E.I. DuPont de Nemours and Company Inc. requests the establishment of tolerances for residues of the herbicide chlorsulfuron (2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl] benzenesulfonamide) in or on the following raw agricultural commodities:

- barley grain 0.02 ppm
- barley straw 0.1 ppm
- kidney and liver of cattle, goats, hogs, horses and sheep 0.03 ppm
- meat, fat and meat by-products of cattle, goats, hogs, horses and sheep 0.02 ppm
- milk 0.02 ppm
- oat grain 0.05 ppm
- oat straw 0.1 ppm
- wheat grains 0.02 ppm
- wheat forage 6 ppm
- wheat straw 0.1 ppm

Temporary tolerances for chlorsulfuron have been established previously on wheat and barley grain at 0.05 ppm (PF#062376). No other chlorsulfuron petitions are pending.

Conclusions

1. The directions for the tank mixes should include a statement which explains that all restrictions on the diuron and metribuzin labels must be observed.

2a. The nature of the residue in wheat, oats, and barley is adequately understood for the purposes of this petition. The terminal residue of concern will consist of chlorsulfuron, 2-chlorobenzenesulfonamide and conjugate of 2-chloro-5-hydroxy-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl] benzenesulfonamide and 2-chloro-5-hydroxybenzenesulfonamide. The chlorsulfuron residues occurring as conjugates must be determined since they comprised a major portion of the radioactivity observed in wheat and straw. Since, however, only low levels of residues were observed in grain and straw, the petitioner may wish to impose a
label restriction prohibiting grazing or feeding of wheat, oat, or barley forage or fodder to livestock in lieu of submitting the studies required above. If this option is desired, the petitioner should submit a revised Section B proposing the label restriction along with a revised Section F withdrawing the proposed wheat forage tolerance. The tolerance levels of wheat, oats, and barley grains and straw should be increased to 0.05 and 0.5 ppm respectively. Those modifications should also be submitted in a revised Section F.

Finally, the petitioner should be informed that any future uses of chlor-sulfuron on wheat, oats, or barley which result in the occurrence of significant residues in grain may require additional metabolism studies which identify the nature of this residue in grain.

2b. The metabolism of chlor-sulfuron in ruminants and poultry is not adequately understood at this time. Additional metabolism studies on a lactating ruminant and poultry are needed. Ideally these studies should reflect consecutive daily doses of radiolabeled chlor-sulfuron at high enough levels so that radioactivity in tissues, milk and eggs can be identified. Animals should be slaughtered within 24 hours of the last treatment and milk and eggs should be sampled during the course of the experiment. Depending on the results of the poultry metabolism study, the need for a poultry feeding study may be obviated.

With regards to the option of withdrawing the wheat forage tolerance request, imposing a forage feeding restriction and raising the tolerance proposal for grain and straw, if this option is adopted the petitioner will need to submit only a poultry metabolism study to be carried out as discussed above. Any future use for chlor-sulfuron, however, which resulted in detectable residues in grain and straw, will reactivate our requirements for additional ruminant metabolism study.

3a. In light of conclusions 2a above, the analytical method which determines only chlor-sulfuron in the subject commodities is not adequate for obtaining residue data or for enforcement purposes. An analytical method which determines the terminal residue of concern discussed above is needed. The method should include a step which will release the conjugates in the subject crops. Validation data including blank crop values and sample chromatograms should be submitted along with the new method. The validation data should reflect fortification of wheat, oats and barley grain and straw, wheat forage, and if needed, wheat milling fractions.

3b. Since the metabolism of chlor-sulfuron in animals is not adequately understood at this time, no final conclusion as to the adequacy of the method for determination of chlor-sulfuron in meat, milk, poultry and eggs is available. The initial indication is that the terminal residue will
consist of parent, metabolite(s) and possible conjugates. Therefore, an adequate analytical method which determines all residues of concern in meat and milk and probably poultry and eggs is needed. Validation data on all four commodities along with control values and sample chromatograms should be submitted along with the method. Recoveries should be consistently >65%. As above the method should include a step which releases any conjugated residues of concern.

3c. If the petitioner chooses to restrict the feeding of wheat, oats, and barley forage as discussed in 2a above, an analytical method which determines parent molecule only in grain and straw would then be acceptable. The present method as submitted, however, showed low recoveries and would probably require modification before we would consider it acceptable for enforcement purposes. As stated above, recoveries should be consistently >65%.

3d. With respect meat and milk, if the forage restriction is adopted, the method submitted must still be modified in such a way that recoveries of parent compound in meat and milk are consistently >65%. This is needed because recoveries using the present form of the method were low. The method will, in all probability, also have to be extended to poultry and eggs. Validation data including control values and sample chromatograms for all four commodities should be submitted.

