# Results of a Survey for the Presence of Daminozide and Unsymmetrical Dimethylhydrazine in Food

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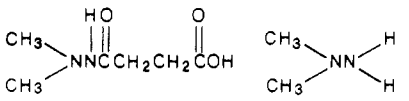
The U.S. Food and Drug Administration (FDA) conducted a survey in 1986 to determine whether samples of fruits and fruit products contained residues of daminozide (Alar), a plant growth regulating chemical, and/or its degradation product, unsymmetrical dimethylhydrazine (UDMH). Maximum daminozide residue levels detected were 0.6 ppm in stored fresh apples, 0.8 ppm in applesauce, 1.1 ppm in apple juice, 3.6 ppm in frozen cherries, and 5.9 ppm in canned cherries. No daminozide was detected in grape juice samples, either single strength or concentrate. Maximum UDMH residue levels were 0.062 ppm in applesauce, 0.041 ppm in apple juice, 0.007 ppm in frozen cherries, and 0.60 ppm in the canned sour cherries. No UDMH was detected in stored, fresh apples or the grape juice products. No findings of daminozide exceeded the tolerances, which are 20 ppm for apples, 55 ppm for cherries, and 10 ppm for grapes.

Daminozide (succinic acid 2,2-dimethylhydrazide; Alar) is a plant growth regulator used to make fresh fruit firmer, control induction of flowering, prevent spoilage and watercore development, reduce fruit drop, and enhance storability and color (Dozier et al., 1985). It has been marketed since 1963 and has been used primarily on apples, although it has also been used on grapes, cherries, and other fruits and vegetables to improve harvest quality and/or reduce harvest cost.

Daminozide (1) has been identified as a possible carcinogen (Toth et al., 1977) and is known to degrade to unsymmetrical dimethylhydrazine (UDMH) (2) when present in an apple homogenate that is subsequently boiled (Newsome, 1980). UDMH residues in food are of particular concern because the compound has been identified not only as a toxin (Tuazon et al., 1981; Chevrier, 1974; Simpson and Barrow, 1972; Rouganne et al., 1962; Cornish and Hartung, 1969; Barth et al., 1967) but also as a potential carcinogen in studies with laboratory animals (Kimura et al., 1984; Sakita et al., 1983; Schmeltz et al., 1977; Christenson and Luginbyhl, 1975; Roe et al., 1967).



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In August 1985, the Environmental Protection Agency (EPA) announced its intention to suspend the use of daminozide because of the concern of possible carcinogenic effects arising from exposure to it and especially to UDMH. However, an EPA Scientific Advisory Panel subsequently found that existing information was insufficient to support banning the product. In view of this finding, the EPA announced in January 1986 that the final decision regarding the continuation of the food uses of daminozide would be delayed until additional data could be developed. To this end, they proposed that the registrant initiate extensive oncogenicity studies for daminozide and UDMH. Other toxicological and chemical data were also requested (*Fed. Regist.*, 1987).

In the interim, restrictions were ordered to reduce both the application rates and allowable daminozide residue levels. There is particular concern over potential residues in processed apple products intended for consumption by infants, since small children consume greater quantities of apple product relative to their body weight than do adults.

Since conclusive scientific data necessary to characterize the risk of dietary exposure to daminozide and/or UDMH were not available, additional information on residue levels of these compounds was needed. Thus, in February 1986, a program was initiated to collect and analyze samples of domestic apple, sour cherry, and Concord grape products (with a known treatment history, if possible) in order to obtain additional estimates of the magnitude and frequency of occurrence of daminozide and UDMH residues.

## EXPERIMENTAL SECTION

**Sample Collection.** All samples were collected in March and April 1986.

(*1*) *Stored, Fresh Apples.* The 11 samples analyzed were Washington State Red Delicious apples collected from three different processors.

(*2*) *Processed Apple Products.* A total of 16 samples of apple juice in retail containers were collected from three different processors in Washington State, seven in Michigan, and three in New York.

Of the 15 samples of applesauce in retail containers, three were collected from processors in Washington State, three in Michigan, and three in New York.

The processed apple products collected in Washington State were obtained from different processors than were the stored, fresh apples.

(*3*) *Concord Grape Products.* Of the grape juice samples, 11 were from bulk storage tanks; one consisted of retail containers. They were obtained from two different processors in Michigan, three in New York, and two in Pennsylvania.

