# Consumption and Fate of Aflatoxin $B_1$ by Lactating Cows

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Four lactating cows, in a Latin square design, were fed concentrate containing either 10, 50, 250, or 1250 ppb of aflatoxin $B_1$ ($AFB_1$) for 14 days with no $AFB_1$ for 56 days between treatment. Within squares the cow on the highest dose received [$^3$H]$AFB_1$ to follow excretion patterns. $AFM_1$ in milk increased to day 4 with little change through day 14 in cows fed 250 and 1250 ppb of $AFB_1$. Two days after treatment cessation no $AFM_1$ was found in milk. Traces of $AFM_1$ were found in the 50-ppb treatment and none at 10 ppb. Regression analyses indicated that concentrate $AFB_1$ must exceed 46 ppb for $AFM_1$ to be detectable in milk. This is equivalent to 15 ppb of $AFM_1$ in the total ration or 230 $\mu$g per day. A lag in tritium excretion in milk relative to urine indicated these excretory routes were served by separate pools.

Aflatoxins are a group of extremely toxic and hepatocarcinogenic compounds that may pose a threat to animal and human health when present in feed and food sources (Purchase, 1972). Aflatoxins are produced by *Aspergillus spp.* (Sargeant *et al.*, 1961) where growth is favored by a warm and humid climate (Kumar and Sampath, 1971; Schindler *et al.*, 1967). Aflatoxin $B_1$ ($AFB_1$) has been detected in peanuts and peanut products, cottonseed-based feeds, and certain other common feed sources (Schroeder and Boller, 1973). Recently, the known incidence of $AFB_1$ in corn has increased (Shotwell *et al.*, 1972, 1973). These feedstuffs represent a major source of nutrients fed all classes of livestock and poultry that are destined for slaughter or milk production. Therefore, it is essential that the disposition and excretory patterns of aflatoxins by food producing animals be understood.

In the early research, the carcinogenic properties of aflatoxin in many species became known. Bile duct proliferation (Newberne and Butler, 1969), alterations in liver mitochondrial activity (Svoboda *et al.*, 1966; Doherty and Campbell, 1972, 1973), fatty infiltration (Hamilton and Garlich, 1971), impaired protein and RNA synthesis (Clifford and Rees, 1966), and reduced liver vitamin A content (Lynch *et al.*, 1971; Allcroft and Lewis, 1963) are all indicative of the metabolic impact of dietary aflatoxins. Sheep appeared to be more tolerant of aflatoxins than other species (Newberne and Butler, 1969). The $LD_{50}$ for a single dose in young calves is approximately 1.5 mg/kg body weight (Lynch *et al.*, 1972). Urinary nitrogen losses increased in calves as chronic aflatoxin intake increased linearly from 0 to 0.08 mg/kg body weight (Lynch *et al.*, 1973).

$AFM_1$, which is the hydroxylated metabolite of $AFB_1$, was first found in milk and resides principally with the protein fraction (Allcroft and Carnaghan, 1963). The quantity secreted in milk was in direct proportion to intake (Allcroft and Roberts, 1968). When high levels are fed, 1–3% of the ingested dose appears in the milk. Milk levels of $AFM_1$ drop rapidly when intake is discontinued (Linde *et al.*, 1965). In view of the likelihood of feed contamination, it therefore becomes important to determine the minimum intake of $AFB_1$ which produces detectable levels of $AFM_1$ in cows' milk. The study reported herein had this as the first objective. Secondary objectives were to measure the excretion patterns and tissue distribution of metabolic products after ingestion of $AFB_1$. Identification and quantification of other possible metabolites in milk and tissues will be reported elsewhere.

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## EXPERIMENTAL SECTION

Four cows were selected by freshening dates, health, and availability from the University herd for this study. Later, one cow ceased lactating from causes not apparently unrelated to treatment and was replaced halfway through the experiment. The experimental design was a 4 × 4 Latin square in which four levels of $AFB_1$ (Makor Chemical Co. Ltd., Jerusalem, Israel) were administered orally at four time periods of lactation.