3e. Any future use of chlorsulfuron which results in significant residues in grain or straw will require submission of adequate methods which determine metabolites of concern as discussed in 3a and 3b above.

3f. At such time as an adequate method for all of the commodities concerned is available and the other deficiencies in this petition are resolved, a method trial will be initiated. This method trial must be completed successfully before any tolerances for chlorsulfuron could be established.

4a. Residue data on wheat, oats, and barley submitted in this petition is not adequate for establishment of tolerances on these crops mainly because only the parent compound was determined and the terminal residue of concern consists of other metabolites/conjugates (see Conclusion 2a above.) The lack of specific information with regards to the number of applications made, whether applications were pre- or postemergence, whether the chlorsulfuron was applied via ground or air equipment, whether a surfactant was employed also constitute deficiencies in the data. The raw residue data sheets for the studies already submitted may allow clarification of these points and therefore should be submitted. The formulation proposed for use on wheat, oats, and barley was used in only 2 of the studies submitted. Additional residue data, and depending on the level of residues found in grain - a wheat milling study - are needed. The residue data should provide information on all three crops, reflect pre- and postemergence applications (i.e., 1 or 2 applications), the maximum proposed use rate, ground and aerial application and use of
the proposed formulation and surfactant. The method of analysis should
determine chlorsulfuron, and metabolites and conjugates of concern in the
subject crops. The additional data need not be as extensive as the
original program given the low application rates involved with this use
of chlorsulfuron. A limited number of bridging studies will be
acceptable as long as they fulfill the requirements listed above. 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. Finally, when the additional data is submitted, a
revised Section F proposing the tolerances in terms of parent,
metabolites/conjugates at appropriate levels should also be submitted.

4b. If the wheat, oats, and barley forage restriction is imposed, the residue
data submitted to date will be adequate to support the tolerances to be
proposed (See Conclusion 2A) in grain and straw only if the raw data
sheets to be submitted resolve the deficiencies in the specific informa-
tion discussed in 4a above. Limited bridging studies employing the formu-
lation proposed for use will be needed in any event. If the deficiencies
listed above for the residue data submitted to date are not resolved by
the raw data sheets, additional residue studies which fulfill the require-
ments above and reflect determination of parent compound only will be
needed. It is stated that in the plant metabolism studies total radio-
activity in grain and straw never exceeded the 0.05 and 0.5 ppm levels
respectively which are to be proposed in a revised Section F. When the
raw data sheets/additional data is submitted, the revised Section F will
also be needed.

4c. If no detectable residues (<0.01 ppm) of chlorsulfuron are observed in
wheat grain in the residue studies discussed above, no wheat milling
study will be needed.

4d. Any future use of chlorsulfuron which results in significant residues in
grain or straw will reactivate all of the residue data requirements
discussed in conclusion 4a above.

5a. Based on the feeding study and limited animal metabolism studies
submitted, this chemical is classed in category 2 of Section 180.6(a)
with respect to meat and milk and therefore tolerances on these
commodities are needed. No such conclusion can be made with respect to
poultry and eggs until the poultry metabolism and feeding studies
required are submitted. The indication is, based on the rat and ruminant
studies, that this chemical will also be classed in category 2 of Section
180.6(a) with respect to poultry and eggs. Until such time as the
metabolism of chlorsulfuron is adequately understood however, no
recommendations with respect to appropriate levels or what compounds
comprise the terminal residue of concern in meat and milk and probably poultry and eggs can be made. When the required studies are presented, a revised Section F proposing tolerances for meat, milk, and probably poultry and eggs should also be submitted. Finally, depending on the results of the required ruminant and poultry metabolism studies additional/new feeding studies for these species may be needed.

5b. The discrepancy between the occurrence of glucuronide conjugates of chlorsulfuron residues in goat urine vs the lack of these conjugates in cattle urine should be addressed in the additional ruminant metabolism study required above.

5c. If the wheat, oats, and barley forage restriction is imposed, this will obviate the need for an additional ruminant metabolism study required above. Also the poultry metabolism study required will probably suffice in lieu of a feeding study depending on the results of the experiment. If the wheat forage deletion option is adopted, a revised Section F proposing an 0.1 ppm tolerance in milk and an 0.3 ppm level in meat (including liver and kidney) should be submitted. These higher levels are needed because of the feeding study but also because of the low recoveries observed in these commodities. Appropriate tolerances for poultry and eggs, if needed, should also be proposed.

