raw and cooked breast and drumstick (with skin) and thigh (without skin) and 2-4 g samples of raw and cooked thigh skin. Aluminum drying cups were loosely covered with perforated aluminum foil caps to prevent loss of fat due to spattering. The percentage of total solids expressed as the dry weight of the samples was used in residue calculations.

Lipid Analyses

Lipid in the raw and cooked chicken tissues was determined using soxhlet extraction (AOAC Method 11.23). The dried samples were placed in the soxhlet thimble and extracted for 5-6 hrs with petroleum ether. Two ten ml aliquots of this extract were transfered separately to predried 50 ml Erlenmeyer flasks. The petroleum ether was removed over a steam bath followed by drying under a vacuum of 711 mm at 70°C. Percentage lipid was calculated as follows:

$$\% \text{ lipid} = \frac{\text{g lipid} \times \frac{\text{total ml petroleum ether}}{10}}{\text{wet sample weight}} \times 100$$

For the broth, duplicate 14-15 g samples were weighed after first homogenizing the entire broth sample in a Waring

blender. The broth was stirred for 30 min with 150 ml petroleum ether using a 2.5 cm magnetic stirrer at medium speed. The broth extracts were then washed with 1% sodium sulfate solution to remove any polar compounds remaining and dried over anhydrous sodium sulfate for 30 minutes. Aliquots of lipid in petroleum ether were taken and treated as previously described.

Residue Extraction and Cleanup

Petroleum ether soxhlet extraction (AOAC Method 11.23) was used for removal of PBBs from tissue samples. The broth was stirred with 150 ml petroleum ether for 30 minutes using a 2.5 cm magnetic stirrer at medium speed. The broth extractions were then washed with 1% Na_2SO_4 solution to remove any polar compounds remaining and dried over anhydrous sodium sulfate for 30 minutes. Both broth and tissue sample extracts were treated with acetonitrile partitioning (AOAC Method 29.001), Kuderna Danish concentration and Florisil cleanup (AOAC Method 29.014). The Florisil column used was 50 cm long x 20 mm id. The packing consisted of 12 mm anhydrous sodium sulfate, 11 cm florisil and again 12 mm sodium sulfate on top. The sample was carefully applied to the top of the packing and eluted with a 6% solution of ethyl ether in petroleum ether collecting 200 ml of eluate.

For the 0 ppm in feed sample the eluate was evaporated to dryness with Kuderna Danish and then the sample was brought

up to 1/2 ml for GLC analyses. The 30, 45, 60 and 90 ppm in feed samples were taken from the 200 ml eluate without evaporation for GLC analyses. Blanks were carried through the complete extraction and cleanup procedure to insure that no contamination of either solvents or glassware was occuring.

GLC Analyses

PBBs were quantitated by comparing the area of the 6-isomer peak produced by the sample and the area of the 6-isomer produced by the standard (Firemaster® BP-6, Lot No. 5143, Michigan Chemical Corporation, Chicago, IL). The samples and standards in petroleum ether solution and nanograde hexane, respectively, were run on a Tracor 560 GLC, equipped with a ^{63}Ni electron capture detector and interfaced with a Digital PDP-8e-Pamila GC Data System. The column for the GLC was a pyrex column, 1.83 m long x 4.0 mm id, packed with 3% OV-1 (Methyl Silicone) on Chromosorb W 60/80 mesh. The carrier gas was nitrogen with a flow rate of 40 ml/min. Temperatures were 270, 240, and 300°C, at the injection port, column and EC detector, respectively. Standards were injected at the beginning of each run, after every 6-7 samples and at the end of the run. Quantitations were based on the peak area of the standards (hexabromobiphenyl peak). Figure 1 shows a GLC tracing of a PBB standard and sample containing PBB.

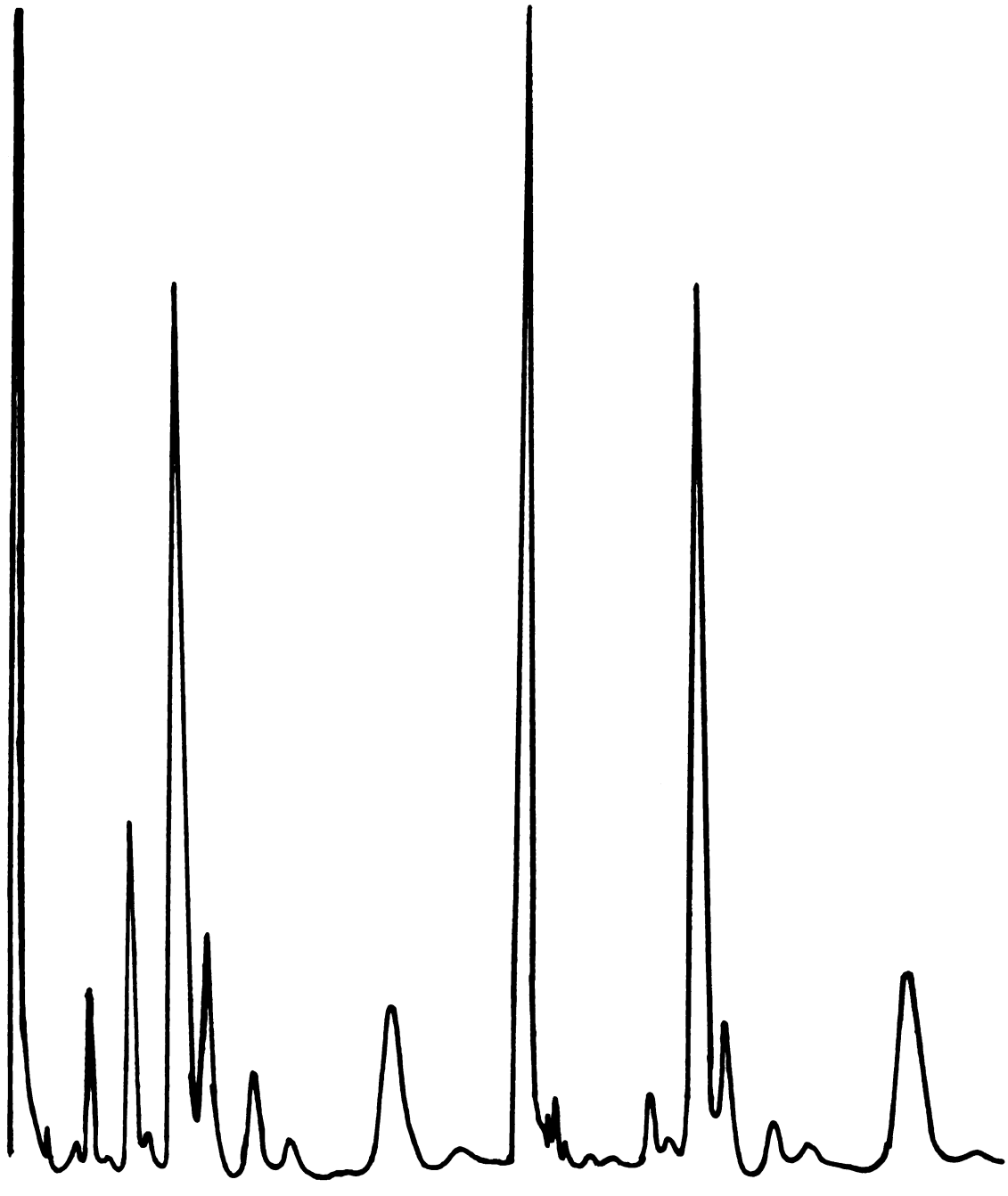

Figure 1. GLC tracing of PBB in standard and poultry tissue sample.

a) 4 g of 1 ppm PBB standard.

b) Sample thigh skin raw from bird fed 60 ppm PBB in diet (162 ppm fat weight basis).

PBB levels were expressed as ppm (wet weight), ppm of solids and ppm of fat. Total micrograms of PBBs in raw and cooked chicken pieces as well as in broth were used to calculate percentage recovery and percentage distribution of PBBs recovered in the cooked meat and broth.

Percentage recoveries of the PBB residues by the utilized method were determined, in spiked samples at levels of 0.02, 50, and 100 ppm, to be 55, 66 and 97%, respectively.

Confirmation of PBBs

The presence of the PBB residues was confirmed by ultra violet irradiation and mass spectrometric analysis. Ultra violet test was carried out in a Rayonet photochemical reactor, model 1162, equipped with short wave UV lamps. Mass spectrometric analysis was run on a pool of all of the extracted samples. A GC-65 gas chromatograph interfaced to a DuPont 21-490 mass spectrometer which was in turn interfaced to a Digital PDP-12 computer was used. The mass spectra was obtained at an ionizing voltage of 70 eV with a source temperature of 210°C.

Data Analyses

PBB levels expressed as ppm wet tissue, ppm in solids, and ppm in the fat as well as percentage fat and percentage solid were analyzed for variance due to state, i.e. raw, cooked, or broth, chicken piece, and level of PBB fed using

the MSU Stat System Version 4.2 for the Michigan State University CDC 6500 computer.

Percentage meat and broth yield as well as percentage of total recovered PBBs and recovered PBBs in the meat were analyzed for variance due to chicken piece and level of PBB fed. The studentized range test (Duncan, 1957) was used to sort out any significant differences established by these analyses.

RESULTS AND DISCUSSION

To investigate the effectiveness of cooking in reducing brominated biphenyl levels in chicken tissue, hens that had been fed five levels of PBB were analyzed. Thigh meat, thigh skin, drumstick with skin and breast with skin from the right half of each bird were analyzed raw; whereas, pieces from the left half, and their resultant broth were analyzed following pressure cooking.

Chicken Meat and Broth Yields

Analyses of variance of percentage meat and broth yield (Table 7) indicate the level of PBBs fed did not significantly affect these parameters. The type of piece cooked, however, did significantly affect both average meat and broth yield.

The cooked meat yield (Table 8) for breast (61.6%) and thigh skin (59.2%) pieces was significantly higher ($P<0.01$) than the drumstick (53.3%) or thigh meat (50.2%) pieces. The average weight in grams of the pieces were 216.1 g, 74.6 g, 85.7g and 13.5 g for breast, drumstick, thigh and thigh skin, respectively. In comparing the average original piece weight, the breast piece weighed 2.5 times more than the next heaviest

Table 7. Analyses of variance for percentages of meat and broth yield.

Source	df	Meat Yield		Broth Yield	
		Mean Square	F Statistic	Mean Square	F Statistic
Total	59				
Level of PBB fed	4	45.24	2.19	93.66	1.61
Piece	3	409.58	19.81***	359.46	6.18**
Level x piece	12	17.13	0.83	26.41	0.45
Within	40	20.68		58.12	

** Significant at the 1% level of probability.

*** Significant at the 0.1% level of probability.

Table 8. Means[1] and standard deviations of the means for percentages cooked chicken meat yield.