The cows were fed a ration consisting of corn silage, hay, and concentrate. The concentrate was pelleted and contained approximately 18% crude protein and sufficient supplemental calcium, phosphorus, and vitamins A and D to assure the recommended allowances of the NRC (National Research Council, 1971) in the total ration. Concentrate was offered at the rate of 1 unit/3 units of milk produced. In early lactation, this provided about 40% of the ration dry matter. First-cutting well-cured full-bloom Orchard Grass was offered at 1.8 kg/da. Corn silage, offered *ad libitum*, contained 36% dry matter and was 8.0% crude protein and 28% crude fiber on a dry basis. Daily intakes of all rations were recorded.

$AFB_1$ was administered on a twice daily basis at levels of 10, 50, 250, and 1250 ppb of the concentrate over a 14-day period. An 8-week period of no $AFB_1$ administration was maintained between treatment periods. Each cow's allotted concentrate was placed in a rubber feeding tub. $AFB_1$ was distributed over the concentrate as a chloroform solution and the chloroform evaporated by placing in front of a fan for a few hours. Cows consumed virtually all of their respective concentrate.

Prior to dosing, control samples of milk were collected and frozen until analysis. On day 7 the cow receiving the 1250-ppb dose had a jugular catheter and an indwelling bladder catheter established to facilitate blood sampling and total urine collection. On day 8, [$^3$H]$AFB_1$ (0.5 mCi) (tritiated by New England Nuclear, Boston, Mass.) purified by thin-layer chromatography was fed as a portion of the usual dose. Milk and urine were collected, weighed, and, together with blood samples, were taken at 0, 2, 4, 8, 14, and 24 hr and then at 12-hr intervals up to 144 hr. Feces were collected for 12–16-hr periods, weighed, mixed, and sampled. All samples were stored at −10° until analysis. The dosing of [$^3$H]$AFB_1$ to the cow receiving the highest level was repeated on day 8 of subsequent periods and to all cows in period 4, 24 hr prior to slaughter. Except for the first cow dosed, all others were given 1.5 mCi/cow.

Aliquots of blood serum, urine, and milk were added directly to Aquasol (tritiated by New England Nuclear, Boston, Mass.) which served as a solvent and fluor, and

**Table I. Feed Intake, Milk Production, and $AFB_1$ Intake of Cows by Treatment Group**

| | $AFB_1$ in concentrate, ppb | | | | Prob > F |
|---|---|---|---|---|---|
| | 10 | 50 | 250 | 1250 | ANOVA |
| Feed intake | | | | | |
| Grain, kg/day | 5.3 ± 0.8 | 5.1 ± 0.4 | 5.2 ± 0.6 | 5.4 ± 0.8 | 0.28 |
| Hay, kg/day | 1.6 ± 0.2 | 1.7 ± 0.2 | 1.6 ± 0.2 | 1.7 ± 0.1 | 0.75 |
| Silage, kg/day | 24.6 ± 5.3 | 24.4 ± 3.2 | 24.5 ± 1.8 | 24.3 ± 3.2 | 1.00 |
| Total dry matter intake, kg/day | 15.7 ± 2.0 | 15.4 ± 1.2 | 15.6 ± 1.0 | 15.7 ± 1.5 | 0.95 |
| Milk production, kg/day | 14.9 ± 3.01[a] | 14.4 ± 2.6 | 15.2 ± 4.7 | 14.7 ± 3.1 | 0.98 |
| $AFB_1$/total feed, ppb | 3.0 ± 0.8[b] | 16.2 ± 1.9[b] | 86.0 ± 10.1[b] | 466.0 ± 124.0[c] | 0.01 |
| Total $AFB_1$ intake, μg/day | 46.0 ± 9.0[b] | 250.0 ± 21.0[c] | 1342.0 ± 212.0[d] | 7313.0 ± 1919.0[e] | 0.01 |

[a] Mean ± standard deviation, $n = 6$. [b-e] Rows with similar superscripts are not different by Duncan's multiple range test ($P > 0.05$).

