

## **MEMORANDUM**

SUBJECT: Streptomycin. List A Reregistration Case No. 0169/Chemical ID No. 006306. Guideline 171-4(k): Magnitude of the Residue in Beans Grown from Treated Seed--Preliminary Report from Gustafson, for Potential Inclusion in the RED; CBRS Position on Data Requirements to Support SLN Registrations of Products Containing Streptomycin on Sugarbeets Grown for Seed. MRID Nos. Not Available for this Preliminary Report. CBRS No. 10453. DP Barcode No. D181855.

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## **BACKGROUND**

Streptomycin is a human antibiotic drug which is also registered for use as a bactericide to control bacterial diseases in plants. Tolerances of 0.25 ppm are currently established under 40 CFR §180.245 for residues of streptomycin in or on the food crops celery, peppers, tomatoes, potatoes, and pome fruits; products containing streptomycin are also registered for use on beans, as well as selected ornamental shrubs/trees. The residue and product chemistry chapters of the streptomycin/streptomycin sulfate Reg. Std. were issued 3/16/88; the Guidance Document for products containing streptomycin/streptomycin sulfate as the active ingredient was issued 9/88.

The Reregistration Eligibility Document (RED) is scheduled to be issued 9/92. Registered labels call for seed treatment of succulent and dry beans; however, the Agency has no data depicting streptomycin residues in beans grown from the treated seed. Data depicting streptomycin residues in or on beans, bean vines, and bean hay following seed treatment according to registered labels were required in the 9/88 Reg. Std. [Guideline Ref. No. 171-4(k)]. The registrant was requested to propose an appropriate tolerance for streptomycin in or on beans (succulent and dried), based on the results of the field trials.

Gustafson has submitted a preliminary report including the residue levels found in beans (succulent and dried), bean forage, bean hay, and bean straw grown from treated seed; field trial protocols; and preliminary storage stability results. SRRD has requested CBRS to determine if the preliminary information is adequate for inclusion of the bean uses in the RED. Furthermore,

SRRD has requested CBRS to determine what (if any) data will be required to support SLN registrations of products containing streptomycin for use on sugarbeets grown for seed.

### **CONCLUSIONS--BEANS**

1. Field Trials: The protocols submitted in the preliminary report indicate that CBRS should have no problem with this portion of the residue study, provided the following apply:
  - a) The seed was in fact treated at the maximum allowable rate.
  - b) The Agency receives complete descriptions of field residue trials (with adequate documentation) in the final report.
  - c) Locations of field trials were obtained in the analytical portion of the report. These constituted **adequate geographic representation for succulent and dry beans**.
  
2. Analytical Method: The modified cylinder plate method used to determine streptomycin residues in bean matrices is acceptable for data collection, even though highly variable recoveries (average = 49%) were obtained from fortified samples.
  - a) The registrant stated that variable recoveries are typical of this type of assay, due to the inherent variability of the method. The registrant must submit additional information regarding the high recoveries obtained from the dry bean samples; a brief explanation will be considered confirmatory information.
  - b) Supporting raw data and calculations were submitted by the registrant, and are acceptable.
  - c) While it is the only method available for quantitation of streptomycin residues at this time, CBRS is not confident as to the accuracy of the streptomycin residue results.
  - d) Based on the data submitted for succulent beans, the detection limit of 0.25 ppm for this matrix is questionable. Due to high background, the detection limit for succulent beans should be 0.5 ppm.
  
3. Residues in Beans:
  - a) There were no streptomycin residues above the 0.25 ppm detection limit in dry beans/forage/hay/straw. Therefore tolerances should be established at 0.25 ppm for streptomycin residues in or on dry beans, forage, and hay as a result of seed treatment, assuming the final report corroborates this conclusion.

- b) Since residue data are translatable from beans, forage, and hay, CBRS will not require any residue data for streptomycin residues in or on cannery waste; no concentration of residues is expected upon processing of beans to cannery waste.
  - c) Residues in succulent beans were above the 0.25 ppm detection limit, but did not differ statistically from residues detected in the untreated succulent beans. Consequently, CBRS considers the residues detected in the succulent beans to be related to background inherent to succulent beans analyzed using the modified cylinder plate method.
  - d) Compliance problems could result from the higher background for streptomycin residues in or on succulent beans. Consequently, a tolerance for streptomycin residues should be established at the 0.5 ppm detection limit for succulent beans, assuming the final report corroborates this conclusion.
4. Storage Stability: CBRS is unable to make any conclusions regarding the storage stability of streptomycin residues in or on beans based on the submitted preliminary data. The issue of stability of streptomycin residues in or on beans will be revisited upon receipt of the final report. These data will be considered confirmatory in nature.

#### **CONCLUSIONS--SUGARBEETS**

1. The current SLN registration, OR850037, calls for foliar treatment of sugarbeets grown for seed. Use restrictions prevent any livestock/human exposure to treated plants/seeds. The foliar application rate is 50 to 200 ppm, 250 times less than the labeled seed treatment rate for beans.
2. Based on the bean data, the dramatic difference in application rates, the great interval between seed crop treatment and root crop harvest, dilution effects, and label restrictions, no tolerances or supporting residue data are required to support the SLN registration for streptomycin on sugarbeets grown for seed, i.e. CBRS considers this a nonfood use.

## **RECOMMENDATION**

CBRS recommends that, provided all confirmatory data and additional storage stability data are provided by the registrant, a tolerance of 0.5 ppm be established [40 CFR §180.245] for streptomycin residues in or on succulent beans grown from treated seed. Tolerances of 0.25 ppm should be established for streptomycin residues in or on dry beans, bean forage, and bean hay grown from treated seed. No tolerances or data depicting streptomycin residues in bean cannery waste are required. No residue data are required to support the SLN registration for streptomycin on sugarbeets grown for seed, which is considered to be a nonfood use.

CBRS has no problem with the inclusion of the bean seed treatment in the RED; however, since the data are extremely preliminary, an amendment may be necessary when the final data are reviewed. If SRRD would have a problem with an amendment, it may be preferable to issue the RED without the bean uses reregistered.

## **DETAILS: BEANS**

### **Succulent and Dry Beans, Field Residue Trials**

The documentation from the field residue trials was not included in the preliminary reports. In addition, there were no summaries of the field trials provided. However, the report did include the Gustafson field residue trial protocols (Succulent Beans, 171-4/AS90-BS; Dry Beans, 171-4/AS90-BD).

### **Succulent Bean Protocol**

Succulent bean seed should be treated at a rate of 0.028 lb ai/100 lbs of seed, the maximum rate allowed on registered labels. The test substance is applied as a 5% water slurry, using appropriate seed treatment equipment. Captan-300 and Lorsban (chlorpyrifos) may be applied as needed. Lots of treated seed should be tested for homogeneity; documentation of the seed treatment, including lot numbers, treatment dates, etc. should be retained for Gustafson. Succulent bean field trials will be conducted at single sites in the following locations: Pacific Northwest, CA, NY, WI, and VA [analytical results submitted in the preliminary report show that field trials were actually conducted in CA, CO, MI, ND, NY, and OR]. Single control and replicate treatment plots should be 4 rows wide, and 40 ft in length. Forage (entire above ground plant with pods) will be harvested 45 days after planting. Entire pods with beans will be harvested at maturity; bean hay will be harvested (entire above ground plant without pods) at maturity. For forage, beans, and hay, the entire harvested samples, taken from at least 12 random locations within the plot, will be composited, and then the 2.5 lb sample will be obtained randomly. Samples should be frozen as soon as possible after harvest, and kept frozen during shipping. All records from the field trial should be retained for Gustafson.