Level of PBB Fed	Piece				PBB Level Average
	Breast	Drumstick	Thigh Meat	Thigh Skin	
0	60.3±3.5	54.0±1.1	51.2±1.2	57.2±4.3	55.7±4.3[a]
30	63.9±2.8	56.1±0.4	51.0±1.2	55.5±8.9	56.6±6.3[a]
45	63.6±2.2	53.4±1.9	51.5±0.8	66.7±8.7	58.8±7.8[a]
60[2]	58.9±2.9	50.9±2.9	47.2±2.2	56.6±2.5	53.4±5.3[a]
90[2]	61.2±1.3	51.8±1.2	50.2±0.9	59.9±13.2	55.8±7.6[a]
Piece Average	61.6±3.0[a]	53.3±2.4[b]	50.2±2.0[b]	59.2±8.3[a]	

[1] Based on 3 hens. Averages with the same superscript are not significantly different at $p<0.01$ (Duncan, 1957). The significant studentized range value for comparing two consecutive means ($p<0.05$) is 7.5.

[2] Fed clean feed 8 additional weeks before slaughter.

piece, and thus had less surface area per unit of weight which could contribute to the reduced cooking losses. Removal of skin may have contributed to greater loss from the thigh meat since Funk et al. (1968) reported ground beef cylinders wrapped in fat exhibited significantly lower cooking losses than did unwrapped cylinders.

The broth yield (Table 9) of the breast piece (89.6%) was significantly higher ($P<0.01$) than that of the thigh skin (77.7%). The breast pieces were comparatively so large that the moisture lost from them contributed more to the drip and therefore increased the broth yield more than did the smaller pieces.

Percentage Fat and Solids

Analyses of variance of percentage fat and solids in raw and cooked chicken pieces and broth (Table 10) indicate a highly significant difference ($P<0.001$) in percentage fat and percentage solids among states analyzed (raw, cooked and broth) and among the four pieces.

The average percentage of fat in the cooked chicken pieces (14.0%) were significantly lower ($P<0.001$) than in the raw pieces (21.1%) showing that rendering had occured during cooking (Table 11). In addition, the average fat content of the drumstick (4.6%) was less ($P<0.05$) than that of the breast piece (7.9%) or thigh meat (7.5%); both which had less fat ($P<0.01$) than the thigh skin (27.7%). Because of

Table 9. Means[1] and standard deviations of the means for percentage chicken broth yield.

Level of PBB Fed	Piece				PBB Level Average
	Breast	Drumstick	Thigh Meat	Thigh Skin	
0	87.3±10.6	83.3±6.1	76.9±17.3	74.0±8.0	80.4±11.1[a]
30	85.7±7.9	79.7±5.1	82.5±3.3	76.1±6.4	81.0±6.3[a]
45	91.7±8.0	80.1±8.0	84.4±5.0	77.6±4.1	83.7±8.3[a]
60[2]	87.9±10.4	83.1±7.2	81.3±1.8	82.7±7.0	83.8±6.7[a]
90[2]	95.4±8.5	85.3±4.0	91.0±2.2	78.1±6.7	87.5±8.3[a]
Piece Average	89.6±8.5[a]	82.3±5.7[b]	83.2±8.5[b]	77.7±6.2[b]	

[1] Based on 3 hens. Averages with the same superscript are not significantly different at $p<0.01$ (Duncan, 1957). The significant studentized range value for comparing two consecutive means ($p<0.05$) is 12.6.

[2] Clean feed 8 additional weeks before slaughter.

Table 10. Analyses of variance of percentage fat and solid in raw and cooked chicken pieces and chicken broth.

Source	df	Fat		Solids	
		Mean Square	F Statistic	Mean Square	F Statistic
Total	179				
State	2	6444.40	163.69***	32919.35	1530.42***
Piece	3	5065.46	128.66***	1912.99	88.93***
State x piece	6	1570.32	39.88***	1138.70	52.94***
Level of PBB fed	4	64.32	1.63	45.99	2.14
State x level	8	30.08	0.76	43.94	2.01
Piece x level	12	15.11	0.38	22.99	1.07
State x piece x level	24	11.76	0.30	15.90	0.74
Within	120	39.37		21.51	

*** Significant at 0.1% level of probability.

Table 11. Percent fat content[1] of raw and cooked chicken and chicken broth.

State[2]	Level of PBB Fed	Piece				PBB Level Average
		Breast	Drumstick	Thigh Meat	Thigh Skin	
Raw	0	11.4±5.2	8.4±5.4	14.9±3.9	54.1±1.6	
	30	14.4±7.2	9.2±4.9	15.9±7.1	55.0±2.1	
	45	10.3±3.2	6.0±1.2	9.6±2.7	53.3±13.3	
	60[3]	14.4±3.4	7.5±1.7	10.1±1.5	48.2±4.5	
	90[3]	12.3±8.3	7.3±2.6	14.2±6.5	56.5±18.5	
Cooked	0	8.4±2.6	6.7±2.2	8.8±2.9	27.3±3.8	11.9±15.2[a]
	30	13.4±6.7	6.4±2.3	11.8±6.0	40.6±4.5	14.1±17.0[a]
	45	8.9±0.5	5.4±2.4	9.1±2.0	30.6±14.0	11.3±15.8[a]
	60[3]	7.8±3.2	5.0±2.0	6.4±0.5	23.4±4.6	10.5±13.4[a]
	90[3]	11.4±4.3	5.9±2.4	8.9±3.0	33.7±18.3	12.0±16.7[a]
Broth	0	1.3±0.6	0.3±0.0	0.6±0.2	0.4±0.0	
	30	0.8±0.3	0.3±0.3	0.7±0.1	0.6±0.0	
	45	1.3±0.7	0.2±0.1	0.7±0.3	0.8±0.4	
	60[3]	1.3±0.6	0.4±0.1	0.5±0.4	0.4±0.1	
	90[3]	1.9±1.3	0.4±0.1	0.7±0.4	0.7±0.9	
Piece Average		7.9±6.1[b]	4.6±3.8[b]	7.5±6.2[b]	27.7±23.4[a]	

[1] Mean and standard deviation of the mean for 3 hens. Averages with the same superscript are not significantly different at $p<0.01$ (Duncan, 1957). The significant studentized range value for compraing two consecutive means ($p<0.05$) is 7.1.

[2] State Averages: raw - 21.1±19.4[a]; cooked - 14.0±11.8[b]; broth - 0.7±0.6[c].

[3] Fed clean feed 8 additional weeks before slaughter.

its higher fat content, the thigh skin also had significantly average higher ($P<0.01$) solids (37.5%) content than did the other chicken pieces which were 26.7, 24.6 and 22.4% for breast, thigh meat and drumstick, respectively (Table 12). Greater rendering of fat from thigh skin during cooking probably contributed to the significant interactions shown.

Neither the average lipid nor solids content were affected by the level of PBB fed to the hens. High standard deviations of the means of especially 45 and 90 ppm PBB fed birds, seen in percentage meat and broth yields (Tables 8 and 9) and percentage fat and solids (Tables 11 and 12) point out the great variability among birds.

Residue Analyses

Data concerning PBB levels in the raw and cooked chicken as well as in the chicken broth, expressed as ppm in wet tissue, ppm in fat and ppm in solids for each of the five levels fed are summarized in Tables 13, 14, and 15. Analyses of variance established a highly significant ($P<0.001$) interaction between state, piece and level PBB fed expressed as ppm wet weight (Table 16). On a ppm solid and ppm fat basis a highly significant difference between levels of PBB fed is seen. The major factor in this interaction was that a marked increase in ppm PBB residue was seen in each tissue with increase in level fed.

In this study control birds had low levels of PBBs in their tissue (Table 13); the highest level occured in the

Table 12. Percent solids content[1] of raw and cooked chicken and chicken broth.

State[2]	Level of PBB Fed	Piece				PBB Level Average
		Breast	Drumstick	Thigh Meat	Thigh Skin	
Raw	0	31.5±2.6	29.3±3.3	37.0±3.1	65.3±2.6	
	30	33.8±2.8	31.0±3.4	33.4±3.7	64.9±3.6	
	45	31.7±2.9	27.6±1.6	30.2±2.5	70.0±3.9	
	60[3]	34.7±1.8	28.4±2.0	30.7±1.5	59.6±3.3	
	90[3]	35.4±7.7	28.7±0.8	30.9±9.1	58.7±17.7	
Cooked	0	40.0±2.8	37.4±1.2	40.4±0.3	43.1±2.9	27.1±20.9[a]
	30	49.3±3.9	38.2±1.3	42.2±3.6	54.2±5.1	29.1±22.5[a]
	45	45.2±7.2	37.7±2.2	40.5±1.6	45.9±9.7	27.6±22.2[a]
	60[3]	41.2±4.4	37.0±1.2	39.3±1.5	40.2±4.4	26.2±19.8[a]
	90[3]	52.1±12.6	39.1±1.3	40.8±2.1	52.0±16.1	28.4±22.6[a]
Broth	0	1.3±0.6	0.3±0.0	0.6±0.2	0.4±0.0	
	30	0.8±0.3	0.3±0.2	0.7±0.1	0.6±0.0	
	45	1.3±0.7	0.2±0.1	0.7±0.3	0.8±0.4	
	60[3]	1.3±0.6	0.4±0.1	0.5±0.1	0.4±0.1	
	90[3]	1.0±1.3	0.4±0.1	0.7±0.4	0.7±0.9	
Piece Average		26.7±19.5[b]	22.4±16.3[c]	24.6±17.7[bc]	37.1±27.9[a]	

[1] Mean and standard deviation of the mean for 3 hens. Averages with the same superscript are not significantly different at $p<0.01$ (Duncan, 1957). The significant studentized range value for comparing two consecutive means ($p<0.05$) is 5.3.

[2] State Averages: raw - 39.6±15.0[b]; cooked - 42.8±7.1[a]; broth - 0.7±0.6[c].

[3] Fed clean feed 8 additional weeks before slaughter.

Table 13. PBB levels[1] expressed as ppm of wet tissue of raw and cooked chicken and chicken broth.