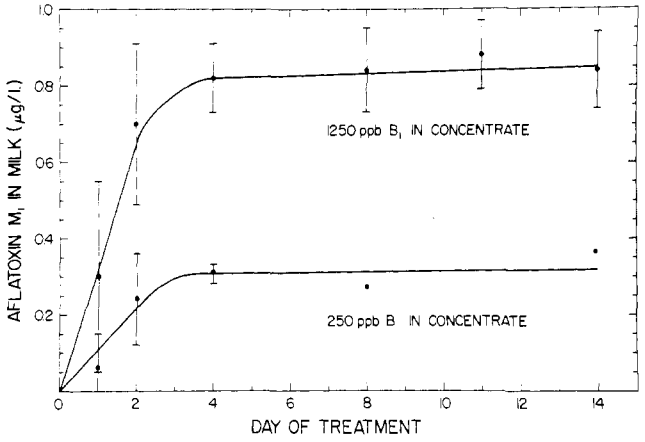


**Figure 1.** $AFM_1$ content in milk during daily consumption of 1250 and 250 ppb of $AFB_1$ in the concentrate. Each point represents the mean ± standard deviation for a minimum of three observations excepting two points which are averages of two observations.

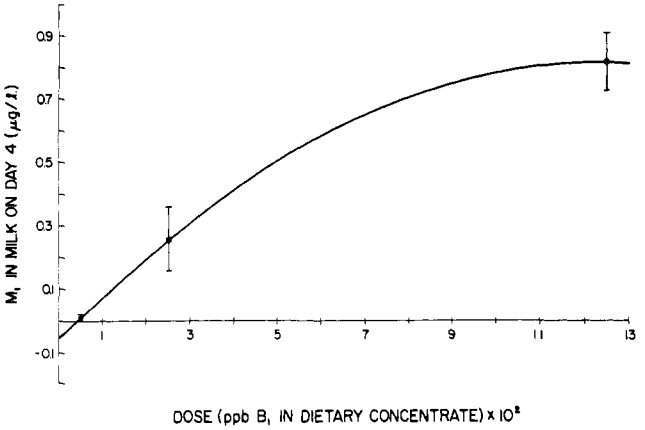


**Figure 2.** The relationship of $AFB_1$ intake on $AFM_1$ concentration in milk. The intercept at 46 ppb represents the level of $AFB_1$ intake above which $AFM_1$ can be detected in milk. This is equivalent to 15 ppb in the total ration of 230 μg/day. The equation describing the plotted curve is in the text.

counted by liquid scintillation. Quench was corrected by an external standard. Fecal samples could not be counted directly and were therefore extracted by the modified Jacobson method (McKinney, 1972). Aliquots of the extract were counted after cleanup and concentration. Some fecal samples were homogenized with known amounts of water and subsequently distilled and the water distillate counted to determine tritium exchange with water.

In the early half of the study both stored and fresh milk samples were extracted for aflatoxins by the modified Jacobson procedure (McKinney, 1972). Later, the methods of Pons *et al.* (1973) and Stubblefield and Shannon (1974) were used because cleanup for thin-layer application and recoveries were more consistent. A sufficient number of stored samples from the early part of the experiment were also analyzed by this second method to confirm that all analytical values were comparable.

Commercially prepared (Adsorbosil-5) thin-layer plates (Applied Science Laboratories, State College, Pa.) were used for the final identification of aflatoxins. The developing solvent system used was isopropyl alcohol–acetone–chloroform (5:10:85) (Pons *et al.*, 1973).

