Pesticide Branch
OC/FA
Division of Pharmacology

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Division of Food

PP #355, Guthion on Brussels sprouts and citrus fruits.
FAP #793, Guthion on citrus peel and in dried citrus pulp.
Chemagro Corporation, Kansas City, Missouri.

The Chemagro Corporation proposes a pesticide tolerance of 2 ppm for residues of O,O-dimethyl S-4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl phosphorodithioate (Guthion) on Brussels sprouts, grapefruit, lemons, oranges, and tangerines.

The petitioner also proposes a food additive tolerance of 5 ppm for residues on citrus peel and in dried citrus pulp which result from the application of Guthion to the growing agricultural crop.

Conclusions

Adequate methods are available for enforcing the proposed pesticide and food additive tolerances.

Residues of Guthion in or on Brussels sprouts, grapefruit, lemons, oranges, tangerines, and in dried citrus pulp will not exceed their respective proposed tolerances, if the pesticide is used as directed.

A food additive tolerance will not be required for residues of Guthion in citrus peel. We are in agreement with the petitioner, who at this writing, has indicated that a tolerance for residues on the peel is not necessary, and that it should be withdrawn.

The use of dried citrus pulp in cattle feeds will result in no Guthion residues (<0.01 ppm) and no significant residues (about 0.01 ppm) of Guthion metabolites in the milk. Trace residues (<0.1 ppm, and probably <0.05 ppm) will be present in fat, liver, and kidney; none (<0.02 ppm) in lean meat.

Recommendations

Pharmacological considerations permitting, it is recommended that a pesticide tolerance of 2 ppm be established for residues of Guthion in or on Brussels sprouts, grapefruit, lemons, oranges, and tangerines; and that a food additive tolerance of 5 ppm be established for residues of Guthion in dried citrus pulp.

Detailed Considerations

Proposed Usage

Guthion is proposed for the control of numerous insects which attack the subject crops. For this purpose, it is available formulated as Guthion Liquid Concentrate (containing 2 lb act/gal), Guthion 25% Wettable Powder, and Guthion 3% Dust. It would be used on:

> Brussels sprouts, at 8-12 oz act/acre in repeat applications, up to 7 days before harvest.
>
> Citrus fruits, at 4-8 oz act in 100 gal water in full coverage sprays (i.e., liquid concentrate and wettable powder formulations only). Only one application is to be made during the year.

Analytical Method

The method of Meagher et al., (J. Ag. Food Chem., 8, 282; 1960) with modifications was used for purposes of this petition. By this method, the residue is hydrolyzed under alkaline conditions to anthranilic acid, which is diazotized and coupled with N-(1-naphthyl)ethylenediamine to yield a color having a maximum absorption at 555 mμ. The modifications involve extraction and the cleanup needed to eliminate plant pigments and other naturally occurring interferences. A special variation is that developed for use with milk and animal tissues, in which the anthranilic acid is estimated photofluorometrically instead of colorimetrically.

The method modification described in Report 7561 is similar to that used in the AOAC collaborative study using apricots, cabbage, cherries, grapes, peaches, and plums (Cox, W. S., JAOAC, 44, 188; 1961, and 45, 406; 1962) and in District tryouts with green beans (PP #314). However, despite satisfactory results with cabbage and green beans, the petitioner found that further modifications were necessary for use on Brussels sprouts because of extraordinary interference (Report 8441).

The procedure of Report 7561 was used on citrus fruits and with minor variations (Report 8762) on citrus by-products as well. Gunther, in his study of residues on citrus fruits and in dried citrus pulp (Report 8681) utilized another modification (Report 5398a) based on that described in Report 3382. The latter procedure performed satisfactorily in trials with grapes in connection with PP #249, but has now been superseded by that of Report 7561.