State[2]	Level of PBB Fed	ppm PBBs in Breast	Drumstick	Thigh Meat	Thigh Skin	PBB Level Average
Raw	0	0.02±0.02	0.02±0.01	0.04±0.02	0.07±0.05	
	30	4.04±0.77	4.76±2.16	4.15±1.61	26.73±8.13	
	45	8.06±3.61	5.24±0.64	5.67±2.12	25.77±14.09	
	60[3]	19.28±12.65	7.80±1.82	11.08±7.67	57.01±14.48	
	90[3]	23.17±11.68	11.23±7.53	13.42±8.95	46.35±15.18	
Cooked	0	0.01±0.00	0.01±0.01	0.04±0.02	0.04±0.01	0.02±0.03[a]
	30	5.42±1.97	5.86±2.48	8.44±5.91	16.00±1.94	6.61±8.06[b]
	45	7.00±2.75	8.16±2.73	8.45±3.96	35.31±10.26	9.02±11.47[b]
	60[3]	5.78±0.51	4.51±0.37	5.27±1.35	29.82±21.55	11.84±17.80[c]
	90[3]	17.70±11.75	10.41±4.23	11.33±7.07	51.30±8.91	15.65±17.96[d]
Broth	0	.003±.001	.001±.000	.006±.002	.001±.000	
	30	.181±.154	.161±.181	.171±.024	.096±.035	
	45	.431±.240	.098±.027	.343±.191	.383±.218	
	60[3]	.744±.327	.187±.025	.309±.213	.234±.202	
	90[3]	1.61±0.71	.311±.037	.572±.102	.390±.350	
Piece Average		6.23±8.83[a]	3.92±4.53[a]	8.06±6.00[a]	19.30±22.03[b]	

[1] Mean and standard deviation of the mean for 3 hens. Averages with the same superscript are not significantly different at $p<0.05$ (Duncan, 1957). The significant studentized range value for comparing two consecutive means ($p<0.05$) is 6.8.

[2] State Averages: raw - 14.03±16.48[a]; cooked - 11.54±14.13[b]; broth - 0.312±0.405[c].

[3] Fed clean feed for an additional 8 weeks before slaughter.

Table 14. PBB levels[1] expressed as ppm in fat of raw and cooked chicken and chicken broth.

State[2]	Level of PBB Fed	PBB expressed as ppm in fat of				PBB Level Average
		Breast	Drumstick	Thigh Meat	Thigh Skin	
Raw	0	0.18±0.11	0.26±0.07	0.26±0.09	0.14±0.09	
	30	32.6±15.7	53.1±10.6	53.5±29.7	46.4±18.2	
	45	154.9±157.0	88.7±10.4	86.9±15.3	51.7±33.6	
	60[3]	133.1±79.4	110.6±50.5	103.9±62.5	121.1±41.1	
	90	266.3±182.3	194.2±198.2	77.1±31.3	147.8±116.6	
Cooked	0	0.08±0.03	0.13±0.13	0.34±0.47	0.16±0.05	0.28±0.34[a]
	30	42.7±7.1	85.4±12.1	71.8±8.5	39.3±3.8	44.4±23.3[b]
	45	80.1±35.1	159.4±45.4	89.6±32.9	106.3±14.1	86.5±62.5[c]
	60[3]	81.4±28.4	103.1±44.9	83.1±26.9	130.8±97.3	91.1±52.1[c]
	90[3]	205.4±225.0	234.6±214.9	164.4±167.0	212.8±158.6	169.2±148.4[d]
Broth	0	0.28±0.24	0.18±0.01	1.15±0.58	0.22±0.10	
	30	19.8±10.2	45.9±26.8	24.3±7.2	18.2±10.2	
	45	34.1±16.7	56.8±34.1	48.0±9.6	48.1±7.8	
	60[3]	60.5±19.5	43.5±7.1	61.7±41.4	57.4±56.5	
	90[3]	163.7±199.4	83.1±10.3	159.0±172.3	122.3±157.3	
Piece Average		85.0±116.6[a]	83.9±94.3[a]	68.6±74.2[a]	73.5±63.5[a]	

[1] Mean and standard deviation of the mean for 3 hens. Averages with the same superscript are not significantly different at $p<0.05$ (Duncan, 1957). The significant studentized range value for comparing two consecutive means ($p<0.05$) is 93.7.

[2] State Averages: raw - 86.1±95.8[a]; cooked - 94.8±74.2[a]; broth - 52.6±75.5[b].

[3] Fed clean feed for an additional 8 weeks before slaughter.

Table 15. PBB levels[1] expressed as ppm in solids of raw and cooked chicken and chicken broth.

State[2]	Level of PBB Fed	PBB expressed as ppm of solids of				PBB Level Average
		Breast	Drumstick	Thigh Meat	Thigh Skin	
Raw	0	0.06±0.05	0.06±0.02	0.11±0.05	0.12±0.07	
	30	12.0±2.2	15.0±5.4	22.1±11.4	43.8±13.5	
	45[3]	26.0±12.4	19.5±1.9	26.9±5.6	37.5±22.1	
	60[3]	55.7±37.3	27.6±7.2	35.6±23.6	99.2±27.9	
	90[3]	63.4±19.3	39.5±27.5	33.4±10.4	79.1±10.2	
Cooked	0	0.02±0.00	0.03±0.03	0.22±0.10	0.10±0.03	0.21±0.34[a]
	30	11.3±4.9	14.4±6.4	19.6±8.3	29.3±2.2	23.0±14.4[b]
	45[3]	16.7±9.0	21.5±6.2	20.3±8.6	77.5±16.0	36.1±21.8[bc]
	60[3]	13.9±1.6	12.2±0.8	13.5±4.0	75.4±56.7	46.6±36.6[c]
	90[3]	41.0±39.1	26.9±11.9	28.4±19.3	107.0±48.1	78.9±89.5[d]
Broth	0	0.28±0.24	0.18±0.01	1.15±0.58	0.22±0.10	
	30	19.8±10.2	45.9±26.8	24.3±7.2	18.2±10.2	
	45[3]	34.1±16.7	56.8±34.1	48.0±9.6	48.1±7.8	
	60[3]	60.5±19.5	43.5±7.1	61.7±41.4	57.4±56.5	
	90[3]	163.7±199.4	83.1±10.3	159.0±172.3	122.3±157.3	
Piece Average		34.6±60.5[a]	27.1±25.7[a]	33.2±54.2[a]	53.0±55.9[b]	

[1] Mean and standard deviation of the mean for 3 hens. Averages with the same superscript are not significantly different at $p<0.05$ (Duncan, 1957). The significant studentized range value for comparing two consecutive means ($p<0.05$) is 50.1.

[2] State Averages: raw - 31.8±29.4[a]; cooked - 26.5±32.7[a]; broth - 52.6±75.5[b].

[3] Fed clean feed for an additional 8 weeks before slaughter.

Table 16. Analyses of variance of PBB levels in hens.

Source	df	ppm Wet		ppm Solid		ppm Fat	
		Mean Square	F Statistic	Mean Square	F Statistic	Mean Square	F Statistic
Total	179						
State	2	3204.18	88.44***	11424.79	5.94**	31301.91	4.65*
Piece	3	2318.21	63.99***	5632.63	2.93*	2669.52	0.40
State x piece	6	602.72	16.64***	2268.80	1.18	5250.21	0.78
Level	4	1420.93	34.25***	30588.78	15.92***	141594.69	21.05***
State x level	8	385.32	10.64***	4564.48	2.37*	3729.42	0.55
Piece x level	12	226.70	6.26***	1251.54	0.65	2615.49	0.39
State x piece x level	24	88.76	2.45***	654.93	0.34	2129.77	0.32
Within	120	36.23		1921.96		6727.51	

* Significant at 5.0% level of probability.
** Significant at 1.0% level of probability.
*** Significant at 0.1% level of probability.

high fat containing thigh skin. This unexpected presence of a low level of contamination may have resulted from PBB particles being airborne on dust. Although all groups of birds were housed in separate cages, they were all in the same cage room and were fed their respective rations at the same time. The blanks that were constantly run showed no PBB contamination and hence the birds themselves must have been contaminated and not reagents or glassware.

The levels of PBBs in the tissues increased progressively with increasing levels in the feed and were highest in the high fat thigh skin (Figure 2). This phenomenon was also shown by Ritchey et al. (1967) where raw tissues were found to contain residue levels reflecting DDT and lindane intake.

It was expected that the PBB levels in the tissues of hens fed feed containing 60 and 90 ppm PBBs and which were subsequently fed "clean food" for eight additional weeks, would be lower than that in tissue from birds fed 30 and 45 ppm PBB in the feed since the half life of eggs, liver and muscle had been found to be approximately 3 weeks (Polin and Ringer, 1975). The samples in this study, however, either contained skin or were higher in fat and thus these factors may have affected our reduced rate of elimination. This could be in agreement with studies that have shown PBB accumulates to a greater degree and is metabolically eliminated more slowly from fatty tissue than from liver and muscle (Gutenman and Lisk, 1975: Fries et al., 1973a). In addition, Stadelman et al. (1965) reported that feeding high levels of