## RESULTS AND DISCUSSION

The effect of $AFB_1$ administration on milk production and voluntary feed intake is shown in Table I. It is evident that intake of any portion of the ration was unaffected by the size of the $AFB_1$ dose. Furthermore, milk production was totally unaffected by level of $AFB_1$ consumption. Lynch *et al.* (1971) found that voluntary intake of hay and grain by ruminating calves decreased dramatically after daily oral doses (80 μg/kg body weight) of crude aflatoxin powder for 1 week. However, no intake response was observed in his studies when 60 μg/kg body weight was administered daily for 6 weeks. Different expressions of $AFB_1$ intake are also shown in Table I. The highest dose in our study (10 mg/day) was equivalent to 20 μg/kg body weight; therefore the absence of a change in appetite agrees with the results of Lynch *et al.* (1971).

Determining the kinetic behavior for the secretion of aflatoxin in milk as related to dosage level was an objective of this study. Based on thin-layer chromatographic observations, $AFM_1$ could be quantitated in milk of those cows receiving the two highest $AFB_1$ intake levels. This was true for milk samples taken at the end of day 1 as well as throughout the 14-day dose period. $AFM_1$ concentration in milk usually reached a maximum by day 4. The $AFM_1$ concentration response with time is shown in Figure 1. Although not shown, 2 days after the dose was discontinued (day 16), no more $AFM_1$ was detected in milk. Only traces of $AFM_1$ (~0.01 μg/l.) were found in milk of cows receiving 50 ppb in the concentrate, but none was ever observed in the 10-ppb treatment.

In order to facilitate regression analysis, trace levels (<quantifiable) were assigned a value of 0.01 ppb (equivalent to the minimum detectable quantity). Regression equations of $AFM_1$ in milk with dose level in the concentrate for either day 4 ($Y = -0.0646 + 0.0014X - 0.0000006X^2$) or day 8 ($Y = -0.0540 + 0.0013X - 0.0000004X^2$), where $Y$ = amount of $AFM_1$ in milk and $X$ = dose in parts per billion, were virtually the same with $R^2$ of 0.96 and 0.95, respectively. Under the feeding conditions of this experiment, the plotted regression for day 4 (Figure 2) shows that $AFM_1$ can be expected to appear in milk when the concentrate exceeds 46 ppb. Extrapolated

**Table II. Level of $AFM_1$ in Milk of Cows after 4 and 8 Days of $AFB_1$ Feeding**

| | $AFB_1$ in concentrate, ppb | | | | Prob > F |
|---|---|---|---|---|---|
| | 10 | 50 | 250 | 1250 | ANOVA |
| $AFM_1$ in milk, day 4 μg/l. | 0.00 ± 0.00[a,b] | 0.01 ± 0.01[b] | 0.26 ± 0.10[c] | 0.82 ± 0.09[d] | 0.01 |
| $AFM_1$ in milk, day 8 μg/l. | 0.00 ± 0.00[b] | 0.01 ± 0.01[b] | 0.23 ± 0.10[c] | 0.86 ± 0.14[d] | 0.01 |
| $AFM_1$ output in milk, day 4 μg | 0.00 ± 0.00[b] | 0.06 ± 0.07[b] | 4.10 ± 2.20[c] | 12.24 ± 2.60[d] | 0.01 |
| $AFM_1$ output in milk, day 8 μg | 0.00 ± 0.00[b] | 0.10 ± 0.07[b] | 3.73 ± 2.17[c] | 12.64 ± 2.45[d] | 0.01 |
| $AFM_1$ in milk, day 4 % of $AFB_1$ daily intake | 0.00 ± 0.00[b] | 0.01 ± 0.00[b] | 0.30 ± 0.16[c] | 0.17 ± 0.03[c] | 0.01 |

[a] Mean ± standard deviation, $n = 4$. [b–d] Means within rows with similar superscripts are not different by Duncan's multiple range test ($P > 0.005$).

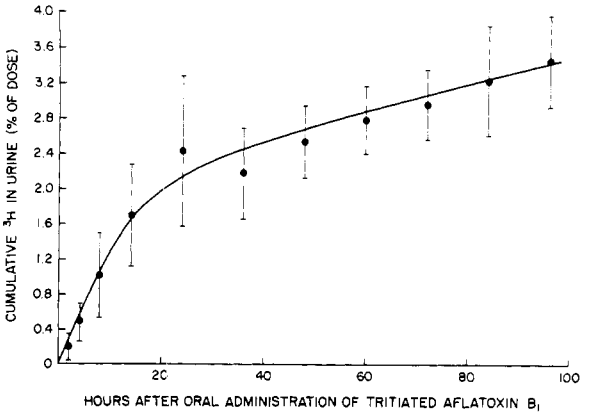


**Figure 3.** Cumulative tritium recoverd in urine as percentage of consumed [$^3$H]$AFB_1$.

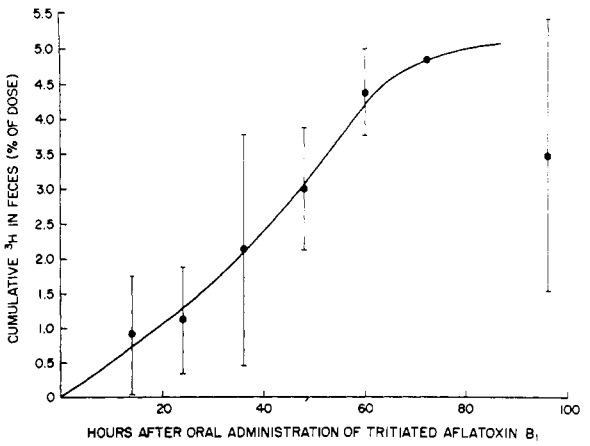


**Figure 4.** Cumulative tritium recovered in feces as percentage of consumed $AFB_1$.

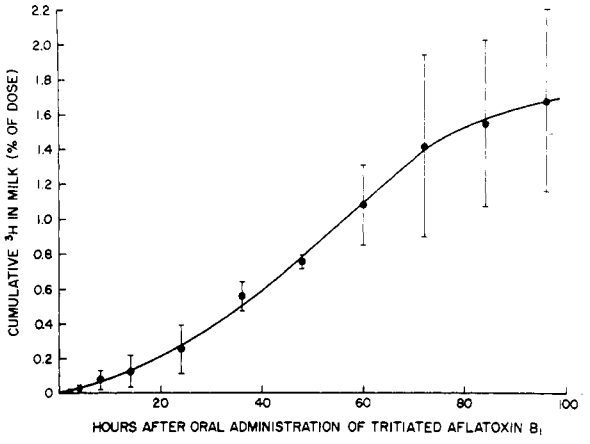


**Figure 5.** Cumulative tritium recovered in milk as percentage of consumed $AFB_1$.

to the total ration intake, this would represent 15 ppb. (See Table I for dosage relative to the total ration intake.)

The results generally agree with Allcroft and Roberts (1968) who suggested that $AFB_1$ should not exceed 50 ppb in the concentrate in order to ensure negligible amounts of aflatoxin milk. Furthermore, the rapid drop in aflatoxin concentration after discontinued treatment agrees closely with Keyl and Booth (1971).

Total $AFM_1$ output in milk expressed as a total quantity and as a percentage of $AFB_1$ intake is shown in Table II. In the 250- and 1250-ppb groups, respectively, 0.30 and 0.17% expressed relative to daily dose were recovered as $AFM_1$. These two values were not significantly different ($P > 0.05$).

With high $AFB_1$ intake, excretion in milk was reported to amount to 1–3% of the dose (Masri *et al.*, 1969) although Linde *et al.* (1965) found less than 1.0% of the total amount of aflatoxin fed to be present in milk. The latter observations agree with our data, perhaps because the dose levels were similar.

The portion of the experiment in which [$^3$H]$AFB_1$ was administered was initiated with the primary objective of detecting unidentified metabolites in milk and tissue. In this report only the rates of excretion of total radioactivity are presented.