(*4*) *Processed Sour Cherries.* Nine 30-lb drums of frozen, sweetened, pitted tart cherries were collected. Each was from a different processor in Michigan. Three canned, pitted sour cherry samples in 1-gal cans were collected from another Michigan processor.

**Sample Preparation.** (*1*) *Stored, Fresh Apples.* The samples were frozen upon receipt; each sample was chopped and mixed in a 40-qt Hobart vertical cutter–mixer to form an icy, granular mix. Aliquots of 1 qt were kept frozen until analyzed in June and July 1986.

(*2*) *Apple Products.* Apple juice and applesauce samples were stored at room temperature in their original unopened containers and composited just prior to the analyses by mixing together equal portions from each of six large or twelve small sealed containers. The first four apple juice

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**Table I. Levels (ppm) of Daminozide and UDMH Detected in Stored Fresh Apples[a]**

| daminozide | UDMH | daminozide | UDMH | daminozide | UDMH |
|---|---|---|---|---|---|
| 0.2 | ND | 0.4 | ND | 0.2 | ND |
| 0.6 | ND | ND | ND | ND | ND |
| 0.3 | ND | 0.4 | ND | ND | ND |
| 0.3 | ND | ND | ND | | |

[a] Location stored, Washington. ND = none detected.

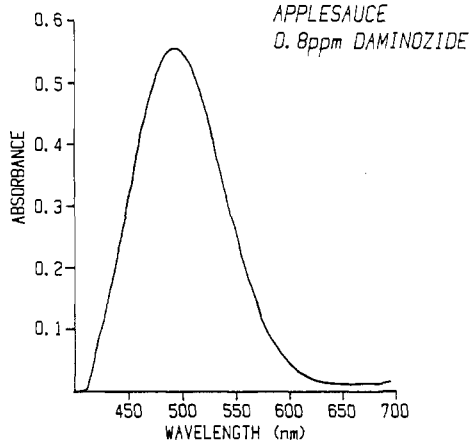


**Figure 1.** Typical visible absorption spectrum for the trisodium pentacyanoaminoferrate derivative of daminozide in a food product.

samples listed in Table I were analyzed for UDMH immediately after preparation of the composites. Those composites were then refrigerated until analyzed for daminozide 1 month later. All the other apple juice samples were analyzed for daminozide immediately after they were opened and composited, and they were analyzed for UDMH within 11 days.

All of the applesauce samples were analyzed for UDMH immediately after they were composited; they were analyzed for daminozide within 19 days.

(3) *Concord Grape Products.* The grape juice samples were refrigerated until analyzed. The analyses for daminozide were done 1–3 months after the analyses for UDMH.

(4) *Processed Sour Cherries.* The top 2–3 cm of each container of frozen cherries was discarded, and 1 kg of the remaining product was blended in a Waring Blendor while frozen. These composites were immediately analyzed for UDMH and kept refrigerated for the daminozide analyses that were completed within 3 weeks. Analyses of the canned cherries were completed within 4 days after the cans were opened and their entire contents composited by blending them in a vertical cutter–mixer.

**Methods of Analyses.** *Daminozide* (*Pesticide Analytical Manual*). One-hundred-gram samples of concentrated grape juice and 250-g aliquots of each of the other sample composites were analyzed. Daminozide residues were hydrolyzed in alkaline media, thus releasing UDMH that was distilled and reacted with trisodium pentacyanoamine ferrate at pH 5. Daminozide levels were determined by spectrophotometric comparisons of samples and standards similarly treated. A typical spectrum is presented in Figure 1.

Recoveries ranged from 65 to 101% for all products except grape juice, where recoveries were as high as 140%.

The minimum detectable level was 0.1 ppm for all products except grape juice. The spectrophotometric base line produced by grape juice samples made determinations at low levels more difficult and consequently increased the minimum detectable level to 0.2 ppm.