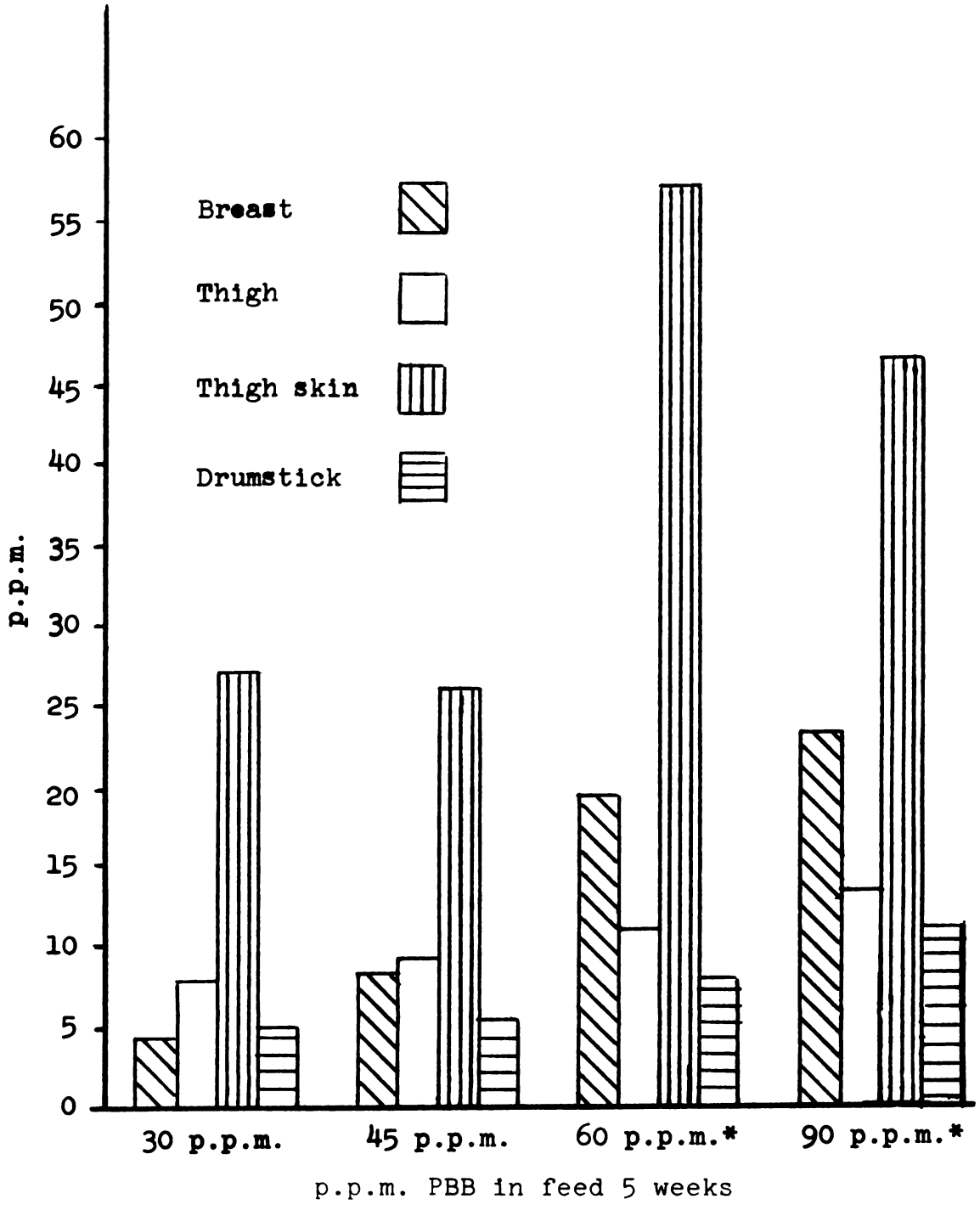

Figure 2. PBBs in raw hen tissue, ppm wet weight basis.

* Birds were fed clean feed for 8 additional weeks.

several pesticides resulted in greater residue persistance in eggs and abdominal fat of hens than was found when low levels were fed.

As can be seen from the high standard deviations of the means in Tables 13, 14 and 15 wide variation in the PBB levels and fat content occured in the birds within each group. However, duplicate values (within 1-10% difference except samples from 0 ppm PBB fed birds where differences varied as much as 50-100% due to limits of sensitivity) on any one tissue gave reasonable agreement.

PBB in Wet Tissue

When the chicken pieces were cooked, the level of PBBs in the wet tissue decreased slightly (Table 13). Part of the PBB lost was recovered in the broth. The average level of 0.74 ppm PBBs in the broth of the breast pieces from birds fed 30, 45, 60 and 90 ppm PBB was higher than the average level in the broth of the other pieces. The drumstick, thigh, and thigh skin contained a mean ppm PBB in their broth of 0.19, 0.35 and 0.28, respectively. Since the amount of cooking water was constant, greater amounts of PBBs in the breast broth resulted from the larger amount of fat rendered from the larger piece.

Figure 3 depicts the reduction of ppm PBB in cooked pieces expressed on a wet weight basis. The greatest reduction is seen in the higher fat thigh skin and large breast pieces, and the least in the low fat drumstick. These data

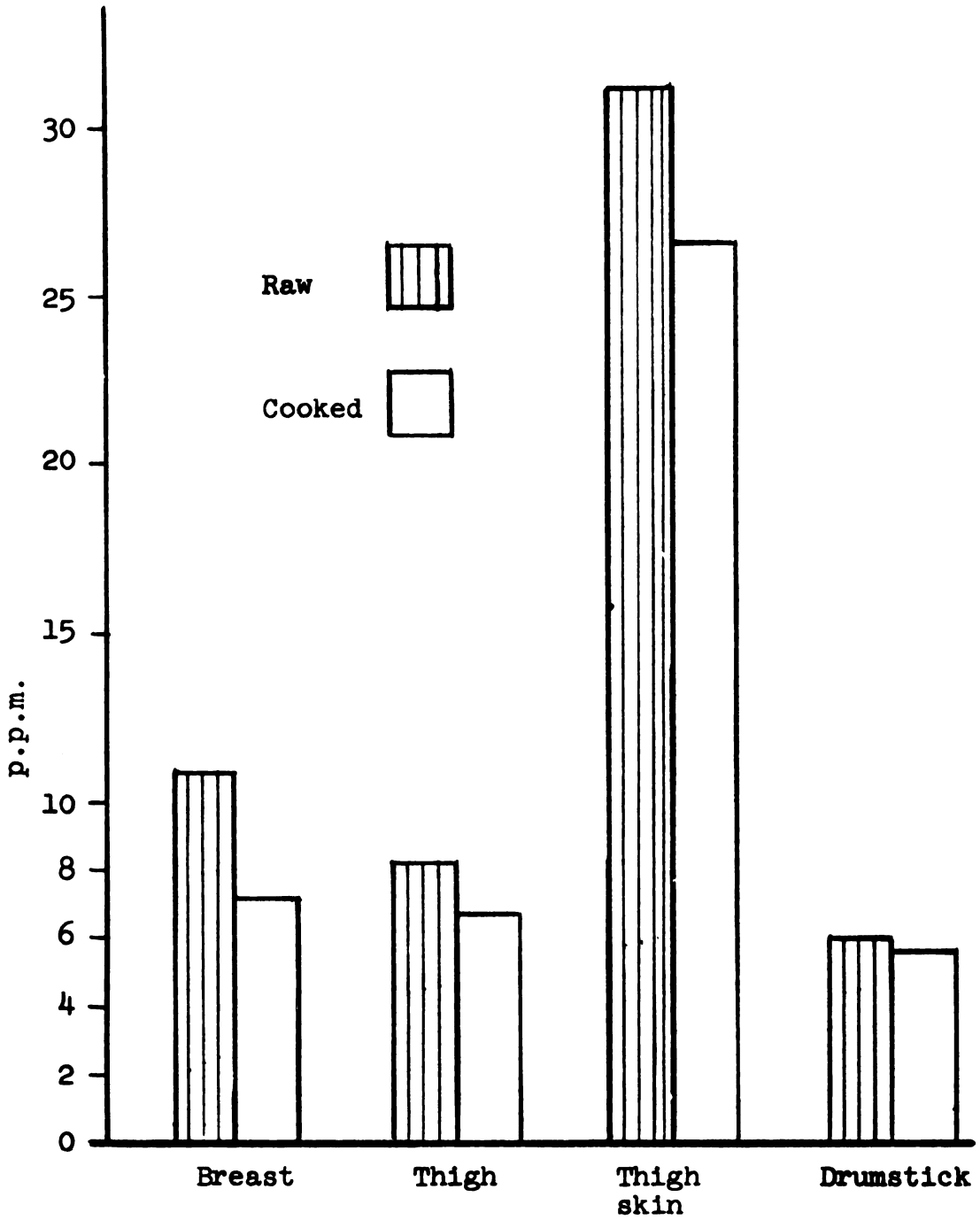

Figure 3. PBBs in wet tissue of raw and cooked hen pieces (data shown are composite of all 15 birds analyzed).

reflect the preferential distribution of PBBs in the lipid phase and rendering of PBBs with the fat appears to be a mode of removal. Several previous studies (Ritchey et al., 1967, 1969, 1972; Liska et al., 1967; Morgan et al., 1972; Zabik, 1974) have indicated that rendering during cooking significantly reduced levels of chlorinated hydrocarbon pesticides in chicken.

PBB Content on a Fat and Solids Basis

When the PBB content was expressed on a fat basis there was no significant difference among chicken pieces (Table 14). On a solids basis the thigh skin had a higher ($P<0.05$) level of PBBs than the other pieces (Table 15). Cooking did not significantly affect the level of PBBs expressed on a solids basis, however, the average PBBs in the fat of the broth (52.6 ppm) was less ($P<0.05$) than that in the fat of the raw (86.1 ppm) or cooked pieces (92.2 ppm). Thus, the average values in the fat of cooked pieces were slightly higher than those in the fat of raw pieces (Figure 4). While rendering of fat is undoubtedly an important mode of PBB reduction, the amount of PBB reduced is not directly proportional to fat removal.

Supporting this finding Lee et al. (1970) also observed this nonproportionality to fat removal in work with several chlorinated hydrocarbon pesticides. Murata et al. (1976) found PBB levels were higher in butter, cheese and freeze-dried cream than levels in buttermilk, cheese whey and cream,

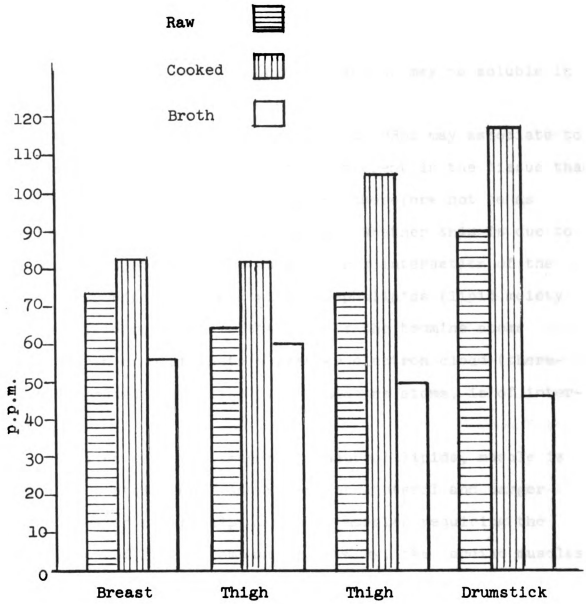

Figure 4. PBBs in the fat of hen pieces; raw, cooked and broth state (data shown are a composite of all 15 birds analyzed).

respectively, reflecting the preferential distribution of PBBs in the lipid phase of the products. However, the researchers also found PBB levels in skim milk, buttermilk, and cheese whey were slightly higher than PBB levels in pasteurized whole milk, per fat unit, suggesting some PBB may be associated with lipoprotein and or may be soluble in the "serum" of milk.

Data from this study suggest that PBBs may associate to a greater extent with lipoproteins present in the tissue than some other lipophilic compounds and therefore not be as easily removed with fat in cooking. Whether this is due to chemical properties, such as greater π interaction of the parent PBB biphenyl rings with phospholipids (lipid moiety of some lipoproteins) or the size of the bromine atoms attached which contribute a greater electron cloud (therefore decreased solubility) than chlorine atoms, is of interest.

Besides variable amounts of neutral lipids, muscle is found to contain small amounts of cholesterol and larger quantities of phospholipid. Those muscles requiring the production of greater amounts of energy, the cardiac muscles and the muscles of locomotion, contain the greater amount of phospholipid, and tend to be darker in color (Orten and Neihaus, 1975). Lawrie (1966) concurs with this and states; dark (red) muscles are characterized by being high in respiratory activity and constantly in action, light(white) muscles generally operate in short bursts of activity with little

capacity for respiratory activity.

