

Polybrominated Biphenyls in Chicken Eggs vs. Hatchability¹ (40298)

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In the fall of 1973, polybrominated biphenyls (PBB) were accidentally introduced into feed for poultry and livestock within Michigan. Millions of chickens and thousands of cattle were destroyed to lessen the contamination of PBB into the food-chain (1). Fries *et al.* (2) had reported that eggs from hens fed 20 ppm PBB averaged 21.5 ppm of hexabromobiphenyl (6-BB) plus heptabromobiphenyl (7-BB), and that 7 weeks after withdrawal of the PBB diet eggs contained 2.2 ppm of these compounds. Fat from these hens contained 69.5 and 62.4 ppm of these PBBs at the respective times of 9 weeks on PBB diets and 7 weeks after their withdrawal. Egg residues were reported to be 1.5× the dietary level of PBB after 4 weeks of feeding PBB diets (3). This steady state effect was confirmed to occur as early as 10 days (4, 5). Based on data obtained from feeding PBB to quail (6), calculations (4) revealed a relationship of PBB in eggs to be 1.3× the level in the diet, similar to the 1.5 value calculated from the dose-response curve (5) and the reported value by Cecil *et al.* (3).

Embryo mortality as a percent of fertile eggs averaged 6.2% and 3.9% for diets containing either 20 ppm PBB or no PBB, respectively (7). Ringer and Polin (8) showed that hatchability declined and chicks from contaminated eggs were less viable when hens were fed 125 ppm PBB in the diet, but not when fed 25 ppm (8) or 30 ppm (4). Quail eggs hatched normally when PBB was fed at 20 ppm, but failed to hatch when 100 ppm was fed (6).

The study reported herein will establish the relationship between PBB in eggs vs. hatchability by two approaches, which will be shown to disagree on the extent of this relationship. The implications of this incompatibility may indicate that analysis for 6-BB may not be a definitive approach to assess

PBB toxicity.

Materials and methods. Adult female White Leghorn chickens 10 months in production (about 60 weeks of age) were assigned at random into one of 7 treatments, or to a control group. Twenty-four hens were in each group. PBB, as Firemaster FF-1 was used in this experiment. This compound differs from that of Firemaster BP-6 used in other studies (2, 3, 6, 7) in that FF-1 has anti-caking substances added and had been milled to obtain a free-flowing compound. In other words, Firemaster BP-6 was an intermediate product. Firemaster FF-1 was the final product sold commercially, the one used in this study, and the chemical involved in the contamination of Michigan's livestock and poultry. It is reported (4) to contain 62.8% 6-BB and 13.8% 7-BB, as compared to 79.2% 6-BB and 14.3% 7-BB for the Firemaster BP-6 (2).

The details of the materials and methods used in the experiment, as well as the procedure for the analysis of PBB in eggs were reported (5). Briefly, the hens were fed FF-1 in the diet at 0.2, 1, 5, 25, 125, 625 or 3125 ppm for 5 weeks, then fed feed without FF-1 for 8 weeks to obtain data on withdrawal effects. Eggs require about 9–10 days to be completely formed, 8–9 of which are for yolk formation. Thus, sampling of eggs started on day 9 of the experiment, and was on every 7th day thereafter until the 37th day after withdrawal (Table I). The experiment started June 17, 1974. Starting on June 18th, eggs were saved. So that the time for egg sampling coincided with the middle of a 7-day collection period, the first setting of eggs in the incubator were those collected in the first 5 days on the experiment. All subsequent settings were from 7-day collections. The midpoint of the 1st collection period was day 9 on and represented equally days 6 through 8 for the accumulation phase and days 10 through 12 of the steady-state phase. Thus, the hatch value for a week's collection would represent the hatch value for the midpoint of

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TABLE I.

