Occurrence of Bromides in the Milk of Cows Fed Sodium Bromide and Grain Fumigated with Methyl Bromide

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Bromide is secreted in the milk of lactating dairy cows when the element is present in their diet as a result of naturally occurring bromides in forage or grain, added sodium bromide, or inorganic bromides arising from methyl bromide fumigations. The amount of bromide in the milk is proportional to that in the diet and appears to be correlated to levels of bromide in the blood. A total diet containing 43 p.p.m. bromide resulted in 10 to 20 p.p.m. bromide in milk with no effect on production.

When grain and other agricultural commodities are fumigated with methyl bromide some of the methyl bromide reacts with the commodity to form methylated compounds, with a concurrent formation of bromide ions (7). The bromide residues are nonvolatile and are not removed by aeration. Most of the methyl bromide is unreacted and because of its high vapor pressure (760 mm. of Hg at 4.5° C.) is quickly dissipated by aeration (2–6). Since grains bearing bromides from methyl bromide fumigations are likely to be fed to dairy cows, an investigation has been conducted to elucidate the relationship between bromide intake by cows and bromide levels in milk. This study included a survey to determine to what extent naturally occurring bromides are found in milk.

Analytical Method

The total bromide residues presented were determined by the method described by S. A. Shrader *et al.* (5). Recovery experiments have shown that the method will determine 95% of bromide added as potassium bromide to natural substrates, such as grain flour, and cheese. Blood samples of 20 grams and milk samples of 25 grams were used in the analytical procedure.

Naturally Occurring Bromides in Milk

Bromides occur naturally and might be expected to occur in feeds grown on native soils. To determine whether or not bromides occur naturally in milk, samples were collected for total bromide analysis from several areas of the United States. The samples of milk and dairy feed used were collected from dairies where no known bromine fumigant had been used either on the soil or on the diet. Results of these analyses (Table I) show that bromides may be present naturally in milk in amounts ranging from nil to 8 p.p.m. Amounts in the

Table I. Total Bromide in Milk and Dairy Feed from Various Locations in the United States

Total Br, P.P.M. Milk	Feed	Feed Description
		FREEHOLD, N. J.
4	3	Green Forage (white clover, blue and brome grass)
	14	Grain Mix
		SALEM, N. J.
3	5	Forage (brome grass)
	11	Grain Mix
	8	Silage (soybean, sorghum)
		SUFFOLK, VA.
8	9	Green Forage (orchard grass, Ladino clover, fescue)
	11	Grain Mix
	8	Hay
4	9	Green Forage (orchard grass, Ladino clover)
	8	Grain Mix
	9	Hay
		LORAHATCHIE, FLA.
2	3	Forage
	2	Mixed Dry Ration
	5	Dried Citrus Pulp
	18	Sun Dried Alfalfa Meal
	5	Purina Milk Chow
		W. PALM BEACH, FLA.
1	<1	Forage
	5	Special Dairy Feed
		CLEARWATER, FLA.
1	2	Millet
	3	Cottonseed Hulls + Citrus Pulp + Alfalfalass
	9	Special Dairy Feed
		SANFORD, FLA.
1	9	Xtra Good Dairy Feed
	3	Dried Citrus Pulp
	2	Grass
	4	Feed (grain)
		CLEARWATER, FLA.
2	10	Vigo-Bulky Dairy Feed
	3	Citrus Pulp + Beet Pulp + Alfalfalass
		SUNFLOWER, MISS.
3	7	Silage
	26	Dry Forage
	11	Oats + Wheat + barley

(*Continued on page 88*)

Table I. (Continued)

Total Br, P.P.M. Milk	Feed	Feed Description
	11	Silage
	15	Forage
	6	Feed Concentrate
		INDIANOLA, MISS.
3	10	Silage
	25	Hay (native clover)
	6	Dairy Concentrate
	4	Silage
	12	Feed Concentrate
	20	Forage
3	5	Silage
	7	Cottonseed + dairy feed
	3	Dry Forage
	5	Ground Corn
	10	Dry Forage
		STONEVILLE, MISS.
3	6	Winter Peas + Vetch
	6	Oats + Dairy Feed
	8	Dry Forage (alfalfa + sudan)
		GLEN ALLEN, MISS.
3	5	Dry Forage
	8	Dairy Feed
		RASHANON, TEX.
2	(Feed samples not received)	
		ALVIN, TEX.
2	(Feed samples not received)	
1	(Feed samples not received)	
		ORANGE CO., CALIF.
2	3	Brewer's Malt
	12	Alfalfa Hay
	17	Mixed Grain
	0.08	Water
<4	4	Alfalfa Hay
	18	Mixed grain
	0.07	Water
4	6	Alfalfa Hay
	10	Oats + Barley + Citrus Peel
	6	Alfalfa Hay
	0.07	Water
3	10	Alfalfa Hay
	5	Mixed Grain
	0.13	Water
1	5	Pasture Grass
	16	Alfalfa + Barley
	0.18	Water