Crop residues (except those of the Gunther study which is described below) were extracted by blending with acetone. The cleanup, consisting of column chromatography, benzene extraction, and zinc reduction sufficed for the citrus fruits but proved unsatisfactory for Brussels sprouts. In this instance, it was found that additional column chromatography, followed by an isoelectric extraction at pH 4.2 into benzene effectively reduced the interference. (Zinc reduction was not needed in this modification for Brussels sprouts.) Recoveries were good, being 96% or more for Brussels sprouts fortified with 0.75 ppm Guthion, or its oxygen analog, and 92% or more for grapefruit, lemons, and oranges fortified with 0.75 ppm and 1.00 ppm Guthion. Blank values were usually reported as being equal to or less than 0.3 ppm apparent Guthion, with occasional values up to 0.5 ppm, and one apparently aberrant value of 1.3 ppm for Brussels sprouts. The sensitivity is, therefore, about 0.3-0.5 ppm, a rather poor showing in relation to the level of the tolerance requested. We consider it adequate, however, in view of the nature of the crops involved.

The data of Gunther (Reports 5398a and 8681) show acceptable recoveries for surface residues on oranges and lemons, extracted with n-hexane, and subjected to only a minimal cleanup. With oranges, recoveries were 88% or more for the peel fortified with 5 ppm Guthion, and for rind-free, peeled pulp fortified with 0.5 ppm Guthion. The sensitivities were said to be 0.2 ppm for the peel and 0.02 ppm for the pulp. With lemons, lower, but consistent and adequate recoveries of 74% or more for the peel and pulp, at undisclosed fortification levels, were obtained. Sensitivities would appear to be 0.4 ppm for lemon peel and 0.1 ppm for the pulp.

Method for Citrus By-Products

The procedure of Report 7561 with minor variations gave good recoveries of 90% or more for citrus pulp, juice, press liquor, and molasses, fortified with 2 ppm Guthion. Lower, but still acceptable recoveries were obtained for the peel (73% or more) and the dried cattle feed (about 82%) fortified with 2 ppm Guthion, and for the oil (about 80%) fortified with 4 ppm Guthion. Recoveries for the oxygen analog, when available, were usually 5-10% lower than the corresponding Guthion recovery values. Sensitivities were 0.3 ppm or better for all by-products except the oil, where the sensitivity was 0.7 ppm.

Method for Milk and Animal Tissues

The procedure for milk and animal tissues includes the isoelectric extraction of hydrolyzate into benzene, followed by measurements of the fluorescence at 425 mμ (with an activating wavelength of 330 mμ). Column chromatography was introduced to improve the sensitivity, but not without a reduction in the recovery. Recovery data are given for Guthion, Guthion oxygen analog, and a number of possible metabolites, and values are generally 75% or better. The question of the identity of the metabolites is still open to some extent, though narrowed down considerably (see Reports 7391, 7392, 7407, and 7559 of PP #336). It has been stated in one of these reports that the recovery in the case of milk is probably no better than 75%, and the same would seem to apply for animal tissues as well, which is adequate in view of the situation regarding the metabolites. The sensitivity is 0.01 ppm for milk, 0.02 ppm for tissues, and 0.03 ppm for fat, in terms of Guthion.

General

It had formerly been believed that the zinc reduction step introduced as part of the cleanup procedure would render the method non-specific. It now appears that the method with this step included is specific for Guthion in the presence of parathion, methyl parathion, and EPN since the benzene extraction will remove these interferences (petitioner's letter of 5/31/62, PP #336). We now have no reason to doubt the applicability of this method with or without the zinc reduction step to the determination of residues on Brussels sprouts and citrus fruits. Since the method splits the benzazimide moiety, it will determine any metabolites which incorporate this moiety. The procedures described are therefore considered adequate for enforcing the proposed tolerances.

A method tryout was not considered necessary in view of the several previous tryouts (PP #249, #314, #336) and the AOAC collaborative work.

Residue Data

It is this petitioner's practice, for reasons of economy perhaps, to postpone residue analysis until a large number of samples have accumulated. For example, according to data in this petition, samples were held in cold storage for 9-14 months and sometimes for as long as 17 months after harvest before being analyzed. This has raised a question concerning the stability of residues during storage.