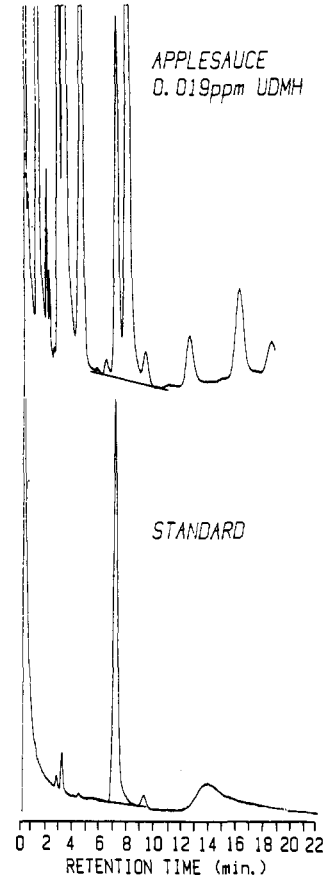


**Figure 2.** Typical gas chromatographic responses for the 2-nitrodimethylhydrazone derivative of UDMH for a food product and a standard. Conditions: stationary phase, 10% SP1000, 1.8 m × 2 mm (i.d.); 200 °C; carrier gas, helium; flow rate, 30 $cm^3$/min; detector, electron capture, 275 °C; injection port, 250 °C.

*UDMH* (*Wright, 1987*). One-hundred-gram aliquots of the sample composites were blended with L-ascorbic acid, which ionizes the UDMH and prevents its oxidation. The homogenate was filtered and an aliquot derivatized with 2-nitrobenzaldehyde and eluted through an alumina column for additional cleanup.

Gas chromatography was used to identify the derivatized extracts and make quantitative determinations. A 1.8 m × 2 mm packed column with 10% SP1000 on 80/100-mesh Supelcoport was used. The column oven was operated at 200 °C, the injection port at 250 °C, and the electron capture detector at 275 °C. The argon/methane carrier gas flow rate was 30 mL/min, and the detector signal was attenuated so 0.5 ng of the derivative resulted in a 50% full-scale deflection (FSD) at the recorder. A 5% FSD was accepted as the minimum level of detection. This is equivalent to 0.006 ppm for the 8 mg of sample generally injected. Typical chromatograms are presented in Figure 2.

Quantitation was done by comparing the sample response with that of an external standard. The standard, which was the 2-nitrobenzaldehyde derivative of UDMH, was prepared from reagent-grade UDMH and purified as directed by the method. Sample and standard concentrations were adjusted so their responses were within the linear range of the detector. Recoveries ranged from 65 to 115%.

This method was further validated by demonstrating that if daminozide is present it is not degraded to UDMH during the analysis. This was done by adding 2, 10, and 20 ppm daminozide to apple juice, apple sauce, and cherries, respectively, and analyzing for UDMH. No

**Table II. Levels (ppm) of Daminozide and UDMH Detected in Apple Juice[a]**

| location processed | daminozide | UDMH |
|---|---|---|
| Apple Juice for the General Population | | |
| WA | 0.2 | 0.007 |
| WA | 0.1 | 0.008 |
| WA | 0.4 | 0.012 |
| MI | 0.7 | 0.013 |
| MI | 0.8 | 0.005 |
| MI | 0.8 | 0.024 (0.018)[b] |
| MI | 0.9 | 0.034 (0.030) |
| NY | 0.1 | ND |
| NY | 0.7 | 0.012 |
| NY | 0.2 | 0.011 |
| Apple Juice for Infants and Juniors | | |
| MI | 0.9 | 0.041 (0.030)[b] |
| MI | 1.1 | 0.031 (0.029)[b] |
| MI | 0.8 | 0.014 (0.011)[b] |
| NY | ND | ND |
| NY | ND | ND |
| NY | ND | 0.007 |

[a] ND = none detected. [b] Presence of UDMH confirmed by GC/MS.

**Table III. Levels (ppm) of Daminozide and UDMH Detected in Applesauce[a]**

| location processed | diaminozide | UDMH |
|---|---|---|
| Applesauce for the General Population | | |
| WA | 0.8 | 0.019 |
| WA | 0.7 | 0.062 (0.074) |
| WA | 0.1 | 0.005 |
| MI | ND | 0.005 |
| MI | 0.5 | 0.029 |
| MI | 0.1 | 0.023 |
| NY | ND | ND |
| NY | 0.2 | 0.005 |
| NY | ND | 0.006 |
| Applesauce for Infants and Juniors | | |
| MI | 0.4 | 0.021 (0.034)[b] |
| MI | 0.3 | 0.020 (0.019)[b] |
| MI | ND | ND |
| NY | ND | ND[b] |
| NY | ND | ND |
| NY | 0.4 | 0.025 (0.023)[b] |

[a] ND = none detected. [b] Presence or absence of UDMH confirmed by GC/MS.