Table II. Total Bromide in Blood and Milk of Lactating Cow Given Oral Dose of Sodium Bromide for 5 Days

Day	P.P.M. Total Bromide Blood[a]	Milk[b]
−1	20	10
0	20	10
1[c]	70	27
2[c]	...	44
3[c]	...	52
4[c]	...	53
5[c]	170	60
1	...	58
2	...	49
3	...	41
4	131	35
5	...	30
6	111	28

[a] P.M. sample.
[b] Composite A.M. and P.M. milking.
[c] Days fed 12.5 grams sodium bromide in A.M.

range of 1 to 5 p.p.m. seem to be most likely, and highest amounts are probable in the tidewater area of Suffolk and vicinity. The amounts of bromide present in hay and other dairy feed grown on untreated soil probably has origin in the natural bromides present in the soil. Untreated soils from the tidewater area of Virginia have been shown to contain from 10 to 30 p.p.m. bromide (*7*).

Bromides in Milk as a Result of Feeding Sodium Bromide

In a pilot experiment, a lactating cow was fed five consecutive daily doses of 12.5 grams each of sodium bromide. Samples of blood and milk were analyzed for total bromide. Results are given in Table II.

Following this experiment, a further study was conducted at Michigan State University where sodium bromide was added to the grain ration of dairy cows at rates equivalent to 50, 100, and 200 p.p.m. bromide. Grain was fed to four cows in the proportion of 1 pound per 4 pounds of milk produced. After the bromide level had equilibrated in the milk from feeding the 50 p.p.m. level, the next highest level was fed, and so on. Milk samples were sent from East Lansing, Mich., to The Dow Chemical Co. laboratories in Midland, Mich., and analyzed for total bromide. Results of this investigation are given in Table III.

Bromide Content of Cow's Milk and Blood Resulting from Feeding Grain Fumigated with Methyl Bromide

A grain ration, consisting of oats and corn, was fumigated with sufficient methyl bromide to yield 53, 100, and 220 p.p.m. bromide residue, respectively, in three separate lots. Each of these rations was fed to a group of four lactating cows, and the milk was analyzed at frequent intervals for total bromide. The ration was fed at the rate of 1 pound for each 4 pounds of milk produced. Cows on this experiment were eating about 18 kilos of food per day. Of this total, 3.5 kilos was the fumigated grain ration. The levels of bromide ingested as expressed in p.p.m. based on the total diet are:

P.P.M. Bromide in Grain Ration	in Total Diet
53	10[a]
100	19
220	43

[a] Basis of calculation $= \frac{3.5}{18} \times 53 = 10.$

The bromide content of the grain ration had no effect on the quantity of milk produced (Table IV). Results of the total bromide analysis in milk are given in Tables V, VI, VII, and VIII, and Figure 1.

The data from blood analyses are given in Table IX.

Ratio Between Amount of Bromide Ingested and Bromide Secreted

The information available from the two sources of dietary bromide, i.e., from the addition of sodium bromide, and from grain fumigated with methyl bromide, have been compared to strike a ratio between the amount of bromide secreted in the milk to that ingested in the diet (Table X). The average ratio of secreted to ingested bromide found where the source of the bromide was sodium bromide was 0.18. Where the source of bromide was from methyl bromide fumigated grain, the average ratio was 0.38, which suggests the possibility that a different situation exists where bromide is formed *in situ* from the methylation reaction. These results are of interest and should be confirmed by additional experiments.