RELATIONSHIP BETWEEN HATCH AND HEXABROMOBIPHENYL (6-BB) IN EGGS

		FIREMASTER FF-1 (FF-1) IN DIET - PPM													
		0.2		1.0		5.0		25.0		125.0		625.0		3125.0 ^A	
DATE	DAY FOR EGG SAMPLE	6-BB IN EGG-PPM	% HATCH	6-BB IN EGG-PPM	% HATCH	6-BB IN EGG-PPM	% HATCH	6-BB IN EGG-PPM	% HATCH	6-BB IN EGG-PPM	% HATCH	6-BB IN EGG-PPM	% HATCH	6-BB IN EGG-PPM	% HATCH
6/27	DAY 9 ON	.14	(90) 92	1.8	(88) 93	7.7	(100) 93	23	(101) 91	232	(60) 28	-	(8) 0	-	-
7/4	16 ON	.26	(91) 95	1.4	(97) 92	11.0	(105) 97	85	(90) 87	304	(60) 5	-	-	-	-
7/11	23 ON	.34	(96) 88	1.3	(101) 84	3.4	(85) 89	46	(76) 96	178	(57) 2	-	-	-	-
7/18	30 ON	.43	(89) 96	1.5	(98) 89	5.6	(93) 95	33	(94) 85	145	(63) 0	-	-	-	-
7/25	2 OFF	.54	(62) 94	1.7	(93) 93	9.5	(86) 94	30	(82) 84	220	(50) 18	-	-	-	-
8/1	9 OFF	.58	(83) 92	0.8	(81) 91	1.9	(81) 94	11.3	(76) 92	58	(66) 74	-	-	-	-
8/8	16 OFF	.12	(74) 92	0.33	(75) 93	1.3	(61) 90	10.9	(61) 92	30	(58) 86	-	-	120	-
8/15	23 OFF	.10	(75) 93	0.25	(67) 84	0.83	(71) 94	6.8	(60) 87	54	(67) 85	78	-	65	(14) 43
8/22	30 OFF	.05	(64) 91	0.13	(60) 88	0.69	(52) 92	6.0	(57) 90	21	(55) 91	71	(18) 33	40	(17) 35
8/29	37 OFF	.07	(56) 95	0.13	(56) 96	0.84	(53) 91	5.3	(41) 83	19	(55) 78	73	(19) 21	48	(14) 21
9/5	44 OFF	-	(53) 93	-	(56) 100	-	(40) 98	-	(38) 92	-	(40) 93	-	(30) 37	-	(8) 63
9/12	51 OFF	-	(65) 94	-	(59) 95	-	(44) 91	-	(41) 83	-	(49) 88	-	(43) 19	-	(6) 67

() = NUMBER FERTILE; % HATCH = (NUMBER HATCH/NUMBER FERTILE) x 100

A = FF-1 WITHDRAWN 7 DAYS SOONER, THEREFORE ADD 7 DAYS TO "DAY FOR EGG SAMPLE"

Hatchability of eggs from White Leghorn chickens fed diets with polybrominated biphenyl, Firemaster FF-1, and the hexabromobiphenyl levels (6-BB) analyzed in eggs representative of each hatch.

that week; in this case, day 9 on. The eggs from the mid-point of the week were opened, pooled, and analysed for 6-BB by the Michigan Department of Agriculture, as previously described (5). Hens were artificially inseminated once a week with semen collected from males housed in a separate room and fed diet without FF-1.

6-BB was assayed by gas liquid chromatography using one or both procedures employing a ³H-foil electronic detector at a temperature of 220° in the column and detector, and 250° in the injector, or a ⁶³Ni-detector at temperatures of 270° in the column, 310° in the detector and 300° in the injectorport. The important aspect for this experiment was that FF-1 was assessed from chromatograms by reading the peak height of the 6-BB peak using Firemaster BP-6 (Lot-#5143) as a standard. Subsequent comparison of this standard with those used by the Food and Drug Administration (FDA) showed comparable

patterns. The standard BP-6 was obtained from Michigan Chemical Company, the former manufacturer of FF-1. Linear and curvilinear regression and analysis of variance were applied to the data (9), after converting percentage values of hatch to arcsin $\sqrt{\%}$ (9).

