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PP# 4G1444

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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CASWELL FILE

SUBJECT: PP# 4G1444. Glyphosate in or on corn, cotton, soybeans, and wheat. Evaluation of analytical methods and residue data. DATE: June 3, 1974

Caswell # 161A

FROM:

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

PC 41730

TO: Coordination Branch and Toxicology Branch, RD

Monsanto Company proposes that temporary tolerances be established for combined residues of the herbicide glyphosate, (Trade name Roundup) CP67573, N-phosphonomethyl glycine, and its metabolite aminomethylphosphonic acid (CP50435), in or on the following:

| | |
|---|---|
| corn fodder and forage (all types) | 0.5 ppm |
| cottonseed and cotton forage and hay | 0.5 ppm |
| corn grain (all types) | 0.1 ppm |
| soybean grain and soybean forage and hay | 0.1 ppm |
| wheat grain and wheat forage and straw | 0.1 ppm |

The proposed experimental program involves the use of 19,430 lbs. of active ingredient on a total of 9715 acres in many of the States which grow significant amounts of these crops.

This is the first tolerance proposal for this compound. Glyphosate is one of the degradation products of glyphosine, N,N-bis (phosphonomethyl) glycine, for which a temporary tolerance on sugarcane has been established (PP# 2G1233) and for which a permanent tolerance (also on sugarcane) is now pending (PP# 4F1439).

Conclusions

1a. The metabolism of glyphosate in the subject crops is adequately defined for the purposes of these temporary proposals. Studies indicate only a limited uptake of herbicide from the soil by the crop; significant degradation of parent compound occurs upon incorporation into the plant. The major metabolic pathway involves the formation of aminomethylphosphonic acid (CP 50435) and glyoxalate via C-N enzymatic bond cleavage. Significant natural product formation then occurs through incorporation of glyoxalate or aminomethylphosphate fragments and/or $CO_2$ fixation.

Most of the residue is extractable with water; the non-extractable portion (ranging from 10% to 32% in the various crop forages) is believed due to ionic bonding of the acidic phosphonates to basic natural products and incorporation of $^{14}CO_2$ and metabolic C fragments into natural plant constituents. We defer to TB as to whether any further identification of the unextractables is needed for permanent proposals.

b. In animals (rat and cow) most of the administered $^{14}C$ glyphosate is excreted (90% within 5-7 days) in the feces (75%) with the remainder in the urine. The major component of the residue is the parent compound; small amounts of CP 50435 and CP 70948 (see Figure) may also be present.

2. Adequate analytical methods are available for the enforcement of the proposed temporary tolerances for glyphosate and CP 50435.

3a. Residues, if any, of parent compound and CP 50435 in corn grain, sweet corn (kernels plus cob with husks removed), soybeans and wheat grain will not exceed the proposed tolerance of 0.1 ppm.

b. Residues, if any, in wheat forage and straw would not exceed the proposed tolerance of 0.1 ppm. Residues in corn fodder and forage would not be expected to exceed the proposed tolerance of 0.5 ppm; on the basis of the available residue data which shows high control values, we cannot conclude whether or not real residues would, in fact, be present. The data are also inconclusive in regard to residue levels in the other forages (soybeans and cotton).

c. There would be no problem of residues of parent compound or CP 50435 in the by-products soybean oil, meal, and soapstock, or in mill fractions of wheat, or in corn oil or meal.

d. Additional residue studies are needed to determine whether or not the proposed tolerance of 0.5 ppm for cottonseed is adequate. Since there is evidence that real residues may be present in the seed, fractionation studies are also needed to determine the fate of the herbicide in processing to the corresponding meal and oil and soapstock. On the basis of the submitted data we cannot conclude whether or not the proposed tolerance of 0.5 ppm for cotton forage and hay is appropriate.

e. Due to the uncertainties as to whether or not real residues are present in corn fodder or forage, we can draw no conclusions re residues in sweet corn cannery waste. See Recommendations.

