CONFIDENTIAL

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

AUG 17 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Use of Chlorine Generator Pads on Fresh Grapes; Evaluation of Residue Data and Analytical Methods; Submission of May 23, 1989 (MRID No. 41105601, DEB No. 5407).

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Frupac International Corporation has submitted data on residues in grapes resulting from the inclusion of chlorine generating pads (containing calcium hypochlorite) in boxes of grapes prior to shipping and storage. This submission is in response to the memo of April 15, 1988, "Akin, Gump, Strauss et al Submission dated 3/11/88. Review and Evaluation of Testing Protocol for Chlorine Generators Applied to Fresh Fruit," Elizabeth T. Haeberer.

Deficiencies Remaining

The subject submission fulfills the objectives of the protocol cited above, therefore no deficiencies remain outstanding in this regard. DEB defers to Toxicology Branch concerning the significance of residues occurring on grapes from this use. A formal petition proposing exemption from tolerance needs to be submitted.

Conclusions

1a. Three separate analytical methods were required to determine total organic halides (TOX), trihalomethanes (THM), and inorganic chlorides. THM was determined by a head-space GC method, TOX by extraction and GC determination, and inorganic chloride by electrolyte specific electrode analysis. Adequate validation data are submitted for the THM and TOX methods. These methods are adequate for their intended use.

1b. Validation data have not been submitted for the inorganic chloride analysis. The registrant claims recoveries of 100 to 117% at the 25 to 35 ppm fortification level. Pending receipt of method validation data, we tentatively conclude that the analytical method is adequate for its intended purpose.

2a. Perfect grape berries, under conditions of commerce, with no temperature abuse, may contain the following residues from the proposed use:

Variety	Inorganic Chloride	$CHCl_3$	TOX
Flame Seedless	53.0 ppm	102.3 ppb	157.0 ppb
Thompson Seedless	81.0 ppm	64.0 ppb	69.0 ppb

2b. Bruised and/or shattered grapes, or grapes which are temperature abused, will contain higher residues.

Variety	Inorganic Chloride	$CHCl_3$	TOX
Flame Seedless	210 ppm	143 ppb	223 ppb
Thompson Seedless	150 ppm	330 ppb	230 ppb

Recommendation

Toxicological considerations permitting, and pending receipt of acceptable inorganic chloride analysis method validation data, DEB could recommend in favor of an exemption from tolerance for the post-harvest use of calcium hypochlorite pads on grapes. The 40 CFR 180.1054 should be amended accordingly. A formal petition should be submitted proposing an exemption.

Detailed Considerations

Manufacturing Process and Formulation

See Confidential Appendix.

Proposed Use

For the control of decay in grapes, mainly fro the fungu botrytis cinerea, calcium hypochlorite (chlorine generator) pads are packed inside the box of fruit. Harvested grapes, in open boxes, undergo fumigation with chlorine gas at room temperature and

40 ppm gas concentration for 20 minutes. Grapes are then selected and packed as follows:

> A polyethylene liner vented with 6 mm diameter holes in a 10 cm square pitch pattern is placed in a wooden box measuring 18 5/8" x 11 1/2" x 5 3/4" (8.2 kg capacity). Fine wood shavings are placed on the bottom of the liner and a sheet of tissue placed over the shavings and over the sides of the box. Bunches of tissue wrapped grapes are placed in the box and the tissue already in the box is folded over the top of the grapes. the chlorine generator pad is placed over the tissue, the polyethylene liner folded over the tissue, and the box sealed. Boxes of packed grapes are stored in refrigeration at 0-2°C for a period of up to 40 days. This use pattern is very similar to the use of sodium metabisulfite pads in grape storage.

In our review of April 15, 1988, cited above, the following comments were made under Detailed Considerations:

> In light of the many clearances for hypochlorites, RCB (DEB) felt that an exemption from tolerance might be appropriate for this use and deferred to Toxicology Branch, HED, as to what data would be needed.
>
> Toxicology Branch wants residue data for total organic halide, trihalomethane and inorganic halides on both treated and untreated grapes. No toxicology studies have been requested at this time.
>
> The stated objectives of the protocol are:
>
> 1. To determine the total chlorine content of grapes packed with a chlorine generator pad and stored under normal handling conditions as a function of time.
> 2. To determine the variation in the total chlorine content of grapes after pad removal as a function of temperature and time.

