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

OCT 5 1982

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: Anne Barton
Acting Deputy Director
Hazard Evaluation Division (TS-769)

SUBJECT: Residue Chemistry Chapter of the Glean Registration Standard.

Attached is a copy of the Residue Chemistry Chapter of the Glean Registration Standard written by RCB. This was to have been completed by Du Pont under an agreement with the company and EPA as part of a pilot effort to save OPP (including RCB) resources.

Although RCB spent significant time (48 days) reviewing industry input on this pilot project, the Residue Chemistry Chapter submitted to the Agency was not acceptable and needed to be rewritten. SPRD subsequently recommended using RCB's nine memoranda on this subject as a substitute for Du Pont's unsuccessful efforts and thus avoided an RCB non-concurrence. PSD later objected and wanted a more coherent summary. Therefore, RCB has written a Residue Chemistry Chapter in order to complete this Registration Standard.

As Bill Dickinson noted in his evaluation memo of this pilot standard, this project was not helpful to RCB in saving Branch resources. Also I wish to point out that in order to complete this standard, RCB did the job that Du Pont was supposed to do for us.

Charles L. Trichilo, Chief
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

Attachment

cc: J. Melone
SPRD P.M.
RD P.M.

DEB

RESIDUE CHEMISTRY

Formulation

The formulation requested for use is DuPont's Glean Weed Killer. Glean contains 79.8% of technical chlorsulfuron. All inerts in the formulation are cleared under Section 180.1001.

Technical chlorsulfuron is a minimum of 94% pure. The manufacturing process and impurities are detailed in (EPA Accesson No. 070471) PP# 2F2604. We expect no additional residue problems with the small amounts of impurities in the formulation.

Proposed Use

To control weeds in wheat, oats and barley, apply chlorsulfuron post-emergence and/or pre-emergence (wheat only) at rates of 3.54 to 14.18 grams active ingredient/acre. The herbicide can be applied via ground or aerial equipment using a minimum of 3 or 1 gallons of solution per acre respectively.

Tank mixtures with diuron and metribuzin for use on winter wheat are recommended. The directions for the tank mixes should include a statement which explains that all restrictions on the diuron and metribuzin labels must be observed.

DuPont surfactant WK or Ortho X-77 surfactants are recommended for postemergence treatments.

Metabolism

Plants

Metabolism studies on wheat and barley grown both in the field and in a greenhouse were submitted. (See PP#2F2604/EPA Accession No. 070471).

The wheat experiments involved treating flats of wheat in the four-leaf stage with ^{14}C-phenyl-labeled chlorsulfuron at a rate of 70 g/ha. Wheat samples were collected at 1 day, 1, 2, and 4 weeks after treatment and at maturity. Treated leaves and new growth were analyzed separately. The samples were washed with acetone to remove surface residues, blended with 80:20 acetone/water, centrifuged and the supernatant removed. The acetone was stripped off and the concentrated

solution was acidificed and extraced first with ether and then with N-butyl alcohol. The ether and alcohol solutions were taken to dryness.

The various fractions contained the following radioactive residues. The initial acetone fraction contained surface residues of chlorsulfuron and hydrolysis products. The other extract contained parent, hydrolysis products and a conjugate. The n-butyl alcohol fraction contained mainly a conjugate of chlorsulfuron. The remaining water extract showed highly polar materials.

The conjugate in the n-butyl alcohol extract was isolated via TLC and hydrolyzed with beta-glucosidase in phosphate buffer at 34°C for 4 hours. This was followed by acidification and ether extraction. Ether extracts were purified via TLC and counted and isolated materials were further identified using mass spectrometry.

The results of the greenhouse study showed that radioactive residues in wheat forage decreased from 1.51 ppm at day 1 to 0.045 ppm at maturity. Wheat grain contained only 0.0072 ppm of radioactivity. Very little translocation to new growth of wheat forage was observed. Radioactivity in the foliage consisted of chlorsulfuron and 2-chlorobenzenesulfonamide. Radioactivity released upon hydrolysis with beta-glucosidase was identified as 2-chloro-5-hydroxy-N-[(4-methoxy-6-methyl-1,3,5-triazine-2-yl) aminocarbonyl] benezenesulfonamide. This chemical was most probably conjugated with glucose since the enzyme employed is highly specific for cleavage of glucose conjugates.