4a. Based on a tracer study with a cow, we place these uses (excldding wheat) in category 2 of Section 180.6(a) with respect to residues in meat and milk. Because of deficiencies in the residue data (see Conclusion 3 above), we cannot determine what tolerance levels would be appropriate. See Recommendations Section. For wheat, since there is no evidence of real residues in the grain, forage or straw, the use falls into category 3.

b. As there is no evidence of any real residues in the poultry feed items (corn and wheat grain, soybean fractions) these uses (excluding cotton) fall into category 3 with respect to poultry tissue and eggs.

5. EEB has concluded that a crop rotation restriction is required in the labelling.

Recommendations

Toxicology considerations permitting we recommend that the proposed temporary tolerance of 0.1 ppm for wheat grain and wheat forage and straw be established.

For reasons cited in the above Conclusions, we recommend against the proposed tolerances for corn and soybeans. However, if the petitioner were to impose label restrictions against the feeding of the treated soybean forage (and hay), we could recommend favorably for the proposed tolerances on soybeans. (The tolerance should however be expressed as soybeans rather than soybean grain.) A tolerance would then no longer be needed for soybean forage and hay.

As for corn, we could recommend favorably for field corn only if a label restriction against feeding of field corn fodder and forage were imposed. (A forage tolerance would then no longer be needed.) The tolerance for grain should be expressed as corn grain, field.

We assume it is the petitioner's intent to include sweet corn in the proposed use (data are presented for both field and sweet corn).

As indicated in Conclusion 3e, we cannot draw any conclusions regarding residues in sweet corn cannery waste and there are no practical ways to restrict its use in animal feeds. We thus cannot categorize this use in sweet corn as far as Sect. 180.6(a) is concerned. We therefore recommend against the establishment of a tolerance for sweet corn or its fodder and forage.

For reasons given in Conclusion 3d, we recommend that the proposed tolerance for cottonseed and cotton forage and hay not be established. The latter tolerance should be expressed in terms of cotton forage and fodder when established.

For future permanent tolerances we will need;

1. An improved residue method (better recoveries) and evidence for the specificity of the analytical method in the presence of other pesticides with tolerances on the subject crops.

2. Data showing the stability of residues under conditions of storage.

3. Additional data to indicate the level of residues, if any, in corn (field and sweet) fodder and forage. This also applies to forages

of soybeans and cotton, unless feeding restrictions are imposed in connection with permanent tolerance proposals.

4. Additional residue studies for soybeans, wheat and cotton reflecting a wider geographical representation.

5. A conventional large animal feeding study. Validated analytical methods will be needed to enforce any meat and milk tolerances. If real residues are present in poultry feed items, a poultry feeding study will also be needed.

6. Contingent upon TB's response to Conclusion 1a above, additional identification of the unextractable plant residues may be needed.

## Detailed Considerations

### Formulation

Glyphosate is formulated as a water soluble concentrate containing 41% of the isopropylamine salt as the active ingredient. The principal inert ingredients are [redacted]. We previously requested information as to the identity of [redacted] (COB letter to petitioner [redacted] has proposed an exemption from the requirements of a tolerance for residues of this material when used as a pesticide adjuvant [redacted]. This petition is currently under review. Monsanto has submitted a report outlining studies with [redacted] which indicate ready biodegradation to $^{14}CO_2$ and rapid disappearance of the material in soil. There is also little evidence of any uptake from the soil by the corn or soybean plant.

[redacted]

### Proposed Use

For all of the subject crops, glyphosate is to be applied at rates ranging from 1-5 lbs. a.i./acre depending upon type of weed to be controlled. Applications are to be made on emerged weeds before crop planting (3-7 days before tillage). A repeat application at 1-2 lbs. a.i./acre (before planting) is permitted, but with no more than 10 lbs. total per acre per crop. Post-harvest treatments in the fall may also be made at the same application rates.

Nature of the Residue

Plant Metabolism

Studies were conducted in an attempt to determine the extent of glyphosate uptake into plants under simulated field conditions, and also to determine the optimum uptake method to facilitate investigation of the metabolism of the herbicide. $^{14}$C-glyphosate labelled in either of the three C atoms in the molecule were used in various studies.

