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REGULATORY ACTIONS AFFECTING THE USE OF ETHYLENE DIBROMIDE IN QUARANTINE FUMIGATION OF CITRUS FRUITS[1]

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Abstract. **Ethylene dibromide (EDB) is the only chemical approved for quarantine fumigation of citrus exported from Florida to other citrus producing states and to Japan. Fumigation is necessary to protect against the spread of the Caribbean fruit fly. The U. S. Environmental Protection Agency (EPA) has proposed banning the use of EDB in quarantine fumigation of citrus and tropical fruits and vegetables because it induced cancer in laboratory rats and mice. This ban, if carried out, would drastically curtail Florida's citrus export trade to Japan and thus might precipitate severe marketing problems for domestic grapefruit. Restrictions imposed by California Occupational Safety and Health Administration (Cal/OSHA) on exposure to EDB has resulted in halting citrus shipments from Florida to California. Concentrations of EDB at fumigation stations were generally low but higher at port warehouses. EDB residues in orange and grapefruit component parts decline rapidly after fumigation. The rate of decline is temperature dependent.**

Ethylene dibromide (EDB) is used in agriculture as a preplant soil fumigant for many crops and as a postharvest fumigant for grain, fruits, nuts and vegetables. A joint report issued by USDA/State and EPA (15) estimated that 14,837,100 pounds (6,729,995 kg) of EDB was used in the U. S. in 1978. Of that amount, only 83,500 lbs (37,875 kg) of EDB was used for quarantine fumigation of various commodities.

During the 1980-81 citrus season, nearly 6.5 million 4/5-bushel (approximately 18 kg) cartons of grapefruit were shipped to Japan (8). Its value was approximately $78 million at destination. Grapefruit exports from Florida to Japan represented 20.0% of all fresh grapefruit shipments, domestic and export, and 62.9% of all grapefruit exports in the 1980-81 season (8).

All citrus destined for export to Japan or other citrus producing areas must be fumigated with EDB to protect importing regions from possible introduction of the Caribbean fruit fly, *Anastrepha suspensa* (Loew). Postharvest fumigation against the Caribbean fruit fly commenced in 1974 and continues to date. Initially, it was conducted inside semi-trailer vans, loaded with packed citrus cartons (2, 12). In 1975, fumigation stations were constructed where loaded semi-trailer vans are placed inside 9000 ft^3 (255 m^3) chambers and fumigated with EDB (3). Research trails (3) indicated than an EDB dosage of 6.5 to 8 oz/1000 ft^3 (6.5-8 g/m^3) for 2 hr was required to assure 99.9968% (Probit 9) mortality of immature flies.

This paper reviews the regulatory actions on the use of EDB and presents data on EDB levels at Florida's 2 fumigation stations, 2 port warehouses and in various components of fumigated fruit.

Regulatory actions affecting the use of EDB

The current Federal permissible exposure limit for EDB is 20 ppm in any 8 hr workshift with a 30 ppm ceiling concentration and an acceptable maximum peak of 50 ppm for a brief period, not to exceed 5 minutes. As a result of a 1974 "Memorandum of Alert" issued by the National Cancer Institute regarding preliminary findings on the carcinogenicity of EDB, the Environmental Defense Fund petitioned EPA to investigate and cancel or restrict the use of EDB (16). In 1975, the office of Pesticide Review of EPA placed EDB on a list of chemicals to be further investigated (16).

In 1977, the EPA published a notice of Rebuttable Presumption Against Registration and Continued Registration (RPAR) of all pesticides containing EDB (7). This was based on preliminary evidence that EDB was a carcinogenic and mutagenic agent and also capable of producing adverse reproductive effects. The EPA invited users and/or registrants of EDB to submit evidence that the use of the chemical was not hazardous. In 1980, EPA issued its Position Document 2/3 (PD 2/3) in which they responded to comments submitted in response to the RPAR notice and proposed the cancellation of use and registration of EDB as a quarantine fumigant for citrus and for tropical fruits and vegetables by July 1, 1983 (16). As an alternative, EPA proposed that gamma irradiation be substituted for EDB.

The State of Florida, Department of Citrus (DOC), issued a rebuttal (13) to the EPA on the grounds that:

1. EDB is used safely in the quarantine fumigation of citrus,

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2. There are no viable alternatives to EDB,
3. There are serious fallacies in EPA's risk assessment of EDB and
4. Cancellation of EDB use in quarantine fumigation of citrus would result in serious economic consequences to the Florida citrus industry and would adversely affect the U. S. balance of payments.