## Dry Bean Protocol

The treatment rate for dry beans is identical to that of succulent beans, 0.028 lb ai/100 lbs of seed. The seed treatment is identical to that of the succulent beans. Field trials are to be conducted in the following locations: Pacific Northwest, CA, NY, ND, MI, and CO [analytical results submitted in the preliminary report show that field trials were actually conducted in CA, CO, MI, ND, NY, and OR]. Plot sizes are identical to those of the succulent beans. Forage (entire above ground plant with pods) will be harvested 45 days after planting. Shelled beans without pods and bean straw (entire above ground plant without pods) will be harvested at maturity. All samples will be harvested from at least 12 random locations within the plot, and composited to obtain the specified 2.5 lb samples.

## CBRS Comment

Provided the protocol has been followed closely by the cooperators, and the seed was indeed treated at the maximum allowable rate, CBRS can tentatively approve the field trial portion of the study, pending receipt of confirmatory field trial reports (including sample harvest dates), to be submitted along with the remainder of the storage stability data. The actual locations of the field trials constitute adequate geographic representation for succulent and dry beans.

## **Analytical Method/Bean Residue Data**

The analytical portion of the preliminary reports was conducted by Microbiological Associates, Inc. of Rockville MD. The method used to determine streptomycin residues in beans (succulent, dry, forage, hay, and straw) was the modified cylinder plate assay, as described by Grove and Randall (1955). Agar plates are overlaid with an additional agar layer containing *Bacillus subtilis* spore culture. On each plate, six dosing cylinders are placed at 60° intervals on a 2.8 mm radius. Each sample or dose level of the streptomycin standard was plated in triplicate, with 3 replicates/plate in 3 alternate cylinders. The remaining cylinders were dosed with 1.0 µg/ml streptomycin. All dosing aliquots were 200 µl. Plates were incubated for 24 ± 4 hours. Following the incubation, zones of inhibition were measured to the nearest 0.1 cm. Irregular shaped zones were measured on an alternate axis, if possible. The grand mean of the 1.0 µg/ml reference zone of inhibition (standard) was determined from the replicate plates. Zones of inhibition for samples and remaining standards were adjusted for the difference between the 1.0 µg/ml zone and the grand mean, in order to compensate for plate-to-plate variability. A standard curve was obtained using least squares regression of the results obtained from standards ranging from 1.0 to 4.0 µg/ml.

Bean samples were extracted by homogenization in a 0.1 M phosphate buffer, followed by shaking on the "high" setting for 30 minutes. The pH was adjusted to 7.8 ± 0.02, and then a 20 ml aliquot was taken and centrifuged; the supernatant was filtered through a 0.45 µm filter to remove contaminating organisms, and the remaining filtrate and homogenate were refrigerated for analysis. To obtain the greatest level of consistency and reproducibility, all of the succulent bean samples (from all 6 sites) were analyzed on the same date and in the same assay. The same was true for forage, hay, dry beans, and straw. All residue values for streptomycin were

calculated based on the assumption that all of the streptomycin was extracted from the bean matrix; streptomycin residues in ppm were calculated taking into account the amount of liquid obtained from the extract. Control samples were fortified at 1.0 and 2.0 µg/ml, in order to obtain recoveries for each assay. Overall recoveries were roughly 49 - 50%, which is typical for this type of assay; similar recoveries were obtained in assays used to generate residue data for other crops which were reviewed in the residue chemistry chapter of the Reg. Std. Dry beans tended to have unusually high recoveries. In general, CBRS is satisfied with these recoveries, given the nature of this biological assay. While it is the only method available for quantitation of streptomycin residues at this time, CBRS is not confident as to the accuracy of the streptomycin residue results.

Recoveries from all bean matrices are presented in Table 1; succulent bean residue results are shown in Table 2, while dry bean residue data are in Table 3.

**Table 1. Succulent/Dry Bean Recovery Data<sup>1</sup>**

Fortification	Recoveries: Succulent Bean Trial			Recoveries: Dry Bean Trial		
	Bean	Hay	Forage <sup>2</sup>	Bean <sup>3</sup>	Forage	Straw
1.0 µg/ml	44	42	61/83	90/184	43	85
	32	40	37/61	92/157	52	47
	24	35	55/73	107/178	34	45
2.0 µg/ml	45	43	50/74	95/198	102	92
	69	48	59/74	95/186	69	89
	62	55	53/72	107/192	47	65

<sup>1</sup> All values are reported as % recovery.

