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Embryotoxic and Teratogenic Effects in Unhatched Fertile Eggs from Hens Fed Polychlorinated Biphenyls (PCBs)

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Beginning with the Swedish investigator JENSEN (1966), ecologists have identified polychlorinated biphenyl (PCB) residues in fish, birds and mammals from widely dispersed locations. Residues have also been reported in human hair, milk, plasma and adipose tissue. (RISEMBROUGH et al. 1970, BIROS et al. 1970, FINKLEA et al. 1972). In 1968, an outbreak of chlorobiphenyl poisoning in Japan (Yusho disease) was traced to the ingestion of rice oil contamination by PCB (KURATSUNE et al. 1972). In the United States inadvertent leakage of PCBs into fish meal resulted in contamination of poultry feedstuffs consumed by millions of chickens (PICHIRALLO 1971). Hatchability of eggs from these chickens was noticeably reduced.

Since accidental PCB feeding caused reproductive failure in chickens, controlled studies involving an extensive series of commercial PCBs were initiated in our laboratory (LILLIE et al. 1973). Six different PCBs (chlorine content 21% to 68%), a polychlorinated terphenyl and a polybrominated biphenyl were fed to White Leghorn chickens, and hatchability and teratogenic effects in the embryos were recorded.1/

MATERIALS AND METHODS

Commercially available PCBs (Monsanto) ranging from 21% to 68% in chlorine content were used. Twelve groups (as listed in Table 1), 35 White Leghorn pullets each, were fed ad libitum 2 or 20 ppm 'Aroclors 1242, 1248 and 1254', 20 ppm 'Aroclors 1221, 1232, 1268 and 5442', 20 ppm of a polybrominated biphenyl (BP-6), and a control group was fed the standard breeder ration. The diets were fed for 9 weeks; the contaminated diet was then withdrawn, and the chickens were fed the uncontaminated breeder ration for 7 weeks.

1/ Aroclors, Monsanto Chemical Co.; BP-6, a polybrominated biphenyl containing 75% bromine, Michigan Chemical Co. Trade names are used solely for the purpose of providing specific information. Mention of a trade name does not constitute a guarantee or warranty of the product by the Federal Government or an endorsement by the Government over other products not mentioned.

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Undiluted semen from White Leghorn males fed the control diet was used to inseminate the pullets once a week and fertile eggs set weekly. Eight hatches were obtained during PCB feeding and six hatches during PCB withdrawal. Residues in egg contents and body fat were determined by gas liquid chromatography using electron capture detection after clean-up using FDA multipesticide methodology (FRIES et al. 1972a).

RESULTS AND DISCUSSION

As reported by LILLIE et al. (1973), hatchability started to decline two weeks after hens were given 20 ppm 'Aroclors 1232, 1242, 1248 or 1254' and after PCB withdrawal, hatchability improved rapidly (Fig. 1). Feeding 2 ppm 'Aroclors 1242, 1248 and 1254' or 20 ppm 'Aroclors 1221, 1268, 5442' or the polybrominated compound 'BP-6' did not affect hatchability. KEPLINGER (1970), MCLAUGHLIN et al. (1963), and SCOTT et al. (1971) have shown that 'Aroclors 1242, 1248 and 1254' reduced hatchability of chicken eggs, either after feeding PCB to hens or after injection of PCB into the egg.

To explore possible causes for the drastic decline in hatchability, unhatched eggs from hens fed treatment feed for 5 to 9 weeks and from the subsequent 7 weeks of uncontaminated feeding, were examined to determine time of embryonic death and incidence of abnormalities. The majority of the hatchability failures occurred during the last 2 days of incubation (Fig. 2). Of 1098 unhatched eggs examined, 210 died on the 20th day of incubation and 523 on the 21st day; 337 of these eggs had been pipped. The chick had developed to the point where it could break the shell but was unable to break the shell completely and emerge.

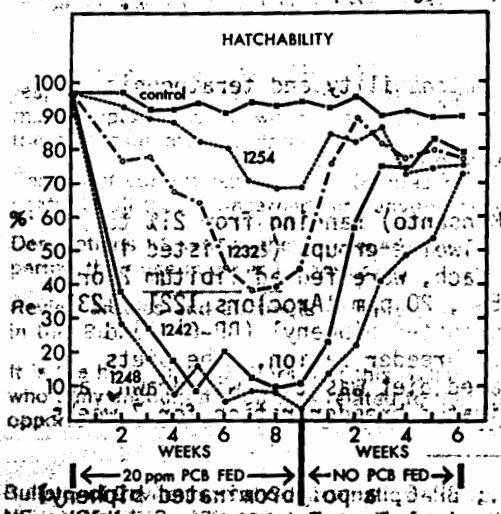


Fig. 1. Hatchability of eggs from hens fed PCBs for 9 weeks followed by 7 weeks of uncontaminated feed (LILLIE et al. 1973).