Table III. Total Bromide Residue in Milk of Cows Fed Grain Ration Containing Sodium Bromide

Days on Feed	P.P.M. Total Bromide in Milk: Av.	Cow 92	Cow 554	Cow 1107	Cow 3012
0	0	<1	<1	<1	<1
		Begin 50 P.P.M. Br^-			
2	1	1	1	1	1
6	1	2	1	1	1
9	2	2	2	2	2
12	2	2	2	2	2
14	2	3	1	2	1
15	2	3	2	2	1
17	2	3	2	2	1
19	2	3	2	2	2
21	2	3	1	1	2
22	3	4	3	3	2
		Begin 100 P.P.M. Br^-			
24 (1)	3	3	3	3	2
27 (4)	4	4	3	4	3
30 (7)	4	5	3	4	3
33 (10)	5	6	4	5	3
35 (12)	4	5	3	5	4
37 (14)	4	5	3	4	3
39 (16)	4	5	3	5	3
42 (19)	5	6	3	6	3
		Begin 200 P.P.M. Br^-			
45 (1)	6	8	5	6	5
48 (4)	8	11	7	7	6
51 (7)	9	13	7	9	6
54 (10)	10	10	7	14	8
57 (13)	11	16	10	12	7
60 (16)	11	17	10	11	7
63 (19)	12	18	10	12	8
66 (22)	12	18	10	12	8
68 (24)	12	18	9	13	8
72 (28)	12	19	9	12	8

Table IV. Average Milk Production of Four Cows in Each Feeding Group

Days on Feed	53 P.P.M.[a] Lb.	53 P.P.M.[a] Kg.	100 P.P.M.[a] Lb.	100 P.P.M.[a] Kg.	220 P.P.M.[a] Lb.	220 P.P.M.[a] Kg.
0	33	15.0	31	14.0	31	14.0
3	33	15.0	31	14.0	30	13.7
6	31	14.0	31	14.0	29	13.2
9	31	14.0	30	13.7	29	13.2
12	31	14.0	29	13.2	30	13.7
15	31	14.0	29	13.2	31	14.0
18	30	13.7	30	13.7	31	14.0
21	28	12.7	29	13.2	29	13.2
24	30	13.7	28	12.7	29	13.2
27	30	13.7	29	12.7	29	13.2
30	30	13.7	28	12.7	29	13.2
33	31	14.0	28	12.7	29	13.2
36	29	13.2	29	13.2	29	13.2
39	30	13.7	29	13.2	29	13.2
− 4	30	13.7	29	13.2	29	13.2
− 9	28	12.7	29	13.2	29	13.2

Feeding Group, Av. Lb. and Kg. of Milk per Cow

[a] P.p.m. bromide in grain ration.

Table V. Total Bromide Residue in Cow's Milk—Cows Fed Grain Ration Containing 53 P.P.M. Bromide—Resulting from CH_3Br Fumigation

Days on Feed	P.P.M. Total Bromide in Milk: Av.	Cow 72	Cow 573	Cow 1109	Cow 3009
0	0	<1	<1	<1	<1
3	3	3	2	3	2
6	3	4	3	3	2
9	4	4	4	3	3
12	4	6	4	5	2
15	5	6	4	6	3
17	5	6	5	5	2
18	6	8	5	6	4
20	5	6	4	7	2
21	5	6	4	7	2
22	5	6	5	6	3
24	6	7	9	5	2
27	6	9	5	6	3
30	7	9	6	7	4
32	9	12	12	8	5
33	8	11	8	8	5
34	8	12	9	9	4
36	8	10	7	8	4
39[a]	8	10	7	8	4
0	7	11	...	7	4
−4	6	9	6	5	2
−9	4	5	4	6	2

[a] Last day on feed.

Table VI. Total Bromide Residue in Cow's Milk—Cows Fed Grain Ration Containing 100 P.P.M. Bromide—Resulting from CH_3Br Fumigation

Days on Feed	P.P.M. Total Bromide in Milk: Av.	Cow 575	Cow 1111	Cow 2001	Cow 3002
0	0	<1	<1	<1	<1
3	3	2	4	2	2
6	4	4	5	3	2
9	6	5	8	5	5
12	6	6	8	5	5
15	5	5	7	4	3
18	7	6	10	6	4
20	7	9	9	5	6
21	6	6	8	6	5
22	6	6	9	5	5
24	6	5	8	5	5
25	7	6	9	5	6
27	8	8	11	6	7
29	7	6	10	8	5
30	7	7	10	5	6
31	7	7	8	6	6
33	7	8	10	6	7
36	8	8	12	7	6
39[a]	8	8	11	7	6
0	8	6	11	9	7
−4	7	7	10	7	5
−9	5	5	5	4	4

[a] Last day on feed.