New data in Report 8682 for Guthion residues on alfalfa, apples, green beans, blueberries, red currants, and raspberries stored at 0 to -10°F show that for the interval 0 to 17 and 31 weeks, values were usually equal to (in 3 out of 4 tries) or higher than those reported initially. During later intervals, i.e., 30-100 weeks, the values were usually higher (in 5 out of 7 tries) than those reported for the beginning of the interval. With the berry crops, two values were lower at the end of the interval, but were offset by two higher values (apiece) in related studies.

Gunther, using duplicate orange peel control hexane stripping solutions fortified with 0.5 and 1.0 ppm Guthion, stored at 10°C for 92 weeks, found apparent increases in residue values of 22, 20, 14, and 10%. He concluded that an evaporative factor was involved.

The weight of the evidence, therefore, is against the loss of Guthion residue in samples held in cold storage for long periods of time.

Residues on Brussels Sprouts

Pertinent data in three of the four available reports of studies conducted in California, New York, and Quebec, indicate that the maximal residue will not exceed about 1 ppm for either single or multiple applications at the maximal proposed dosage and with a preharvest interval of 7 days. The dust formulation, certainly, and the wettable powder formulation, probably, will yield residue values somewhat lower than this.

Guthion does not appear to be very persistent on this crop. We are unable with the data at hand to ascertain the half-life, but data for cabbage in PP #207 indicate a half-life of 3-5 days for this related crop.

Previously in PP #207, the petitioner had proposed the same tolerance for residues resulting from substantially the same usage as that now proposed, except for a preharvest interval of 21 days. The proposal, which was rejected, was based on data for cabbage, none being available for Brussels sprouts at that time. Generally speaking, preharvest intervals for Brussels sprouts more nearly correspond to those for broccoli, cauliflower, and kohlrabi with which it is generally grouped, and some of these may have been assigned simply on the basis of this association in

the same commodity group. However, since the present Guthion data for Brussels sprouts indicate an ample margin of safety with respect to the proposed 2 ppm tolerance, we see no need to increase the preharvest interval of 7 days merely on the basis of the longer intervals that apply to other crops in the same commodity grouping.

Residues on Citrus Fruits

Pertinent data in six of ten available reports of studies conducted in Arizona, California, and Louisiana, indicate that residues resulting from a single application at the maximum proposed dosage, will not, on the day of application exceed:

0.8 ppm on grapefruit (from use of the wettable powder formulation).

0.9 ppm on lemons (from the liquid concentrate).

0.6 ppm on lemons (from the wettable powder formulation).

1.2 ppm on oranges (from the wettable powder formulation).

0.9 ppm on oranges (from the liquid concentrate).

The residue values do not bear any obvious relationship with the size of the fruit. Tangerines, which may be slightly smaller than oranges, but equal in size to lemons might be expected to contain residues of similar magnitude.

Florida, which produces a large portion of the citrus crop is represented by only one value which is not pertinent to this usage. The petitioner has stated (in his letter of 5/7/62) that because of less rainfall, the residues on fruit grown in California will be higher than those on fruit grown in Florida. Reference is made to Gunther's study of residues on lemons and oranges in California. Since no rain fell during the experimental period of this study, it was concluded and we agree that the results are indicative of the maximum possible residues.

Gunther, using a surface stripping technique, determined the residues on the rind-free, peeled pulp and on the peel, and found practically all of the residue to be on the peel. This is not unexpected since Guthion is not known to translocate,

and would be absorbed in the oil in the peel. Using average peel values, we find calculated, adjusted values of about 1.6 ppm and 4.0 ppm in whole lemons on zero day, from the application of 1500 gals/acre at dosages of 4 oz act/100 gals and of 16 oz act/100 gals (respectively). From a plot of residue vs dosage, we find a calculated residue value on whole lemons on zero day of about 2.2 ppm at a dosage of 8 oz/100 gals under these conditions. Similarly for oranges, where the gallonage was 2500 gals/acre, we find a calculated value of 1.6 ppm.

The gallonage used in the Gunther study represents the maximum that would be applied to citrus. Allowance has been made for the usage of wettable powder formulation by projecting the data back to zero day at which time there should be little difference in the level of residues from the emulsion concentrate or the wettable powder formulations. Thus, it would appear that under the extreme conditions of the Gunther experiment, the residues on whole lemons and oranges will not exceed a calculated value of 2.2 ppm and 1.6 ppm. Under more normal conditions, it is most unlikely that they will exceed the level of the proposed tolerance. The same consideration applies to residues on grapefruit and tangerines.