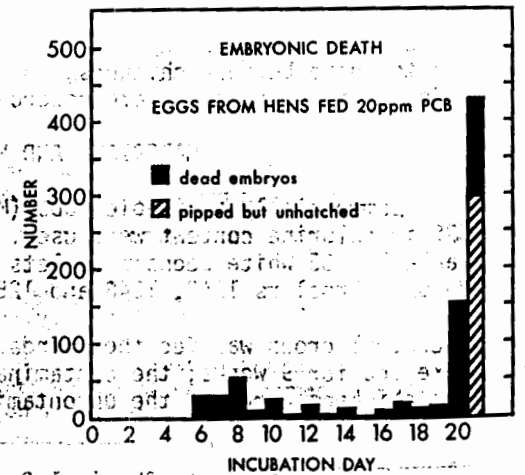


Fig. 2. Occurrences of embryonic death of incubated eggs from hens fed 20 ppm 'Aroclors 1232, 1242, 1248 or 1254'.

Upon gross examination, many of the unhatched embryos were abnormal (Table 1). Those PCB treatments that produced the greatest incidence of dead embryos also produced the highest number of abnormal embryos, indicating a close relationship between total dead embryos and abnormal embryos. After withdrawal of contaminated feed, recovery was slowest in birds previously fed 'Aroclor 1248' (Table 2); after seven weeks, hatchability and numbers of abnormal unhatched embryos approached that of the control group. In all groups, approximately 1/3 to 1/2 of dead embryos were abnormal, irrespective of treatment.

During the 5th to 9th week, 843 dead embryos were examined in the 20 ppm 'Aroclors 1232, 1242, 1248 and 1254' treatment groups. Upon gross examination, there were 286 abnormal embryos with 394 abnormalities (Table 3). The most common abnormality observed was edema. Many of the embryos had large edematous cysts on the rump (Fig. 3) and side, and subcutaneous edema about the neck and head area. Twelve percent of the embryos had unabsorbed yolk (Table 3). A smaller percentage had small bodies, external hemorrhage, skinny legs, rotated ankles or short, crossed beaks. Other abnormalities that occurred to a lesser extent were cleft palate, abnormal eye formations and external viscera. Similar abnormalities occurred in the control group (Table 3); subcutaneous edema accounted for 29% of the abnormalities but was limited to the head, with no large edematous cysts on the head or rump.

Feeding PCBs appears to increase the number of abnormalities inherent in this flock of chickens. GOLDSTEIN et al. (1968) suggested that chemical teratogens act by bringing out "concealed weaknesses" of the developmental processes and that many induced defects are really phenotypic changes brought about through the same biochemical disturbances produced by faulty genes. None of the dead embryos in the control group had unabsorbed yolk. Since the PCBs are lipid soluble and concentrated in the yolk portion of the egg, the unabsorbed yolk in the PCB groups may be due to a local effect of the PCBs on metabolizing systems in the yolk sac. Although the progeny that hatched did not have gross abnormalities, progeny growth was impaired by feeding 20 ppm of 'Aroclors 1232, 1242, 1248 or 1254' in the maternal diet (LILLIE et al. 1973).

PCB residue level in fertile eggs was not related to the incidence of embryotoxic and teratogenic effects in unhatched embryos. Total PCB residue in eggs generally increased as the chlorine content of the PCB increased (Fig. 4). This increase was expected because in these chickens the less chlorinated PCBs are readily metabolized while more chlorinated PCBs are not (FRIES et al. 1973b). The residue level in body fat paralleled the level in eggs.

TABLE 1

Abnormal embryos of eggs from hens during the 5th to 8th weeks of 'Aroclor' and BP-6 feeding and the subsequent 7 weeks of uncontaminated feeding.

Diet	PPM	Fertile Eggs No.	Embryos Dead No.	Embryos Abnormal No.	Abnormal Embryos as % of Fertile Eggs	Abnormal Embryos as % of Dead Embryos
Cont	0	819	32	13	2	41
1221	20	882	36	15	2	42
1232	20	737	195	63	8	32
1242	2	805	44	16	2	36
1242	20	741	365	134	18	37
1248	2	900	40	21	2	52
1248	20	703	429	141	20	33
1254	2	822	51	23	3	45
1254	20	714	109	33	5	30
1268	20	755	51	23	3	45
5442	20	790	52	26	3	50
BP-6	20	627	39	18	3	46

TABLE 2

Abnormal/Unhatched embryos ratios during the 8th week of 20 ppm 'Aroclors' and subsequent 7 weeks of uncontaminated feeding.