UDMH was detected in these fortified samples.

The presence of the UDMH derivative was confirmed in selected samples by capillary gas chromatography/mass spectrometry with an electron impact source. Confirmation was accomplished by selected ion monitoring for the five significant atomic masses at $m/z$ 58, 77, 91, 104, and 193. The samples selected for confirmation are noted in the accompanying tables.

## RESULTS AND DISCUSSION

Levels of daminozide and UDMH residues found in the samples are presented in Tables I–IV. No daminozide nor UDMH residues were detected in the grape juice samples.

As found in previous FDA monitoring, daminozide residue levels in the sampled raw agricultural commodities (apples, sour cherries) were much lower than the established tolerance levels of 20 and 55 ppm, respectively (Code of Federal Regulations, 1975). The maximum daminozide residue levels in apple juice, applesauce, and grape juice, whether single strength or concentrate, were also well below established EPA tolerances. (CFR 180.246 tolerance for grapes is 10 ppm.)

The level of UDMH relative to that of daminozide varied considerably from one product to another, suggesting that the level of UDMH is dependent not only on the concentration of daminozide present in the product but also on the type of product and/or amount of heat processing. For example, no UDMH was detected in the stored, fresh apple samples, which contained 0.2–0.6 ppm daminozide (Table I); however, apple juice and applesauce samples with similar levels of daminozide contained up to 0.013 (Table II) and 0.062 ppm UDMH (Table III), respectively. In addition, only 0.006 and 0.007 ppm UDMH were detected in the frozen cherries, which contained 3.5 and 3.6 ppm daminozide, whereas the canned cherries with comparable levels of daminozide (2.5 and 5.9 ppm) contained 0.32 and 0.60 ppm UDMH (Table IV).

**Table IV. Levels (ppm) of Daminozide and UDMH Detected in Cherries[a]**

| location processed | daminozide | UDMH |
|---|---|---|
| Frozen Cherries | | |
| MI | 0.2 | ND |
| MI | 0.4 | ND |
| MI | 1.8 | ND |
| MI | 0.6 | ND |
| MI | 0.2 | ND |
| MI | 3.6 | 0.007 |
| MI | 3.5 | 0.006 |
| MI | 0.4 | ND |
| MI | ND | ND[b] |
| Canned Cherries | | |
| NY | 0.3 | 0.025 (0.021)[b] |
| NY | 2.5 | 0.324 (0.345)[b] |
| NY | 5.9 | 0.599 (0.637)[b] |

[a] ND = none detected. [b] Presence or absence of UDMH confirmed by GC/MS.

As noted previously, no daminozide nor UDMH was detected in any of the grape juice samples. However, only two samples of the 12 collected may reflect use of daminozide-treated grapes. Representatives of the various processors sampled in Michigan, New York, and Pennsylvania indicated to FDA investigators that daminozide was infrequently used on the 1985 crop due to increasing grower awareness about its potential health effects. Furthermore, some processors required growers/suppliers to guarantee that fruit had not been treated with daminozide.

Processors' records showed that in mid-July of 1985 2 lb of daminozide (active ingredient) was used/acre (1.9 kg/ha) on the orchards from which the first five samples listed in Table I were obtained; daminozide was found within tolerance limits in all of these samples. No specific treatment history could be obtained for the remainder of the stored fresh apple samples. Some of the Michigan and New York processors of the apple juice and applesauce samples related that daminozide had been used in some orchards, but because several varieties and coded lots of apples were often comingled in those two products, the spray history was not pertinent to individual samples.

The presence of the 2-nitrobenzaldehyde derivative of UDMH was confirmed in the sample extracts of all 13 samples tested by capillary gas chromatography/mass spectrometry. This included all the baby/junior foods in which UDMH was detected above 0.01 ppm. The particular samples in which its presence or absence was confirmed are identified in Tables II–IV.

Most heat-processed products had detectable UDMH residues, generally less than 0.05 ppm. The data substantiate findings of other investigators that if commodities bearing daminozide residues are subjected to thermal processing, some of the daminozide degrades to form UDMH in the product. The ratio of the level of UDMH to daminozide in heat-processed products differed considerably from one product to another. The variability

may be caused by the difference in the extent and/or type of heat processing.

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**Registry No.** UDMH, 57-14-7; daminozide, 1596-84-5.

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