Soil uptake studies (using both labelled parent compound and the major metabolite, aminomethylphosphonic acid (CP 50435), with all four subject crops)indicated only about 0.1-0.2% of the amount applied was incorporated into the plant. The treatment rates (4 lbs./A for the parent and 1.5 lbs./A for the metabolite) are roughly equivalent to the proposed usage (preplant at a maximum rate of 5 lbs./A). Samplings of plant tissues were made at 4, 6, and 8 weeks after treatment. Maximum residues (based on total activity measurement) were about 0.3-0.4 ppm for the parent compound in cotton and wheat foliage. CP 50435 showed less propensity for uptake with maximum residues in all cases <0.1 ppm.

The optimum method for uptake into the plant was found to be by hydroponic growing of the plants in nutrient media treated with labelled material. Studies using this technique were made with all four crops. Both aerial and root portions of the plant were analyzed.

The results can be outlined as follows: The plant metabolites in the forages were mostly water soluble-thus 73-90% of the activity was extractable in the various experiments. Additional amounts of extractable material were obtained by subsequent extractions with dilute alkali (4-15%) and dilute acid (0.4-3%); an average over-all extractability of 88% was obtained. Extractability in the roots was less than in the foliar parts-ranging from 17-70% with water; dilute alkali and acid treatments increased the amounts extracted to an overall average of 68-88%.

There was no discernible pattern of change in extractibility with time. (In one study in which plants were hydroponically treated for 6 days and then removed to untreated fresh nutrient media, there was a decrease in extractibility from 67% at 6 days to 48% at 28 days).

Further investigation of the residue in foliar portions reveals the following composition of the plant-contained $^{14}$C material.

| | % Activity | | | | | |
|---|---|---|---|---|---|---|
| | CP67573 | CP50435 | CP70948 | Natural Products | Indeterminate* | Nonextractable |
| Corn forage | 21.1 | 27.9 | ____ | 4.0 | 20.0 | 26.6 |
| Wheat forage | 55.3 | 4.2 | ____ | 1.0 | 8.0 | 31.5 |
| Cotton forage | 61.5 | 6.8 | 2.0 | 8.8 | 10.9 | 10.0 |
| Soybean forage | 69.2 | 9.0 | 1.1 | 9.0 | 2.3 | 9.5 |

*Indeterminate activity is defined as the extractable $^{14}C$-activity which was lost (unaccountable) during the chromatographic measurements carried out.

As can be seen, the major component of the residue is the parent compound in all cases except for corn forage. The major metabolite is CP 50435. A minor metabolite was identified by chromatography as CP 70948 (See Figure 123); however, its presence may be only an artifact on the basis of studies with highly purified $^{14}C$ glyphosate.

A natural products screening methodology was developed and utilized to confirm the incorporation of metabolite fragments into plant constituents. This involves a preliminary fractionation of the plant extract into four components (basic, neutral, and acid-1 and acid-2 phases) through resin column chromatography followed by elution with appropriate solvents, Further identification was made by TLC techniques.

From this work the following conclusions were drawn: 1) significant degradation of parent compound occurs upon incorporation into plants; the metabolite CP 50435 appears to be degraded also. However the high extractibility indicates that conjugation of parent or CP 50435 probably represents only a minor mode of detoxification; 2) the major metabolic pathway involves the formation of CP 50435 and glyoxalate via C-N enzymatic bond cleavage. Significant natural product formation occurs through incorporation of glyoxalate or aminomethylphosphonate fragments and/or $CO_2$ fixation. It is believed by the petitioner that most of the non-extractable $^{14}C$ residue results from permeability problems, ionic bonding of the acidic phosphonates to basic natural products, and incorporation of $^{14}CO_2$ and metabolic carbon fragments into natural plant constituents. See Fig. 123.

We consider the plant metabolism of glyoxalate to be adequately defined.