Diet	PCB Wk.	Weeks after withdrawal of contaminated feed						
	8	1	2	3	4	5	6	7
Cont	2/3	1/2	0/1	1/2	1/5	0/2	1/4	3/6
1232	12/36	10/17	5/14	4/6	2/8	1/8	1/6	1/7
1242	19/53	17/40	14/53	6/17	2/11	5/14	2/8	2/10
1248	19/57	16/43	12/49	13/24	4/38	5/28	7/27	4/11
1254	8/23	1/7	2/8	4/5	0/5	3/9	2/8	1/5

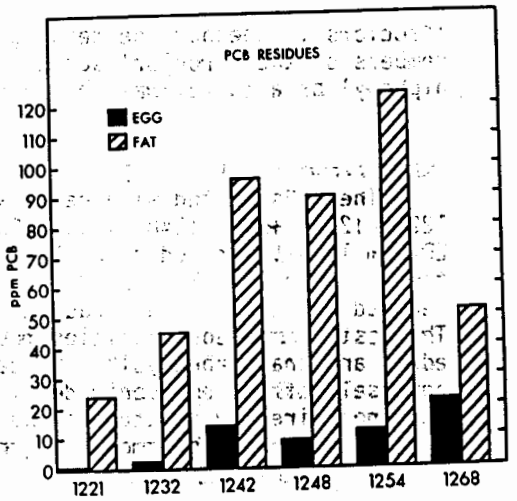
Fig. 1. Hatchability of eggs from hens fed PCBs for 9 weeks followed by 7 weeks of uncontaminated feed (LILLIE et al. 1972)

TABLE 3

Abnormalities in dead embryos of eggs from hens fed 20 ppm 'Aroclors 1232, 1242, 1248 and 1254' for 5 to 9 weeks.

	PCB		Control	
	Number	%	Number	%
Dead Embryos	843	100	32	100
Abnormal Embryos	286	34	13	41
Abnormalities	394	100	17	100
Edema	198	50	5	29
Unabsorbed Yolk	49	12	0	0
Small Body	34	9	2	12
External Hemorrhage	30	8	0	0
Skinny Legs	28	7	2	12
Rotated Ankle	12	3	1	6
Short or Crossed Beak	11	3	1	6
Others	32	8	6	35

Fig. 3. Abnormal embryo after PCB feeding to White Leghorn hens. The 20-day-old embryo has a large edematous cyst on the rump.



% DEPLETION:	1221	1232	1242	1248	1254	1268
EGG	0	17	80	67	42	92
FAT	66	48	93	56	26	17

Fig. 4. PCB residues after 9 weeks of feeding 20 ppm 'Aroclors 1221 to 1268' and % depletion of PCB residues after a subsequent 7 weeks of feeding uncontaminated feed.

The deleterious effects of the 'Aroclors' were not directly related to the percent chlorination. 'Aroclor 1221' (21% chlorine) and 'Aroclor 1268' (68 % chlorine) exerted no adverse effects on embryonic development while marked embryotoxic and teratogenic effects occurred after feeding PCBs with intermediate percentages of chlorine, 'Aroclors 1242 and 1248'. MCLAUGHLIN (1963) reported similar teratogenic effects after injecting 'Aroclor 1242' into eggs.

Specific biphenyl component(s) common to 'Aroclors 1232, 1242, 1248 and 1254' may be responsible for the teratogenicity of these 'Aroclors'. The picture is complicated by the fact that we are not dealing with a single compound. WIDEMARK (1968) has calculated that of the 210 possible chlorinated compounds of a biphenyl, 102 are probable. WEBB and MCCALL (1971) have identified 16, 32, 42, and 26 chlorinated components in 'Aroclors 1232, 1242, 1248 and 1254' respectively. A single component or a combination of components may be responsible for the deleterious effects of these 'Aroclors'.

The possibility of a contaminant also exists. VOS (1972) examined three PCBs, 'Phenoclor DP6', 'Clophen A60' and 'Aroclor 1260' for a toxic impurity. Although 'Aroclor 1260' contained no toxic impurity, 'Phenoclor' and 'Clophen' contained dibenzofurans which were embryotoxic. Dioxins are also very embryotoxic and cause edema when fed to young chicks (FLICK et al. 1973, HIGGINBOTHAM et al. 1968). However, dioxins have not been reported to be present as an impurity in PCBs. Micro quantities of dioxins or polychlorinated-dibenzofurans could possibly be contaminants of 'Aroclors'. Whether the teratogenic producing agent in the middle members of the 'Aroclor' series is a specific polychlorinated biphenyl or a contaminant has yet to be proved.

SUMMARY

The PCBs tested with caged White Leghorn hens were 'Aroclors 1221, 1232, 1242, 1248, 1254, 1268, 5442 and BP-6', fed at the 20 ppm level. In addition, 1242, 1248 and 1254 were also fed at the 2 ppm level. Feeding 20 ppm 'Aroclors 1232, 1242, 1248 and 1254' reduced hatchability and caused teratogenic effects in the embryos. The most common abnormalities found in the unhatched embryos were edema and unabsorbed yolk. Since 'Aroclors 1221 and 1268' did not adversely affect embryonic development, adverse effects of the PCBs were not directly related to the degree of chlorination of the biphenyls, or to the amount of total residue.

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