Sampling Procedure

The sampling procedure is described in detail in our review of April 15, 1988. Grape samples were taken from the top 1/4 of the box, i.e., grapes closest to the generator pad. Samples were placed in air tight Mason jars and shipped to the laboratory in dry ice, where they were placed into a freezer at -17 to -20°C until analyzed. All grape samples were analyzed in triplicate.

Analytical Methods

Three analytical methods were required for the determination of total organic halide (TOX), trihalomethane (THM), and inorganic halide. Prior to processing, the sample jars were fitted with a blender blade assembly, and placed at 0°C for 10 minutes. The samples were blended briefly, approximately 2-5 seconds. The resulting grape homogenate was immediately weighed into vials for the TOX and THM analyses. A portion was also taken for the chloride analyses.

Total Organic Halide - A method was developed for the determination of TOX at the ppb level. The method consists of partitioning the organic halogens from the grape homogenate with hexane. The sample is analyzed on a gas chromatograph equipped with a Hall Electrolytic Conductivity Detector. The GC is fitted with a column consisting of a short piece of uncoated fused silica tubing to discourage any separation of compounds. In addition, a high oven temperature is used, 260°C. The injected sample is delivered to the detector as a single mass and allows the resulting detector response to be interpreted as total organic halogens.

The principle was verified through the analyses of various halogenated compounds such as trihalomethane mixtures, trichlorophenol, heptachlor, toxaphene, and chlordane. Using Heptachlor the following recoveries were obtained: at 10 ppb-66 to 75%, 100 ppb-77 to 87%, 200 ppb-90 to 100%. When controls were fortified at the 200 ppb level with a mixture of trihalomethanes the recoveries were 95 to 110%.

The method appears to be adequate for this use.

Trihalomethanes - The analysis follows US EPA Method 5020/8010 with a modification to enhance precision and accuracy. The modification consists of the use of a automatic headspace sampler, which results in more reproducible sampling. The sealed vial containing the sample is placed in the sample carousel which is temperature controlled to 90°C. The samples are equilibrated for 60 minutes prior to analysis. One ml of headspace is injected onto the GC equipped with a Hall Electrolytic Conductivity Detector.

A trihalomethane mixture was used for standards and fortifications. The following recoveries were obtained in control grapes:

	TRICHLORO-METHANE	DIBROMO-CHLOROMETHANE	BROMODI-CHLOROMETHANE
10 PPB	100-110%	49-75%	92-110%
40 PPB	83-88%	27-33%	65-70%

As would be expected, the heavier brominated compounds have lower recoveries by the headspace method. Since the proposed use deals with the generation of chlorine and potential for chloro-residues, we conclude that the analytical method is adequate for this proposed use.

Inorganic Chloride - This analysis utilizes a specific ion electrode. Approximately 10 grams of homogenate is weighed into a beaker and 10 ml of Chloride Ion Strength Adjustor (CISA) solution is added. This reagent adjusts the ionic strength of the solution and oxidizes certain known interfering compounds.

Standards were prepared using aqueous sodium chloride solutions with CISA added, in the range of 1, 10, and 100 ppm. A direct reading Orion 404A meter equipped with an Orion Scientific combination chloride electrode was used to determine the chloride content of the grape homogenate. The registrant states that fortified samples were analyzed at 25 and 35 ppm, with recoveries ranging from 100 to 117%. No method validation data were included with this submission. David Holzworth, counsel for Frupac, was notified of this omission (telecommunication, 8/9/89). Mr. Holzworth, in response to my inquiry, arranged a conference call to include Dr. Michael Wei, science advisor for Frupac. Dr. Wei agreed to submit validation data for the specific ion electrode method.

Pending receipt of the validation data, we tentatively conclude that the method is adequate for the proposed use.

Residue Data

The residues studies were performed on two grape varieties, Flame Seedless and Thompson Seedless. All analysis were performed in triplicate. All samples were analyzed for TOX, THM (Chloroform), and chloride. In addition, the air in each box and the air surrounding each box was tested for chlorine gas. No chlorine gas was ever found outside the boxes. Traces of gas were found within the boxes, 1-23 ppb, and quickly dissipated when the boxes were opened and the generator pads removed.