5d. Any future use of chlorsulfuron which results in significant residues in grain and/or straw may require submission of additional ruminant and/or poultry feeding studies as well as proposals for higher/new tolerances in meat, milk, poultry and eggs.

6. The International Tolerance Sheet is attached. There are no Codex or foreign tolerances established for chlorsulfuron. Therefore no compatibility questions are involved with this petition.

Recommendation

We recommend that the proposed tolerances not be established for the reasons given in conclusions 1, 2a, 2b, 3a, 3b, 3c, 3d, 4a, 4b, 5a, 5b, and 5c above. The requirements for resolution of these deficiencies are also discussed in the appropriate conclusions above: The petitioner should also be informed of the additional requirements discussed in conclusions 2a, 2b, 3e, 3f, 4c, 4d and 5d.

Detailed Considerations

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 Attachment A at the end of this review. 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 postemergence 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 post-emergence treatments.

Nature of the Residue

Plants

Metabolism studies on wheat and barley grown both in the field and in a greenhouse were submitted.

The wheat experiments involved treating flats of wheat in the four-leaf stage with 14C-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 acidified and extracted 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]benzene-sulfonamide. 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. Treatment (=SOUTH=)100 g/ha and application was made when plants were about 12 inches high. Samples were taken daily for six days 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 radioactivity was identified as parent compound along with two conjugated metabolites. Parent compound accounted for 26% of the total radioactivity initially but this decreased 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-triazine-2-yl)aminocarbonyl]benzene-sulfonamide was released from metabolite A.

The metabolism study on greenhouse or field-grown 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-chlorobenzenesulfonamide were also observed. Most of the surface residue (acetone wash fraction) was chlorosulfuron. 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 chlorosulfuron 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 chlorosulfuron, its hydrolysis product 2-chlorobenzenesulfonamide and conjugates of 2-chloro-5-hydroxy-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) aminocarbonyl] benzene sulfonamide and 2-chloro-5-hydroxybenzene sulfonamide. The chlorosulfuron residues occurring as conjugates must be determined since they comprised the major portion of the radioactivity observed in wheat and barley. Since, as discussed below, the analytical method does not include a step which would release chlorosulfuron metabolites from conjugates nor allows quantitation of these compounds, we will need submission of a validated method which determines metabolites along with additional residue data. A revised Section F proposing the tolerances on the subject commodities in terms of parent and metabolites of conjugates will also be needed. The petitioner should be so informed.

Since, however, only low levels of residues were observed in grain and straw, the petitioner may wish to impose a label restriction prohibiting grazing or feeding of wheat, oat, and barley forage or fodder to livestock in lieu of submitting the studies required above. If this option is desired, the petitioner should submit a revised Section B proposing the label restriction along with a revised Section F withdrawing the proposed wheat forage tolerance. The tolerance levels for wheat, oats, and barley grains and straw should be increased to 0.05 and 0.5 ppm respectively. These modifications should also be submitted in a revised Section F.

The petitioner should also be informed that any future uses of chlorosulfuron which result in the occurrence of significant residues in grain may require identification of this residue in the grains of wheat, oats, and barley.
Aninal Metabolism

Metabolism studies in rats and lactating goats were submitted in this petition. These are discussed below. An earlier rat study was submitted in conjunction with PP# 062376 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 61.14.6 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-chlorobenzene-sulfonamide 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 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 were 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$R_f$ 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 injected 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 at this time.

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 recovered.

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-hydroxychlor-sulfuron (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-hydroxychlor sulfuron (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-hydroxychlor sulfuron, 2-chlorobenzenesulfonamide, and 2-chloro-5-hydroxybenzenesulfonamide.
We do not consider the nature of the residue in ruminants and poultry to be adequately understood at this time. We will need an additional metabolism study in a ruminant.

This study should reflect consecutive daily doses of radiolabeled chlorsulfuron at high enough feeding levels so that radioactive residues in tissue and milk can be identified. This should be possible since high levels of residues were observed in rat tissue at high dosages. A poultry metabolism study is also needed. This also should reflect consecutive doses at high levels. Depending on the results of this experiment, the poultry metabolism study may obviate the need for a poultry feeding study. (See our discussion in Meat, Milk, Poultry, and Eggs section below.)

With regards to the option of withdrawing the wheat forage tolerance request, imposing a feeding restriction for wheat, oats, and barley forage, and raising the tolerance proposals for grain and straw: if this option is adopted the petitioner will need to submit only a poultry metabolism study to be carried out as discussed above. Any future use for chlorsulfuron, however, which results in detectable residues in grain and straw, will reanimate a requirement for an additional ruminant metabolism study.