Table VII. Total Bromide Residue in Cow's Milk—Cows Fed Grain Ration Containing 220 P.P.M. Bromide—Resulting from CH_3Br Fumigation

Days on Feed	P.P.M. Total Bromide In Milk: Av.	Cow 576	Cow 1131	Cow 2014	Cow 3010
0	0	<1	<1	<1	<1
3	3	3	3	4	3
6	7	8	8	7	6
9	9	11	10	8	5
12	12	13	12	12	9
15	13	14	15	13	10
18	14	15	16	15	11
21	14	15	15	15	11
24	14	13	17	14	11
26	14	15	15	14	10
27	15	17	16	12	...
28	16	17	20	17	10
30	14	15	17	15	10
33	15	15	18	16	11
36	15	16	16	16	12
39[a]	15	16	19	15	11
0	16	17	20	17	10
−4	14	16	14	14	10
−9	9	11	10	8	7

[a] Last day on feed.

Table VIII. Bromide Secreted in Milk—Cows Fed Grain Ration Containing 53, 100, and 200 P.P.M. Bromide

Day	P.P.M. Bromide in Milk[a]: 53[b]	100	220	Mg. Br^- Secreted/Cow/Day in Milk[a]: 53	100	220
0	0	0	0	0	0	0
3	3	3	3	45	42	41
6	3	4	7	42	56	92
9	4	6	9	56	82	119
12	4	6	12	56	79	164
15	5	5	13	70	66	182
18	6	7	14	82	96	196
21	5	6	14	64	79	185
24	6	6	14	82	77	185
27	6	8	15	82	102	198
30	7	7	14	96	89	185
33	8	7	15	112	89	198
36	8	8	15	105	105	198
39	8	8	15	110	105	198
−4	6	7	14	82	93	185
−9	4	6	9	51	66	119

[a] Average of 4 cows.
[b] P.p.m. bromide in grain ration. 53 p.p.m. in diet ≠ 185 mg. bromide ingested per cow per day. 100 p.p.m. in diet ≠ 350 mg. bromide ingested per cow per day. 220 p.p.m. in diet ≠ 770 mg. bromide ingested per cow per day.

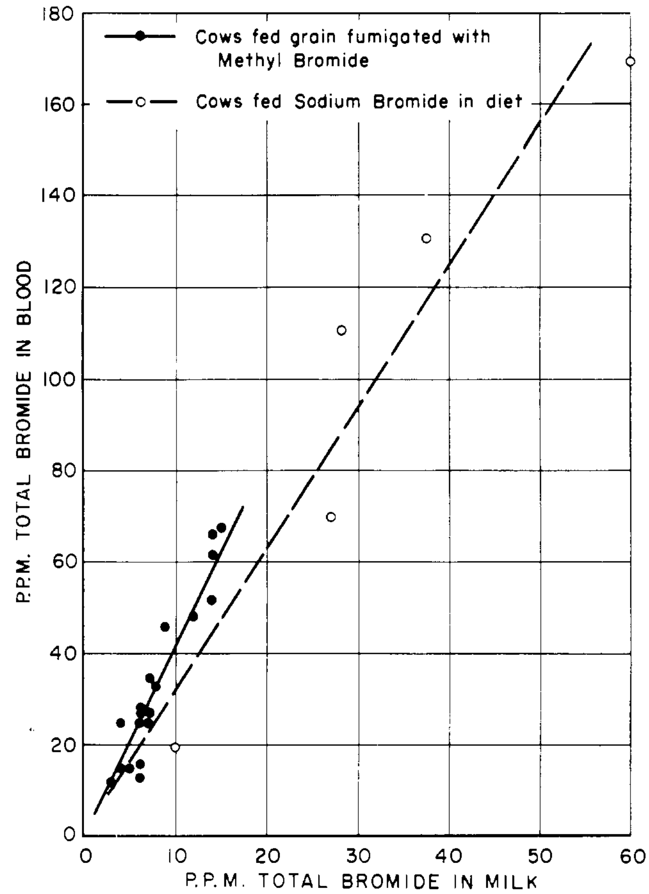

Figure 1. Correlation of blood and milk bromide in cows

Table IX. Total Bromide in Blood of Cows Fed Grain Ration Containing Bromide from Methyl Bromide Fumigation

Days on Feed	0[a]: Cow 101	Cow 572	Av.	53[a]: Cow 573	Cow 1109	Av.	100[a]: Cow 575	Cow 1111	Av.	220[a]: Cow 576	Cow 1131	Av.
0	...	...	...	1	1	1	1	2	2	1	2	2
4	3	3	3	10	13	12	15	20	18	22	27	25
11	2	3	3	12	17	15	20	34	27	40	52	46
18	4	2	3	13	18	16	20	33	27	46	58	52
25	6	5	6	11	15	13	14	36	25	54	68	61
32	...	...	...	22	33	28	27	42	35	55	76	66
39	...	...	...	22	30	26	28	38	33	55	80	68
−6	3	4	4	14	16	15	20	30	25	41	55	48

P.P.M. Total Br^- in Blood

[a] P.p.m. of bromide in grain ration.