In a work by Peng (1965) it was found the phospholipid content of total lipids in chicken leg muscle (dark meat) tend to be higher than in lipids of the breast muscle (white meat). Katz et al. (1966) found the opposite to be the case with the phospholipid content of poultry white meat being higher than the phospholipid content of dark meat. However, the poultry tissue contained by far the greater percentage of phospholipids than the skin and depot fat.

Beecher et al. (1968) in studying pork muscle, stated total lipid content tends to be higher in light (white) muscle than in dark (red) muscle and muscles with lower total lipids possess a greater proportion of phospholipids. In agreement with this, Luddy et al. (1970) found the dark portion of the semitendinosis muscle of pork contained a greater proportion of phospholipids than did the white; that is the light muscle contained 20% more lipid, 20% more glycerides, and 40% less phospholipids than the the dark muscle.

In addition, Yadrick et al. (1971) reported that dark pork muscles with a lower percentage neutral lipid content possessed the greater percentage of phospholipids compared to the light pork muscle studied. The authors also found increased dieldrin levels in dark muscle and lower percentage reduction of dieldrin the cooking process. This may mean dieldrin follows phospholipid disproportionately.

Looking at the fatty acid content of phospholipids present in muscle it was shown by Luddy et al. (1970) that the

fatty acids of light pork muscles are predominately monoenes while the dark muscle phospholipid fatty acids were higher in polyunsaturates. Kutz et al. (1966) found poultry tissue, both white and dark meat, to be higher in the fatty acid arachidonic acid than was skin or depot fat. In addition, Peng (1965) noted fatty acid composition of the phospholipid sphingomyelins was such that the dark meat contained a number of the longer chain fatty acids from C_{21}-C_{25} whereas the white muscle had none. Polyenoic acids may have greater π interactions with the parent rings of PBB and hence hold them tighter than monoenoic acids. The conclusion could be drawn that structure may contribute to increased bonding with halogenated hydrocarbons and perhaps more so with brominated hydrocarbons and decreases their liklihood of following the fat proportionately in rendering out of a meat during cooking. In this study there was less reduction of PBBs in cooking of the drumsticks which consist of dark muscle with increased levels of phospholipids.

Recovery and Distribution of PBBs After Cooking

Total micrograms of PBBs in the cooked chicken and broth were compared to the level in the respective raw chicken piece to calculate the percentage PBB recovery. No significant differences occurred among the percentage of PBB recovered in any of the four pieces; total percentage recoveries from raw were 68.1% in thigh skin, 75.8% in the breast piece,

83.9% in the thigh meat, and 84.6% in drumsticks. PBB recoveries, however, did tend to be higher in the chicken pieces which contained less fat (i.e. drumstick and thigh meat) even though these pieces had lower percentage meat yields (greater cooking losses) (Table 8) than did the higher fat breast piece or thigh skin. This may again be pointing to greater association of PBBs with phospholipids in tissue.

The distribution of the recovered PBBs between the cooked meat and broth is illustrated in Figure 5. The percentage of recovered PBBs in the meat did not differ significantly among the four pieces evaluated and ranged from 65.5% in the cooked thigh skin, 67.1% in cooked thigh, 71.2% in cooked breast to 72.9% in the cooked drumstick. The proportion of the recovered PBB in the cooked meat is considerably higher than that found in previous studies. Recovered PCBs were about equally distributed between the cooked meats and broth (Zabik, 1974) while only 1/4 to 1/3 of recovered lindane, dieldrin and DDT compounds occurred in the cooked hen pieces (Morgan et al., 1972). The bulkier size and higher molecular weight of the PBB molecules in comparison to the organochlorine pesticides studied, may contribute to a smaller proportion of the recovered PBB material being found in the broth.

Another factor in the recovery of greater amounts of PBBs in the meat as compared to other studies, might be the level of contamination of the tissue from which the PBBs are being extracted. Murata et al. (1976) observed losses of PBB from spray dried skim milk (61-69%) were greater than from

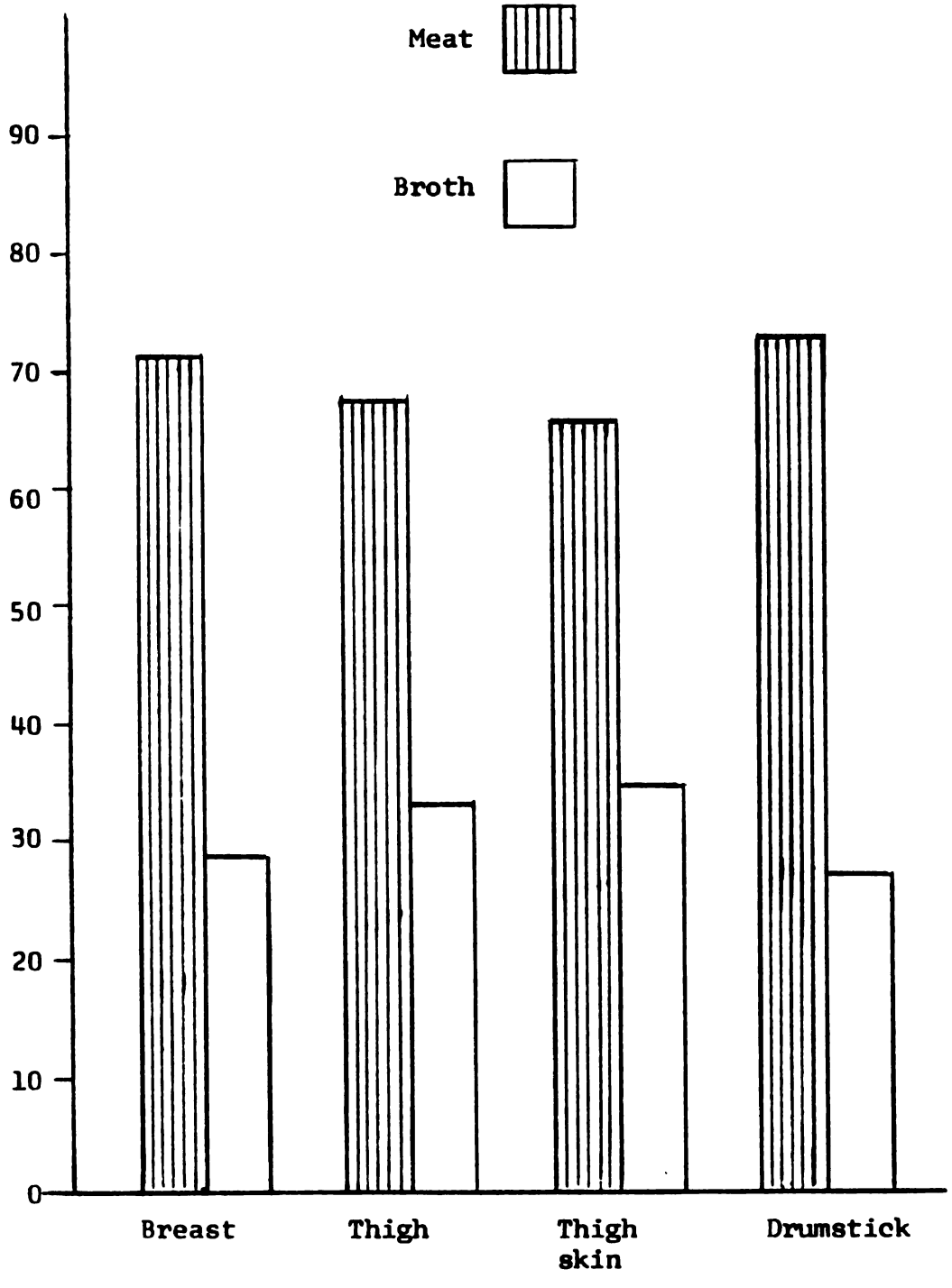

Figure 5. Distribution of recovered PBBs between cooked tissue and broth (data shown is a composite of all 15 birds analyzed).

spray-dried whole milk (30-36%). These results suggested to the authors that PBBs may be more easily removed from low fat products and/or when PBB levels in the original product were low. In this study, the levels of contaminants fed were quite high (up to 90 ppm) compared to the 25 ppm fed by Zabik (1974). Thus, these high levels of PBB contamination may have influenced the distribution recovered. Also of interest, for pieces from control hens which showed low level contamination (0.02-0.07 ppm wet, 0.14-0.26 ppm fat basis in raw tissue, Figures 14 and 15) the proportion of recovered PBBs in the meat was slightly less, ranging from 39.8% in the drumstick to 58.7% in the thigh meat. Therefore, it is possible cooking has an even greater potential for reducing low levels of PBBs such as might have reached the market during 1973-1974.

SUMMARY AND CONCLUSIONS

Thigh meat, thigh skin, drumsticks with skin, and breast with skin were analyzed to investigate the effectiveness of cooking in reducing PBB residue levels in contaminated chicken tissue. Pieces from the right half were analyzed raw whereas pieces from the left half, and their resultant broth, were analyzed following pressure cooking (15 psi for 15 min). These hens had been fed either 0, 30, 45, 60, and 90 ppm PBBs.

Analyses of variance of percentage meat and broth yield indicate the level of PBBs fed did not significantly affect these parameters. However, the type of piece cooked did significantly affect both average meat and broth yields, with the breast and thigh skin (the higher percentage fat pieces, 7.9 and 27.7%, respectively) producing the greatest yields of cooked meat (61.6 and 59.2%, respectively). Also the broth yield of the breast pieces was significantly higher than the broth yield of the thigh skin pieces.

Analyses of variance of percentage fat and solids in raw and cooked chicken pieces and broth indicated a highly significant difference in percentage fat and percentage solid between states analyzed (raw, cooked and broth) and among four pieces. The percentage fat of raw and cooked and broth was 21.1, 14.0, and 0.7%, respectively; and of the four

pieces, breast with skin was 7.9%, drumstick with skin was 4.6%, thigh meat was 7.5%, and thigh skin was 27.4% fat. These data indicate a rendering out of fat in the cooking process and show that breast meat and thigh skin had the highest percentage fat. The breast was considered higher in fat than thigh meat even though it was very close in percentage fat mainly because of its large size. The breast pieces averaged 2 1/2 times the size of the next smallest piece which was the thigh piece.

PBB residue analysis on a wet basis revealed that PBB levels increased with increasing levels fed, were the highest in high fat pieces and were reduced in the cooked sample from the paired raw sample. These data reflect the preferential distribution of PBBs in the lipid phase and rendering of PBBs with the fat appears to be a mode of removal.

