Table IV. Methanol-Ethanol Re-extraction of Radioactivity from Solids[a] Unremovable by Extraction D

Age of Residues, Days	Radioactivity, %[b]		
	Chloroform-soluble	Water-soluble	Inextractable
0	43.9 ± 11.5	32.5 ± 7.7	23.6 ± 7.2
7	5.7 ± 1.4	54.5 ± 27.5	39.8 ± 26.2

[a] 2 to 3 replicate samples. [b] ± mean deviation.

Table V. Malathion Residues[a] (P.P.M.[b]) Extracted from Gooseberries

Days after Application[c]	Extraction A[d]	Extraction D[e]	Extraction A/ Extraction D
0	132.0 ± 6.3[e]	125.0 ± 6.0	1.056
3	68.0 ± 1.8	63.0 ± 4.7	1.079
7	41.7 ± 1.5	39.5 ± 3.4	1.056

[a] Each value is mean of duplicate determinations on four samples.
[b] ± standard deviation.
[c] Concentration of dip emulsion: 570 p.p.m. actual malathion.
[d] Extraction with benzene from unmacerated berries.
[e] Extraction with chloroform and methanol–acetone mixture from macerated berries.

different for very aged residues and for other kinds of plant material—e.g., citrus fruits.

The decrease in efficiency of the stripping method after the passage of time is thought to be due primarily to the penetration of malathion into the plant tissue, from which it is difficult to remove without prior maceration with a solvent. The fixation of malathion to the plant constituents in a firmly chemically bound and therefore inextractable form is evidently of minor importance (Tables III and IV).

It is assumed that after malathion is applied to the crop surface, it starts to penetrate through the cuticle into the outermost cell layers. Here the chemical is at first rapidly degraded into water-soluble metabolites (Table III), and therefore no malathion can be accumulated in the plant tissue during this initial period. At this time, stripping the crops without maceration removes malathion quantitatively. Later, the breakdown of malathion into water-soluble products (*4, 8, 18, 19*) decreased (Table III), probably because of inhibition of the malathion-degrading enzymes (*3, 7, 8, 9, 18*) by malathion itself or its antiesterase derivatives (*7, 12, 13, 15, 18*). When the rate of degradation of malathion in the plant tissue is thus reduced, its lifetime and amount are gradually increased and it may penetrate further into the tissue. At this time, maceration of the crop somewhat increases the efficiency of the extraction methods.

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FUMIGANT RESIDUES

Bromide Residues in Chicken Tissues and Eggs from Ingestion of Methyl Bromide–Fumigated Feed

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EARLIER reports have dealt with bromide residues in milk from cows fed on diets containing bromide residues. Young *et al.* (*4*) fed peanut vines grown in soil which had been fumigated with ethylene dibromide and Lynn *et al.* (*2*) fed rations to which sodium bromide had been added or which had been fumigated with methyl bromide. Another consideration, when crops are fumigated with organic bromide fumigants or are produced from fumigated land, is that chickens eating such feed could produce eggs and meat containing inorganic bromide residues. This study was carried out to determine what residues would occur in eggs and chicken tissues as the result of feeding rations which contain bromide residues resulting from fumigation with methyl bromide.

Laying hens have been fed diets containing from <5 to 410 p.p.m. of bromide residue. Eggs were collected over a period of 70 days, and hens were sacrified at 28, 44, 56, and 70 days. Total bromide residues were determined on whole eggs, egg whites, yolks, and shells, and light meat, dark meat, liver, kidney, skin, feathers, and blood. At equilibrium, ratios of average bromide residues in the tissues to the feed content were: whole eggs, 1.0; yolks, 1.2; whites, 0.8; egg shells, 0.3; light meat, 0.2; dark meat, 0.3; skin, 0.4; liver, 0.5; feathers, 0.6; kidneys, 0.8; and blood, 1.7.