Animal Metabolism

Tracer studies with the rat show that 90% of the administered dose of $^{14}$C-glyphosate is eliminated within 5 days. Most of the compound (75%) is eliminated in the feces, the remainder in the urine. Identification studies (TLC, NMR, GLC and GC-MS) indicate the major component is the parent compound. Minor peaks corresponding to CP 50435 and CP 70948 were found in some feces extracts but they are believed to be due to impurities in the glyphosate administered rather than a product of metabolic transformation.

In one study a lactating cow was fed $^{14}$C-glyphosate (methyl C labelled) at a dose level equivalent to 10 ppm in the diet for 7 days. Daily milk, urine, and feces samples were collected. At the end of the test period the animal was sacrificed and the tissues analyzed for $^{14}$C content.

The test material was again excreted mainly in the feces where residues reached a plateau within 3 days; residue levels in urine plateaued after 2 days. Approximately 78% of the administered dose was recovered, mainly (74%) in the feces. No identification of the components of the residue was made. Total residues (by activity measurement, as parent compound) were 0.009-0.015 ppm in milk. Residues in liver, muscle, and fat ranged up to 0.05 ppm. Higher levels (up to 0.2 ppm) were detected in the kidney.

Analytical Method

Residue methods for the parent compound and CP 50435 were developed. Ground samples (seed and grain samples are first treated with either butanol or a chloroform methanol mixture) are extracted with water and residues of the two components isolated by elution from ion exchange resins. After further purification by charcoal treatment the two compounds are separated by resin column chromatography. Finally the parent compound and its metabolite are separately converted to the coresponding N-trifluoroacetyl methyl ester for quantitation by GC using phosphorous specific flame photometric detection.

Recoveries of parent compound ranged from 50-105% in the various forages at fortification levels of 0.05-1.5 ppm. Recoveries in grains and seed ranged from 45-137% at the same level. Comparable recoveries for the metabolite were obtained at 0.1-0.4 ppm levels. Controls were in all cases <0.05 ppm.

We consider this method adequate for enforcement of these temporary tolerances. For a future permanent proposal an improved method (with better recoveries) should be developed. We would also need evidence of the specificity of the method in the presence of other pesticides with tolerances on the subject crops.

Residue Data

General

No information is given on length or conditions of storage of treated samples before analysis. For these temporary proposals we can accept the validity of the residue data reported. For a permanent tolerance we should have some data on stability of residues under conditions of storage.

Treatments in all of the residue studies were at the rate of 8 lbs. a.i./A. This is a 1.6X exaggeration of the maximum recommended rate of 5 lbs./A. Applications were made pre-plant or pre-emergent to bare ground [just before or at seeding]. Normally applications are made early pre-plant (3-5 weeks) to existing undesirable weeds (not to bare ground) since the product controls by foliar contact rather than by soil residual characteristics. This would presumably enhance the opportunity for residue pick-up by the subject crops.

No studies reflecting post-harvest treatments were presented. However we would not expect such uses to lead to any significant residues in crops harvested the following season. For a permanent proposal, however some data for post-harvest treatments should be furnished.

All samples were analyzed for both parent compound and CP 50435.

Corn

All samples of field corn grain and sweet corn (kernels plus cob with husks removed) showed no detectable residues (<0.05 ppm) of either parent compound or CP 50435. In one sample of field corn fodder 0.07 ppm of glyphosate was found but the corresponding control was 0.14 ppm; in sweet corn forage residues of 0.18-0.38 ppm were also reported with 0.3 ppm found in the controls. The petitioner believes that the apparent residues in the controls are due to cross-plot contamination since treated plots are in some cases adjacent to controls. As these are pre-plant treatments this could seemingly be accounted for only by soil movement from treated plots into adjacent check plots.

We conclude that residues, if any, in corn grain and sweet corn (kernels plus cob with husks removed) will not exceed the proposed tolerance of 0.1 ppm for combined residues of parent compound and metabolite. Residues in the forages and fodder would not be expected to exceed the proposed tolerance of 0.5 ppm; from the above results, however, we cannot determine whether or not real residues would be present in the foliar portions. This will be discussed further in the Meat and Milk Section.