The following studies and analyses were conducted for each variety:

1. Control
2. Box of grapes fumigated once with chlorine gas at 40 ppm level for 20 minutes, no pad include. Samples were analyzed after 0,20 and 40 days in cold storage. Samples were taken 0, 2, 4, 8 and 12 hours after removing boxes from storage and opening.

   THE REMAINING STUDIES INCLUDED A SINGLE FUMIGATION AS IN #2, AND PACKING WITH A CHLORINE GENERATOR PAD.

3. Samples were taken after 0, 5, 10, 15, 20, 30 and 40 days in storage.
4. Boxes removed from cold storage after 20 days, opened and pads removed, left at 22°C and sampled at 0, 2, 4, 8 and 12 hours.
5. Same as #4 except that boxes were left in cold storage for 40 days prior to removal.
6. Same as #4 except that box was maintained in cold storage after opening and removal of pad.
7. Same as #4 except that boxes were stored 40 days prior to opening and then maintain in cold storage after opening and removal of pads.
8. Boxes were kept in cold storage for 20 days. They were then maintained at 22°C for 24 hours prior to opening and pad removal. After opening sampling was conducted at 0, 2, 4, 8 and 12 hours.
9. Same as #8 except that boxes were kept in cold storage for 40 days prior to the 24 hour holding period at 22°C.
10. Bruised grapes were kept in cold storage 20 days and sampled in triplicate.
11. Same as #10 except that grapes were kept in storage 40 days.
12. Same as #10 except that shattered grapes were used.
13. Same as #12 except stored for 40 days.

Total organohalides analyses included both the more volatile trihalomethanes and the heavier halogenated organics, while trihalomethane included the volatiles trichloromethane (chloroform), bromodichloromethane and dibromochloromethane. THM was reported in terms of chloroform only, since there would be no increase in the latter two compounds. TOX and THM residues were reported in parts per billion, while chloride residues were reported as parts per million.

The residue levels in the control grapes were essentially the same for both varieties: Flame Seedless, TOX - 15, 15, 25 ppb; $CHCl_3$ - 2, 2, 2 ppb; chloride - 34, 42, 38 ppm; Thompson Seedless, TOX - 15, 15, 15 ppb; $CHCl_3$ - 2.0, 2.1, 2.0 ppb; chloride - 35, 34, 39 ppm. Residues of bromodichloromethane and dibromochloromethane occur in each variety at 5 ppb, for each species.

Levels of inorganic chloride in Flame Seedless grapes remained at or near control levels (34-53 ppm) throughout most of the study. Higher levels were observed in bruised and/or shattered grapes which were stored 40 days. These grapes contained levels of inorganic chloride ranging from 96 to 210 ppm.

Thompson Seedless grapes had levels of inorganic chloride at or near control levels for all of the 20 day storage studies. Levels were elevated for the 40 day studies and ranged from 48 to 81 ppm. Residue levels for the bruised and/or shattered grapes, after 40 day storage, were higher, and ranged from 63 to 150 ppm.

From these data we can conclude that, under the conditions of commerce, perfect grapes may contain inorganic chloride residues at 53 ppm and 81 ppm for Flame Seedless and Thompson Seedless grapes respectively. If bruised and/or shattered grapes are involved, residues may be as high as 210 ppm in Flame Seedless and 150 ppm in Thompson Seedless.

For both grape varieties, residues of $CHCl_3$ remained near control levels from a single fumigation with no pad included in storage, i.e., 2.0-2.2 ppb. When the chlorine generator pad was included, higher residues were observed. For Flame Seedless residues were at or near control for the first 10 days of storage, after which $CHCl_3$ residues rose to 6.9 ppb at day 20, 51.7 ppb at day 30, and 102.3 ppb at day 40. These residues dropped to 69.5 ppb after 12 hours at 22°C with the pad removed. For Thompson Seedless grapes $CHCl_3$ residues remained close to control levels through day 15. Levels rose to 5.1 ppb at day 20, 22.0 ppb at day 30, and 64 ppb at day 40. These residues dropped to 24.3 ppb after 12 hours at 22°C with pad removed. In the studies where the pad was removed but the boxes were kept in cold storage, $CHCl_3$ residue levels did not drop after 12 hours.