Analytical Methods

Most samples were analyzed by an x-ray fluorescence measurement as described by Getzendaner (*1*). For calibration purposes, selected samples at different concentration levels were analyzed chemically by the method of Shrader *et al.* (*3*). In this manner a "sensitivity factor" was derived to convert peak heights to concentration. Because of the bulky nature of feathers it was not possible to press them into a dense pad suitable for x-ray fluoresence analysis. The feathers were consequently analyzed chemically (*3*).

Experimental Procedure

One ton of a complete layer ration was obtained from a local supplier. Five 100-pound burlap bags of this feed were fumigated with methyl bromide in a large fumigation vault at the rate of 0.7 pound per 27 cu. feet. After 48 hours of aeration and thorough mixing of the feed, it analyzed 410 p.p.m. of bromide residue. The experimental feeds were prepared in 225-pound batches from this. After mixing, each batch was bagged in 25-pound portions in polyethylene bags, which were stored under refrigeration, until needed for feeding. During bagging, three samples were collected randomly for analysis. Table I gives the data on preparation and analysis of feeds used.

Twelve groups of four hens each were put into individual pens and fed on the untreated feed for about 2 weeks prior to initiation of the experimental period. All eggs were collected, starting with the pretreatment feeding until the end of the experiment. After 28, 44, and 56 days on the fumigated feed, one bird from each treatment was sacrified. After 70 days the remaining hens were sacrified. All of the light and dark meat, liver, kidneys, skin, and feathers were collected from all hens at slaughter. Blood was collected from those sacrified at 70 days. Each tissue type from each hen was analyzed separately. Representative samples of the light meat and dark meat, and all the skin, were ground in a small meat grinder to give homogeneous masses from which aliquots were removed for analysis. Whole liver and the kidneys were macerated by hand.

Eggs from each pen on selected days were blended together for analysis. As indicated in Table II, whites and yolks of individual eggs from the 51- and 410-p.p.m. feeding rate were analyzed to determine the partition of bromide into these parts. These samples were selected after the level of bromide residue had reached a maximum. Shell samples were made of a composite of several days' eggs, selected as above. Each sample consisted of the shells of 6 eggs, which were dried overnight at room temperature, then crushed into small pieces for analysis. Because of the high mineral content of the shells, sensitivity of the x-ray fluorescence method was reduced from the usual 5 p.p.m. to 13 p.p.m.

Results and Discussion

Bromide residues in whole eggs collected during the feeding period are shown in Figure 1. The maximum level of residue attained in the eggs was approximately equal to the concentration of bromide in the feed. There was a tendency for a maximum to be reached at around 20 to 40 days while on feed, with a gradual drop beyond this time. Wide variations in residue content were found.

Table II gives the results of analyses of individual egg whites and yolks over the period of 32 to 36 days on the experimental diets. At the lower level of feeding the yolks contained an average of more than twice the amount in the whites. In eggs from the 410-p.p.m. bromide feeding, the ratio of bromides in yolks and whites was 3 to 2.

The results of analyses of chicken tissues are given in Table III. The averages of tissue residues found after 70 days are plotted in Figure 3. All of the tissue residues were directly proportional to the dietary level. A wide variation of levels in the same tissue from different individuals on the same feed (Table IV) was noted. The fact that the points for feathers and skin are more widely scattered than those for the other tissues