Residue analyses on a fat basis revealed a highly significant difference between levels of PBB fed - as level fed increased, ppm PBB residue increased. The birds fed 60 and 90 ppm PBB in their ration for five weeks and then on "clean feed" for 8 additional weeks before slaughter were expected to show levels similar to the 30 and 45 ppm level fed birds since the half life in eggs, liver and muscle had been found to be approximately 3 weeks. However, the samples either contained skin or were higher in fat content and the slower rate of elimination of residue shown here would be in agreement with studies showing that PBB accumulates to a greater degree and is metabolically eliminated more slowly from fatty

tissue than from liver and muscle.

On a fat basis there was no significant difference among pieces in PBB residue content. In cooking the average PBB residue content of the fat in the broth was less than in the fat of the raw or cooked pieces, i.e. average values in the fat of cooked pieces were slightly higher than those in the fat of the raw pieces. Therefore, while rendering of fat is an important mode of PBB reduction, the amount reduced is not directly proportional to the amount of fat removed as has been shown in some work with PCBs. The reasons for nonproportionality of fat and PBB removal may be due to increased π bonding of parent PBB biphenyl rings with phospholipids in the tissue, or the larger size of the bromine atom which would contribute a larger electron cloud with increased association and possibly reduced fat solubility.

Total micrograms of PBBs in the cooked chicken and broth were compared to the level in the respective raw chicken piece to calculate the percentage recovery. Percentage recoveries were 68.1, 75.8, 83.9 and 84.6% for thigh skin, breast, thigh meat and drumstick, respectively. PBB recoveries tended to be higher in the chicken pieces with less fat (i.e. drumstick and thigh meat) even though these pieces had greater cooking losses.

The percentage of recovered PBBs in the cooked meat did not differ significantly among the four pieces analyzed, the range was from 65.5% in thigh skin to 72.9% in drumstick pieces. The proportions of recovered PBBs in cooked meat

was considerably higher than that recovered in previous studies. The high levels of contamination may have contributed to the lower amounts found in the broth since several studies have indicated halogenated hydrocarbons may be more easily removed from low fat products and/or when PBB levels in the original product were low. Thus, the percentage of recovered PBBs in the tissue of control birds (low level contamination samples) was slightly less ranging from only 39.8% in drumsticks to 58.7% in thigh meat. It appears cooking may have an even greater potential for reducing low levels of PBB such as might have reached the market during 1973-1974.

SUGGESTIONS FOR FUTURE RESEARCH

In this study the lipophilic compound PBB did not completely follow the fat in the process of rendering out of fat in cooking. The reasons for this and under what conditions more PBBs could be removed in processing should be investigated. Studies with light and dark meat which contain different lipoproteins and different levels of lipoproteins might be used in such a study.

The correlated higher reduction of PBBs in lower fat content tissue should be elaborated further with a larger number of samples with varying PBBs and lipid concentrations.

Greater percentage reduction of PBBs from low PBB residue level tissue was observed. This was shown in 0 ppm fed birds which were contaminated possibly due to airborne dust carrying PBB. An evaluation of this result could be done with the two above studies.

Studies done with DDT elimination from poultry tissue have found some retention of DDD (the converted DDT isomer) in the meat. Some unknown peaks appeared in this study, particularly at low levels where the sample was highly concentrated. This may possibly be due to partial debromination of the PBBs in the processing or perhaps through metabolism. These peaks should be further investigated. This

should then be extended to include a toxicological evaluation of lower brominated biphenyl compounds.

This study as observed differences in PBB and PCB in ease of removal from tissue, and the maintenance of high tissue levels following a clean diet. This may be due to chemical differences between chlorine and bromine atoms or it may be due to solubility differences between chlorine and bromine atoms. Some of the answers to these differences could be elucidated in the above suggested studies, but further work looking at these specific questions should be pursued.

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APPENDIX A

Layer Ration for PBB Experiments[1]

Ingredient	Parts/1000
Corn, #2 yellow	572.2
Soybean meal, 50%	200
Meat and bone meal	30
Alfalfa, 17%	40
Tallow	55
Methionine hydroxy analogue	0.8
Dicalcium phosphate	18
Limestone	69
Salt, iodized	3
Choline chloride, 50%	2
Vitamin mix[2]	5
Mineral mix[3]	5

[1] Calculated analysis: protein - 17.5%; ME - 3.02 kcal/g; Ca - 3.5%; P (available) - 0.6%; fat - 8.3%; fiber - 2.8%; methionine - 2.0% of protein.

[2] Vitamin mix supplies per kg: vitamin A - 10,000 IU; vitamin D_3 - 1,000 ICU; dl-x-tocopherol - 10 IU; menadione sodium bisulfite - 4.0 mg; riboflavin - 4.0 mg; niacin - 10.0 mg; biotin - 100 mcg; vitamin B_{12} - 8 mcg; pyridoxine - 4.0 mg; ethoxyquin - 125 mg.

[3] Mineral mix supplies per kg: magnesium - 500 mg; manganese - 50 mg; zinc - 50 mg; copper - 5 mg; iodine - 1.0 mg; cobalt - 3 mg.

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Polybrominated Biphenyl Levels in Raw and Cooked Chicken and Chicken Broth[1]

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ABSTRACT S.C.W.L. laying hens were fed either 0, 30, 45, 60 or 90 p.p.m. PBBs for five weeks. Hens fed 0, 30 and 45 p.p.m. of PBBs were sacrificed at the end of this five week period whereas those hens fed 60 and 90 p.p.m. of PBBs were fed a clean standard laying ration for an additional eight weeks at which time they were sacrificed.

Thigh meat, thigh skin, drumstick and breast (with skin) from half of each bird were analyzed raw whereas pieces from the other half were analyzed following pressure cooking separately in 500 ml. of deionized water at 15 p.s.i. for 15 min. Cooking yields were obtained, and the cooked meat and broth were analyzed separately for PBBs.

PBB residues were found in all tissues analyzed from birds receiving each level of PBB treatment with considerable bird variation in quantity found. Residue levels in tissue increased with increased levels of PBB in feed, and were related to fat level in tissue (wet basis). The level of PBBs expressed as p.p.m. on a solids basis were lower in the cooked sample than in corresponding raw piece with part of the PBBs lost being found in the drip. Recoveries of PBBs in cooked tissue and broth ranged from 68.1% in the thigh skin to 84.6% in drumstick with approximately two-thirds of the recovered PBBs found in the cooked meat itself.

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INTRODUCTION

The growing concern over environmental contamination most likely stems from the rapid influx of synthetic compounds and their accompanying waste materials. Of these compounds, much attention has been focused on halogenated hydrocarbons. Organochlorine pesticides and polychlorinated biphenyl (PCBs) with their lipophylic properties have been found in the fat of virtually all animals, and have been detected in human tissues as well (Biros *et al.*, 1970; Fujiwaru, 1975; Musial *et al.*, 1974). These compounds resist biological degradation (Fries *et al.*, 1973) and consequently are expected to be present in the ecosystem for an extended period of time. Occurrences of contamination in human tissue are generally associated with ingestion of food containing low levels of these compounds. Although precise information concerning toxicity of halogenated compounds at very low levels is not known, the concern is for possible chronic effects of their continual assimilation and accumulation in body fat.

Polybrominated biphenyls (PBBs) are members of the halogenated hydrocarbon class of compounds with structure, reactivity, use and toxicity similar to those attributed to PCBs of higher chlorination. Michigan Chemical Corporation manufactured a product called Firemaster® BP-6. BP-6 has an average of 6 bromine atoms attached to the biphenyl rings and to meet specifications is brought up to 75% bromine by weight. General properties include: solid at room temperature with a softening point of 72°C.; decomposes at 300–400°C.; very low solubility in water, 11 p.p.b. at 25°C.; soluble in most organic solvents; and a vapor pressure of 5.2×10^{-6} mm. Hg at 25°C. (Kerst, 1974). PBBs are industrial compounds used mostly as fire retardants and plasticers. Some examples of major uses are business machines, electrical uses, and fabricated products. PBBs are not used in food or feed, nor are they used in products that come in contact with human skin as in flame retarding fabrics (Kerst, 1974).

However, in May, 1973, an incredible error was made during feed manufacture in Michigan: the PBB compounds, Firemaster® FF-1[2] was mistaken for magnesium oxide (trade name Nutrimaster) and mixed into high protein dairy pellets (Jackson and Halbert, 1974; Dunckel,

[1] Michigan Agricultural Experiment Station Journal Article No. 7895.

[2] Firemaster® BP-6 mixed with the anticaking agent "Flo-Gard" which is manufactured by PPG Industries and which contains 83% silicone dioxide and a maximum of 7% calcium oxide.

1975). Through this contamination and consequent cross contamination of other feeds, dairy animals, poultry and swine were affected. This mistake has cost Michigan farmers untold dollars in loss of animals, income from those animals, and the cost of clean-up procedures to rid their farms of this contaminant. In the poultry industry alone, approximately two million birds and nearly five million eggs were destroyed.

Toxicity studies have shown PBBs to have a similar mode of action to other halogenated compounds in that they produced porphyria, liver injury, tremor and loss of weight (Strik, 1973; Jackson and Halbert, 1974; Prewitt *et al.*, 1975; Babish *et al.*, 1975; Lee *et al.*, 1975; Aftosmis *et al.*, 1972). Feeding studies have shown that bromine levels in biological systems elevate rapidly and then plateau but when the animals are fed on "clean feed" again the levels decrease. This decrease occurs more rapidly in milk, eggs, liver and muscle than in fatty tissue which loses its PBB store much more slowly.

Studies concerning the possibility of removing halogenated compounds from food stuffs upon processing are of great interest. Several studies have been conducted concerning the removal of lipophylic compounds from such products as poultry (Zabik, 1974; Morgan *et al.*, 1972; Ritchey *et al.*, 1967, 1969, 1972); eggs (Zabik and Dugan, 1971); sausage patties (Funk *et al.*, 1971); bacon (Yadrick *et al.*, 1971); pork loins (Maul *et al.*, 1971); pork muscles (Yadrick *et al.*, 1972); and beef loaves with texturized soy (Shafer and Zabik, 1975). Most of the losses seen were attributed to fat rendering or leaching out during cooking—the more severe the rendering the greater the loss. Different levels of success in elimination of the contaminants were seen depending upon the compound worked with, the levels of contamination and the tissue from which the extraction was accomplished.

The purpose of this study was to investigate the effect of cooking on the PBB levels in chicken tissue and chicken broth. The ease of residue removal from skin in contrast to tissue was evaluated by cooking thigh meat and skin separately.