**Analytical Method**

The method used to obtain residue data determines chlorsulfuron only using hplc equipped with a Tracar Model 965 photoconductivity detector. 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 cleanup 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 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 concen-
trated, 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
<65%. Control values were again all <0.01 ppm. Sample chromatograms were
submitted.

Since we have concluded above that the technical residue of concern in wheat,
oats, and barley consists of chlorsulfuron, its hydrolysis product 2-chloro-
benzenesulfonamide, and conjugates of 2-chloro-5-hydroxy-N-[(4-methoxy-6-
methyl-1,3,5-triazin-2-yl)aminocarbonyl]benzenesulfonamide and 2-chloro-5-hy-
droxybenzenesulfonamide, we do not consider a procedure which measures parent
only adequate for obtaining residue data or for enforcement purposes. The
petitioner should be informed that an adequate method which determines the
terminal residue of concern in the subject crops should be submitted along
with appropriate validation data, control values and sample chromatograms.
The validation data should reflect fortification of wheat, oats, and barley
grain and straw, wheat forage and, if needed, wheat milling fractions. The
method should include a step which releases the glucoside conjugates of chlors-
sulfuron metabolites.

With respect to meat and milk, since the metabolism of chlorsulfuron in animals
is not adequately understood at this time, we can make no final conclusions
with respect to the hplc method used to determine parent compound in meat and
milk except that we suspect that the terminal residue will consist of meta-
bolites of chlorsulfuron as well as possible conjugates. Based on this, the
petitioner should be informed that an adequate analytical method which deter-
mines all residues of concern in meat, milk, and probably poultry and eggs will
be needed.

Validation data on all four commodities as well as control values and sample
chromatograms should be submitted along with the method. The recoveries
should be consistently >65%. As above, the method should include a step which releases any conjugated residues of concern.

If the petitioner chooses to restrict the feeding of wheat, oats, and barley forage as discussed above, an analytical method which determines parent molecule only in grain and straw would then be acceptable. The present method as submitted, however, showed low recoveries and would probably require modification before we would consider it acceptable for enforcement purposes. As stated above, recoveries should be consistently >65%. With respect to meat and milk, if the forage restriction is adopted, the method submitted must still be modified in such a way that recoveries of parent compound in meat and milk are consistently >65%. This is needed because recoveries using the present form of the method were low. The method will, in all probability also have to be extended to poultry and eggs. Validation data including control values and sample chromatograms for all four commodities should be submitted.

Any future use of chlorsulfuron which results in significant residues in grain or straw will require submission of adequate methods which determine metabolites of concern as discussed above.

At such time as adequate methods for all of the commodities concerned are available and the other deficiencies in this petition are resolved, a method trial will be initiated. This method trial must be successfully completed before any tolerances for chlorsulfuron could be established. The petitioner should be so informed.

Residue Data

Residue data submitted on barley, oats, and wheat involved 26 studies 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), and Washington (2). These studies reflected application of 14.175 to 907.2 gms active ingredient/acre. These rates are 1 to 64X of the proposed use. Chlorsulfuron was applied in 11 to 50 gallons/acre using an 80% wettable powder or a 75% dry flowable formulation. Most of the data, however, is for the wettable powder as opposed to the dry flowable formulation proposed for use. It is not clear whether the two applications permitted by the label were performed or whether applications were pre- or post-emergence. Also, no indication of whether the pesticide was applied via ground or air or with a surfactant is given. We will require, therefore, submission of the raw residue data sheets in order to clarify the way in which these residue studies were performed. The petitioner should be so informed.

Residues 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.
We do not find this data adequate for the establishment of permanent tolerances in wheat, oats, and barley mainly because only parent compound was determined and we have concluded above that the terminal residue of concern consists of parent 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. Also, the lack of specific information with regards to the number of applications made, whether applications were pre- or post-emergence, whether the chlorsulfuron was applied via ground or air equipment, and whether a surfactant was employed also constitute deficiencies in the data. The raw residue data sheets for the studies already submitted may allow clarification of these points and therefore should be submitted. The formulation proposed for use on wheat, oats, and barley was used in only 2 of the studies submitted. Additional residue data, and depending on the level of residues found in grain, a wheat milling study, are needed. The residue data should provide information on all 3 crops, reflect pre- and post-emergence applications (i.e., 1 or 2 applications), the maximum proposed use rate, ground and aerial application and use of the proposed formulation and a surfactant. The method of analysis should determine chlorsulfuron, and metabolites and conjugates of concern in the subject crops. The additional data need not be as extensive as the original program given the low application rates involved with this use of chlorsulfuron. A limited number of bridging studies will be acceptable as long as they fulfill the requirements listed above. 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. Finally, when the additional data is submitted, a revised Section F proposing the tolerances in terms of parent, and metabolites/conjugates at appropriate levels should also be submitted.