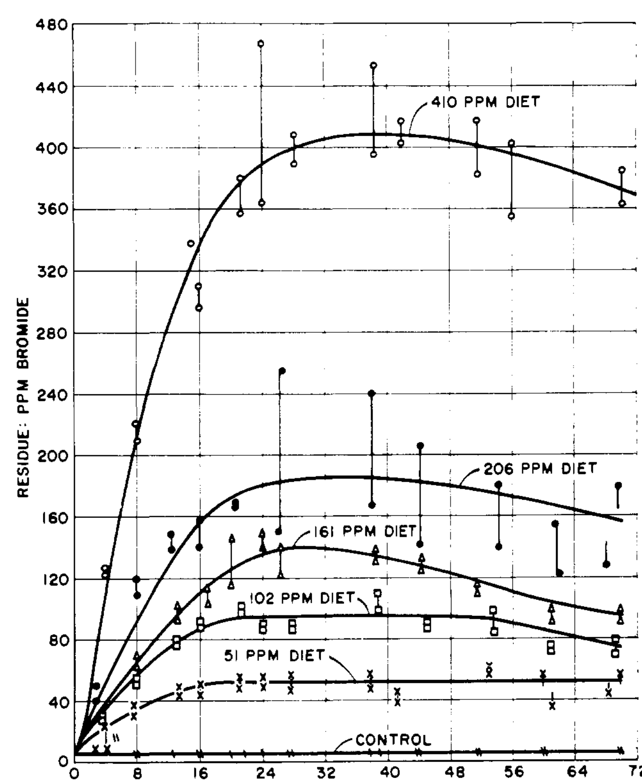

Figure 1. Bromide residues in eggs from chickens on fumigated diets

╲ Control	△ 161-p.p.m. diet
× 51-p.p.m. diet	● 206-p.p.m. diet
□ 102-p.p.m. diet	○ 410-p.p.m. diet

Table I. Preparation of Feed for Laying Hens

Feed Mixed, Lb.		Bromide Content, P.P.M.			
Fumigated	*Unfumigated*	*1*	*2*	*3*	*Av.*
0	225	<5	<5	<5	<5
28	197	48	50	54	51
56.3	168.7	104	104	98	102
84.3	130.7	163	165	155	
		150	148	161	161
112.5	112.5	209	210	199	206
220	0	402	416	413	410

Table II. Bromide Residues Found in Egg White and Yolk

Days on Fumigated Feed	Br Found, P.P.M. — 51 P.P.M. in Feed: In white	51 P.P.M. in Feed: In yolk	410 P.P.M. in Feed: In white	410 P.P.M. in Feed: In yolk
32	..	..	324	506
			280	480
33	32	57	293	450
			290	441
34	28	62	428	550
	26	71	...	...
35	26	77	276	392
	24	56	...	...
36	34	76	...	...
Av.	30	67	315	473

Table III. Bromide Residues Found in Chicken Tissues

Treatment and Tissue	Bromide Residues, P.P.M., Found after: 28 Days, Bird 1	44 Days, Bird 2	56 Days, Bird 3	70 Days, Bird 4	5	6	7	8	Av.
Control									
Light meat	<5	<5	<5	<5	<5	<5	<5	<5	...
Dark meat	<5	<5	<5	<5	<5	<5	<5	<5	...
Liver	6	<5	<5	<5	<5	<5	<5	<5	...
Kidneys	8	<5	<5	<5	<5	<5	<5	<5	...
Skin	<5	<5	<5	<5	<5	<5	<5	<5	...
Feathers	6	3	41	17	1	0.5	20	1.2	...
Blood	...	...	...	...	<5	<5	<5	<5	...
51 p.p.m. Br									
Light meat	11	9	10	12	15	14	14	15	14
Dark meat	13	13	14	13	18	18	19	18	17
Liver	33	28	39	21	28	21	26	30	25
Kidneys	45	29	47	36	44	39	44	35	40
Skin	30	28	26	12	12	13	18	14	14
Feathers	23	33	51	77	61	53	70	53	63
Blood	...	...	...	90	86	96	96	103	94
102 p.p.m. Br									
Light meat	16	23	19	23	24	23	28	23	24
Dark meat	28	28	26	29	39	33	42	33	35
Liver	40	61	53	36	39	50	39	32	37
Kidneys	65	92	71	68	74	81	66	72	72
Skin	50	36	26	21	25	29	41	22	28
Feathers	10	94	85	38	33	85	117	47	64
Blood	...	...	...	146	157	171	162	144	156
161 p.p.m. Br									
Light meat	31	25	25	27	40	25	31	30	31
Dark meat	41	36	40	42	70	40	58	36	49
Liver	80	62	65	66	104	64	73	56	73
Kidneys	116	102	95	40	109	72	130	102	91
Skin	56	65	47	32	73	34	46	52	47
Feathers	15	86	83	61	34	82	158	174	102
Blood	...	...	...	222	232	189	241	219	181
206 p.p.m. Br									
Light meat	<5	38	33	44	42	46	44	42	43
Dark meat	8	49	32	49	64	64	71	70	64
Liver	18	108	74	62	121	65	112	144	101
Kidneys	26	140	113	163	122	134	152	172	149
Skin	19	95	62	80	47	47	53	66	59
Feathers	22	166	58	24	39	115	33	95	61
Blood	...	...	...	262	263	308	331	379	309
410 p.p.m. Br									
Light meat	66	63	60	95	88	108	...	...	74
Dark meat	100	86	101	145	113	144	...	...	134
Liver	172	130	195	77	164	219	...	...	153
Kidneys	344	176	288	308	292	304	...	...	301
Skin	290	176	145	206	135	144	...	...	162
Feathers	34	61	312	133	515	102	...	...	250
Blood	...	...	...	702	703	670	...	...	692