METHODS AND MATERIALS

Sample: Fifteen White Leghorn hens approximately 9 months of age were fed a standard cage layer ration for 5 weeks which was contaminated with 0, 30, 45, 60, and 90 p.p.m. Firemaster® FF-1 (3 hens/feeding level). At the end of five weeks the first three groups (0, 30 and 45 p.p.m. levels) were slaughtered. The remaining two groups were placed on clean feed for an additional 8 weeks and then slaughtered. At the time of slaughter the hens were picked, eviscerated and chilled overnight in ice water before being carefully dissected to provide breast pieces, drumsticks, thigh meat, and thigh skin from the right side for raw analyses and those from the left side for cooking and subsequent analyses. The breasts were split so the keelbone remained with the half to be cooked. All samples were wrapped in aluminum foil, labeled as to bird identification, p.p.m. in feed, piece and state in which they were to be analyzed. The samples were then frozen and held at −20°C. until analyzed.

Samples, as they were analyzed, were removed from the freezer and thawed at 4−5°C. for approximately 24 hours. The raw samples were deboned, the meat and skin of the breast and drumstick were combined, while the thigh and thigh skin were kept separate.

Samples were homogenized using a Waring blender; the amount necessary for analysis was removed, and the remainder frozen in glass jars with foil-lined lids.

Cooking: Each chicken piece to be cooked was processed under 15 p.s.i. pressure for 15 minutes in 500 ml. deionized water, in a 3.8 l. aluminum pressure sauce pan. After the pan had cooled 5 minutes the pressure was released. Percentage yields of cooked chicken pieces were calculated as the weight of cooked chicken pieces minus bone divided by the raw piece weight times 100.

Broth percentage yields were calculated as the cooked broth volume divided by 500 ml. times 100. Cooked samples were homogenized, placed in glass jars, with foil-lined lids and then frozen and held at −20°C. pending analysis. The broth was not frozen to eliminate the possible problem of bottle breakage upon freezing; but rather held 4−5°C. in glass bottles and analyzed within one month.

Analyses: Moisture and lipid determinations were carried out in duplicate by drying at 100°C. and 70°C., respectively, under vacuum of 711 mm. (28 in.) Hg. Lipid samples dried were aliquots of the petroleum ether extracts used for PBB analyses.

Duplicate 4−6 g. samples of raw and cooked breast and drumstick (with skin) and thigh

TABLE 1. - *Means[a] and standard deviations of the mean for the percentage chicken meat and broth yield*

Measure	Level of PBB fed	Piece			
		Breast	Drumstick	Thigh meat	Thigh skin
Meat yield %	0	60.3 ± 3.5	54.0 ± 1.1	51.2 ± 1.2	57.2 ± 4.3
	30	63.9 ± 2.8	56.1 ± 0.4	51.0 ± 1.2	55.5 ± 8.9
	45	63.6 ± 2.2	53.4 ± 1.9	51.5 ± 0.8	66.7 ± 8.7
	60[b]	58.9 ± 2.9	50.9 ± 2.9	47.2 ± 2.2	56.6 ± 2.5
	90[b]	61.2 ± 1.3	51.8 ± 1.2	50.2 ± 0.9	59.9 ± 13.2
Broth yield %	0	87.3 ± 10.6	83.3 ± 6.1	76.9 ± 17.3	74.0 ± 8.0
	30	85.7 ± 7.9	79.7 ± 5.1	82.5 ± 3.3	76.1 ± 6.4
	45	91.7 ± 8.0	80.1 ± 8.0	84.4 ± 5.0	77.6 ± 4.1
	60[b]	87.9 ± 10.4	83.1 ± 7.2	81.3 ± 1.8	82.7 ± 7.0
	90[b]	95.4 ± 8.5	85.3 ± 4.0	91.0 ± 2.2	78.1 ± 6.7

[a]Based on 3 hens. Significant studentized range values for comparing two consecutive means ($P<0.05$) are 7.5 and 12.6 for percentage meat and broth yield, respectively (Duncan, 1957).

[b]Fed clean feed 8 additional weeks before slaughter.

TABLE 2. – *Fat and solids content[a] of raw and cooked chicken as well as chicken broth*

Measure	State	Level of PBB fed	Piece			
			Breast	Drumstick	Thigh meat	Thigh skin
Fat %	Raw	0	11.4 ± 5.2	8.4 ± 5.4	14.9 ± 3.9	54.1 ± 1.6
		30	14.4 ± 7.2	9.2 ± 4.9	15.9 ± 7.1	55.0 ± 2.1
		45	10.3 ± 3.2	6.0 ± 1.2	9.6 ± 2.7	53.3 ± 13.3
		60[b]	14.4 ± 3.4	7.5 ± 1.7	10.1 ± 1.5	48.2 ± 4.5
		90[b]	12.3 ± 8.3	7.3 ± 2.6	14.2 ± 6.5	56.5 ± 18.5
	Cooked	0	8.4 ± 2.6	6.7 ± 2.2	8.8 ± 2.9	27.3 ± 3.8
		30	13.4 ± 6.7	6.4 ± 2.3	11.8 ± 6.0	40.6 ± 4.5
		45	8.9 ± 0.5	5.4 ± 2.4	9.1 ± 2.0	30.6 ± 14.0
		60[b]	7.8 ± 3.2	5.0 ± 2.0	6.4 ± 0.5	23.4 ± 4.6
		90[b]	11.4 ± 4.3	5.9 ± 2.4	8.9 ± 3.0	33.7 ± 18.3
	Broth	0	1.3 ± 0.6	0.3 ± 0.0	0.6 ± 0.2	0.4 ± 0.0
		30	0.8 ± 0.3	0.3 ± 0.2	0.7 ± 0.1	0.6 ± 0.0
		45	1.3 ± 0.7	0.2 ± 0.1	0.7 ± 0.3	0.8 ± 0.4
		60[b]	1.3 ± 0.6	0.4 ± 0.1	0.5 ± 0.4	0.4 ± 0.1
		90[b]	1.9 ± 1.3	0.4 ± 0.1	0.7 ± 0.4	0.7 ± 0.9
Solids %	Raw	0	31.5 ± 2.6	29.3 ± 3.3	37.0 ± 3.1	65.3 ± 2.6
		30	33.8 ± 2.8	31.0 ± 3.4	33.4 ± 3.7	64.9 ± 3.6
		45	31.7 ± 2.9	27.6 ± 1.6	30.2 ± 2.5	70.0 ± 4.9
		60[b]	34.7 ± 1.8	28.4 ± 2.0	30.7 ± 1.5	59.6 ± 3.3
		90[b]	35.4 ± 7.7	28.7 ± 0.8	30.9 ± 9.1	58.7 ± 17.7
	Cooked	0	40.0 ± 2.8	37.4 ± 1.2	40.4 ± 0.3	43.1 ± 2.9
		30	49.3 ± 3.9	38.2 ± 1.3	42.2 ± 3.6	54.2 ± 5.1
		45	45.2 ± 7.2	37.7 ± 2.2	40.5 ± 1.6	45.9 ± 9.7
		60[b]	41.8 ± 4.4	37.0 ± 1.2	39.3 ± 1.5	40.2 ± 4.4
		90[b]	52.1 ± 12.6	39.1 ± 1.3	40.8 ± 2.1	52.0 ± 16.1

[a]Mean and standard deviation of the mean for 3 hens. Significant studentized range values for comparing two consecutive means ($P<0.05$) are 7.1 and 5.3 for percentage fat and moisture, respectively (Duncan, 1957).

[b]Fed clean feed 8 additional weeks before slaughter.

TABLE 3. – *PBB levels[a] of raw and cooked chicken as well as chicken broth*

State	Level of PBB fed	PBB expressed as p.p.m. in wet tissue of				PBB expressed as p.p.m. of solids of				PBB expressed as p.p.m. in fat of			
		Breast	Drum-stick	Thigh meat	Thigh skin	Breast	Drum-stick	Thigh meat	Thigh skin	Breast	Drum-stick	Thigh meat	Thigh skin
Raw	0	0.02 ± 0.02	0.02 ± 0.01	0.04 ± 0.02	0.07 ± 0.05	0.06 ± 0.05	0.06 ± 0.02	0.11 ± 0.05	0.12 ± 0.07	0.18 ± 0.11	0.24 ± 0.07	0.26 ± 0.12	0.14 ± 0.09
	30	4.04 ± 0.77	4.76 ± 2.16	4.15 ± 1.61	26.73 ± 8.13	11.96 ± 2.16	14.99 ± 5.36	22.07 ±11.39	43.77 ± 13.51	32.63 ± 15.74	53.13 ± 10.58	53.47 ± 29.73	46.37 ± 18.20
	45	8.06 ± 3.61	5.24 ± 0.64	5.67 ± 2.12	25.77 ±14.09	26.02 ±12.43	19.46 ± 1.88	26.89 ± 5.59	37.51 ± 22.08	154.92 ±157.04	88.66 ± 10.44	86.86 ± 15.27	51.67 ± 33.57
	60[b]	19.28 ±12.65	7.80 ± 1.82	11.08 ± 7.67	57.01 ±14.48	55.69 ±37.32	27.60 ± 7.17	35.58 ±23.63	99.18 ± 27.91	133.08 ± 79.37	110.61 ± 50.46	103.90 ± 62.48	121.08 ± 41.13
	90[b]	23.17 ±11.68	11.23 ± 7.53	13.42 ± 8.95	46.35 ±15.18	63.38 ±19.33	39.47 ±27.51	33.35 ±10.42	79.07 ± 10.17	266.30 ±182.30	194.23 ±198.18	77.12 ± 31.25	147.77 ±116.61
Cooked	0	0.01 ± 0.00	0.01 ± 0.01	0.04 ± 0.02	0.04 ± 0.01	0.02 ± 0.00	0.03 ± 0.03	0.22 ± 0.10	0.10 ± 0.03	0.08 ± 0.03	0.13 ± 0.13	0.34 ± 0.47	0.16 ± 0.05
	30	5.42 ± 1.97	5.86 ± 2.48	8.44 ± 5.91	16.00 ± 1.94	11.33 ± 4.94	14.35 ± 6.41	19.61 ± 8.32	29.27 ± 2.23	42.69 ± 7.14	85.43 ± 12.09	71.83 ± 8.49	39.33 ± 3.75
	45	7.00 ± 2.75	8.16 ± 2.73	8.45 ± 3.96	35.31 ±10.26	16.65 ± 9.00	21.51 ± 6.19	20.28 ± 8.60	77.45 ± 16.00	80.12 ± 35.06	159.43 ± 45.36	89.57 ± 32.94	139.59 ± 71.83
	60[b]	5.78 ± 0.51	4.51 ± 0.37	5.27 ± 1.35	29.82 ±21.55	13.93 ± 1.64	12.17 ± 0.82	13.48 ± 3.98	75.43 ± 56.69	81.35 ± 28.44	103.08 ± 44.90	83.05 ± 26.91	130.79 ± 97.26
	90[b]	17.70 ±11.75	10.41 ± 4.23	11.33 ± 7.07	51.30 ± 8.91	40.98 ±39.12	26.91 ±11.92	28.44 ±19.34	107.02 ± 48.07	205.35 ±225.02	234.61 ±214.89	164.44 ±166.97	212.78 ±158.60
Broth	0	.003 ± .001	.001 ± .000	.006 ± .002	.001 ± .000					0.28 ± 0.24	0.18 ± 0.01	1.15 ± 0.58	0.22 ± 0.10
	30	.181 ± .154	.161 ± .181	.171 ± .024	.096 ± .035					19.77 ± 10.18	45.91 ± 26.75	24.25 ± 7.17	18.24 ± 10.23
	45	.431 ± .240	.098 ± .027	.343 ± .191	.383 ± .218					34.12 ± 16.73	56.75 ± 34.13	48.04 ± 9.57	48.10 ± 7.79
	60[b]	.744 ± .327	.187 ± .025	.309 ± .213	.234 ± .202					60.47 ± 19.54	43.52 ± 7.11	61.69 ± 41.37	57.39 ± 56.54
	90[b]	1.61 ± 0.71	.311 ± .037	.572 ± .102	.390 ± .350					163.70 ±199.40	83.06 ± 10.32	159.01 ±172.33	122.25 ±157.27