In March 1981, the Federal Insecticide, Fungicide, Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) examined all evidence presented and submitted by the EPA staff and by EDB users and registrants, for and against registration of EDB. The Panel issued a brief report (14) in which it acknowledged the carcinogenicity and mutagenicity of EDB in animal tests, but did not approve the proposed phase-out of EDB use on citrus and tropical fruits by July 1983. It also recommended reevaluation by EPA of the risks and benefits of irradiation as an alternative to EDB. The report also indicated that "The Panel finds it difficult to evaluate whether it is feasible for the citrus industry to move to irradiation as an alternative to EDB control of fruit flies".

The EPA is currently evaluating all the comments submitted by individuals and groups, and in light of the recommendations made by SAP, a Position Document No. 4 will be issued. This document will incorporate the final administrative action on EDB use as a quarantine fumigant.

Actions by California Occupational Safety and Health Administration (Cal/OSHA)

Due to the 1980 and 1981 outbreaks of Mediterranean fruit fly in California and the subsequent requirement for fumigation of citrus and other fruits with EDB (10), the State of California Occupational Safety and Health Standards Board attempted to institute an emergency standard on exposure to EDB, not to exceed 15 parts per billion (ppb). This standard was rejected by the California Office of Administrative Law. However, on September 2, 1981, the same Board adopted another emergency standard to regulate exposure to EDB (5). The new standard included the following provisions:

1. Exposure to EDB shall not exceed 130 ppb (0.130 ppm).
2. Employers shall monitor EDB in each work place, including warehouses where fumigated commodities are stored. Each work operation shall also be monitored.
3. Each employee should be notified by the employer of his exposure levels and whenever these levels exceed 130 ppb, a statement to that effect should be included in the notification along with a description of the corrective action being taken to reduce exposure to or below the 130 ppb limit.
4. If the level of EDB in the work place exceeds 130 ppb, protective clothing, respirators or self-contained breathing apparatus should be used.
5. Employers are required to provide training to employees relating to the hazards of EDB and precautions for its safe use.
6. Signs in English and Spanish shall be posted by the employer to read: DANGER, ETHYLENE DIBROMIDE, CANCER HAZARD, MAY CAUSE STERILITY. Such signs are to be posted in work areas where exposure to EDB may take place but are not required in areas where produce is sold at retail outlets.

The above regulatory requirements triggered major supermarket chains in California to halt purchase of fumigated citrus fruits from Florida and Texas and tropical fruits, mainly papaya, from Hawaii.

So far, it appears that the strict regulation imposed on exposure to EDB in California has succeeded in virtually halting citrus shipments to that state from Florida. The future of EDB as a quarantine fumigant is yet to be determined by EPA, OSHA and the Food and Drug Administration.

EDB levels at Florida citrus fumigation stations

Monitoring of EDB by the DOC and the Florida Department of Agriculture and Consumer Services (FDACS) at Wahneta and Fort Pierce fumigation stations was conducted routinely using personnel air samplers and activated carbon tubes. EDB was measured by electron capture gas chromatography.

Results of monitoring EDB at the 2 principal fumigation stations in Florida (Wahneta and Fort Pierce) are listed in Table 1. EDB concentrations are generally, but not always, below the 0.130 ppm ceiling suggested by the National Institute of Occupational Safety and Health, (NIOSH) (11). However, EDB concentrations higher than 0.130 ppm are sometimes encountered, particularly inside fumigation chambers, in which case the wearing of a protective full-face respirator is required.

Table 1. EDB levels measured during October, November and December, 1980 at Fort Pierce and Wahneta fumigation stations.

Sample description	Number of samples	Average ppm	Standard deviation
Fort Pierce			
Hallway (15 minn)	4	0.044	0.073
Hallway (TWA[z])	4	0.026	0.020
Outside operator (15 min)	1	0.044	—
Outside operator (TWA)	3	0.064	0.018
Inspectors office (TWA)	3	0.001	0.001
Trucker, inside chamber with face respirator (15 min)	4	0.750	0.966
EDB storage area	1	0.025	—
Wahneta			
Hallway (15 min)	17	0.047	0.050
Outside operator (TWA)	2	0.090	0.005
Inspectors office (15 min)	6	0.014	0.022
Truckers lounge (15 min)	3	0.005	0.006
Trucker going into chamber (face respirator used)	3	0.482	0.270
EDB storage area	5	0.032	0.025

[z]Time weighted average.

EDB levels in citrus warehouses

Monitoring of EDB by the DOC and the FDACS was also conducted at port warehouses. Fumigated citrus fruit are usually held at port warehouses for 1 to 6 days before being loaded on ships. In 1978, Arthur D. Little, Inc. (1) published the results of their monitoring of EDB at Port Canaveral. When the warehouse contained newly fumigated fruit, the EDB level was 0.97 ppm. This level declined to 0.048 ppm 72 hr after fumigation.

