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Effect of Congeners of Polybrominated Biphenyls on Hatchability of Chicken Eggs. I. 2,2′,4,4′,5,5′-Hexabromobiphenyl vs. PBB[1, 2] (40486)

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The accidental contamination of feeds in 1973 with polybrominated biphenyls (PBBs), a flame retardant (fireMaster FF-1), led to the removal from the food chain of thousands of cattle, about 1½ million chickens, and considerable dairy and poultry by-products within Michigan (1). The presence of PBBs into the ecosystem persists today and is constantly monitored. Assays for PBB residues in milk, meat, eggs, and biological samples, including those from humans, are based on gas liquid chromatography (2) using the major hexabromobiphenyl (HBB) peak to monitor PBB levels (Fig. 1, Peak 4). Most laboratories monitor PBBs using Peak 4. This peak accounts for an estimated 62.8% of fireMaster FF-1 (FF-1) (3), the commercial product, or an estimated 79% of the product fireMaster BP-6 (BP-6) (4). BP-6 is the mixture to which anticaking substances is added and milled to obtain the product FF-1. The FF-1 was the culprit in the accidental contamination of feeds.

Residue levels of HBB - Peak 4, hereafter referred to as HBB-4, in chicken eggs were established over a wide range of feed levels from 0.2 to 3125 ppm for FF-1 (5), and 20 to 2000 ppm of BP-6 (6) with excellent agreement by the two laboratories on steady-state values being 1.5 times the feed level. In addition, a comparison of the data from our laboratory (5), using FF-1, to those from the USDA laboratory (6), using BP-6, reveals excellent agreement on the levels of these two products to produce toxic effects in chick embryos and chicks.

Depletion of HBB-4 from chicken eggs appears to be biphasic, with the early phase having a biological half-life estimated at 17 days (2, 5) or 28 days (7), and the second phase estimated at 16 weeks (6). Thus, movement of these congeners from tissues to newly-formed yolk within the contaminated hen is not necessarily a constant process; instead the postulate is that the mixture is subject to differential distribution. Recent data (8) showed that following withdrawal of FF-1 from the diet, hatchability returned to normal, yet in some eggs high levels of HBB-4 were found. As a consequence a derived equation for HBB-4 in eggs versus hatchability was shown to be invalid (8). Based on these data and evidence by Fries *et al.* (7) on differential distribution of PB-6 congeners, the conclusion reached (8) was that not all congeners gave equivalent toxicity. In this experiment we examined the effect of HBB-4 on hatchability of chicken eggs.

Material and methods. Starting June 5, 1978, 10 laying hens were fed *ad libitum* for 21 days, a diet containing 80 ppm FF-1, 10 hens a diet containing 52 ppm HBB-4, isolated from FF-1 (9), and 10 hens fed the same stock diet without any PBBs. The diet with 80 ppm FF-1 was expected to reduce hatchability to about 25% (2). The level of 52 ppm of HBB-4 was based on 65% of FF-1 being HBB-4.

Particular caution was exercised to avoid contamination of our laboratory facilities so hens were moved to an isolated room at the Michigan State University's Poultry Science Research and Teaching Center (PSRTC). There the birds were housed singly in suspended, colony-type, wire cages, 12 cages per unit, and each group of hens was in a separate unit. The hens were moved from our laboratory in the Department of Poultry Science to the PSRTC 2 weeks prior to the experiment. Artificial inseminations were done once a week using semen pooled from males maintained on a stock diet but held in the laboratory within the department. Thus, the semen was transported to the PSRTC with a delay of 30–60 min before inseminations were

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performed. This was not an ideal situation, but a necessary consequence for isolating the PBB experiment. The semen was used without extenders, and held at ambient temperature. We believe these factors, especially the delay, accounted for the relatively lower fertility values given in Table 1. Cecil and Bitman (6) reported that no effect on fertility was observed using PB-6.

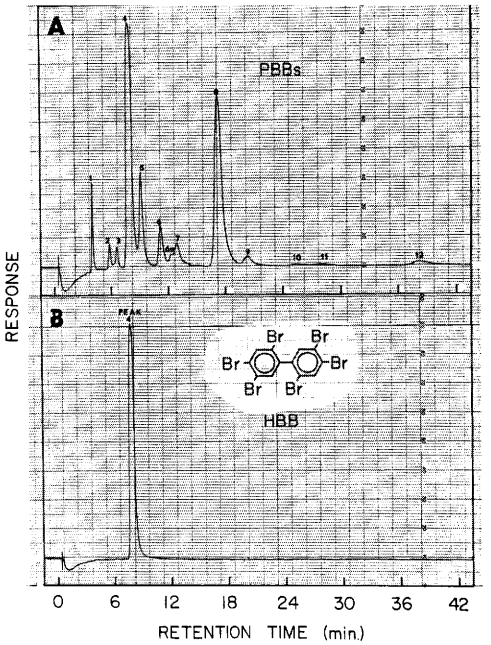

FIG. 1. A. The chromatograph obtained on fireMaster FF-1, a polybrominated biphenyl, according to the procedure in (12), showing 12 components. B. The 2,2′,4,4′,5,5′-hexabromobiphenyl accounts for Peak 4, the compound used in this study.

Eggs were collected daily, held in a room maintained at 10° and set weekly so that, the longest time that eggs were held in storage was 8 days. The hatcher was used only for the PBB experiment in order to prevent PBB contamination elsewhere in the incubation room. Incubated eggs were checked on day 7 for infertiles and these were opened to determine if embryos died at an early stage of development. Incubation was terminated on day 21, as normally practiced. On day 21, all eggs that failed to hatch were opened to assess embryo development and viability. Both chicks and live embryos were examined for edema (5, 6, 10) which occurs at levels of FF-1 or BP-6 exceeding 40 ppm in the diet (2, 8).

Results and discussion. On the average, the intake of FF-1 was comparable to prior experiments (2); although feed intake, and thus intake of chemical, were variable. FF-1 did produce the expected deleterious effects of a poor hatch, as low as 5% during Week #3, and a high incidence of edema, 61.5% in the cervical and/or abdominal areas of both the embryos alive and pipping through the shell at the time the hatch was harvested, and the chicks in the hatching trays (Table I). In marked contrast, the eggs from hens fed 52 ppm HBB-4, a level equivalent to the amount

TABLE I. EFFECT OF FIREMASTER FF-1 AND 2,2′,4,4′,5,5′-HEXABROMOBIPHENYL (HBB-4) ON HATCHABILITY OF CHICKEN EGGS.

	No treatment			80 ppm fireMaster FF-1			52 ppm HBB-4		
	Week #1	Week #2	Week #3	Week #1	Week #2	Week #3	Week #1	Week #2	Week #3
No. hens	10	10	10	10	10	10	10	10	10
Feed intake—g/b/d	161[a]	78	118	119	95	108	82	120	101
No. eggs	41	36	46	40	27	26	30	21	22
% production based on settable eggs	58.6	51.4	65.7	57.1	38.6	37.1	42.9	30.0	31.4
% fertile of eggs set	90.2	83.3	84.8	90.0	74.1	76.9	76.7	47.6	77.3
% hatch of fertile	83.8	73.3	59.0	66.7	35.0	5.0	82.6	60.0	58.8
% alive pips at hatch of fertile	10.8 (4)[b]	23.3 (7)	28.2 (11)	13.9 (5)	35.0 (7)	60.0 (12)	13.0 (3)	0.0	23.5 (4)
% pipped embryos and hatchlings with edema at hatch	0.0	0.0	2.9 (1)	10.3 (3)	35.7 (5)	61.5 (8)	0.0	0.0	14.3 (2)

[a] Excessive spillage from overfeeding.
[b] No. of items.

of HBB-4 in 80 ppm FF-1, hatched as well as the controls, and had a very low incidence of edema, an incidence comparable to that found in controls (Table I). The higher feed intake during Week #2 by the group fed HBB-4 accounted for sufficient chemical intake for a possible effect to occur.

The effect of 80 ppm FF-1 in this experiment is approximately what would be expected based on prior data (2, 6). Egg production declined from Week #1 to Week #3 about 19 percentage units for hens fed 80 ppm FF-1 as compared to a decline of 11 percentage units for hens fed HBB-4 (Table I). During this time the controls showed a net gain of 7 percentage units. Previous studies (2) showed that FF-1 at levels up to 125 ppm had no effect on egg production during Week #1. With only 10 hens per group and an experiment of short duration because of the limited availability of HBB-4, the data on egg production are difficult to interpret. In groups of this size a change in 7–11 percentage units in either direction is not unexpected. In our previous studies (2, 5) egg production data varied this much in groups of 25 hens not showing effects on production. Cecil and Bitman (6) used groups of 10 hens and showed that production can vary that much in hens fed BP-6 at less than toxic levels. They found that 64 ppm BP-6 caused production to decline from an initial value of about 68% in Week #1 to about 40% for Weeks #3 through #8, a decline of 28 percentage units. In our experiments (2) egg production changed the following percentage units from Week #1 to Weeks #3–5: control = +6.1; 30 ppm (a no effect level on hatch) = −10.7; 45 ppm (a borderline effect on hatch) = −29.9; 60 ppm = −3.3; 90 ppm = −23.9; 120 ppm = −35.6. Therefore, the data obtained with HBB-4 indicate that it had no effect or a very slight effect, but in no way near the magnitude of the change caused by FF-1 containing an equivalent level of HBB-4. Apparently the HBB-4 effect on egg production correlates with its lack of effect on the more sensitive indicators, namely embryo toxicity, and edema of chicks and embryos.

There was no decisive effect of FF-1 on fertility (Table I), and this agreed with data from prior experiments (2), and by Cecil and Bitman (6), who used PB-6. Thus, an adverse effect by HBB-4 on fertility was ruled out. The generally lower fertility values throughout reflected our procedural problem of the delay between collection and insemination of the semen.

Some other component(s), other than the main component 2,2′,4,4′,5,5′-hexabromobiphenyl appeared to account for the toxicity of FF-1 at the level evaluated. Biocca *et al.* (11) reported that the 2,2′,4,4′,5,5′-hexachlorobiphenyl fed to young chicks at 400 ppm in the diet was very much less toxic than 3,3′,4,4′,5,5′-hexachlorobiphenyl at 3 or 10 ppm.

HBB-4 is used to monitor PBBs in the ecosystem where levels of less than 1 ppm predominate at the present time. Differential distribution of the congeners was shown (4, 7), so one must consider the possibility that the presence of HBB-4 does not necessarily indicate that the toxic substance is present in proportional quantity to each other as the PBBs pass through the food chain, particularly after withdrawal (4). An example that this occurs can be noted from the hatchability data following withdrawal of PBB diets from chickens (2). Hatches return to normal; yet, eggs have high levels of HBB-4 (2, 8).

We realize that negative data are being reported and are aware that considerable experimentation on isolated congeners of PBBs remain in order to identify and determine their relative toxicity. The importance of chickens as a source of food necessitated that the HBB-4 be evaluated in it as soon as possible. Other reports (9, 14) have indicated that the two major components of FF-1, HBB-4 and 2,2′,3,4,4′,5,5′-heptabromobiphenyl (HBB-8), comprising 83% of the mixture, may be relatively nontoxic. One congener, 2,3′,4,4′,5,5′-hexabromobiphenyl (Fig. 1, Peak 6) (12) would appear to be of primary interest. It was reported (13) to be similar to FF-1 in its ability to produce a mixed-type induction of liver microsomal drug metabolizing enzymes in rats. Both HBB-4 and HBB-8 were strictly phenobarbital-type inducers (9, 14).

Summary. The major component, 2,2′,4,4′,5,5′-hexabromobiphenyl (HBB-4) of the polybrominated biphenyl fireMaster FF-1, involved in the contamination problem in Michigan, did not produce either an edema

in embryos and newly-hatched chicks or a decline in hatchability when fed to laying chickens. However, fireMaster FF-1, containing an equivalent level of the HBB-4 isomer caused the expected deleterious effects.

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