[a]Mean and standard deviation of the mean for 3 hens. Significant studentized range values for comparing two consecutive means ($P \leq 0.05$) are 6.8, 50.1 and 93.7 for p.p.m. wet, p.p.m. solids and p.p.m. fat, respectively (Duncan, 1957).

[b]Fed clean feed for an additional 8 weeks before slaughter.

(without skin) and 2–4 g. samples of raw and cooked thigh skin were weighed to the nearest 0.1 mg. and analyzed for PBB content. Duplicate 14–15 g. samples of broth were taken after first homogenizing the total broth recovered.

Petroleum ether Soxhlet extraction (A.O.A.C. Method 11.23) was used for removal of PBBs from tissue samples. The broth was stirred with 150 ml. petroleum ether for 30 minutes using a 2.5 cm. magnetic stirrer at medium speed. The broth extractions were then washed with 1% $NaSO_4$ solution to remove water, and dried over anhydrous sodium sulfate for 30 min. Both broth and tissue sample extracts were treated with acetonitrile partioning (A.O.A.C. Method 29.001), Kuderna Danish Concentration and Florisil cleanup (A.O.A.C. Method 29.014) with 6% solution of ethyl and petroleum ethers collecting 200 ml. of eluate.

PBBs were quantitated by comparing the area of the 6-isomer peak produced by the sample and the area of the 6-isomer produced by the standard (Firemaster® BP-6, lot #5143, Michigan Chemical Corporation, Chicago, Illinois).

The samples and standards in petroleum ether solution and nanograde hexane, respectively were run on a Tracor 560 GLC, equipped with a ^{63}Ni electron capture detector and interfaced with a Digital PDP-8e-Pamila GC Data System. The column for the GLC was a pyrex column, 1.83 m. long x 4.0 mm. i.d., packed with 3% OV on Chromosorb W 60/80 mesh. The carrier gas was nitrogen with a flow rate of 40 ml./min. Temperatures were 270°C., 240°C., and 300°C., at the injection port, column and EC detector, respectively. Standards were injected at the beginning of each run, after every 6–7 samples and at the end of the run. Quantitations were based on the peak area of the standards (hexabromobiphenyl peak). The presense of the PBB residues was confirmed by ultraviolet irradiation and mass spectrometric analysis. Ultraviolet test was carried out in a Rayonet photochemical reactor, model 1162, equipped with short wave UV lamps. Mass spectrometric analysis was run on a pool of all of the extracted samples. A GC-65 gas chromatograph interfaced to a DuPont 21-490 mass spectrometer which was in turn interfaced to a Digital PDP-12 computer was used. The mass spectra was obtained at an ionizing voltage of 70 eV. with a source temperature of 210°C.

Percentage recoveries of the PBB residues by the utilized method were determined, in spiked samples at levels of 0.02, 50, and 100 p.p.m., to be 55, 66 and 97%, respectively.

PBB levels were expressed as p.p.m. (wet weight), p.p.m. of solids and p.p.m. of fat. Total micrograms of PBBs in raw and cooked chicken pieces as well as in broth were used to calculate percentage recovery and percentage distribution of PBBs recovered in the cooked meat and broth. The data were analyzed for variance and the studentized range test (Duncan, 1957) was used to sort out any significant differences established by these analyses.

RESULTS AND DISCUSSION

Analyses of variance of percentage meat and broth yield indicate the level of PBBs fed did not significantly affect these parameters. The type of piece cooked, however, did significantly affect both average meat and broth yield (Table 1). The cooked meat yield for breast (61.6%) and thigh skin (59.2%) pieces was significantly higher ($P<0.01$) than that of drumstick (53.3%) or thigh meat (50.2%) pieces. In addition, the broth yield of the breast piece (89.6%) was significantly higher ($P<0.01$) than that of the thigh skin (77.7%). The breast piece weighed 2.5 times more than the next heaviest piece and thus had less surface area per unit of weight which would contribute to the reduced cooking losses.

The average percentage of fat in the cooked chicken pieces (14.0%) were significantly lower ($P<0.001$) than in the raw pieces (21.1%) showing that rendering had occurred during cooking (Table 2). In addition, the average fat content of the drumstick (4.6%) was less ($P<0.05$) than that of the breast piece (7.9%) or thigh meat (7.5%); both of which had less fat ($P<0.01$) than the thigh skin (27.7%). Because of its higher fat content, the thigh skin also had significantly average higher ($P<0.01$) solids (37.1%) content than did the other chicken pieces used which were 26.7, 24.6, and 22.4% for breast, thigh meat and drumstick, respectively (Table 2). Neither the average lipid nor solids content were affected by the level of PBB fed to the hens.

PBB levels in the raw and cooked chicken as well as in the chicken broth for each of the five levels fed are summarized in Table 3. The control birds had low levels of PBBs in their tissues; the highest level occurred in the high fat containing thigh skin. This unexpected low

level of contamination may have resulted from PBB particles being airborne on dust. Although all groups of birds were housed in separate cages, they were all in the same cage room and were all fed their respective rations at the same time.

The levels of PBBs in the tissues increased progressively with increasing levels in the feed and were highest in the high fat thigh skin. It was expected that the PBB levels in the tissues of hens fed feed containing 60 and 90 p.p.m. PBBs and which were subsequently fed clean food for eight additional weeks, would be lower than that in tissues from birds fed 30 and 45 p.p.m. PBB in the feed since the half life in eggs, liver and muscle has been found to be approximately 3 weeks (Polin and Ringer, 1975). These samples, however, either contained skin or were higher in fat and thus these factors may have affected the rate of elimination. As can be seen from the high standard deviations of the mean in Tables 2 and 3, wide variation in the PBB levels and fat content occurred in the birds of each group. Duplicate values on any one tissue, however, gave reasonable agreement.

When the chicken pieces were cooked, the level of PBBs in the wet tissue decreased slightly (Table 3). Part of the PBBs lost was recovered in the broth. The level of PBBs in the broth of breast pieces was higher than that in the broth of the other pieces. Since the amount of cooking water was constant, greater amounts of PBBs in the breast broth resulted from a larger amount of fat rendered from the larger piece.

When the PBB content was expressed on a solids or fat basis, there was no significant difference among chicken pieces. Cooking did not significantly affect the level of PBBs expressed on a solids basis, however, the average PBBs in the fat of the broth (52.6 p.p.m.) was less ($P<0.05$) than that in the fat of the raw (86.1 p.p.m.) or cooked pieces (96.2 p.p.m.). Average values in the fat of cooked pieces were slightly higher than those in the fat of the raw pieces. Thus, while rendering of fat is undoubtedly an important mode of PBB reduction, the amount of PBB reduced is not directly proportional to fat removal.

Total micrograms of PBBs in the cooked chicken and broth were compared to the level in the respective raw chicken piece to calculate the percentage recovery. No significant differences occurred among the percentage of PBB recovered in any of the four pieces, percentage recoveries were 68.1% in thigh skin, 75.8% in the breast piece, 83.9% in the thigh meat, and 84.6% in drumsticks. Recoveries, however, did tend to be higher in the chicken pieces which contained less fat (i.e. drumstick and thigh meat) even though these pieces had lower percentage meat yields (Table 1) than did the higher fat breast piece or thigh skin.

The distribution of the recovered PBBs between the cooked meat and broth is illustrated in Figure 1. The percentage of recovered PBBs in the meat did not differ significantly among the four pieces evaluated and ranged from 65.5% in the cooked thigh skin to 72.9% in the cooked drumstick. The proportion of the recovered PBB in the cooked meat is considerably higher than that found in previous studies. Recovered PCBs were about equally distributed between the cooked meats and broth (Zabik, 1974) while only 1/4 to 1/3 of recovered lindane, dieldrin, and DDT compounds occurred in the cooked hen pieces (Morgan *et al.*, 1972). The bulkier size and higher molecular weight of the PBB molecules may contribute to a smaller proportion of the recovered material being found in the broth. Moreover, Stadleman *et al.* (1965) reported that feeding high levels of several pesticides resulted in greater residue persistance in eggs and abominal fat of hens than was found when low levels were fed. Thus, the high levels of contamination may have influence this distribution. For pieces from control hens, the proportion of recovered PBBs

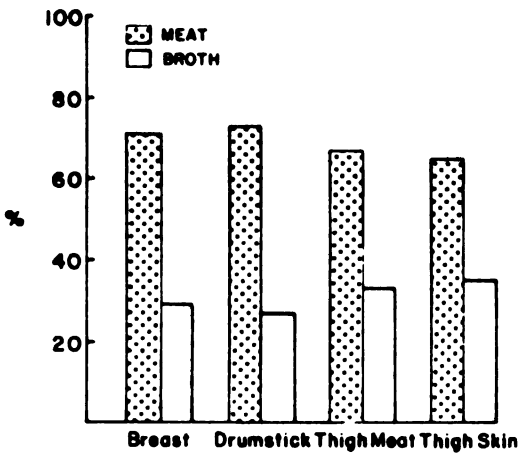

FIG. 1. Distribution of recovered PBBs in cooked chicken and chicken broth.

in the meat was slightly less, ranging from 39.8% in the drumstick to 52.7% in the thigh meat.

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