The EDB levels monitored by DOC and FDACS in the warehouses at the ports of Tampa and Fort Pierce are shown in Table 2. Levels measured were higher than the proposed NIOSH ceiling of 0.13 ppm (11).

EDB residues in fumigated citrus fruit

EDB was measured in various components of fumigated 'Valencia' oranges and 'Marsh' grapefruit after various storage durations. Tables 3 and 4 list the concentrations of

Table 2. EDB levels at Port of Fort Pierce and Port of Tampa warehouses during storage of fumigated citrus fruit.

Location sampled	EDB Conc. (ppm)
Port of Fort Pierce	
Open space, 2 feet from nearest carton	0.13
Open space, between 2 adjacent pallets	0.270
Open space, in corner between 3 pallets	0.230
Open space, in middle of warehouse,	0.187
Port of Tampa	
Open space in warehouse, between pallets	0.359
Open space, walking with warehouse clerk	0.150
Samples placed near middle stack of palletized cartons, 4 hr, TWA[z]	0.546
Average and standard deviation of three 15-min samples collected from same location of TWA sample.	0.479 ± 0.047

[z]Time weighted average.

EDB detected in peel, pulp and whole 'Valencia' oranges and 'Marsh' grapefruit, respectively, held at 70°F (21.1°C). Table 5 lists the levels of EDB measured in commercially fumigated grapefruit and held in storage at 55°F (12.8°C) for 42 days. Initial levels of EDB varied between grapefruit and oranges. However, these levels declined with time in storage. The rate of decline is faster at 70° than at 55°F. These results are similar to those reported by King et al. (9).

Table 3. EDB conc in fumigated 'Valencia' orange fruit after various storage periods at 70°F (21.1°C).

Hours after fumigation	EDB conc. (ppm)[z]		
	Whole fruit	Peel	Pulp
0	4.85 ± 0.89	13.30 ± 2.4	0.64 ± 0.09
24	3.60 ± 0.64	8.49 ± 1.22	0.74 ± 0.11
48	1.31 ± 0.16	3.00 ± 0.25	0.34 ± 0.05
72	0.75 ± 0.11	1.75 ± 0.19	0.29 ± 0.03
144	0.156 ± 0.017	0.28 ± 0.03	0.07 ± 0.01
168	0.086 ± 0.029	0.15 ± 0.04	0.029 ± 0.005

[z]Average of 3 replicates ± standard deviation.

Table 4. EDB conc in fumigated 'Marsh' grapefruit after various periods of storage at 70°F (21.1°C).

Hours after fumigation	EDB conc. (ppm)		
	Whole fruit	Peel	Pulp
1	9.15	31.10	1.31
24	4.41	16.40	1.67
72	1.18	4.45	0.59
144	0.16	0.33	0.14
168	0.08	0.07	0.07
288	0.014	0.011	0.022
336	0.013	0.007	0.023

EDB is a fumigant which has, over many years been proven effective against many insects particularly fruit flies (4). Infestation of certain parts of California by the Mediterranean fruit fly (10) increased the need for EDB. Any severe restriction or cancellation of its quarantine uses could result in world-wide disruption of movement of fresh fruits and vegetables. A ban on EDB in the U. S. would likely be followed by similar actions in other countries. In Florida alone, a ban on EDB, in the absence of a suitable substitute, would result in 6.8 million cartons grapefruit surplus in the domestic market (6).

Table 5. EDB levels in fumigated 'Marsh' grapefruit after various periods of storage at 55°F (12.8°C).

Time in storage (days)	EDB conc in whole fruit[z] (ppm)
0	3.92 ± 0.5
1	3.26 ± 0.5
3	1.99 ± 0.4
7	0.81 ± 0.16
14	0.18 ± 0.004
42	0.008 ± 0.005

[z]Average of 3 replicates.

The levels of EDB detected at Florida fumigation stations were generally low. However, in port warehouses where citrus fruit are stored prior to loading on ships, the levels are generally higher. Further monitoring is needed and additional ventilation might assist in reducing EDB buildup. EDB residue in fruit declined gradually with time after fumigation and was temperature dependent (9). This information can be used in determining optimum time after fumigation for marketing.

Imposing unrealistically low EDB exposure limits or residue tolerances on the citrus industry would be economically damaging if these limits cannot be met practically and economically.