Results and discussion. Table I contains the weekly hatchability data (number hatch per number fertile) and the 6-BB levels of eggs representative of the day and week that the eggs were collected. Not included in Table I were the hatchability data for the first 5 days on the experiment for which no egg samples were obtained. These hatch values were 95.3, 80.0, 83.1, 88.1, 88.5, 90.0, 69.7 and 61.5% for the eggs from FF-1 levels of 0, 0.2, 1, 5, 25, 125, 625 and 3125 ppm in the diet, respectively. When these values were considered with those of Table I for the first 5 weeks that FF-1 was fed, hatchability of control eggs averaged 89.9 (\pm 4.1)%, mean (\pm SD), and 91.2 (\pm 3.1)% for the entire 13 weeks of the

experiment. Hatchability during 5 weeks of feeding FF-1 at 0.2, 1, 5 and 25 ppm were 90.6, 88.5, 92.8, and 89.9%, respectively; none of these values were significantly different, $P \leq .05$, from the control value. On the other hand, poor hatches were obtained when 125 ppm FF-1 was fed, but not until the hatch representing day 9 on (actually days 6–12) was obtained. Within the first 5 days of feeding FF-1 at 625 and 3125 ppm, hatchability was significantly ($P \leq .05$) below normal. None of the eight fertile eggs hatched that were obtained from the hens fed 625 ppm PBB and representing day 9 on; and no eggs were laid by those hens fed 3125 ppm FF-1. A subsequent experiment revealed (4, 5) that during the steady-state phase of days 9 to 35 for feeding FF-1, the minimum effective level for FF-1 in the diet to produce a significant effect on hatchability was between 30 and 45 ppm.

6-BB was detected in whole egg samples, based on the dose-response curve for steady state values (5), at 0.3, 1.5, 7.4, 43.4, and 215 ppm for the treatment levels of 0.2, 1, 5, 25 and 125 ppm FF-1 in the diet, respectively. The latter level produced a high mortality in chick embryos (Table I) during the last few days of hatch. Edema of the abdominal and cervical regions was the prevalent pathological sign observed in embryos and newly hatched chicks from FF-1 treatment (Fig. 1a and 1b). The clinical signs resemble those of embryos from polychlorinated biphenyl treatment (7). The edema was the only side effect to be observed that was increased in incidence above abnormalities detected in control embryos.

Estimated $t_{1/2}$ values were obtained from the 6-BB data in Table 1 and found to be 10 and 21 days for depletion time from prior treatment with FF-1 at 0.2–1.0 ppm, and