The petitioner should also be informed that, if a wheat milling study is needed, the grain to be milled should contain detectable residues of chlorsulfuron/metabolites/conjugates so that if any concentration occurs in the fractions the factor can be accurately determined.

If the wheat, oats, and barley forage restriction is imposed, the residue data submitted to date will be adequate to support the tolerances to be proposed (see Conclusions 2a) on grain and straw only if the raw data sheets to be submitted resolve the deficiencies in the specific information discussed in 4a above. Limited bridging studies employing the formulation proposed for use will be needed in any event. If the deficiencies listed above for the residue data submitted to date are not resolved by the raw data sheets, additional residue studies which fulfill the requirements above and reflect determination of parent compound only will be needed. It is noted that, in the plant metabolism studies, total radioactivity in grain and straw never exceeded the 0.05 and 0.5 ppm levels, respectively, which are to be proposed in a revised Section F. When the raw data sheets/additional data are submitted, the revised Section F will also be needed.
If no detectable residues (<0.01 ppm) of chlorsulfuron are observed in wheat grain in the residue studies discussed above, no wheat milling study will be needed.

Any future use of chlorsulfuron which results in significant residues in grain or straw will reactivate all of the residue data requirements discussed above.

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. 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 glucuronide 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 should be addressed in the additional ruminant metabolism study required.

Based on the discussions above, we class this chemical in category 2 of Section 180.6(a) with respect to meat and milk and therefore tolerances on these commodities are needed. No such conclusion can be made with respect to poultry and eggs until the poultry metabolism and feeding studies required above are submitted. We suspect, however, based on the rat and ruminant studies, that this chemical will also be placed in category 2 of 180.6(a) with respect to poultry and eggs. Until such time as the metabolism of chlorsulfuron is adequately understood we can make no recommendations with respect to appropriate levels or what compounds should be included in the tolerance regulations for meat, milk, and possibly poultry and eggs. The petitioner should be informed that we will need a revised Section F proposing tolerances in these commodities at such time as the animal metabolism studies required are submitted. He should also be advised that, depending on the outcome of these metabolism studies, additional feeding studies may be needed.

If the wheat, oats, and barley forage restriction is imposed this will obviate the need for an additional ruminant metabolism study required above. Also, the poultry metabolism study required will probably suffice in lieu of a feeding study depending on the results of the experiment. If the wheat forage deletion option is adopted, a revised Section F proposing an 0.1 ppm tolerance in milk and an 0.3 ppm level in meat (including liver and kidney) should be submitted. These higher levels are needed because of the feeding study but also because of the low recoveries observed in these commodities. Appropriate tolerances for poultry and eggs, if needed, should also be proposed.

Any future use of chlorsulfuron which results in significant residues in grain and/or straw may require submission of additional ruminant and/or poultry feeding studies as well as proposals for higher/new tolerances in meat, milk, poultry, and eggs.
Other Considerations

The International Tolerance Sheet is attached. There are no Codex or foreign tolerances established for chlorsulfuron. Therefore, no compatibility questions are involved with this petition.
Chlorsulfuron scientific reviews

Page 19 is not included in this copy.
Pages _____ through _____ are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients
___ Identity of product impurities
___ Description of the product manufacturing process
___ Description of product quality control procedures
___ Identity of the source of product ingredients
___ Sales or other commercial/financial information
___ A draft product label
X The product confidential statement of formula
___ Information about a pending registration action
___ PIFRA registration data
___ The document is a duplicate of page(s) _________
___ The document is not responsive to the request

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.
INTERNATIONAL RESIDUE LIMIT STATUS

CHEMICAL: 2-chloro-4-cyano-6-[3-(4-methoxyphenyl)-2-methyl-2-propyl]-thiazole

CCPR NO: None

Codex Status:

✓ No Codex Proposal
Step 6 or above

Proposed U.S. Tolerances:

Residue: chlorantraniliprole

Crop(s) Limit (mg/kg)
none

Crop(s) Tol. (ppm)

<table>
<thead>
<tr>
<th>Crop(s)</th>
<th>Tol. (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kale, spinach</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Cabbage</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Beet, beet seed</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Melon and cantaloupe</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Meat, fish, meat by-products</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Milk</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Oat grain</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Oat straw</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Wheat grain</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Wheat bran</td>
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</tr>
<tr>
<td>Wheat straw</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

MEXICAN TOLERANCIAS

Residue:

Crop Limit (ppm)
none

Crop Tolerancia (ppm)
none

Notes: