# Metabolic Studies with Alar Growth Regulator in the Dairy Cow

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No residues of Alar (*N*-dimethylaminosuccinamic acid) were found in the milk of a cow fed 25 p.p.m. of the compound in the feed. Alar was excreted in the urine and feces, representing 11.9 and 81.4%, respectively, or 93.3% of the total Alar fed. Alar was stable in the presence of fresh rumen fluid and beef liver slices and homogenate. Appropriate procedures were devised for isolation of Alar from various body fluids and excreta prior to colorimetric analysis.

Uniroyal Chemical's growth regulator, *N*-dimethylaminosuccinamic acid (Alar, B-nine), is an effective compound for limiting vegetative growth, tree size, and fruit drop in the culture of apples and for the promotion of flowering in several plants. The metabolism of $C^{14}$-labeled Alar has been studied in rats (Ryer and Sullivan, 1967). Based on the measurement of total radioactivity, excretion of the compound or metabolite occurred almost entirely in the urine and feces. Small amounts of radioactivity were also present in various organs and expired carbon dioxide. These workers did not attempt to separate Alar from possible metabolites prior to analysis. The use of apple pomace in cattle feed and the possibility of drift contamination from orchards onto adjacent forage prompted the present investigation of the metabolism of the compound in a lactating cow.

## EXPERIMENTAL PROCEDURE

A Holstein cow was catheterized and fed 25 p.p.m. (based on a daily ration of 50 pounds) of pure, recrystallized Alar daily for 4 days. This amounted to 0.57 gram of the compound per day or a total of 2.28 grams for the 4-day feeding period. The compound in water was thoroughly mixed with the grain. Morning and evening subsamples of the total mixed milk were taken one day prior to feeding (control samples), daily throughout the feeding period, and for 6 days thereafter. The total daily urine and feces samples were similarly collected, weighed, mixed, and subsampled during the same test period. The feces samples were collected in specially constructed trays. All samples were immediately frozen prior to analysis.

For determination of Alar in milk, well-mixed whole milk were centrifuged at 1000 r.p.m. for 30 minutes. To 40 grams of the skim milk were added 2 ml. of a 100% aqueous solution of trichloroacetic acid. After the solution stood 10 minutes, 6 grams of Celite 521 were added and the mixture was filtered with light suction through a sintered glass funnel. The filter was rinsed with 10 ml. of water and the filtrate was transferred to a 250-ml. Erlenmeyer flask with a 24/40 standard taper joint. The sample was chilled and a chilled slurry of 15 grams of sodium hydroxide in 17 ml. of water was added. A condenser was attached and the solution was distilled and analyzed as previously described (Edgerton *et al.*, 1967), except that during color development, the solution was heated for 15 minutes at 60° C. The absorbance measurements were referred to a standard curve developed by alkaline hydrolysis of 0- to 100-μg. amounts of pure Alar followed by color development of the distilled dimethylhydrazine. The standard curve had a *y* intercept of 0.03 absorbance unit (A.) and a slope of 0.0065 A. per μg.

Determination of Alar in urine was conducted as follows: Five to 10 ml. of urine were transferred to a beaker and water was added to give a total volume of 30 ml. The solution was adjusted to pH 1 with 6*N* hydrochloric acid. The solution was transferred to a 125-ml. separatory funnel and partitioned twice with 30-ml. portions of diethyl ether. The ether layers were discarded. The aqueous solution was then adjusted to pH 10 with 6*N* sodium hydroxide and again extracted twice with 30 ml. of ether. The chilled aqueous solution was transferred to the distillation flask which contained 15 grams of sodium hydroxide pellets. The remainder of the analysis was performed as described above, except that the solution was allowed to stand 10 minutes without heating during color development. Also the final dilution of this solution with distilled water (before measuring absorbance) was to 12 ml. rather than 10 ml. (Edgerton *et al.*, 1967). The absorbance measurements were referred to the respective recovery curves (see Figure 1).

**Determination of Alar in Feces.** A 100-gram sample of well-mixed feces was transferred to a beaker containing 200 ml. of 50% sodium hydroxide. The contents were mixed and allowed to stand overnight at room tempera-

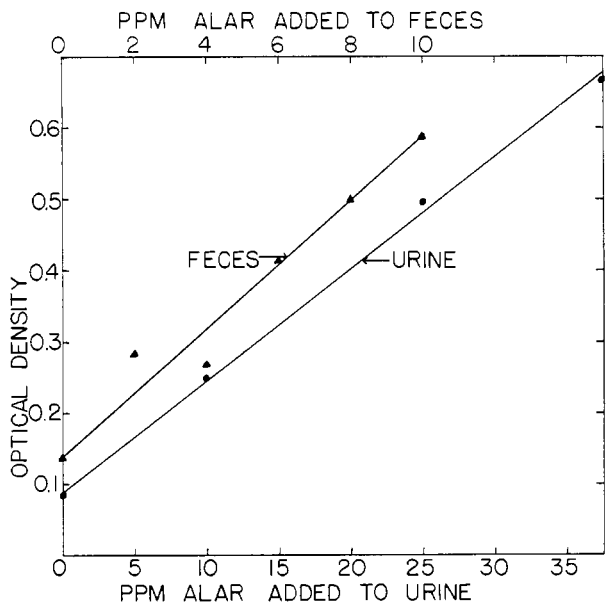


**Figure 1. Recovery of Alar added to urine and feces**

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