Based on direct counting of blood serum, absorption of tritium was quite rapid. Blood tritium increased from 20 cpm/ml at 2 hr to 40 cpm/ml at 4 hr, and then increased at a slower rate to about 80 cpm/ml at 24 hr with only moderate increases to 48 hr in some cows. This early absorption (detectable with 2 hr) indicates rapid transport from the rumen. Whether this is due to actual movement of $AFB_1$ or some tritiated metabolite was not established.

The extent of early absorption is supported in part by the cumulative tritium in urine as the per cent of dose (Figure 3). Approximately one-half of the tritium excreted in urine over 96 hr occurred during the first 24 hr.

Fecal recovery of tritium was greatest between 36 and 60 hr (Figure 4) as indicated by the steep slope of the cumulative curve. This might be expected in the ruminant since the peak effluent of undigested residues would appear in the feces during this period of time (Balch and Campling, 1965) and would be expected to carry some of the tritiated compounds through the digestive tract.

In contrast to the excretion pattern in urine, the cumulative tritium content of milk percentagewise was increasing at its fastest rate between 40 and 60 hr (Figure 5). The shape of the curve for milk secretion was similar to the

fecal tritium curve rather than to the urine curve. $AFM_1$ is apparently associated with the protein fraction in milk (Allcroft and Carnaghan, 1963). $AFM_1$ may also associate with protein fractions in the ruminal environment and be carried through the intestine in a form similar to that secreted in milk. According to $AFM_1$ analysis, the content in milk increased up to 3 or 4 days during chronic dosing at which time it plateaued. The radioactive secretion in milk reflects a similar pattern. Considering both radioactivity and chemical analysis, milk is obviously not a principal excretory route for aflatoxin. Evaluation of total distribution patterns is not possible with these data in that labeled materials by all excretory routes after 96 hr accounted for less than 15% of the $AFB_1$ dose in each animal. The tritium unaccounted for may be present in tissue. A variety of tissues are currently being analyzed.

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# Accumulation of Dietary Polychlorinated Biphenyls (Aroclor 1254) by Rainbow Trout (*Salmo gairdneri*)

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The accumulation of PCB's (Aroclor 1254) by a Mt. Shasta strain of rainbow trout (*Salmo gairdneri*) from a dietary level of 15 ppm was determined using a gas chromatograph equipped with an electron capture detector. The relative concentration (parts per million) of PCB's in the fish stabilized while absolute quantities (micrograms of PCB/fish) increased as the fish grew. The total retention of PCB's from the diet was 68% for a 32-week feeding period. The distribution of PCB's was fairly constant in the lipid portion of various tissues. PCB's did not appear to be eliminated from the trout after PCB exposure ceased even when the fish were starved. The fish did not appear to be adversely affected by the PCB's and no mortalities were attributed to PCB toxicity.

Polychlorinated biphenyls (PCB's) are industrial chemicals which are widely distributed in the environment (Jensen *et al.*, 1969; Risebrough *et al.*, 1968). Due to their chemical and physical characteristics, PCB's are persistent, and they accumulate in the food chain in much the same manner as the organochlorine pesticides. Many species of fish and wildlife contain concentrations of PCB's that possibly could cause adverse effects. Of particular importance are those species of fish used for human consumption and animal feed. Atlantic salmon (*Salmo salar*) caught off Canada contained 0.45–0.62 ppm of PCB's (Zitko *et al.*, 1972). Lake Erie fish analyzed during 1970–1971 contained detectable residues of PCB's with average levels for different species ranging from 0.08 to 4.4 ppm. Coho salmon from Lake Erie averaged 2.1 ppm of PCB's (Carr *et al.*, 1972). This study was initiated to follow the accumulation in and to determine possible adverse effects of dietary PCB's on rainbow trout.

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EXPERIMENTAL PROCEDURES

**Preparation of Diets.** The control diet was prepared by the method of Castell *et al.* (1972), which consists of mix-