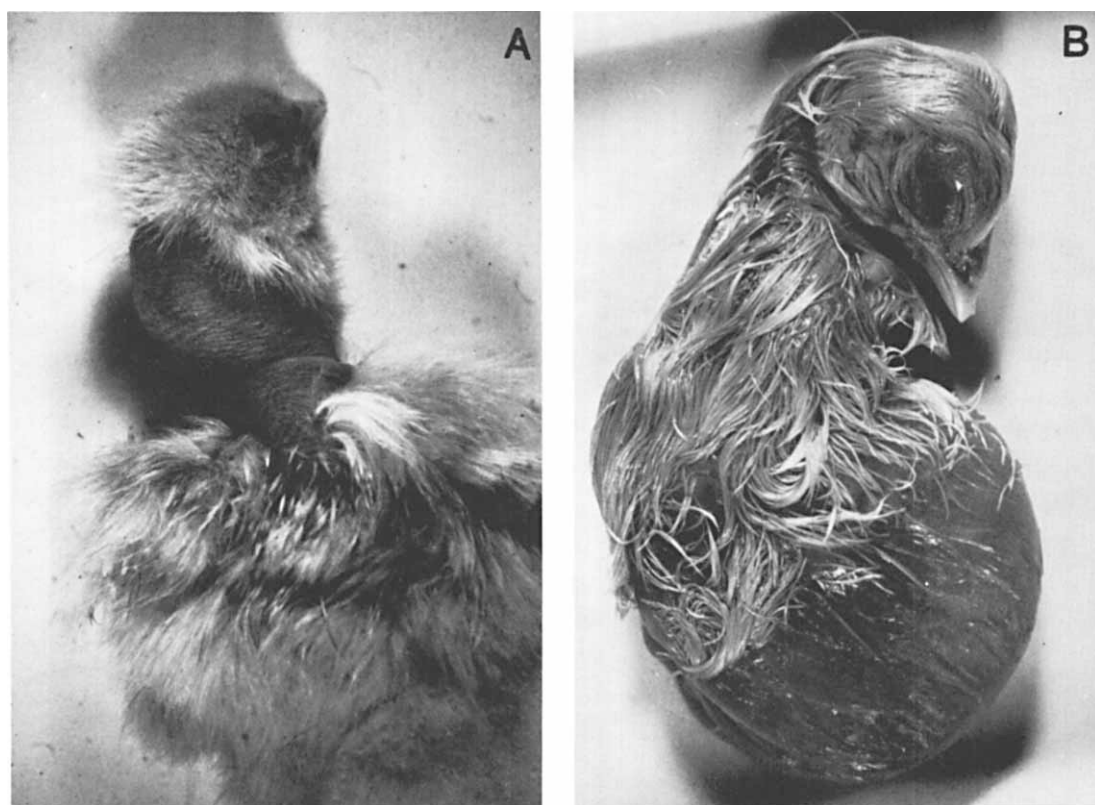


FIG. 1. Edematous condition of embryo (1b) and chick (1a) from feeding polybrominated biphenyl, Firemaster FF-1, to hens at dietary levels higher than 42 ppm. Note the accumulation of fluid typically seen in abdominal and head region of embryos, and cervical area of chicks.

TABLE II.^a

Level of FF-1 withdrawn	Depletion curve	t _{1/2}
0.2 ppm	Y = -0.2024 - 0.0317X	9.5
1.0 ppm	Y = -0.0647 - 0.0286X	10.5
5.0 ppm	Y = 0.3287 - 0.0137X	22.0
25.0 ppm	Y = 1.1870 - 0.0131X	23.0
125.0 ppm	Y = 1.8818 - 0.0158X	19.1

^a Dose-response lines based on depletion curves for 6-BB from chicken eggs after removal of diets with FF-1. The relationship is $Y = a + bx$, where x = days of withdrawal starting at day 9 off, and Y = log ppm 6-BB in whole egg.

5–125 ppm, respectively (Table II). Thus, higher levels of FF-1 treatment required a longer time for depletion based on 37 days of measurements during withdrawal. Furthermore, factorial analysis of these depletion data revealed a significant linear and quartic, but not cubic, effect. This suggests that the slopes of the depletion curves are flattening to some extent and that with depletion beyond 37 days the $t_{1/2}$ values will be greater.

As previously pointed out (5), the relationship between FF-1 in the diet and 6-BB levels in eggs during the steady-state phase, was expressed by the equation $Y = 0.1763 - 1.012X$, where $X = \log$ ppm of FF-1 in the diet, $Y = \log$ ppm 6-BB in whole egg. This is in good agreement with the data by other investigators (3, 6). The response of hatchability to FF-1 in the diet (3), during the steady-state phase, was estimated to be $Y = 297.14 - 140.74X$, where $X = \log$ ppm FF-1, and $Y = \arcsin \sqrt{\%$ of hatchability.

The algebraic summation of these two regressions derived to relate log ppm 6-BB in egg, as X , to $\arcsin \sqrt{\%$ hatch, as Y , is given in Figure 2, line "a", along with a plot of the values from Table I for treatments with FF-1 at 25, 125 and 625 ppm. The regression lines under comparison are: (a) the line based on the derived steady-state values, (b) the line based on the depletion phase ("off" data), (c) the line based on the steady-state phase ("on" data), (d) the line representing both the steady-state and depletion phase of the data from Table I, and (e) a regression line based on egg residues between 30 and 85 ppm of 6-BB.

The lines for "b", "c", and "d" above were calculated on the basis of $Y = a + bx + cx^2$. From these regressions, a linear regression

can be calculated to represent the apparent linear portion of these curvilinear lines. Forcing the data into response lines for either "b", "c", or "d" revealed slopes and intercepts very unlike the derived equation, "a", but whose linear slopes were very similar (Fig. 2-legend). The line calculated for "e" above, and a non-significant, $P > 0.05$, slope indicated by the lack of correlation ($r = 0.363$) between hatchability and 6-BB levels of 30 to 85 ppm in eggs.

Considering all of these comparisons to the derived regression, the conclusion must be reached that the derived equation relating hatchability to egg residues is not valid. Interestingly, the range of 6-BB levels from 30 to 85 ppm supposedly covered a range of hatchability from no effect down to a hatch of 64%, based on the derived equation.

Fries *et al.* (10) reported that 7-BB declined more rapidly in chicken eggs than 6-BB upon withdrawal of diets with BP-6. Also, they noted that the concentrations of these components in fat of hens being fed 20 ppm were 4 times that of diet for 6-BB and 1.5 times that of diet for 7-BB. Thus, there is evidence

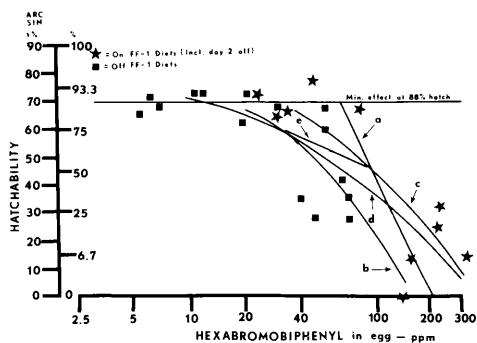


FIG. 2. Relationship between hexabromobiphenyl (6-BB) in whole egg and the hatchability of eggs collected during and after the feeding of diets with 25, 125, 625 or 3125 ppm Firemaster FF-1, a polybrominated biphenyl. Where $X = \log$ ppm 6-BB and $Y = \arcsin \sqrt{\%$ hatchability, the response lines are: (a) A derived equation of $Y = 321.66 - 139.07X$; (b) a response line for data from the depletion phase where $Y = 24.0 + 95.8X - 48.1X^2$, whose linear portion is described by $Y = 160.9 - 68.8X$; (c) a response line for data from the steady-state phase, where $Y = 96.8 + 10.7X - 18.8X^2$ whose linear portion is described by $Y = 173.4 - 65.8X$; (d) summation of curves "b" and "c" where $Y = 56.8 + 37.6X - 23.3X^2$ whose linear portion is $Y = 167.2 - 67.3X$, and; (e) a response line for egg samples with 6-BB between 30–85 ppm where $Y = 127.6 - 43.6X$.

for differential metabolism of the isomers that comprise BP-6, and thus FF-1. The supposition to consider is that not all isomers of these PBBs gave equivalent toxicity and that this would account for our inability to find a close correlation between 6-BB and embryo toxicity over that wide range of 6-BB levels in eggs, and the incompatibility between derived and actual curves of 6-BB in eggs vs. hatchability. On this basis, these hatch and residue data indicated that analysis for FF-1 based on the analysis of only the 6-BB peak was not a definitive approach toward assessing toxicity of BP-6 or FF-1.

Other isomers, and their metabolites will have to be considered in the overall relationship of ingested PBBs to the residues in tissues and their toxicity of the compounds.

Summary. A relationship between polybrominated biphenyl, Firemaster FF-1 (FF-1), in the diet, and eggs, as monitored by hexabromobiphenyl (6-BB), and embryo toxicity, as measured by hatchability, were examined. The minimum dietary level of FF-1 for an effect on hatchability was estimated at 42 ppm, which produced an egg residue estimated at 65.9 ppm 6-BB. Generally, as FF-1 in the diet increased, egg residues increased and hatchability decreased. Regression equations were established for these relationships. After withdrawal of FF-1 in the diet, hatchability returned to normal when FF-1 treat-

ments had been <625 ppm. Over a range of 30–85 ppm 6-BB in eggs there was poor correlation to an effect on hatchability. These latter data were discussed in terms that the 6-BB peak may not be a definitive approach to assess FF-1 toxicity.

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