In the study which included maintaining the sealed boxes at 22°C for 24 hours prior to opening and removal of generator pads, much higher residues of $CHCl_3$ were found. The Flame Seedless contained 43.7 and 88.3 ppb after 20 and 40 day storage. These levels reduced to 25.3 and 86.3 ppb respectively 12 hours after pad removal. Thompson Seedless grapes had $CHCl_3$ residues of 5.3 and 143.0 ppb after 20 and 40 days storage. These residues were 6.5 (constant) and 94.0 ppb 12 hours after pad removal.

For bruised Flame Seedless residues of $CHCl_3$ ranged from 3.9 to 16.0 ppb after 20 days and 85.0 to 140 ppb after 40 days. Bruised Thompson Seedless maintained control level residues after 20 days but rose to 13.0 - 47.0 ppb after 40 days. Shattered Flame Seedless had residues ranging from 2.0 to 18.0 ppb after 20 days and 140 to 330 ppb after 40 days. Shattered Thompson Seedless again had residue at control level after 20 days, but elevated to 100 - 200 ppb after 40 days.

From these data we can conclude that, under the conditions of commerce, with no temperature abuse, perfect grapes may contain 102.3 and 64.0 ppb $CHCl_3$ for Flame Seedless and Thompson Seedless respectively. If the sealed boxes containing generator pads are temperature abused, or if bruised and/or shattered grapes are involved, residues may be significantly higher, i.e., 143 ppb in the former instance and 330 ppb in the latter.

Total halogenated organics (TOX) were present above control levels in both species after a single chlorine gas fumigation, excluding the use of a generator pad in storage. For Flame Seedless at day 0, the residue level was 19 ppb, at day 20 it was 64 ppb, and at day 40 it was 60 ppb. Residue levels did not drop significantly within 12 hours of box opening. Thompson Seedless grapes had control level TOX residues day 0 and 20. At day 40 levels had risen to 34 ppb and did not drop off within 12 hours of box opening.

Flame Seedless grapes showed higher levels of TOX in boxes packed with the generator pads than from a single fumigation. Also data indicate that TOX residues did not dissipate as readily with time as THM residues. The 20 day storage samples which were kept at 22°C after the boxes were opened and pads removed, had initial residues of 63 ppb which dropped to 43 ppb after 12 hours. The 40 day samples had initial residues of 157 ppb which dropped to 108 ppb after 12 hours. The samples which were maintained in cold storage after pad removal had no dissipation of residues.

Thompson Seedless grapes under the above conditions had control level residues at 20 days. After 40 day storage the TOX level was 69 ppb and did not dissipate significantly after pad removal, either at 22°C or in cold storage.

Flame Seedless grapes which were kept in storage 20 and 40 days, then held at 22°C for 24 hours prior to opening boxes and removal of pads had higher TOX residues. The 20 day samples had 111 ppb residues which did not dissipate over a 12 hour period. The 40 day samples had initial residues of 223 ppb which dropped to 98 ppb after 12 hours.

Similarly the Thompson Seedless grapes after 20 day storage had initial residues of 32 ppb which dropped to 22 ppb after 12 hours, and 40 day samples had residues of 99 ppb which did not dissipate after 12 hours.

Bruised and shattered Flame Seedless had TOX levels ranging from 60 to 140 ppb after 20 day storage and 150 to 230 ppb after 40 day storage. Bruised and shattered Thompson Seedless had TOX levels ranging from 15 to 27 ppb (control levels) after 20 day storage and 33 to 120 ppb after 40 day storage.

From these data we can conclude that under the conditions of commerce, with no temperature abuse, perfect grapes may contain 157 ppb and 69 ppb of TOX residues in Flame Seedless and Thompson Seedless grapes respectively. If the sealed boxes containing generator pads are temperature abused, or if bruised and/or shattered grapes are involved, residues may be significantly higher, i.e., 223 ppb in the former instance, and 230 ppb in the latter.

Toxicological considerations permitting, and pending receipt of acceptable inorganic chloride analysis method validation data, DEB recommends in favor of an exemption from tolerance for the post-harvest use of calcium hypochlorite pads on grapes. The 40 CFR 180.1054 should be amended accordingly. A petition proposing an exemption from tolerance should be submitted.

Attachment 1: Confidential Appendix

cc: (With attachment) RF, PMSD/ISB, E. Haeberer, TOX, PP#7E3473 only
cc: Circu, FDA,
RDI: Robert S. Quick, 8/17/89; Richard A. Loranger, 8/17/89

RIN-1344-06

HED Letter/Memo. Dated 8/17/89

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