Table IV. Bromide Residues in Egg Shells

Bromide in Feed, P.P.M.	Days on Fumigated Feed		Bromide in Egg Shells, P.P.M.
Control	...		<13
			<13
			<13
		Av.	<13
51	46–66		<13
			36
			17
		Av.	19
102	41–54		13
			<13
			27
			21
		Av.	17
161	42–54		23
			25
			39
			29
			29
		Av.	29
206	42–56		44
			33
			42
			34
		Av.	38
410	42–70		103
			116
			96
			85
		Av.	100

Table V. Rate of Bromide Accumulation after 70 Days on Feed

Tissue	Ratio: Av. P.P.M. in Tissue / P.P.M. in Feed	Ratio: Max. P.P.M. in Tissue / P.P.M. in Feed
Whole eggs (no shells)	1.0	1.3
Yolks	1.2	1.5
Whites	0.8	1.1
Egg shells	0.3	0.5
Light meat	0.2	0.23
Dark meat	0.3	0.5
Skin	0.4	0.5
Liver	0.5	0.7
Feathers	0.6	1.5
Kidney	0.8	0.9
Blood	1.7	2.1

in Figure 3 can probably be ascribed to external contamination of these tissues by the feed. Table V shows the relationship of bromide accumulation in all of the tissues to that ingested at the 70-day sacrifice period. The second column gives the ratio, taken from Figure 3, of the average residue in each tissue to that ingested. Each ratio is the slope of its corresponding curve in Figure 3. The third column gives the ratio based on maximum residue in each tissue.