Table X. Ratio Between Amount of Bromide Secreted by Cows[a] in Milk and Amount of Bromide Ingested

Commodity Descrip.	P.P.M. Br^-	Mg. Br^- Ingested per Day	Kg. Milk Produced per Day	P.P.M. Br^- in Milk	Mg. of Br^- Secreted in Milk per Day	Ratio Mg. of Br^- Secreted in Milk/Mg. of Br^- Intake	
Grain–CH_3Br	53	185	13.7	8	109	0.59	
	100	350	13.2	8	106	0.30	Av. 0.38
	220	770	13.2	15	198	0.26	
Grain–NaBr	50	175	13.2	2	26	0.15	
	100	350	13.2	4	53	0.15	Av. 0.18
	200	700	13.2	12	159	0.23	

[a] Four cows at Michigan State University, fed 3.5 kg. per day.

Correlation of Blood and Milk Bromide Levels

The correlations between blood and milk bromide levels where the cows were fed sodium bromide or grain fumigated with methyl bromide are plotted for comparison in Figure 1. Although the slopes of the regression curves are slightly different, they indicate similarity between the sources of bromide with respect to blood–milk bromide level relationships.

Conclusions

When dietary bromide is held at a steady concentration, an equilibrium is established with respect to milk bromide levels. The time of equilibration appears to be about 20 to 30 days.

Lactating cows fed a methyl bromide fumigated grain ration at the rate of 1 pound of ration to each 4 pounds of milk produced will secrete milk having increased levels of bromide as follows:

P.P.M. Total Bromide in Grain	P.P.M. Total Bromide in Milk
53	4 to 12
100	7 to 12
220	10 to 20

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SOLVENT EFFECTS ON TOXICITY

Reaction of Certain Phosphorothionate Insecticides with Alcohols and Potentiation by Breakdown Products

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Most pesticides are formulated with solvents, wetting agents, and other materials which increase the opportunity of the pesticide to contact the pest. Formulating agents are selected on the basis of their chemical, physical, and toxicological properties so as to achieve maximum pest control with minimal toxicity to other organisms. Careful toxicological studies are made on the pesticide before and immediately after formulation. As the formulated pesticide is often stored for long periods, and sometimes is exposed to elevated temperatures, the toxicological properties of the formulation must be rechecked on samples undergoing various storage conditions.

Organophosphorus insecticides often pose difficulties in formulation, particularly when a high degree of systemic activity is desired. The instability of certain organophosphates may allow degradation to a variety of toxic agents other than the insecticide as manufactured. Phosphorothionates may undergo oxidation or isomerization (*18*). Transalkylation may yield degradation products of greater mammalian toxicity (*12*). Hydrolysis may destroy the biological activity of the insecticide (*18*), or may yield ions which reattack the organophosphate to yield highly toxic materials, such as the formation of tetraethyl monothionopyrophosphate from Diazinon (*15*). These reactive organophosphoric ions might also combine with certain of the formulation constituents.

The solvent characteristics and toxicological properties of 2-methoxyethanol suggested it as a solvent for formulation of dimethoate [Rogor, *O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate] for use as a plant systemic. Routine checks on toxicological properties of formulated material revealed an unexpected toxic hazard. A preliminary report on the potential hazard from formulating dimethoate in 2-methoxyethanol has been published (*4*). Experimental details are considered here.

Materials and Methods

Insecticides and Solvents. Technical grade solvents were used for the formulation studies, although highly purified 2-methoxyethanol gave the same results as technical material. The insecticides, as obtained from their basic manufacturers, were of technical purity except for dimethoate where samples of varying purity were investigated. Dimethoate-P^{32} was prepared, purified, and characterized by a described procedure (*6*).

Fractionation of Dimethoate Derivatives. To the decomposed dimethoate in solvent was added 20 or more volumes of water. The aqueous solution was then neutralized and extracted twice with equal volumes of chloroform to remove the neutral phosphorus esters. The percentage hydrolysis was calculated from the proportion of the total phosphorus which appeared in the aqueous layer as phosphoric acid salts following the first extraction. After drying the chloroform with anhydrous sodium sulfate and evaporating the solvent, the neutral esters were fractionated on a silica gel column with *n*-hexane and chloroform (*6, 7*). Hydrolysis products were fractionated on an ion-exchange column (*6, 7, 19*), but remained largely uncharacterized. The insecticides other than dimethoate were similarly fractionated by