Gunther had observed an unusual and unexplained persistence of residues on oranges in contrast with those on lemons, and stated that he would repeat his study. Since his orange data probably represent the extreme, we feel justified in basing our evaluation on the results of his study as well as on those of other studies included in this petition.

Residues on Fresh Citrus Peel

Fresh citrus peel is washed and/or steamed as it comes from a citrus segment processing plant.

The peel of Gunther's study above calculated, adjusted values of 7.6 ppm and 8.8 ppm in unwashed lemon and orange peels from one application of 1500 gals or 2500 gals/acre at a dosage of 8 oz/100 gals. Gunther elsewhere in his report, indicated sizeable reductions of 63% and 84% in residue values when lemon and orange peel were washed. Thus, we would expect to find residue values of about 2.8 ppm and 1.4 ppm in washed, fresh lemon and orange peels treated as described above.

In the large scale experiment conducted at the Florida Citrus Experiment Station, described in Report 8782, it was found that whole oranges containing a residue of 1 ppm and whose peel bore residues of 2.7 ppm yielded washed, fresh peel containing 1.9 ppm. The reduction in residue values on the washed peel was only 30%. The close attention given the samples in the Gunther study apparently resulted in greater reductions on washing.

Starting with whole fruit containing 2 ppm, we might expect to obtain washed, fresh peel with a residue of about 3.8 ppm as a result of commercial processing. It would appear then that a tolerance of 5 ppm might be appropriate for residues in washed, fresh citrus peel, except for the following considerations.

The petitioner's interest in the proposed food additive tolerance for the fresh peel is in the production of candied citrus peel.

Some candied peel is actually eaten as such. However, the loss of residue which would occur in the processing of fresh to candied peel makes it quite unlikely that the residue in the candied peel would exceed the proposed tolerance of 2 ppm for the fresh fruit. Fresh peel is boiled three times for 5-10 minutes in fresh water to remove most of the oil in which the residue concentrates. The peel is boiled once more for 40 minutes with syrup which serves further to reduce and dilute the residue which remains. Moderate heating alone, of grape mash at 150°F for 1 hour, caused a reduction of 67% in Guthion residue (PP #249).

When used at a maximum of 25% by weight in cakes and confectionery, the residue in the ready-to-eat item could not exceed 1.25 ppm (25% of 5 ppm) even if no loss occurred in the processing.

When fresh peel is used at a maximum of 40% by weight in marmalade, the residue could not exceed 2 ppm (40% of 5 ppm) again even if no loss occurred in the processing.

We conclude, therefore, that a food additive tolerance is not required for residues in fresh citrus peel.

Residues in Dried Citrus Pulp

Practically all citrus waste is now being processed into the dried pulp. Small amounts of the wet pulp have been fed free choice to beef cattle, but it has proved so troublesome that we may expect even less of this in the future.

A laboratory scale experiment described in the Gunther report shows residues of 5.6 ppm and 0.7 ppm in dried pulp from the conversion of fresh, washed lemon and orange peel containing 3.5 ppm and 0.7 ppm. While indicating the possibility of exceeding the proposed food additive tolerance, the conditions probably were not comparable to a large scale test. In a later test, Gunther found residues of 6.0 ppm, 7.0 ppm, and 4.8 ppm in dried pulp from the conversion of fresh ground, washed lemon and orange peel fortified with 8.8-9.3 ppm Guthion. With 5 ppm in the starting material, the probable maximum residue in peel to be converted, we might expect to find about 3.5 ppm in the dried pulp.

In the more reliable large scale test conducted by the Florida Citrus Experiment Station, whole unwashed (Pineapple var.) oranges containing 1 ppm Guthion yielded a dried citrus pulp containing 1.5 ppm. With 2 ppm in the original whole fruit, we might expect residue values of about 3 ppm in the dried citrus pulp.

We conclude then that a tolerance of 5 ppm is appropriate for residues of Guthion in dried citrus pulp, resulting from application of the pesticide chemical to the growing crop.