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A RAPID METHOD FOR ANALYSIS OF CITRUS FRUIT FOR RELEASE®[1,2]

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Abstract. **Citrus fruit can be analyzed for 5-chloro-3-methyl-4-nitro-1H-pyrazole (Release®), a potential chemical aid for fruit harvest, in less than 2 hours. The method employs the use of a Sep-pac, followed by quantitative Gas-liquid Chromatography (GLC). Samples are silylated for the latter. Uses of the technique were applied to analyzing immature and mature citrus peel for Release® and it was demonstrated that immature fruits metabolized the compound faster.**

Release® (5-chloro-3-methyl-4-nitro-1H-pyrazole) is an effective chemical aid for harvesting citrus fruits (1, 3, 9). It apparently stimulates citrus peel tissue to produce ethylene, thus promoting fruit abscission (1, 5). Ethylene production depends on the concentration of Release® in the peel (2) and this, in turn, is influenced by internal and external factors (4, 6, 7, 8), particularly temperature (6), and the turnover rate at which the parent compound is metabolized to components non-active in stimulating ethylene synthesis (1, 2).

This study was undertaken because it was apparent that a simpler, more efficient method for purification of Release® was needed. The easiest method to reduce losses and decrease the analysis time is to reduce the steps required for partial purification. This was done by using a small cartridge packed with silica gel produced by Waters Associates (Framingham, Mass.). These cartridges (Sep-pac) allowed us to decrease the number of steps before Release® could be quantified easily by GLC-FID.

Materials and Methods

Plant Material. Mature and immature fruits of *Citrus sinensis* (L.) Osbeck cv. 'Valencia' were treated with 300 ppm of Release®. Samples were taken periodically for the purification procedure to follow tissue levels of parent compound.

Purification. A summary of the separation procedure is as follows: one-gram quantities of freeze-dried flavedo portions of the orange peel were homogenized for 1 minute in 100 ml of 80 percent acetone. To each extract was added 6 pg of ^{14}C-labelled 5-chloro-3-methyl-4-nitro-1H-pyrazole with the ^{14}C at the 3-position in the pyrazole ring to allow for the estimation of the final recoveries of parent compound and initially to validate the procedure. This mass of Release® was less than could be detected in the aliquot analyzed by GLC. Cellular debris was filtered from the extracts using Whatman No. 1 filter paper in a Büchner funnel with suction. The filtrates were taken to dryness *in vacuo* at 35°C followed by freeze-drying. Two ml of methylene chloride was added to the dry extracts and sonicated. One ml of the resulting extract was then placed, using a glass syringe, onto a Sep-pak prewashed with 5 ml methylene chloride. A series of organic solvent mixture were used to partially purify Release® before it was eluted. The sequence of solvents used for this fractionation procedure is as follows: the original extract of 1 ml methylene chloride displaced 1 ml of methylene chloride from the Sep-pak cartridge. The loaded cartridge was then washed with a series of 5-ml aliquots of organic solvents. A flow rate of 60 s/5 ml fraction was maintained throughout. Faster or slower flow rates will alter retention time. Five aliquots of freshly distilled n-hexane dried over Na_2SO_4 were followed by 5 aliquots of methylene chloride which was followed by 3 aliquots of 5% diethyl ether in methylene chloride. Release® is normally eluted in the first and second aliquots of the latter. Occasionally it will start eluting in the last aliquot of methylene chloride and its altered retention time seems to be related to the amount of terpenoids in the samples. The elution position was established by radioactive tracer techniques.

These steps using the Sep-pak require less than 30 minutes for completion. Fractions 10, 11, and 12 containing Release® were then combined and quickly taken to dryness. Addition of 10 ml of 0.5 M phosphate buffer (pH 8.0) to the dried residue will dissolve the Release®, leaving in the residue much of the pigmented materials. The Release® was then partitioned back into ethyl acetate by lowering the pH to 1 with 1 N HCl. The ethyl acetate fraction was dried using anhydrous Na_2SO_4 before ethyl acetate was removed using a stream of dry N_2 gas. The residue can be silylated and analyzed using a gas chromatograph equipped with a 1.50 m glass column x 3.4 mm od and packed with OV-17 at 3 percent on 100/120 mesh Gas Chrom Q. The gas chromatograph equipped with a FID can be operated isothermally at 250°C or programmed from 100° to 300°C with helium as the carrier gas at 30 ml min^{-1} at 2.10 bar of column inlet pressure. In the programmed mode, the silylated Release® elutes as 2 isomeric forms with retention times of 10.5 and 10.7 minutes.

To validate the above initial, fast clean-up procedure, isotopic dilution analysis was applied to each step. Radioactivity was determined using scintillation techniques and a Packard Tri-carb scintillation counter.

[1]Release® is a trademark registered by Abbott Laboratories.

[2]Florida Agricultural Experiment Stations Journal Series 3546.
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