<sup>2</sup> Recoveries were verified; both values were reported.

<sup>3</sup> Recoveries were verified; the registrant cited the inherent variability of the assay as the reason for the high/variable recoveries from dry bean.

**Table 2. Streptomycin Residues in Succulent Bean/Forage/Hay\***

State	Beans		Forage		Hay	
	Control	Sample	Control	Sample	Control	Sample
CA	0.22	0.15 - 0.25 (2)	0.18	0.21 - 0.28(2)	0.11	0.11 - 0.12(2)
FL	0.30	0.31 - 0.33 (2)	0.11	0.14 - 0.15 (2)	0.18	0.13 - 0.18(2)
VA	0.41	0.30 - 0.33 (2)	0.20	0.18 - 0.19 (2)	0.12	0.12 - 0.15(2)
NY	0.28	0.25 - 0.28 (2)	0.21	0.23 - 0.24 (2)	0.12	0.12(2)
OR	0.38	0.30 - 0.39 (2)	0.20	0.16 - 0.20 (2)	0.13	0.08 - 0.13(2)
WI	0.23/0.36	0.25 - 0.46 (3)	0.16 - 0.22	0.14 - 0.25 (4)	0.05/0.13	0.05 - 0.13(3)

\* Residues are reported in terms of ppm streptomycin, calculated from the concentration of streptomycin in the extract, multiplied by the volume of the extract, and divided by the weight of the sample extracted. The range of values reported, as well as the number of values included in the range, is presented in the table. The in front of all values indicates that the assay does not yield precise residue levels.

**Table 3. Streptomycin Residues in Dry Beans/Forage/Straw\***

State	Dry Beans		Forage		Straw	
	Control	Samples	Control	Samples	Control	Samples
CA	0.19	0.14 - 0.16 (2)	0.17/0.20	0.16 - 0.20 (3)	0.12	0.11 - 0.15 (2)
CO	0.13/0.14	0.14 (4)	0.15	0.17 - 0.19 (2)	0.09	0.12 - 0.15 (2)
MI	0.11	0.09 - 0.11 (2)	0.22	0.17 - 0.19 (2)	0.10	0.10 - 0.14 (2)
ND	0.16	0.09 - 0.10 (2)	0.18	0.18 - 0.20 (2)	0.12	0.05 - 0.09 (2)
NY	0.14	0.13 - 0.14 (2)	0.18	0.15 - 0.16 (2)	0.10	0.08 - 0.09 (2)
OR	0.16	0.17 (2)	0.20	0.16 - 0.20 (2)	0.07/0.12	0.09 - 0.10 (3)

\* Residues are reported in terms of ppm streptomycin, calculated from the concentration of streptomycin in the extract, multiplied by the volume of the extract, and divided by the weight of the sample extracted. The range of values reported, as well as the number of values included in the range, is presented in the table. The in front of all values indicates that the assay does not yield precise residue levels.

## CBRS Comment Regarding Streptomycin Residues in Beans

CBRS is concerned that the level of streptomycin residues in succulent bean samples was much higher than residues detected in either the forage or the hay of succulent beans, or in any of the treated dry bean matrices. A statistical comparison of residues found in the succulent bean control samples and residues in the treated succulent bean samples indicates that there is no statistical difference between residue levels in treated and untreated samples. However, this information calls into question the adequacy of the 0.25 ppm detection limit for succulent beans, especially when the variability of the method is taken into consideration. For succulent beans, the detection limit appears to be much higher than 0.25 ppm due to some sort of background effect, possibly due to the large quantity of liquid extracted from the succulent beans relative to other bean matrices, an inhibitory substance in bean fruit, or antibiotic production on succulent beans by epiphytic microbes. The 0.25 ppm detection limit appears to be adequate for all other bean matrices (hay/straw/forage/dry beans). Since the registrant has provided adequate data for streptomycin residues in all bean RACs, CBRS will translate these residue data to bean cannery waste; no concentration in cannery waste is expected.