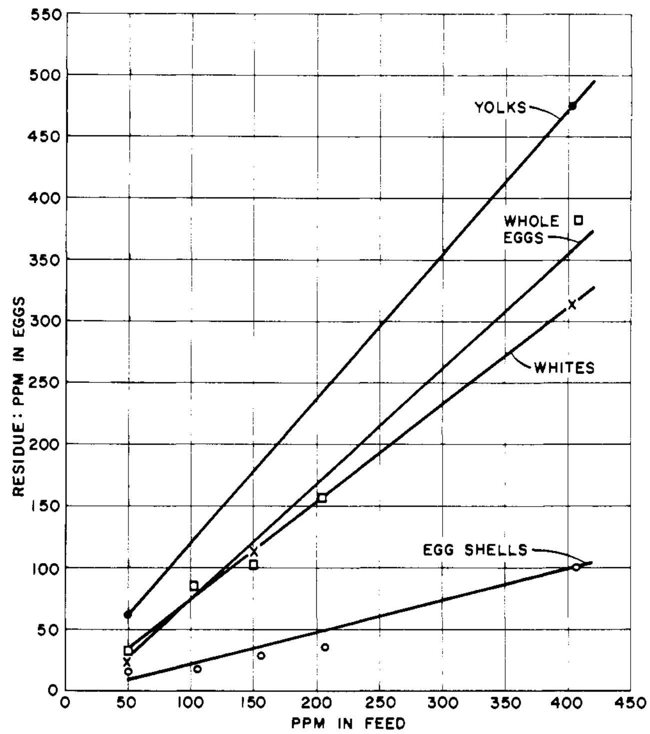

Figure 2. Residues of bromide in eggs vs. bromide content of feed

□ Whole eggs
O Egg shells
● Yolks
× Whites

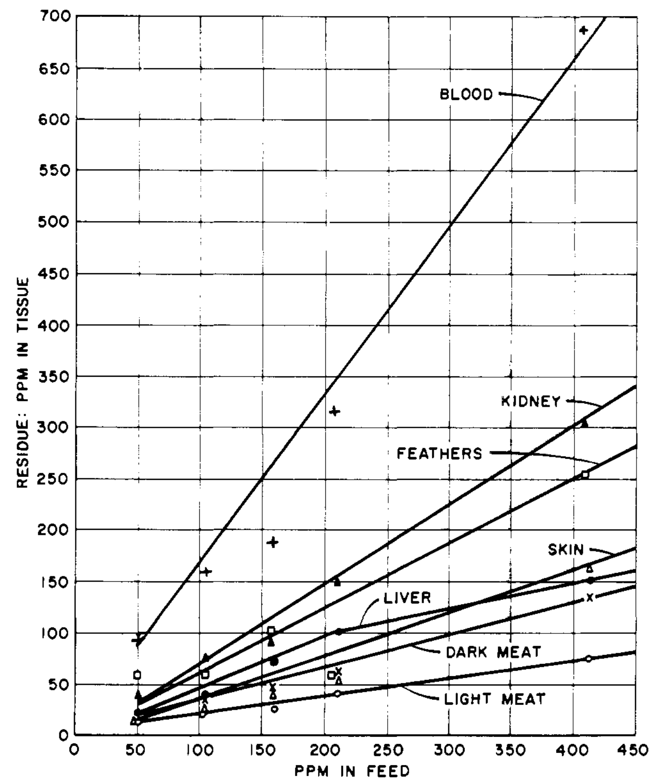

Figure 3. Residues of bromide in chicken tissues after 70 days on fumigated diets

O Light meat
× Dark meat
● Liver
△ Skin
□ Feathers
▲ Kidney
– Blood

Egg shells (Table IV) and feathers (Table III) were analyzed to determine their contribution when included in the diet of chickens. Figure 2 compares the residue content of whole eggs with yolks, whites, and shells. Shells contained only about 25% of the level of bromide residue in the feed. Thus, they would not increase the total intake of bromide appreciably. Feathers contained an average of two-thirds the level of bromide in the feed (Figure 3 and Table V). Modern practice in chicken feeding is to collect the feathers from the pickers, hydrolyze them in basic medium under pressure, and dry the hydrolyzate. This may be included in chicken feed at up to 5% of the diet. Since the bromide content in feathers is generally below the level in the feed being consumed, there would be no buildup of bromide residues in feed because of added feather meal.

In like manner, feather meal may be included in cattle-fattening rations, where it usually comprises not more than 2% of the diet. Feather meal would contribute but insignificant amounts of bromide to the diet of cattle.

Conclusions

The bromide content of eggs and of chicken tissues reaches a maximum in 30 to 40 days when the dietary intake is maintained at a fixed level. Although there are individual variations, a straight-line relationship exists between the dietary intake and the average bromide residue content of each tissue at equilibrium.

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