Residues in Citrus Juice and Citrus Peel Oil

The data of the Florida Citrus Experiment Station showed no detectable residue (i.e., residues no higher than 0.3 ppm) in the juice. Since Guthion is not translocated, and is absorbed into the oil of the peel, we would not expect any detectable residue in the juice of treated citrus fruits.

A residue value of 30.3 ppm was reported for the peel oil, equivalent to 60.6 ppm with Guthion at 2 ppm in the original whole fruit.

Citrus oil is not eaten as such, but is incorporated at various levels up to about 800 ppm in food products. With oil containing 60.6 ppm we might expect 0.05 ppm, at most, in food products as they are eaten.

Residues in Milk

Dried citrus pulp may be fed the year round to dairy cattle in amounts of up to 30%, but more usually at 5-15% of the total dry diet (PP #316, Ethion). A dairy cow, weighing 1000 lbs with a daily, total solids intake of 35 lbs may thus consume up to 10.5 lbs/day of the dried pulp. For pulp containing 5 ppm Guthion, the intake of this chemical would be 24 mg/day, or 0.053 mg/day per kg of body weight.

There are no Guthion or Guthion oxygen analog residues, less than 0.01 ppm in milk of animals fed 0.05-0.20 mg/kg/day. This conclusion is based on P^{32} studies given in Report 7391 (PP #336).

In Report 7077 on the low level feeding of Guthion, there are residues of Guthion metabolites (benzazimide derivatives) which show a linear relationship with the amount of Guthion fed in the range of 0.05-0.20 mg/kg/day. These data indicate an average net residue in the milk of lactating dairy cattle of about 0.004 ppm resulting from the intake of 24 mg/day Guthion in dried citrus pulp. On occasion, however, net values as high as 0.011 ppm were observed in individual samples. Since values levelled off after about 2 days in this 17 day test, the use of average rather than maximum values is justified. All values were calculated as Guthion, but in terms of the benzazimide metabolites, may be only half the indicated amount. Applying a correction of 75% for recovery, we find average and maximal values of 0.006 ppm and 0.015 ppm for Guthion metabolites in the milk. Approximately these same values are obtained by extrapolating the data of Table II, Report 7076, on the high level feeding study. Other data of this report indicate that the residue disappears from the milk within three days after Guthion is withdrawn from the diet.

Studies submitted with PP #336 indicate four unknown metabolites. One of these, possibly the oxidation product of bis(N-methyl benzazimide)disulfide, comprises 85% of the residue. Another may be a second oxidation product of the same disulfide.

We conclude that at the level of intake commensurate with that of the feeding of dried citrus pulp, there will be no Guthion residues (<0.01 ppm), and no significant residues (about 0.01 ppm) of Guthion metabolites in the milk.

Residues in the Meat

Dried citrus pulp may be fed the year round to beef cattle in amounts of up to 50%, but more usually at 30% of the total dry diet. A steer weighing 775 lbs with a daily total solids intake of 25 lbs may thus consume under usual conditions 7.5 lbs/day of the dried pulp. For pulp containing 5 ppm Guthion, the intake of this chemical would be 17 mg/day, or about 0.049 mg/day per kg of body weight.

Referring again to Report 7077, we find that with an intake of 0.10 mg/kg/day, there were very small net residues in the liver (0.06 ppm max) and kidney (0.04 ppm max), the method being sensitive to 0.02 ppm. With a correction of 75% for recovery, these values become 0.08 ppm for the liver and 0.05 ppm for the kidney. An average net corrected value of 0.04 ppm was obtained for the three kinds of fat, the method being sensitive to 0.03 ppm. Assuming residue to be proportional to intake, the residues in animal tissues from the feeding of 0.049 mg/kg/day would be less than 0.05 ppm.

There are no data on the identity of the residue in animal tissue.

We conclude, therefore, that insignificant residues (<0.1 ppm and probably <0.05 ppm) will result in fat, liver, and kidney; none (<0.02 ppm) for lean meat from a level of intake commensurate with that of the feeding of dried citrus pulp.

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