Because of the uncertainties in the residue picture for the forage and fodder, we cannot make any conclusions re residues in sweet corn cannery waste.

Cotton

There are only two studies with cotton-one in Missouri and in California. In the California study no detectable residues were found in cottonseed. In the Missouri study however, values of 0.14 and 0.34 ppm were reported for parent compound in treated seed; corresponding controls were <0.05 ppm (residues of CP 50435 were all <0.05 ppm), Before we can make any final conclusions re residue levels in cottonseed, we will require additional residue studies. If these further studies substantiate the presence of real residues in the seed, fractionation studies will be needed to determine the fate of the herbicide in processing to the corresponding meal and oil.

Residues of 0.2 ppm were found in treated cotton forage in the Missouri study-however the corresponding control showed 1.0 ppm. Residues in both treated samples and controls were <0.05 ppm in the California study. Here again the data are inconclusive as to possible residue levels in the forage.

Soybeans

No detectable residues of either component were found in treated soybeans from studies in Mississippi and Missouri. Detectable residues were found in the controls in these studies, which again are ascribed by the petitioner to contamination in the field. On the basis of findings of no detectable residues in treated samples we conclude that there is little likelihood of any real residues in soybeans from this use, For a future permanent proposal, additional residue data with greater geographical representation should be furnished. If detectable residues are found in the beans, fractionation studies will then be needed.

Positive residues (0.1-1.5 ppm) were found in both treated samples and controls for soybean forage and hay in these studies. On the basis of the data available, we cannot determine an appropriate tolerance level for the forages. See Meat and Milk Section.

Wheat

Except as noted below, no detectable residues (<0.05 ppm) of either parent compound or CP 50435 were found in either wheat grain or wheat forage and straw in studies conducted in Canada and in Montana. (In one straw sample, apparent residues of 0.06-0.07 ppm of parent compound were reported.) We consider these data adequate for this temporary proposal; for a permanent tolerance additional studies (including other geographical areas) will be needed.

We conclude that combined residues, if any, in either wheat grain or wheat forage or straw will not exceed the proposed tolerance of 0.1 ppm.

Residues in Meat, Milk, Poultry and Eggs

There are no conventional livestock or poultry feeding studies. In the tracer study with the cow (discussed in the Nature of the Residue Section) detectable activity was found in the milk (0.01-0.02 ppm) and tissues (up to 0.2 ppm in kidney) from feeding at the 10 ppm level.

Corn and wheat grain, cottonseed fractions, soybeans and soybean fractions, as well as the forages, hays, and straw are all items of livestock feed. (In the case of cotton, the limited data indicate the possibility of residues in the seed and there are no data for the cottonseed fractions. We therefore cannot make any conclusions re residues in meat and milk (or poultry and eggs) as far as the use on cotton is concerned.)

As regards the proposed uses on the other crops in this petition, there is evidence that real residues may be present in the forages of corn and soybeans. Based on the cow tracer study above, we place these uses in category 2 with respect to Section 180.6(a). Because of deficiencies in the residue data (see Residue Data Section) we cannot determine what tolerance levels would be appropriate for meat and milk. If feeding restrictions were imposed in the labelling any problem with residues in meat and milk from this source would be removed. Then, and in the absence of any evidence of real residues in the corn or wheat grain or soybeans, this would become a category 3 situation with respect to 180.6(a).

For future permanent proposals, a conventional large animal feeding study will be needed to determine appropriate meat and milk tolerances. Validated chemical methods of analysis will also be required.

Since there is no evidence of residues in the poultry feed items (corn and wheat grain and soybean fractions), we place these uses (excluding cotton) in Category 3 for poultry tissues and eggs.

Other Considerations

Soil persistence studies indicate dissipation of the residue to less than 10% of the amount initially applied during the growing season (5-7 months). EEB (R. Ney memo of 3-8-74) has requested a crop rotation restriction of 1 year for planting of other crops.

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