Wheat plants were also treated with ^{14}C-phenyl-labeled chlorsulfuron in a field situation. The treatment rate was 100 g/ha and application was made when plants were about 12 inches high. Samples were taken daily for six days and at 2 weeks, 1 month and 2 months (maturity). The samples were extracted and handled as described above for the greenhouse samples.

Radioactivity in the wheat plants ranged from 0.38 to 10.8 ppm (dry weight basis) during the experiment. Again very little translocation to the new growth of the plants was observed. The grain contained ca 0.03 ppm of radioactive residue. The radioactivcity was identified as parent compound along with two conjugated metabolites. Parent compound accounted for 26% of the total radioactivity initially but this decreaed to 5% at maturity. Metabolite A made up 57% of the total radioactivity at Day 1. Metabolite B comprised 5% of the radioactive residue on the first day. As the experiment continued the percentage of A decreased to ca 28% with the

amount of B correspondingly increasing to~30%. In all, 60 to 90% of the total radioactive residue was identified depending on the length of time between treatment and sampling. The metabolites were hydrolyzed again with beta-glucosidase and 2-chloro-5-hydroxybenzene sulfonamide was released from metabolite B and 2-chloro-5-hydroxy-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl] benzenesulfonamide was released from metabolite A.

The metabolism study on barley involved treating both greenhouse and field-grown barley with ^{14}C-phenyl-labeled chlorsulfuron at rates of 100 and 40 gms/hectare respectively. For the field-grown barley, the plants were about six inches high when treated and the plants were sampled at 1, 2, and 4 weeks post-treatment and at maturity. Treated leaves and new growth were analyzed separately.

For the greenhouse barley experiment, the plants were treated at the four leaf stage and sampled one week after application.

Both sets of samples were worked up for quantitation and identification of radioactive residues as described above for wheat.

Radioactivity in the treated field-grown barley foliage ranged from <0.067 to 0.117 ppm. Residues in new growth, including grain, ranged from <0.005 to 0.034 ppm. Metabolites A and B comprised the major portion of the radioactive residue in the barley samples, although some parent and 2-chlorobenzene-sulfonamide were also observed. Most of the surface residue (acetone wash fraction) was chlorsulfuron. Metabolite A accounted for the major portion of the radioactivity after 1 week but decreased at longer times. This decrease was accompanied by corresponding increases in metabolite B. After hydrolysis with beta-glucosidase, metabolites A and B released the same hydroxylated compounds observed in treated wheat.

The metabolism of chlorsulfuron in wheat and barley probably involves both a minor pathway reflecting initial hydrolysis to form 2-chlorobenzenesulfonamide which is then hydroxylated in the phenyl ring followed by conjugation with glucose moieties. The major pathway for metabolism in the subject crops, however, appears to be initial hydroxylation of the phenyl ring followed by glucose conjugation through this hydroxy group. This conjugate (metabolite A) can then undergo hydrolysis to form the conjugate form of 2-chloro-5-hydroxy-benzene-sulfonamide (metabolite B).

Based on the wheat and barley metabolism studies, we conclude that the nature of the residue in the subject crops is adequately understood. The terminal residue of concern will consist of chlorsulfuron, its hydrolysis product 2-chlorobenzenesulfonamide and conjugates of 2-chloro-5-hydroxy-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) aminocarbonyl] benzenesulfonamide and 2-chloro-5-hydroxybenzenesulfonamide.

Tox Branch has concluded (Memo of 6/10/82, C. Frick, PP# 2F2604) however, that they would not be concerned over the presence of residues of 2-chlorobenzene sulfonamide or 2-chloro-5-hydroxy benzenesulfonamide in small grains, therefore chlorsulfuron and 2-chloro-5-hydroxy-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl] benzesulfonamide (5-hydroxychlorsulfuron) including its conjugates will comprise the terminal residue of concern in small grains.

Any future uses of chlorsulfuron which result in the occurrence of significant residues in crops may require additional metabolism studies.

Animals

Metabolism studies in rats and lactating goats were submitted in this petition (See PP# 2F2604/EPA Accession No. 070471). These are discussed below. An earlier rat study was submitted in conjunction with PP# 0G2376 (EPA Accesson No. 099459) and reviewed therein. (See memo of October 17, 1980, E. Zager.)