Since there is no statistical difference between the treated and untreated succulent bean residue levels, CBRS concludes that there are no detectable streptomycin residues in or on the succulent beans resulting from pesticidal applications of streptomycin [CBRS conclusion regarding statistical relationship between residues in untreated and treated succulent beans was reached following personal communication with Maureen Clifford, HED/SAB, 8/31/92]. However, since the detection limit in succulent beans is 0.5 ppm, rather than the typical 0.25 ppm, succulent bean samples could be erroneously flagged during compliance monitoring. In order to prevent such occurrences, CBRS recommends that, provided all confirmatory data and additional storage stability data are provided by the registrant, a tolerance of 0.5 ppm be established [40 CFR §180.245] for streptomycin residues in or on succulent beans grown from treated seed. In addition, tolerances of 0.25 ppm should be established [40 CFR §180.245] for streptomycin residues in or on dry beans, bean forage, and bean hay grown from treated seed.

### **Storage Stability--Preliminary Results**

The preliminary storage stability results include stability data for dry beans/forage/straw for up to 4 weeks, and succulent bean/forage/hay stability data for up to 8 weeks. These data are presented in Table 4.

**Table 4. Storage Stability of Streptomycin Residues in Beans<sup>1</sup>**

Week	Fortification Level <sup>2</sup>	Succulent Beans			Dry Beans		
		Beans	Forage	Hay	Beans	Forage	Straw
0	Low	76	18	17	130	46	20
	High	88	24	21	123	63	83
2	Low	137	100	100	119	72	80
	High	125	118	114	119	57	68
4	Low	37	21	38	152	38	19
	High	70	53	37	171	74	74
8	Low	86	21	36	--	--	--
	High	74	58	51	--	--	--

<sup>1</sup> All values presented in the table represent % recovery from the fortified sample.

<sup>2</sup> The low fortification level was approximately 1.0 µg/ml, while the high fortification was approximately 2.0 µg/ml.

CBRS Comment

The submitted data are preliminary results of the storage stability study. CBRS is unable to make any conclusions regarding the storage stability of streptomycin residues in or on beans based on these data. Assays performed during week 2 all had high recoveries. The remainder of the assays exhibited the typical range of variability; both low and high recoveries were found, but did not indicate any trend/loss of stability. The issue of stability of streptomycin residues in or on beans will be revisited upon receipt of the final report. These data will be considered confirmatory.

**DETAILS--SUGARBEETS**

The LUIS report identified a special local need 24(c) registration for streptomycin-containing products on sugarbeets grown for seed. SRRD has requested CBRS to determine if residue data should be required to support the SLN registration. CBRS has examined the label of SLN OR850037, which calls for the use of Agri-Strep Type D, EPA Reg. No. 618-28, on sugarbeets grown for seed. Under the SLN label, the sugarbeets may be treated foliarly with 50 - 200 ppm streptomycin in early spring or fall. Repeat applications may be necessary, if conditions favor the spread of the bacterial blight. A use restriction states that following

treatment with Agri-Strep, do not graze or cut green forage or hay for livestock feed. Do not feed aftermath or screenings to livestock.

Sugarbeets are treated foliarly at a much lower rate than the seed treatment used on beans (50,000 ppm). CBRS would expect streptomycin residues in beans grown from treated seed to be much higher than residues in sugarbeets grown from seed harvested from treated plants. Since residues in beans were generally nondetectable, and since beans were treated at a rate 250 time higher than sugarbeets (assuming that all of the applied streptomycin ended up in the sugarbeet seed), CBRS would not expect to find detectable residues of streptomycin in or on sugarbeets grown from treated seed. Use restrictions assure that there will be no dietary exposure to streptomycin via the treated plants. Based on the bean data, the dramatic difference in application rates, the great interval between seed crop treatment and root crop harvest, dilution effects, and label restrictions, no residue data are required to support the SLN registration for streptomycin on sugarbeets grown for seed, i.e. CBRS considers this use to be a nonfood use requiring no tolerances.

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