The rat study reflected giving 4 groups of rats the following doses of ^{14}C-phenyl-labeled chlorsulfuron. Group A received a single intravenous dose of 0.11 mg/kg. Groups B, C, and D received a single oral dose of the labeled chlorsulfuron at levels of 16, 16, and 3000 mg/kg. Groups B and D rats were not preconditioned with chlorsulfuron while Group C rats received a diet of unlabeled chlorsulfuron at 100 ppm in the diet for 21 days prior to the radioactive dose. Immediately after medication all rats were placed in metabolism chambers.

Urine and feces were collected at 6 and 24 hours post-dosing and then daily until sacrifice. Carbon dioxide and any volatile material were collected from certain animals in Groups A and B. Group A rats were sacrificed at 96 hours. Groups B and C rats were sacrificed after 72 hours and two Group D rats were killed at 72 hours and two were sacrificed after 168 hours. At sacrifice, blood, organs, and tissue were sampled. All samples were counted, extracted, cleaned-up and identified via mass spectrometry and TLC.

The major portion of the radioactive dose was excreted in the urine and feces of all the groups of rats with the larger percentage of the dose occurring in the urine. Most of the radioactivity was excreted within 48 hours of treatment.

Radioactive residues in blood and tissues ranged from <0.001 to 0.006 ppm in Group A rats, from <0.02 to 2.57 ppm in Group B rats, from 0.03 to 0.31 ppm in Group C rats and from 0.9 to 6114.6 ppm in Group D rats. Radioactivity was fairly uniformly distributed over the various organs and tissues for all groups of rats with somewhat higher values being observed in liver and kidney. No labeled CO_2 or volatiles were detected from any of the rats where the expired air was collected.

Metabolites which were observed in the rat groups included 2-chlorobenzenesulfonamide and two other materials labeled A and B. The major portion of the radioactivity in urine consisted of parent (67.6 to 98.5%) in all rat groups. In the four groups, 2-chlorobenzenesulfonamide comprised 0.1 to 7.3% of the total radioactivity and metabolites A and B made up 1.3 to 24.4% of the total radioactivity. In all, at least ca 75% of the total radioactivity in urine was identified as parent or 2-chlorobenzenesulfonamide.

In rat feces radioactive residues were comprised of 69.2 to 91.4% of chlorsulfuron, 0.6 to 8.5% 2-chlorobenzenesulfonamide and 5.3 to 20.1% metabolite B. In all, >70% of the residue was identified again as parent and 2-chlorobenzenesulfonamide.

Rat tissues were extracted with n-hexane, ethyl acetate and methanol and 86.8 to nearly 100% of the radioactivity was extracted in the last two solvent fractions for all samples. TLC of the concentrated ethyl acetate and methanol extracts showed that the only radioactivity on the plates was comprised of chlorsulfuron and 2-chlorobenzenesulfonamide.

The metabolism of chlorsulfuron in rats occurs mainly via hydrolysis to 2-chlorobenzenesulfonamide. The rate of hydrolysis appears to be rather slow since over 50% of the residue in tissue was observed to be parent compound. Chlorsulfuron and its hydrolysis product 2-chlorobenzenesulfonamide are excreted mainly in the free form in the rat.

The first lactating goat experiment involved five daily doses of ^{14}C-phenyl-labeled chlorsulfuron at approximately 7.1 ppm in the diet. Milk was collected twice daily and urine and feces wree sampled each day. The animal was sacrificed 24

hours after the last treatment and various tissues were sampled. Approximately 85% of the radioactivity administered was excreted in the urine with another 9% excreted in the feces.

Radioactive residues appeared to plateau in the milk after the first day with levels ranging from 0.007 to 0.011 ppm. Residues in tissues and blood ranged from <0.005 to 0.028 ppm (blood). The highest level in tissue was 0.022 ppm observed in the kidney. Radioactivity was characterized only in the urine and feces. No attempt to identify the small amounts of radioactivity in tissues was made.

Analysis of the radioactivity in the urine involved TLC which indicated a very polar material. This material was adjusted to pH 5.0 and treated with beta-glucuronidase at 36° for 24 hours. The hydrolyzed sample was diluted with water, neutralized and extracted with ethyl acetate. The ethyl acetate was removed and the sample was taken up in methanol and applied to a TLC plate. Radioactive bands were scraped from the developed plate and further identified via mass spectrometry. From the TLC_{Rf} values and mass spectroscopic data, it was determined that enzymatic hydrolysis released parent and 2-chlorobenzenesulfonamide in the ratio of 92% to ca 8% respectively. Thus it appears that chlorsulfuron residues are excreted in the urine as glucuronides.

Feces samples were extracted with ethyl acetate and water. Both extracts were concentrated and applied to TLC plates in methanol. The ethyl acetate and water removed 26 and 47% of the radioactivity in the feces. The ethyl acetate extract consisted of free chlorsulfuron (23%) and some 2-chlorobenzenesulfonamide (ca 3%). Radioactivity in the water extract was comprised of polar material and a small amount of free chlorsulfuron. The water extract was hydrolyzed with beta glucuronidase as described for urine above. This enzymatic hydrolysis released parent compound (66% of radioactivity residues) and also provided some 2-chlorobenzenesulfonamide and other unidentified materials.

Excretion of ingested chlorsulfuron in ruminants appears to occur primarily as glucuronide conjugates of parent and its hydrolysis product 2-chlorobenzenesulfonamide as opposed to elimination as free compounds in the rat. This conjugation prior to excretion indicates that chlorsulfuron is absorbed by ruminants with subsequent conjugation occurring in the liver. Since no identification of radioactivity in milk or tissues was attempted, no conclusions regarding the nature of the residue in these commodities when chlorsulfuran is fed to ruminants can be made from this experiment.

The second lactating goat study reflected feeding of ^{14}C-phenyl-labeled chlorsulfuron treated wheat forage for six consecutive days. The total radioactive residue in the foliage reflected 1.5 ppm in the diet. The radioactivity was comprised of approximately 55% of the conjugated 5-hydroxyphenyl analog of chlorsulfuron, (metabolite A), about 14% of the conjugate of 2-chloro-5-hydroxybenzenesulfonamide (metabolite B), and ca 20% of parent compound. Milk, feces, and urine were collected daily. The goat was sacrificed the morning of the 7th day and blood and various tissues were sampled.

Radioactive residues plateaued in milk after ca 24 hours. Levels ranged from 0.0009 to 0.002 ppm in milk. About 67 and 27% of the administered dose was excreted in the feces and urine respectively. Radioactivity in blood and various tissues ranged from <0.001 to 0.01 ppm (blood and bladder). In all, 96.3% of the radioactivity given the goat was recovererd.

Radioactive residues were characterized only in the urine and feces. Urine samples were chromatographed directly on TLC plates or acidified and heated with beta-glucuronidase as described in the initial goat study above. Another sample of urine was hydrolyzed with beta-glucosidase at pH 5.0 as described in the wheat metabolism study above. Feces samples were extracted with 80:20 acetone water, centrifuged, decanted and the excess acetone was stripped off on a rotary evaporator. The solution was acidified and extracted with ether and then with n-butanol. The ether and n-butanol extracts were taken to dryness and the residue dissolved in methanol and applied to TLC plates for identification of the radioactivity.

Radioactive residues identified in urine were parent (44%), 5-hydroxychlorsulfuron (40%), 2-chloro-5-hydroxybenzenesulfonamide (13%) and 2-chlorobenzenesulfonamide.

Most of the radioactivity in feces was found in the ether solution indicating that the radioactive residues were not conjugated. Residues identified were 5-hydroxychlorsulfuron (80%), 2-chloro-5-hydroxybenzenesulfonamide (15%), and chlorsulfuron (4%).

When wheat containing weathered residues of chlorsulfuron is fed to a ruminant, conjugates of the metabolites are hydrolyzed and the residues are excreted mainly in the feces in free form. However, some residues are absorbed and converted to corresponding glucuronides in the liver. Glucuronides produced in the liver include those of parent, 5-hydroxychlorsulfuron, 2-chlorbenzenesulfonamide, and 2-chloro-5-hydroxybenzenesulfonamide.

The metabolism of chlorsulfuron in ruminants and poultry is not adequately delineated at this time since no identification of radioactive residues in tissues and milk was performed. Nor was a poultry metabolism study conducted.

TOX has concluded (Memos of 4/14/82, R. Perfetti and 6/10/82 C. Frick in PP# 2F2604/EPA Accession No. 070762) however that they would not be concerned over the identity of up to 0.22 ppm (kidney) of radioactivity in cow tissue and up to 0.011 pm in milk based on the feeding of 7.1 ppm of radiolabeled chlorsulfuron in the diet of a lactating goat. Additionally, they do not consider toxicologically significant expected low levels of unidentified residues in poultry and eggs which may result from the feeding of grain containing non-detectable residues of chlorsulfuron. Based on these conclusions, no additional animal metabolism studies are required at this time. Any future uses of chlorsulfuron which result in significant residues in feed items will engender the need for additional animal metabolism studies. For the purposes of tolerances in meat and milk, the residue in these commodities will be regulated in terms at chlorsulfuron per se for the present.

Analytical Methods

The method used to obtain residue data determines chlorsulfuron only using hplc equipped with a Tracor Model 965 photoconductivity detector (PP# 2F2604/EPA Accession No. 070471). Briefly, the method involves extraction with ethyl acetate followed by removal of the ethyl acetate on a rotary evaporator. The residue is then taken up in methylene chloride and pH 10 phosphate buffer is added. The methylene chloride is stripped off and the solution is cooled and filtered on a Millipore glass filter.

At this point, certain samples (green plant and oat grain samples) require a size exclusion clean-up step. This clean-up is as follows:

The aqueous filtrate is acidified to pH 2 and extracted with chloroform. The sample is taken to dryness, dissolved in 25% toluene:ethyl acetate and applied to a size exclusion chromatographic column with 25% toluene:ethyl acetate as the mobile phase. The sample is collected, the solvent is stripped off, and the residue is dissolved in pH 10 phosphate buffer. These samples are then taken through the solvent extraction cleanup as with other substrates.

The solvent extraction clean-up involves washing the aqueous buffer with chloroform followed by cyclohexane. The aqueous phase is then acidified to pH 2 and extracted with chloroform. The chloroform extracts are taken to dryness, redissolved in the hplc mobile phase, and analyzed for chlorsulfuron.

Validation data submitted reflected fortification of barley and wheat seeds, straw and wheat, oat and rye plants at 0.01 to 0.2 ppm. Recoveries ranged from 50 to 90% with <50% of the recoveries being greater than 70%. Blank crop values ranged from <0.01 to <0.05 ppm for grain, straw, and green foliage. Four typical chromatographs were submitted.

This method (PP# 2F2604/EPA Accession No. 070471) was modified to determine chlorsulfuron in bovine tissues, feces, urine and milk in conjunction with a feeding study. These modifications are as follows: for milk and urine, the samples are adjusted to pH 2, extracted with toluene and the toluene solution is taken to dryness. The sample is taken up in pH 10 buffer and the solvent extraction steps are continued as above for plant substrates. For feces, liver and muscle sample, the procedure described for grain is used with very minor differences.

For kidney and fat, the samples are extracted with ethyl acetate and concentrated, refrigerated at 5°C overnight, centrifuged and decanted. The samples are then concentrated and taken through the size exclusion clean-up followed by the rest of the normal procedure.

Validation data for milk reflect fortification at 0.01 to 0.08 ppm. Recoveries ranged from 48 to 130% with most values <70%. Control values were all given as <0.01 ppm. Validation data for tissue samples involved fortification at 0.04 to 0.1 ppm and recoveries ranged from 42 to 70% with all values but one $\leq$65%. Control values were again all <0.01 ppm. Sample chromatograms were submitted.

A second method which determines chlorsulfuron, and 2-chloro-5-hydroxy-N-[(4-methoxy-1,3,5-triazin-2-yl)aminocarbonyl]benzenesulfonamide(5-hydroxychlorsulfuron)(metabolite A) after hydrolysis of its glucoside conjugate (PP# 2F2604/EPA Accession No. 070762). Briefly, the method involves blending with aqueous acidic acetone, the solution is then centrifuged and decanted from solids. The procedure is then repeated and all acetone:water solutions are combined. The acetone is stripped under vacuum and the aqueous solution is extracted with methylene chloride. The methylene chloride layer contains metabolite A. The aqueous layer is acidified, chloroform is added, and the sample is treated with beta-glucosidase at

35°C for 6 hours with shaking. After cooling, the chloroform layer is separated and the chloroform is stripped in vacuo.

The residue is dissolved in acetonitrile, washed with hexane and the sample is again taken to dryness. The residue is then dissolved in a solution containing 750 ml of cyclohexane, 125 ml of isopropanol, 125 ml of methanol and 1 ml of 9 parts glacial acetic acid and 1 part water (called Solution R), further cleaned-up on a Sep-Pak® column and the eluate is taken to dryness. The residue is redissolved in Solution R and analyzed via hplc using a photoconductivity detector with a mercury lamp.

The chlorsulfuron residue in methylene chloride is worked-up by addition of glacial acetic acid and the sample is taken to dryness. The residue is then dissolved in Solution R added to a buffer solution and excess organic solvents are removed under a stream of nitrogen. This aqueous solution is washed with methylene chloride, acidified and extracted with toluene. The toluene extract is taken to dryness after addition of 1 ml of glacial acetic acid and the residue is redissolved in Solution R, cleaned-up on a silica Sep-Pak® column and the eluate is evaporated to dryness. The residue is again dissolved in Solution R and analyzed via hplc as above for metabolite A.

Validation data submitted for metabolite A reflected fortification of green wheat at 0.05 to 2 ppm and recoveries ranged from 68 to 115%. Green wheat forage was also spiked with 0.05 to 10 ppm of chlorsulfuron and recoveries ranged from 75 to 119%. All check values for metabolite A and chlorsulfuron using the procedure above were given as <0.05 ppm in wheat forage and grain. Sample chromatographs were submitted.

Data for wheat grain reflected fortification levels of 0.05 to 00.2 ppm and recoveries ranged from 73 to 102%.

Successful method trials on meat, milk, kidney and grain have been completed. Recoveries in grain fortified at 0.05 and 0.1 ppm ranged from 64 to 98% for chlorsulfuron and 5-hydroxy-chlorsulfuron conjugate. Recoveries of chlorsulfuron in meat, milk and kidney ranged from 61 to 104% when fortified at the same levels. The methods employed in the trials were AMR 70-82 and AMR 85-82.

Adequate methods for enforcement of tolerances in wheat, oats and barley forage, grain and straw in terms of parent and 5-hydroxychlorsulfuron and in meat and milk in terms of chlorsulfuron per se are available. Future uses of chlorsulfuron which require additional metabolism studies may also engender the need for submission of additional methodology including validation data, blank crop values and sample chromatograms. Another method trial may also be needed.

Residue Data

Residue data submitted (PP# 2F2604/EPA Accession No.'s 070471, 070762 and 070891) involved 31 studies on wheat, oats and barley carried out in the states of Nebraska (3), Wyoming (1), Kansas (4), Delaware (2), South Dakota (1), Minnesota (5), Oregon (6), North Dakota (2), California (1), Washington (2) and Oklahoma (4). Twenty six of the studies reflected analysis for chlorsulfuron only with the remaining five involved analysis for parent and 5-hydroxychlorsulfuron.

The experiments reflected post-emergent application of 14.175 to 907.2 gm active ingredient/acre (1 to 64X) in 11 to 50 gallons/acre using ground equipment. In most cases an 80% wettable powder formulation was applied as opposed to the 75% dry flowable formulation proposed for use. No surfactant was employed in general. One trial involved two treatments to wheat one week apart while 5 other studies reflected 2 applications to wheat, barley or oats 4 to 6 months apart. Residues of chlorsulfuron in wheat and barley were all <0.01 ppm for grain and <0.05 ppm for straw after 50 to 257 days. Residues in oat grain and straw were given as <0.02 and <0.05 ppm, respectively, after PHI's of 69 to 249 days. Residues in wheat forage ranged from <0.05 to 5.2 ppm after 0 to 7 days, from <0.05 to 0.15 ppm after 12 to 15 days, and were given as <0.05 ppm for PHI's of 28 to 200 days.

Residue data for chlorsulfuron and 5-hydroxychlorsulfuron involved sampling of green wheat forage grown in Oklahoma (4 studies) and treated with 1 post-emergence application of 0.5 oz of active ingredient/acre (1X rate) in 10 to 28 gallons of water using ground equipment. In three of the studies a surfactant was used and the 75% of Dry Flowable Glean formulation was applied in all cases. Residues of chlorsulfuron and metabolite A ranged from 0.5 to 6.2 ppm and from 0.07 to 1.4 ppm respectively after 0 or 1 day PHI's. After 3 to 14 days residues of chlorsulfuron ranged from <0.05 to 3.8 ppm and those of metabolite A ranged from 0.3 to 2.9 ppm. The final study involved treatment of wheat grown in Delaware with 0.62 to 5 oz. active ingredient/acre of chlorsulfuron

using the 75% Dry Flowable Glean formulation and a surfactant. After 85 days (at maturity) wheat grain was analyzed for metabolite A only, and values were given as <0.05 ppm.

Raw data sheets for all of the residue trials were submitted. Residue trial B-3-L (Hays, Kansas) has conflicting dates appearing on it. It is assumed however that the dates given on a second study carried out in Hays, Kansas (A2-R) also reflect the correct dates for B-3-L.

The raw data sheets submitted for the residue studies have indicated that the wheat, barley and oat samples were stored for periods of 157 to 615 days prior to analysis (Note: In some of the studies we were unable to determine how long the samples were stored but the indication was that they were also stored for substantial periods of time prior to analysis). In only the Minot, North Dakota experiment were the samples analyzed within 61 days of sampling.

A 1 year storage stability study (PP# 2F2604 EPA Accession No. 070471) in which wheat grain and straw samples were spiked with 0.04 ppm and 0.2 ppm respectively of chlorsulfuron per se was submitted. Samples were analyzed at day zero and at various intervals up to 363 or 364 (straw) days. Either substrate showed <20% of a decrease after the 1 year storage period. Based on this marginally adequate study we are accepting residue data for grain and straw only in those studies in which samples were stored for less than 365 days.

With respect to forage, documentation that some of the forage samples were analyzed within 3 to 4 months of sampling was submitted. These data show residue levels which are significantly higher than those observed for forage samples stored for longer periods of time indicating that chlorsulfuron residues are degraded during storage.

With respect to residue data for wheat, oats and barley grain and straw, of all of the studies submitted, 12 had grain and straw samples which were known to have been stored for 1 year or less. These studies reflected the states of Kansas, Oregon, Nebraska, Minnesota, North Dakota and Washington. Residue levels in grain and straw are discussed above. While the number of acceptable residue studies on the grain and straw of wheat, oats and barley is rather limited, in light

of the low application rates, we conclude that combined residues of chlorsulfuron and its metabolite 5-hydroxychlorsulfuron would not be expected to exceed 0.1 ppm in grain and 0.5 ppm in straw. The higher tolerance level in grains was needed for the following reasons:

1) No storage stability study for 5-hydroxychlorsulfuron in grains was available and the only residue samples of grain analyzed for this metabolite may have been stored up to 10 months.

2) The detection limit for 5-hydroxychlorsulfuron in grain was given as 0.05 ppm.

3) Only 1 residue study was performed in which 5-hydroxychlorsulfuron glycoside was determined in grain.

Even though we have no residue data or storage stability data for 5-hydroxychlorsulfuron in straw it is concluded, based on ^{14}C-studies, that the 0.5 ppm level is adequate to cover residues which may occur.

With respect to forage, since there was some evidence that chlorsulfuron residues degrade during storage and based on available residue data an appropriate tolerance level in wheat, oats and barley forage was estimated to be 20 ppm.

No wheat milling study is needed at this time since, at most, negligible residues of chlorsulfuron in grain, i.e., <0.1 ppm are expected.

Any future uses of chlorsulfuron which result in significant residues in wheat oats and barley will engender the need for additional residue studies. These experiments should reflect adequate varietal and geographical representation, the maximum number of treatments and application rates, ground and aerial application, use of the proposed formulation with and without a surfactant and analysis of the appropriate raw agricultural commodities for the terminal residue of concern. A wheat milling study may also be required along with additional metabolism studies. With respect to the wheat milling study, if it is needed, the grain should contain detectable residues of chlorsulfuron/metabolites/conjugates so that for any concentration which occurs in a fraction, a factor can be accurately determined.

Meat, Milk, Poultry and Eggs

Wheat, oats, and barley grain are major feed items comprising up to 80% of the diet of cattle and up to 50% of the diet of poultry. In addition, wheat forage can make up 70% of the diet of dairy cattle. Straw can also be fed to cattle. The only animal feeding study submitted in this petition involved feeding dairy cattle 2, 10, or 50 ppm of chlorsulfuron in the diet for twenty eight days (PP# 2F2604/EPA Accession No. 070471). This study is discussed in detail below.

Three groups of two cows were fed chlorsulfuron for 28 days. A second group of 2 cows was kept as a control. Twenty-four hours after the last treatment, one cow from each group was sacrificed and samples of blood and various tissues were taken. The remaining cow in each feeding group was withdrawn from chlorsulfuron for 8 days and then sacrificed and sampled as above. Milk was sampled daily with one sample each week being separated into cream and skim milk fractions. A urine and feces sample was taken from each cow once weekly.

Residues of chlorsulfuron per se in composited (AM + PM) whole milk samples were <0.01 ppm at the 2 ppm feeding level, <0.01 to 0.019 ppm at the 10 ppm feeding level, and 0.021 to 0.10 ppm at the 50 ppm level. Residues in milk decreased to <0.01, <0.01, and 0.072 ppm for the 2, 10, and 50 ppm feeding levels, respectively, within 24 hours of withdrawal from chlorsulfuron. All milk samples showed <0.01 ppm of chlorsulfuron after 48 hours of withdrawal. Residues appeared to be higher in evening milk samples than in morning samples ranging up to 0.11 ppm in one PM sample of a cow being fed 50 ppm of chlorsulfuron in the diet. Residues in milk plateaued at ca 3 days after initiation of medication. Separate analyses of cream and skim milk indicated that there is no preference for chlorsulfuron in the fat portion of the milk.

In urine and feces, residues of free chlorsulfuron ranged from 3.9 to 31 ppm and from 0.06 to 0.74 ppm, respectively, depending on the feeding level. The petitioner contends that chlorsulfuron is excreted from the cow as the intact molecule. This is consistent with the goat study discussed earlier in which hydrolysis of urine excreted glucuronide conjugates released parent and 2-chlorobenzenesulfonamide in the ratio of 92 to 8%.

Residues of parent molecule in various tissues ranged from <0.01 ppm to 0.26 ppm in the cow fed 10 ppm of chlorsulfuron and from <0.01 to 0.25 ppm in the cow fed 50 ppm of this

compound. The highest residues were observed in liver and kidney. However, detectable residues of chlorsulfuron were observed in muscle. All tissues at either the 10 or 50 ppm feeding levels showed <0.01 ppm of parent molecule after the 8-day withdrawal period. That the residue determined by the method was indeed chlorsulfuron was confirmed by gas chromatography mass spectrometric analysis of methylated sample extracts.

An experiment which attempted to determine whether glucronide conjugates of chlorsulfuron occurred in cattle urine or milk was performed. This experiment involved taking duplicate sets of urine and milk samples, hydrolyzing only one set with beta-glucuronidase, and omitting hydrolysis in the other set of samples. Analysis of the duplicate sets of samples indicated slightly larger amounts of chlorsulfuron in the hydrolyzed samples but these differences could be accounted for by differences in recovery of chlorsulfuron. The same situation was observed in urine. Thus it would appear that chlorsulfuron does not occur as a glucuronide conjugate in milk nor is it excreted as such a conjugate in urine. This observation of the lack of conjugated chlorsulfuron in cattle urine is in direct conflict with the results of the goat metabolism study discussed above. This discrepancy was satisfactorily addressed during a conference with DuPont held on 3/31/82.

Based on the discussions above, this chemical is classed in category 2 of Section 180.6(a) with respect to meat and milk. Also, based on the rat and ruminant studies submitted it is probable that chlorsulfuron would be placed in category 2 with respect to poultry and eggs, thus requiring establishment of tolerances in terms of parent molecule and metabolites of concern in all four commodities. However, in light of the TOX conclusions discussed in the Nature of the Residue Section above, tolerances on meat and milk are to be established in terms of parent compound only at levels of 0.3 ppm in meat and 0.1 ppm in milk and no tolerances on poultry and eggs are needed at this time.

Any future use of chlorsulfuron which results in significant residues in feed items may require submission of additional ruminant and/or poultry feeding studies, methodology, proposals for higher/new tolerances in meat, milk, poultry and eggs and identification of the terminal residue of concern in these commodities.

Other Considerations

There are no Codex or foreign tolerances established for chlorsulfuron. Therefore, no compatability questions are involved with this petition.

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